FAO Symposium on

“The role of agricultural biotechnologies for production of bio-energy in developing countries”

ETHANOL PRODUCTION VIA ENZYMATIC HYDROLYSIS OF SUGAR-CANE BAGASSE AND STRAW

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Presentation Outline

• Overview of the Current Brazilian Ethanol Production

• Biomass Ethanol

• The BIO-ETHANOL Project - Ethanol Production from Sugarcane Biomass via Enzymatic Hydrolysis
Main Crops in Brazil

*Brasil*: 851 x 10^6 hectares

- Soya: 21.5
- Corn: 12.3
- Sugar cane: 5.6

**Area [10^6 ha]**

- Paraná: 20.0 x 10^6 ha
- Paraíba: 5.7 x 10^6 ha
- Ceará: 14.6 x 10^6 ha
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 until 2010

70,000 farmers produced 428 million tons of sugarcane (2006/2007 harvest)

The amount of dry bagasse annually produced by the ethanol and sugar industry is of 64 million tons

Refineries made 4 billion gallons of alcohol fuel.

Ethanol production replaces 460 million barrels of oil.
Sugar-cane Plantation

Harvest

Non mechanized → Mechanized → Straw
Straw and/or Bagasse $\rightarrow$ ETHANOL FROM AGROINDUSTRIAL RESIDUE
Sugar Cane Bagasse Composition

Cellulose
~37%

Hemicellulose
~28%

Lignin
~21%

→ C\textsubscript{\textit{6}} Sugars for ETHANOL
(Renewable liquid fuel)

→ C\textsubscript{\textit{5}} Sugars for BIO-REFINERIES
or ENZYMES PRODUCTION

→ Poly aromatic hydrophobic structure
(Renewable Solid Fuel)
Presentation Outline

• Overview of Ethanol Production from Sugar-cane (sucrose)

• Biomass Ethanol

• The BIO-ETHANOL Project - Ethanol Production from Sugar-cane Biomass
No longer oil....biomass!
CELLULAR STRUCTURE OF BIOMASS

Source: Himmel et al. in collaboration with the CSM EM Facility (2004)
Cell wall structure

Buffer treated corn stover

Enzyme treated corn stover

Enzymatic hydrolysis of cellulose to glucose

Note: zone around vascular bundle is eroded compared to native (suggests enzymes leak through pores in bundle)
1st Challenge: Cell Wall Recalcitrance

- Lignocellulose cell walls contain *intermeshed* carbohydrate and lignin polymers and other minor constituents

- The major structural polymers – cellulose, hemicellulose, and lignin – exhibit differential reactivity to thermal, chemical, and biological processing

- By natural design, cell wall polysaccharides are more difficult to break down than storage carbohydrates like starch
CELL WALL STRUCTURE

Lignin

Hemicellulose

Cellulose
Bioconversion of cellulose to ethanol

- Pre-treatment
- Cellulases Production
- Enzymatic Hydrolysis
- Ethanol Fermentation
Enzymes: Endoglucanases e Exoglucanases
Presentation Outline

• Overview of the Current Ethanol Production: Sucrose Sugarcane

• Biomass Ethanol

• The BIOETHANOL Project - Ethanol Production from Sugar-cane Biomass
A RESEARCH NETWORK has been organized to develop in Brazil the technology for the conversion of the sugarcane biomass (bagasse and straw) into fuel ethanol. The hydrolysis of the lignocellulose biomass to release the C₆ fermentable sugars will be carried out via enzymatic hydrolysis.
Brazilian Institutions / International Collaboration

• 14 Brazilian Universities: UNICAMP/Campinas, USP/Lorena, USP/São Paulo, UFRJ/Rio de Janeiro, UENF/Campos, UEM/Maringá, UnB/Brasília, UCS/Caxias do Sul, FURB/Blumenau, UFPE/Recife, UFPB/João Pessoa, UFPR/Curitiba, UFG/Goiânia, UFSc/Florianópolis

• 2 Research Centers: (IPT/São Paulo and INT/Rio de Janeiro)

• Center for Sugarcane Technology Development - CTC/Piracicaba (congregates around 100 sugar and ethanol industries)

• CENBIO (National Centre for Biomass Development)/São Paulo

Foreign Collaboration

• LUND UNIVERSITY - Sweden

• The project is open to the establishment of new collaborations.
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
ETHANOL FROM BIOMASS

Bagasse or Straw → Pre-treatment → Enzymatic Hydrolysis → GLUCOSE

Pentoses (hemicellulose) Bio-Refineries/Enzyme Production

Enzyme

Lignin (Solid Residue)

Sucrose Juice or Molasses → Ethanol Fermentation → Distillation → Ethanol

Sucrose Ethanol

Ethanol Fermentation

Stillage

Yeast
Steam Pre-treatment
Steam Pre-treatment

Fig. 1. Schematic of goals of pretreatment on lignocellulosic material
Sugar-cane Bagasse

Sugar-cane Bagasse

Treated Sugar-cane Bagasse
Enzymes Production for Biomass Ethanol - Principles

- Enzyme cost contribution and effectiveness depends on the biomass source and pre-treatment conditions.
- Development of “tailored made” enzyme blends for sugar-cane biomass.
- Use of crude “cellulase/xylanase/accessory enzymes” preparations.
- “In house” production to reduce the enzymes cost.
Cellulolytic enzymes are mainly produced by various microorganisms:

- Aerobic bacteria
- Anaerobic bacteria
- Fungi
- Actinomycetes
- Clostridium
- Aspergillus
- Trichoderma
Studies on Cellulolytic/Xylanolitic Enzymes

- Enzyme production using fed batch fermentation
- Enzyme production using semi-solid fermentation
- Production and use of inducers (sophorose)
- Selected genes cloning and expression
- Microorg. and genes identification and preservation
- Culture supernatants concentration
- Enzyme activity stabilization
- Evaluation of the effectiveness of different enzyme blends
- Enzymes chemical and biochemical characterization
Cellulase production

Cellulases production

BIOETHANOL cellulases blend
Enzymes Production and Uses
Industrial application of cellulases

The major challenge: to convert, economically, biomass residues into glucose!
Enzymatic Hydrolysis Experiments

- 130 g/L of treated sugar-cane bagasse
- Enzyme blend
- Sodium Citrate Buffer pH 4.8
- Temperature 45ºC
- Agitation 200 rpm
Enzymatic Hydrolysis Kinetics

Bagasse concentration = 130 g/L (dry weight)

% cellulose in bagasse = 55%

Cellulose hydrolysis yield using commercial enzyme: 61%

Cellulose hydrolysis yield using BIOETHANOL enzyme = 90%
Sugar Syrups and Lignin

Treated bagasse + BIOETHANOL Enzymes

Glucose Syrup (65 g/L)

Ethanol fermentation

Residue: Lignin

Burning for Energy
Lignocellulosic hydrolysate

Effects of lignocellulosic inhibitors on the yeast cells (*Saccharomyces cerevisiae*).

Ethanol fermentation

- *Saccharomyces cerevisiae* industrial strain
- Hydrolyzate sugar syrup – 50 g/L glucose
- Inoculum: 200 mg cell dry weight/15 mL

**Experiments:**

- Growth medium (400mM total sugar)
  - *Biomass sugar syrup*
  1. Sugar mollases
  2. Yeast extract dextrose
Ethanol fermentation

Ethanol quantification
### BAGASSE PRODUCTION AND AVAILABILITY/YEAR (KG)

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sugarcane production</td>
<td>428.000.000.000</td>
</tr>
<tr>
<td>Total bagasse production (30% of sugarcane - 50% moisture)</td>
<td>128.400.000.000</td>
</tr>
<tr>
<td>Total dry bagasse production</td>
<td>64.200.000.000</td>
</tr>
<tr>
<td>Surplus bagasse (12% dry bagasse)</td>
<td>7.700.000.000</td>
</tr>
</tbody>
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### THEORETICAL ETHANOL YIELD FROM SUGAR-CANE BIOMASS CELLULOSE

- **1 Kg of bagasse:** 0,24L
- **7.700.000.000 Kg of bagasse:** 1.849.000.000 L

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**Enzyme cost contribution**

Enzyme load: 10 FPU (filter paper unit) per gram of bagasse  
Industrial Enzyme: 140,000 FPU/L

<table>
<thead>
<tr>
<th>Enzyme price (US$/L)</th>
<th>Enzymes cost contribution per L of ethanol (US$/L)</th>
<th>Enzyme market (US$/year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7,00 (FOB price)</td>
<td>2,03</td>
<td>2,290,000,000</td>
</tr>
<tr>
<td>0,17</td>
<td>0,05 *</td>
<td>56,400,000</td>
</tr>
</tbody>
</table>

* Target enzyme cost contribution*
Summary of results: BIOETHANOL Project

- Environmental friendly steam based pre-treatment (no use of chemicals)
- Efficient BIOETHANOL enzyme blend
- Biomass hydrolysate in high sugar yields – concentration will be improved further
- The C₆ sugars syrup are fermentable by usual industrial *Saccharomyces cerevisiae* strain (no GMO)
- The syrup composition favors ethanol yield
On going work

Pilot Plant

- Biomass steam pretreatment
- "In house" enzymes production
- Enzymatic biomass hydrolysis
- Syrup blend to sugar cane juice or molasses
- Current fermentation process
- Use of lignin as fuel
The historically old *Saccharomyces cerevisiae*!
BIOETHANOL RESEARCHERS
Thank You!