FEDERAL UNIVERSITY OF RIO DE JANEIRO
SCHOOL OF CHEMISTRY
LABORATORY OF BIOPROCESS DEVELOPMENT

NEXT GENERATIONS BIOFUELS AND THE CONCEPT OF BIOREFINERY: EMERGING AND FUTURE-BEARING TECHNOLOGIES

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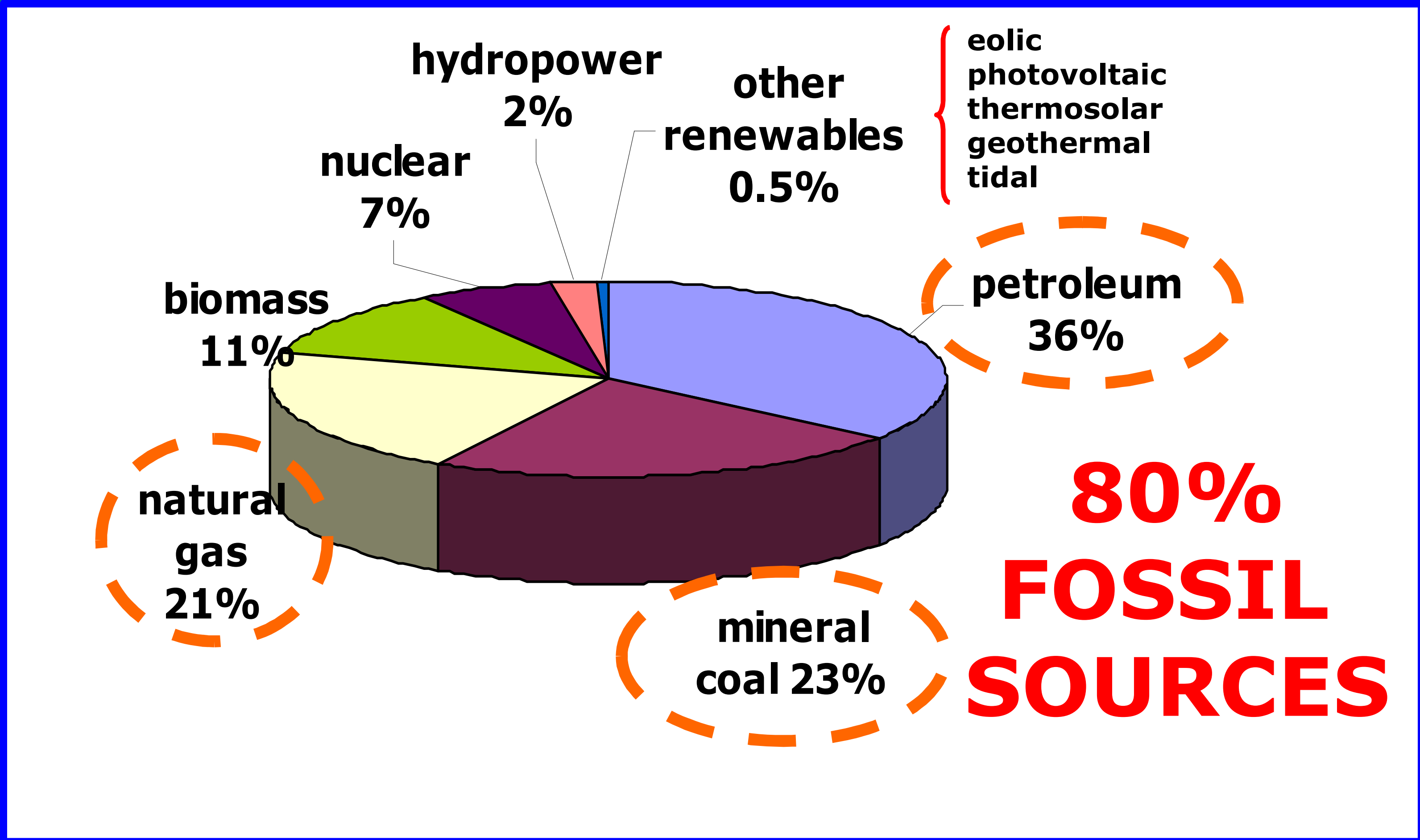
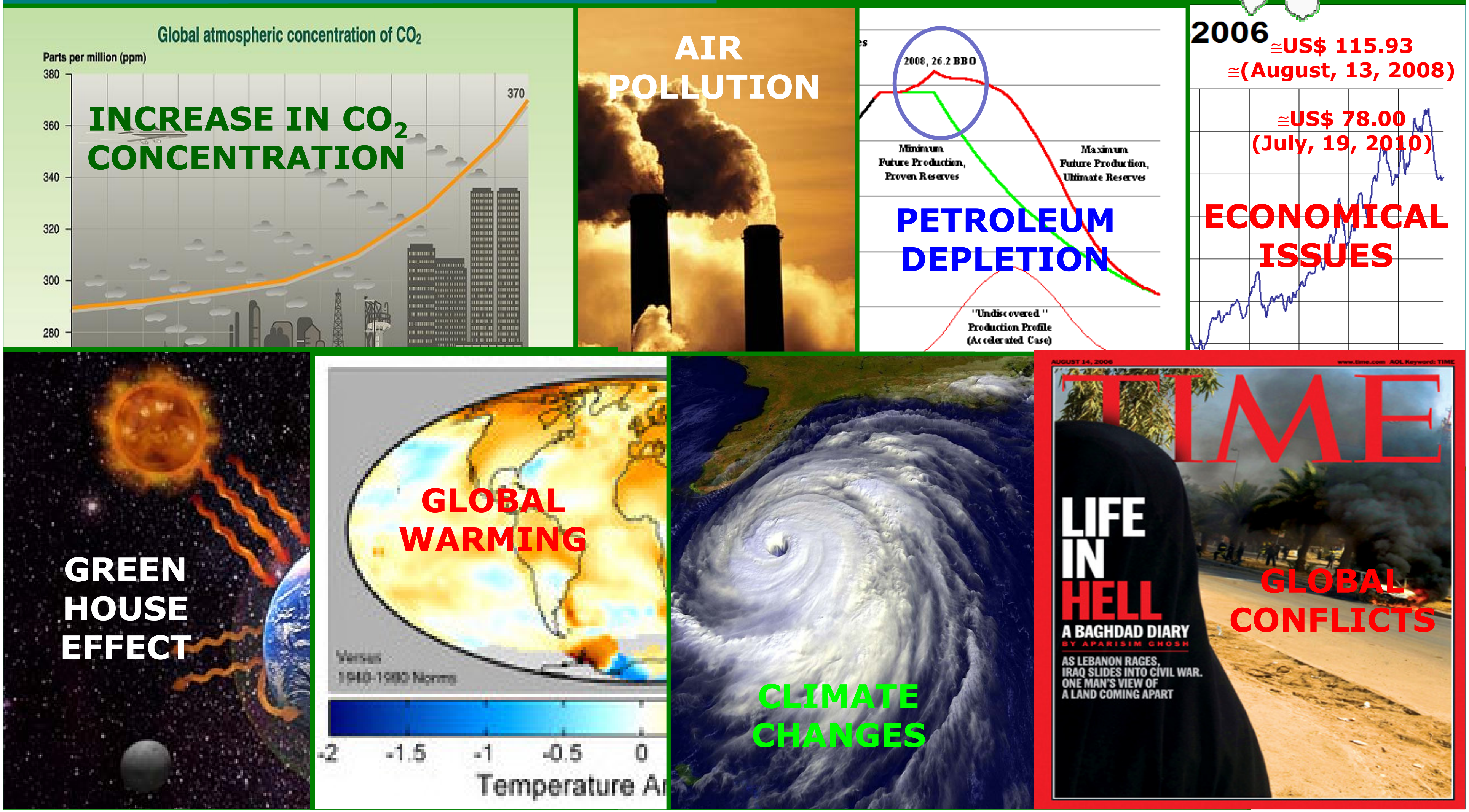
World Demand of Energy

Growth rate 2.3%/yr



Source: MAPA (2007)

SCENERIES ASSOCIATED WITH FOSSIL SOURCES



PARADIGM SHIFT

RETHINK OUR ENERGY MATRIX AND FEEDSTOCK SOURCES

⬇

SEARCH FOR ALTERNATIVE AND RENEWABLE SOURCES LESS POLLUTANT

⬇

DIVERSIFY THEIR USE

⬇

REDUCE OUR DEPENDENCY ON FOSSIL SOURCES

Source Share of Primary Energy in Brazil (2005)

Renewable	%	Non-renewable	%
Hydropower	14.4	petroleum	39,1
Sugar cane	13.5	Natural gas	8.9
Wood and vegetal coal	13.2	Mineral coal	6.7
Others	2.7	Nuclear	1.5
Total	43.9		56.1

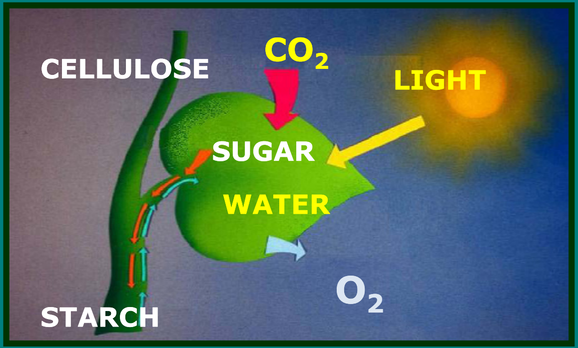
THE COUNTRY HAS ONE OF THE CLEANEST ENERGY MATRIX IN THE WORLD

Fonte: BEN/MME

The Brazilian Context

BIOMASS is an attractive candidate as an alternative to petroleum, since:

- **The only abundant source of renewable carbon;**
- **Lower CO_2 footprint than petroleum;**
- **Domestically available.**



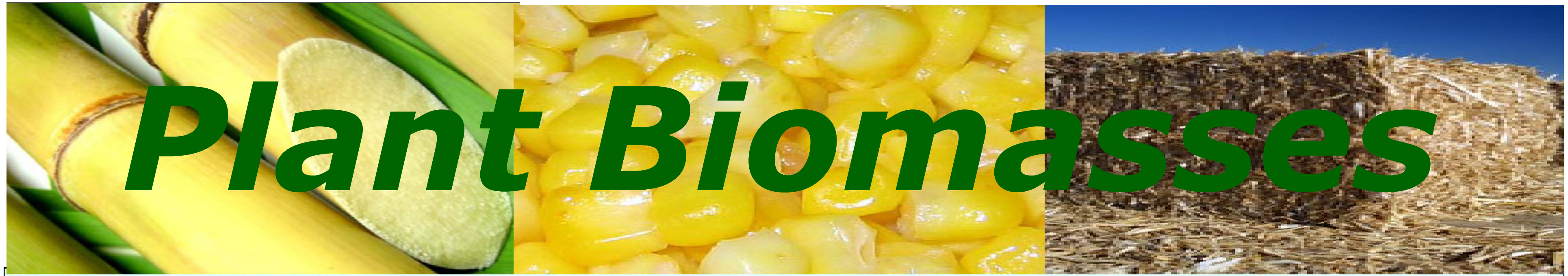
What are Biomasses and Why to use them in substitution and/or in association to fossil sources ?

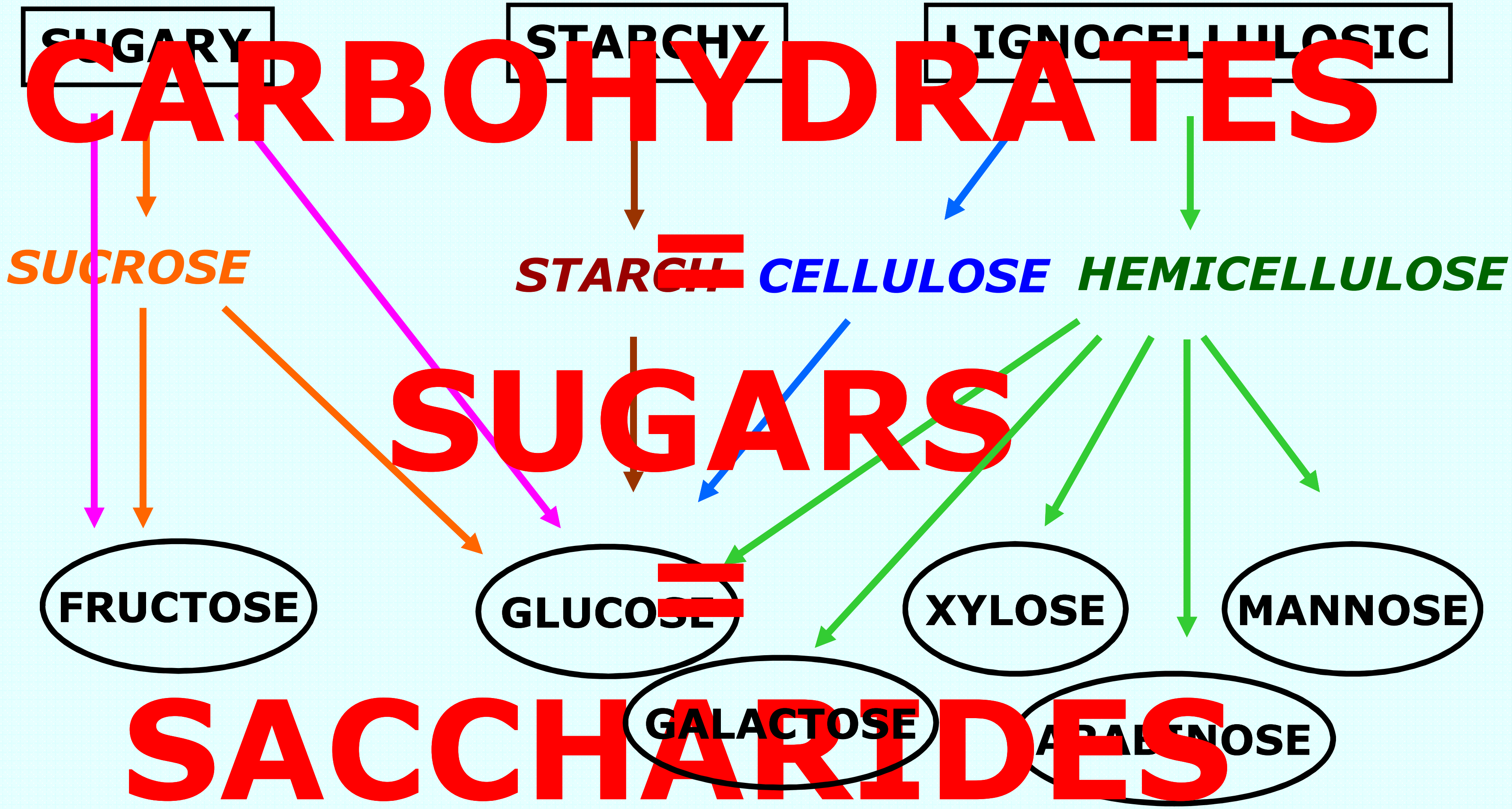
- ***ORGANIC MATTER*** from ***PLANT*** or ***ANIMAL*** sources*;*
- Direct or indirect origin from the ***PHOTOSYNTHESIS PROCESS***;
- They are ***RENEWABLE***.

Plant Biomass

- ***Natural Biomass***
- ***Food Biomass***
- ***Energetic Plantation Biomass***
- ***Residual Biomass***

Plant Biomasses





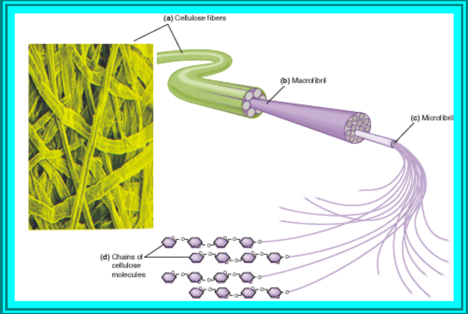
CARBOHYDRATES

=

SUGARS

=

SACCHARIDES



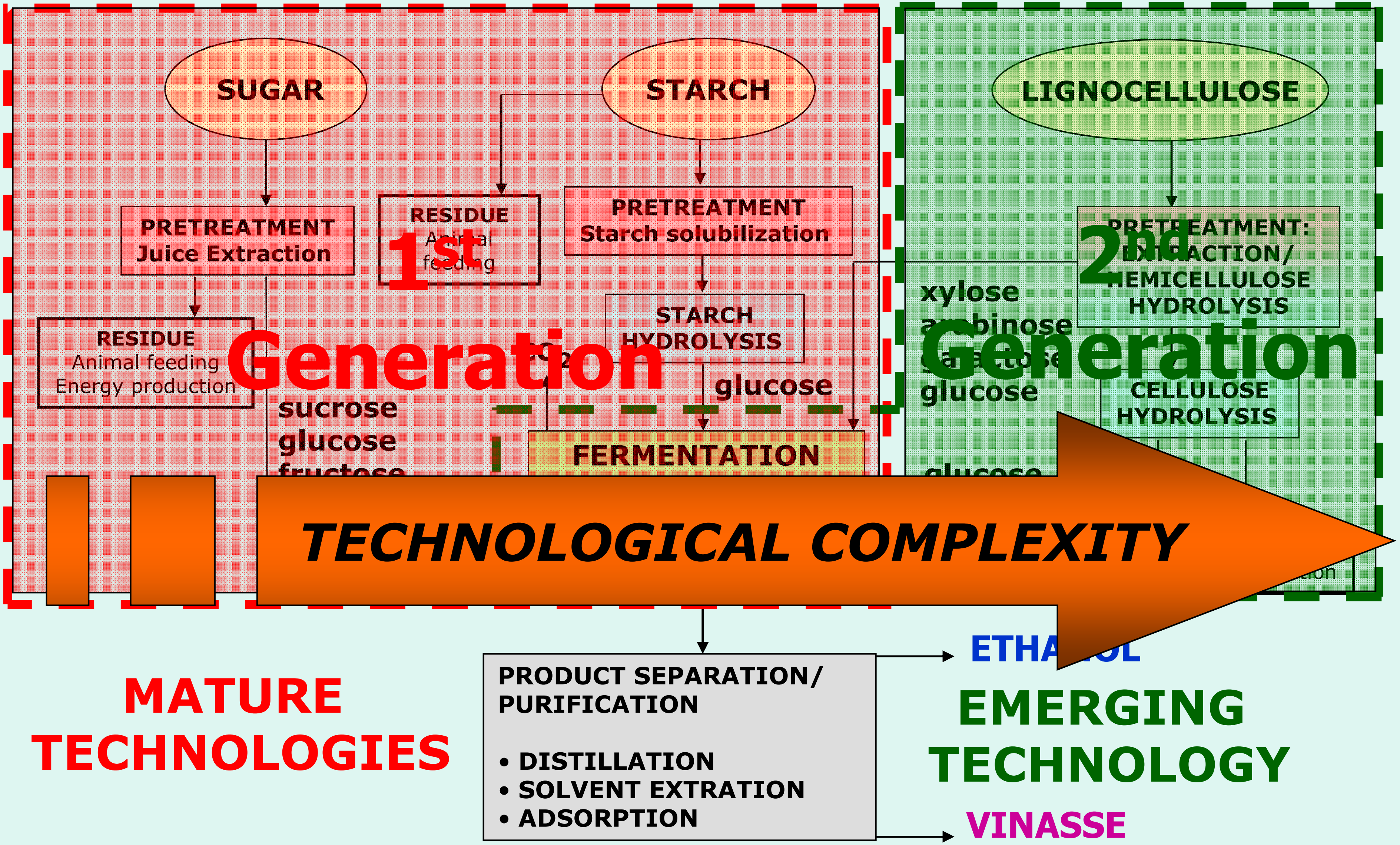
Composition of Some Lignocellulosic Residues

	Corn cobs	Wheat straw	Rice straw	Sugar cane bagasse	Cotton seed	Newspaper	Urban residues
Carbohydrate (%)							
Glucose	39.0	36.6	41.0	**38.1**	20.0	64.4	40.0
Mannose	0.3	0.8	1.8	**n.d.**	4.1	16.6	8.0
Galactose	0.8	4.4	0.4	**1.1**	0.1	n.d.	n.d.
Xylose	14.8	19.2	14.8	**24.3**	4.6	4.6	14.0
Arabinose	4.2	4.4	4.5	**4.5**	4.3	0.5	4.0
Non-carbohydrate (%)							
Lignin	15.1	14.5	9.9	18.4	17.6	21.0	20.0
Ashes	4.3	9.6	4.4	4.8	14.8	0.4	1.0
Proteins	4.0	4.0	n.d.	4.0	4.0	n.d.	n.d.

~ 70% carbohydrate

Source: Lee (1997)

TECHNOLOGIES FOR ETHANOL PRODUCTION FROM BIOMASSES



ETHANOL AND ITS DIFFERENT GENERATIONS

- **1st GENERATION ETHANOL** → **SUGAR CANE JUICE & GRAINS/CEREALS**

MATURE TECHNOLOGIES

☹ **Fuels *vs* Food.**

- **2nd GENERATION ETHANOL** → **LIGNOCELLULOSIC BIOMASS**

EMERGING (GROWING) TECHNOLOGIES

☺ **Does not compete with food production.**

- **3rd GENERATION ETHANOL** → **ALGAE BIOMASS**

FUTURE BEARING TECHNOLOGIES

☺ **Faster growth than traditional crops;**

☺ **Does not compete with agricultural cultures.**

Production Costs of Bioethanol with Different Technologies

1. Sugar Cane Ethanol (Brazil)

Sugar Cane Juice → **Fermentation** → Distillation

0.28 US$/L

2. Corn Ethanol (USA, others)

Corn Starch → Cooking → **EH + Fermentation** → Distillation

0.55 US$/L

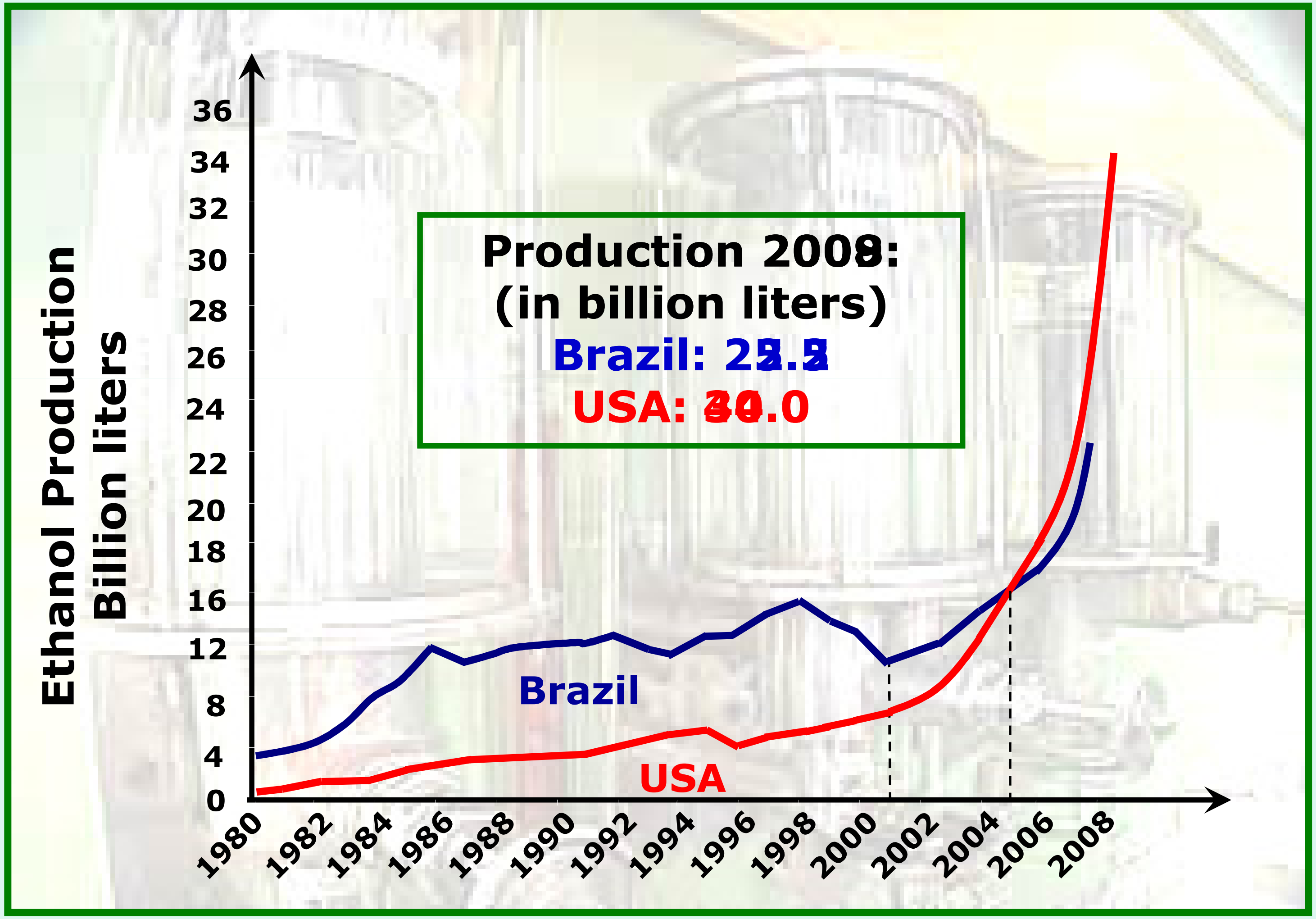
3. Ethanol from Lignocellulosics

Lignocellulosic Biomass → Diluted Acid Pretreatment → **Cellulose + Cellulases + Hemicellulose Hydrolysate FERMENTATION** → Distillation

NREL Estimates ≅ 0.52-0.80 US$/L

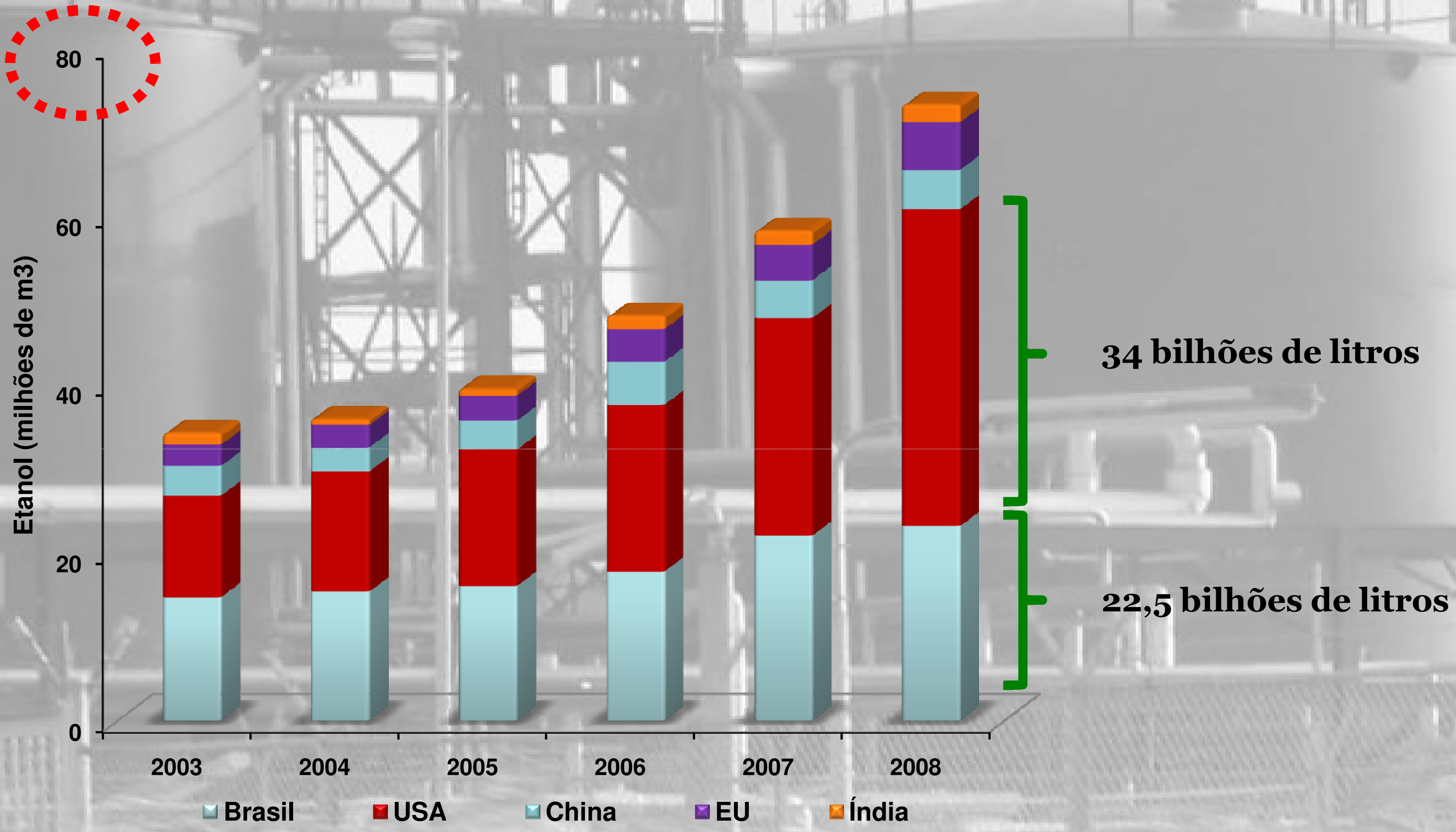
Source: EarthTrends Update (2007)

Brazilian and USA
1st Generation Ethanol Production



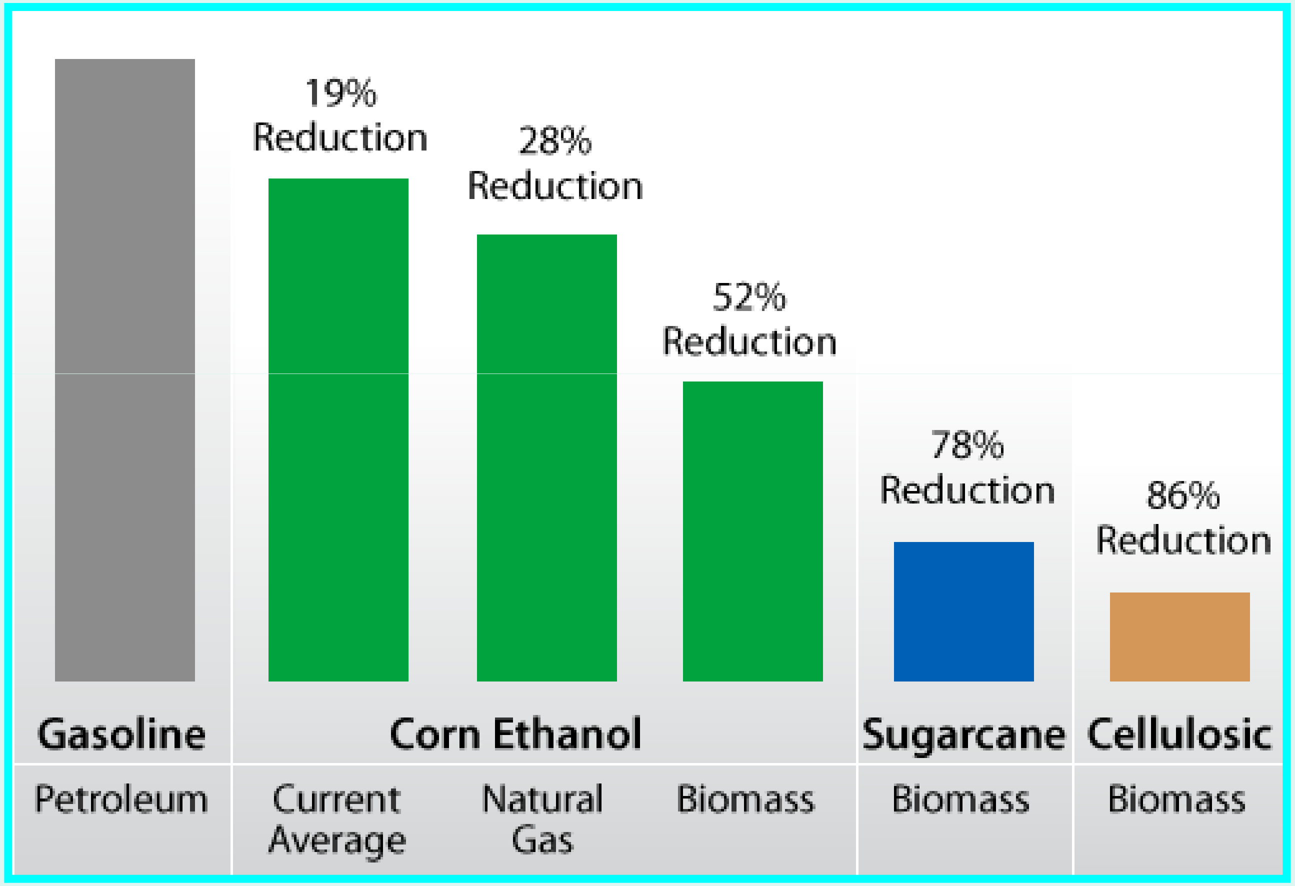
Source: LMC International (2009); RFA –Renewable Fuels Association (2010); SINDAÇUCAR & SIAMIG (2010)

Ranking of Ethanol Producing Countries



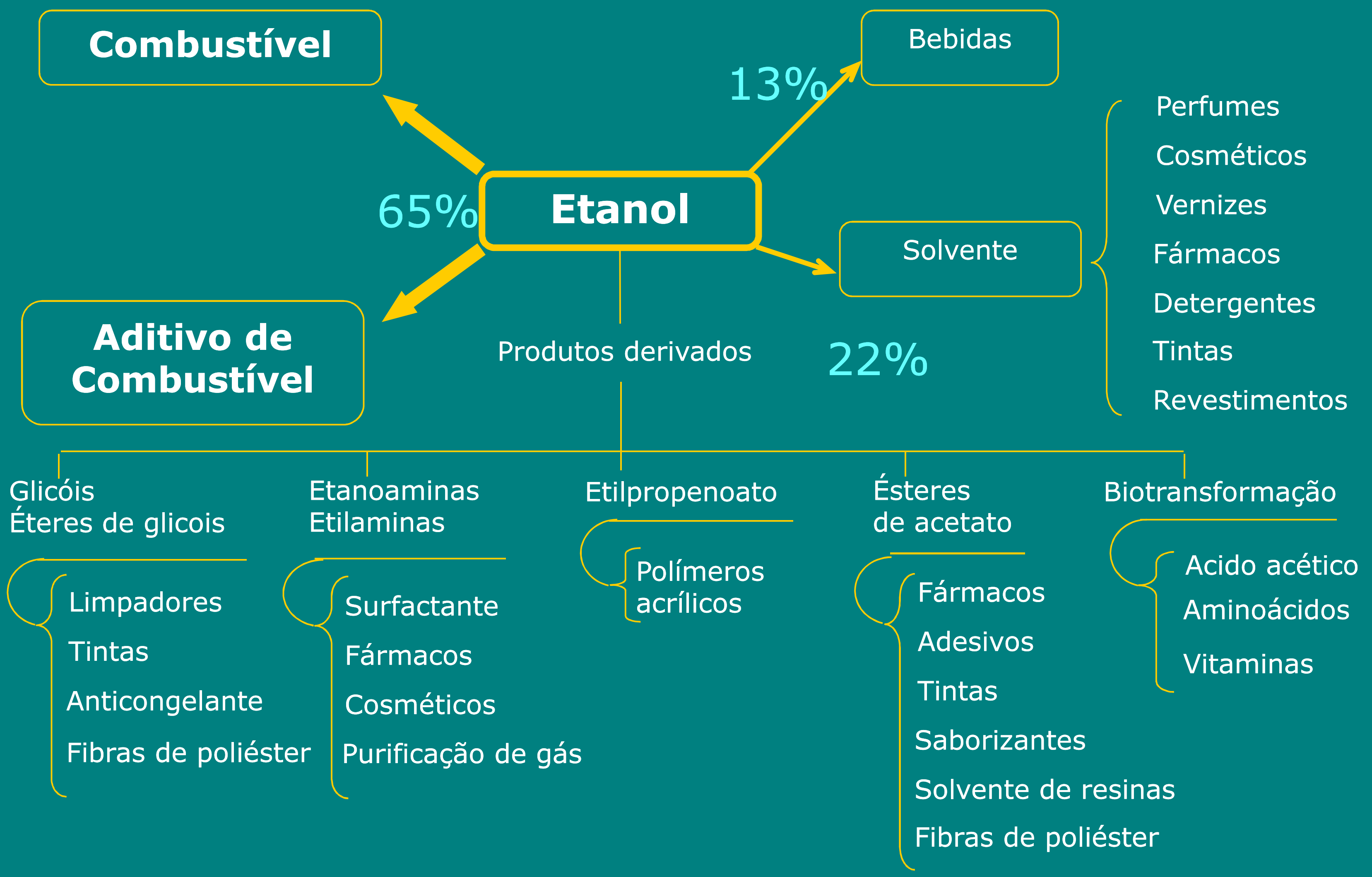
Fonte: LMC International (2008).

Greenhouse Gas Emissions of Transportation Fuels by Type of Energy Used Processing



Source: http://www.afdc.energy.gov/afdc/ethanol/emissions.html

O etanol e suas diferentes aplicações



LAND OCUPATION AND AVAILABLE AREA WITHOUT IMPACT

Crops/Activities	Area (million ha)
Cane (≈11%)	7.2
Soya	22
Corn	14
Planted wood	6.6
Pasture/cattle-raising	200
Available area without impact	100-120
Cultivated area	65
Brazil – total area	855

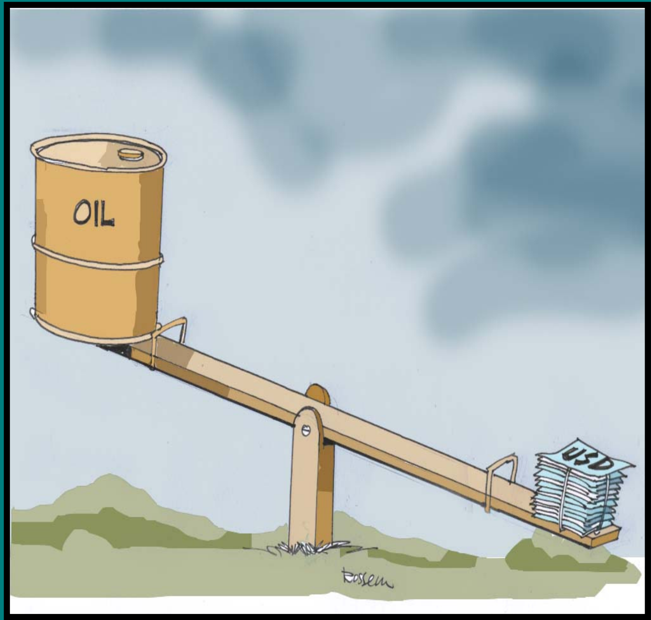
Sugar cane plantation in center-south region of Brazil

Corresponding to 4-5 x total area of Great Britain (24.1 million ha)

Source: Cortez (2006); IBGE (2008); MAPA (2009).

Importance of Bioetanol and its Production from Lignocelulosic Biomass

- **The oil barrel prices reached values which make unfeasible the self-sustainable growth of the nations;**

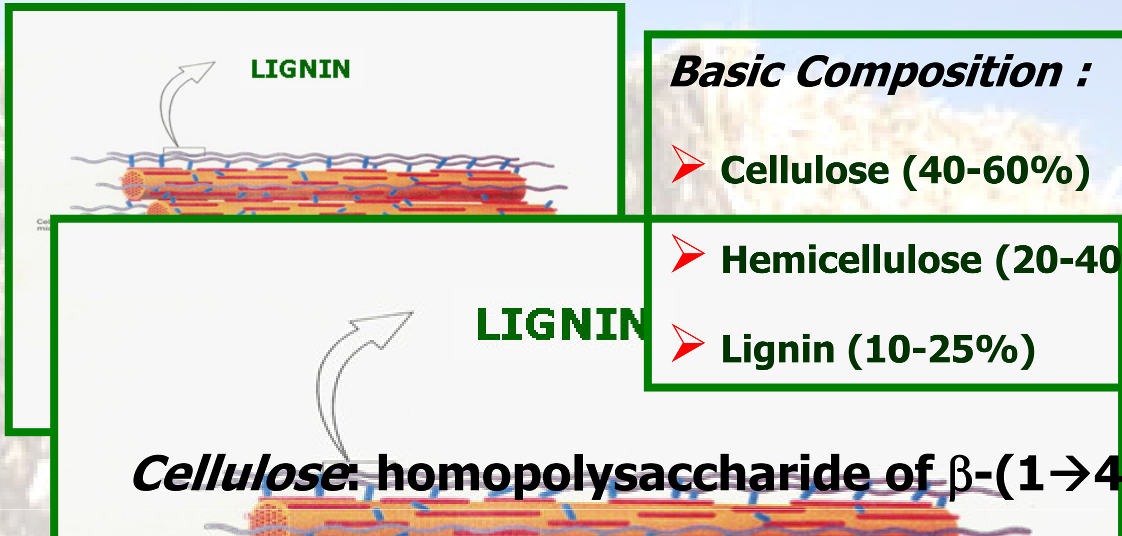


- **Increasing interest for alternative sources of energy;**

- **Necessity in aggregating value to residual biomass, whose generation tends to grow;**

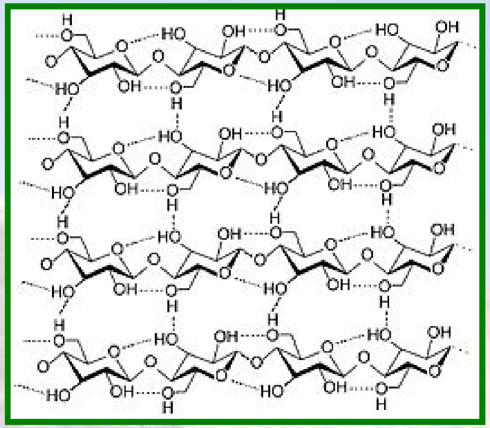
- **Possibility for increasing the ethanol production without the need to expand the availability of agricultural area for feedstock production.**

The Lignocellulosic Complex



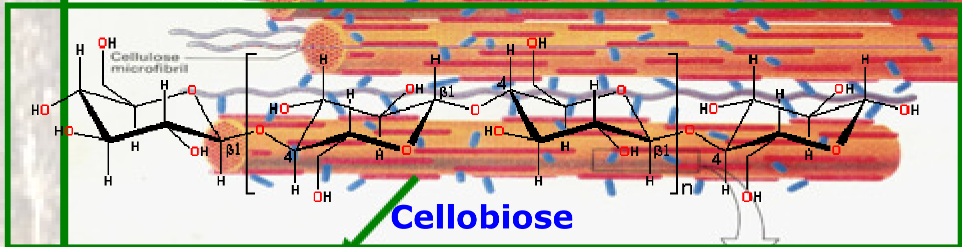
Basic Composition :

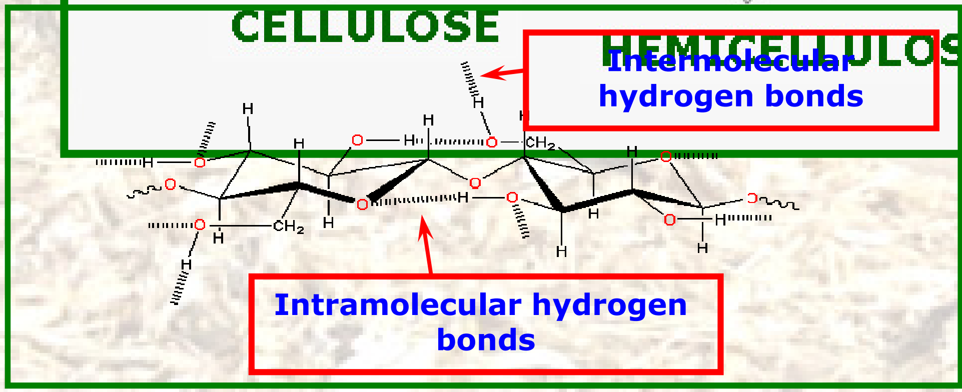
- **Cellulose (40-60%)**
- **Hemicellulose (20-40%)**
- **Lignin (10-25%)**

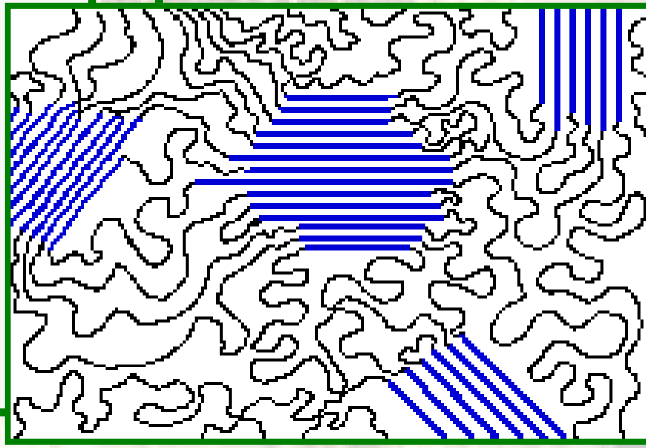


***Cellulose*: homopolysaccharide of β-(1→4)-D-… units**

Basic Composition :
)
40%)

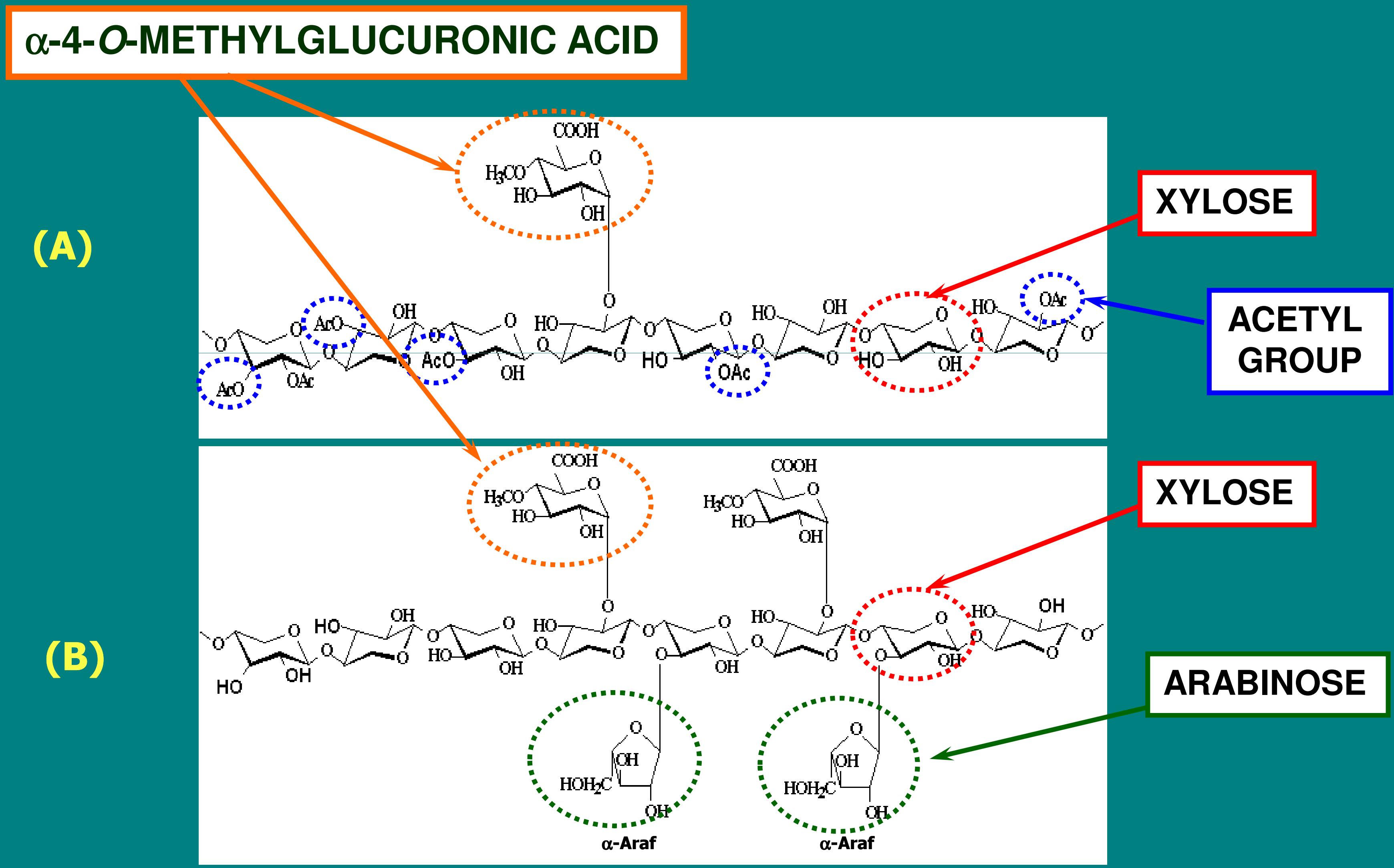






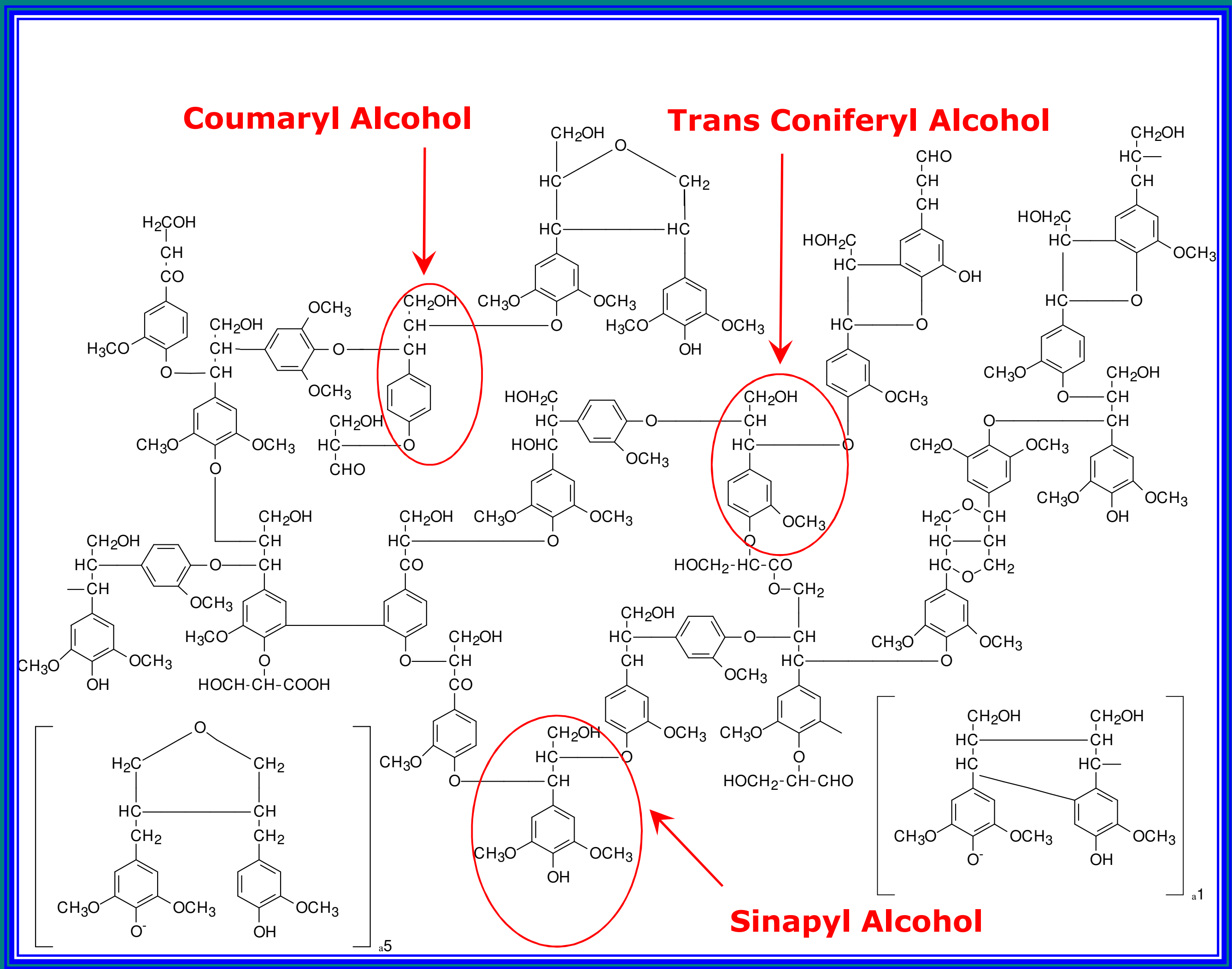
part of a cellulose preparation is amorphous between these crystalline sections

Typical Structures of Hemicellulose (Xylans) in Angiosperm (A) and Gymnosperm (B)



Ac: acetyl group; **α-Araf**: α-arabinofuranose; **α-4-o-Me-GlcA**: α-4-o-methylglucuronic acid

Lignin: Macromolecule of Aromatic Alcohols



Main Differences between Cellulose and Hemicelluloses

CELLULOSE	HEMICELLULOSES
Homopolysaccharide composed of **GLUCOSE** units	Heteropolysaccharides composed of several units of **PENTOSES** and **HEXOSES**
High degree of polymerization (2,000 to 18,000)	Low degree of polymerization (50 to 300)
Produces fibrous arrangements	Do not produce fibrous arrangements
Presents crystalline and amorphous regions	Present only amorphous regions
Slowly hydrolyzed by diluted inorganic acid in high temperatures	Rapidly attacked by diluted inorganic acid in high temperatures
Is alkaline insoluble	Are alkaline soluble

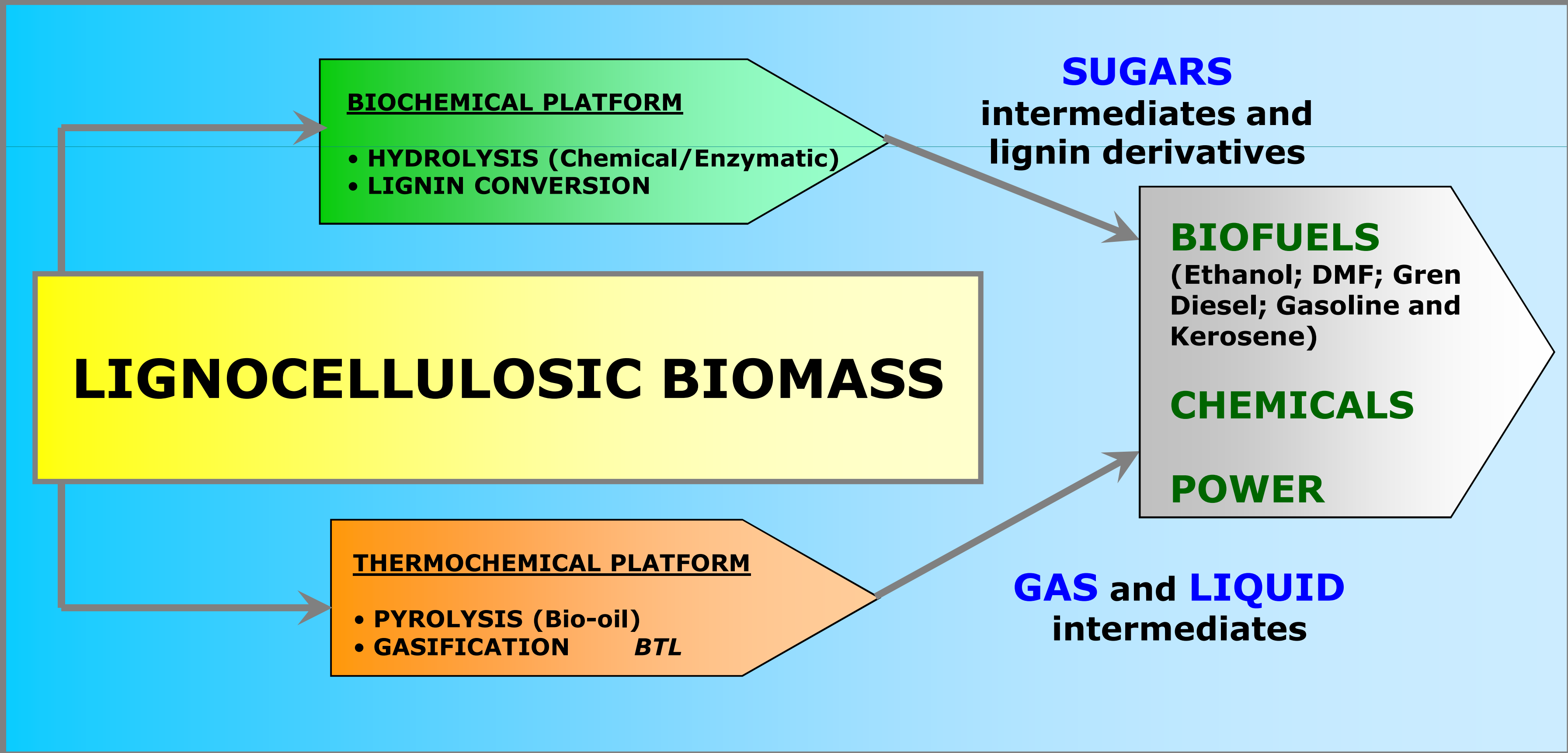
Benefits of Lignocellulose Conversion Technologies through Biological and/or Chemical Routes for the Producition of Bioetanol & other Chemicals

- ☺ Abundant and cheap renewable resources;
- ☺ Their generation does not compete with the use of land for food production;
- ☺ No necessity of expanding the agricultural land for feedstock production;
- ☺ Opportunity for industrial development based on the concept of BIOREFINERY;
- ☺ Reduction in gas emissions that cause the so called "*green house effect*";
- ☺ Cleaner technologies;
- ☺ Macroeconomic benefits for rural communities and for Society as a whole;
- ☺ They are within the perspective of Sustainable Development.

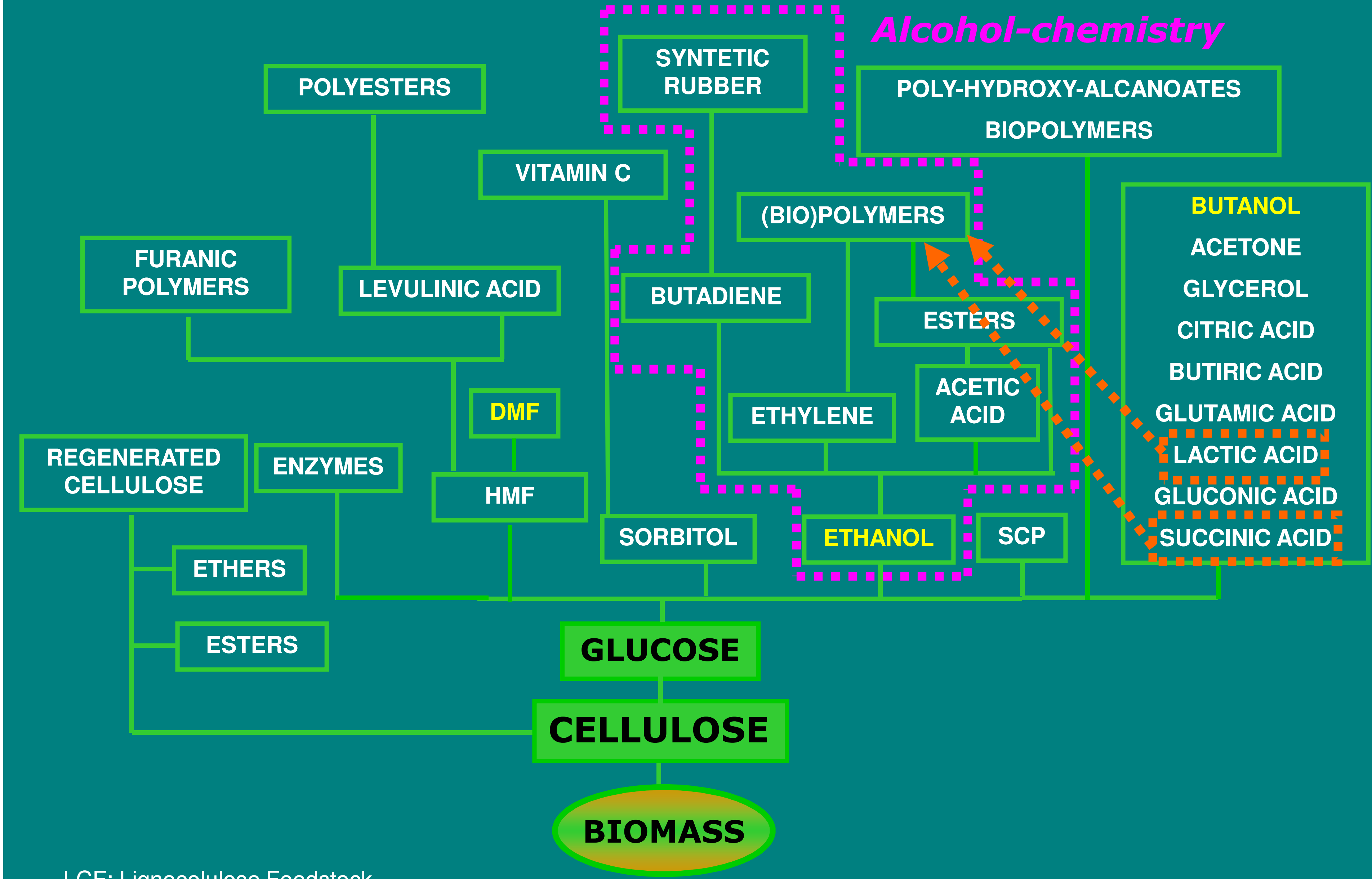
What is a Biorefinery?

BIOREFINERIES are similar to petroleum refineries in concept; however, biorefineries use biological matter (as opposed to petroleum or other fossil sources) to produce TRANSPORTATION FUELS, CHEMICALS, and HEAT and POWER.

INDUSTRIAL BIOREFINERIES have been identified as the most promising route to the creation of a new industry based on renewable resources.

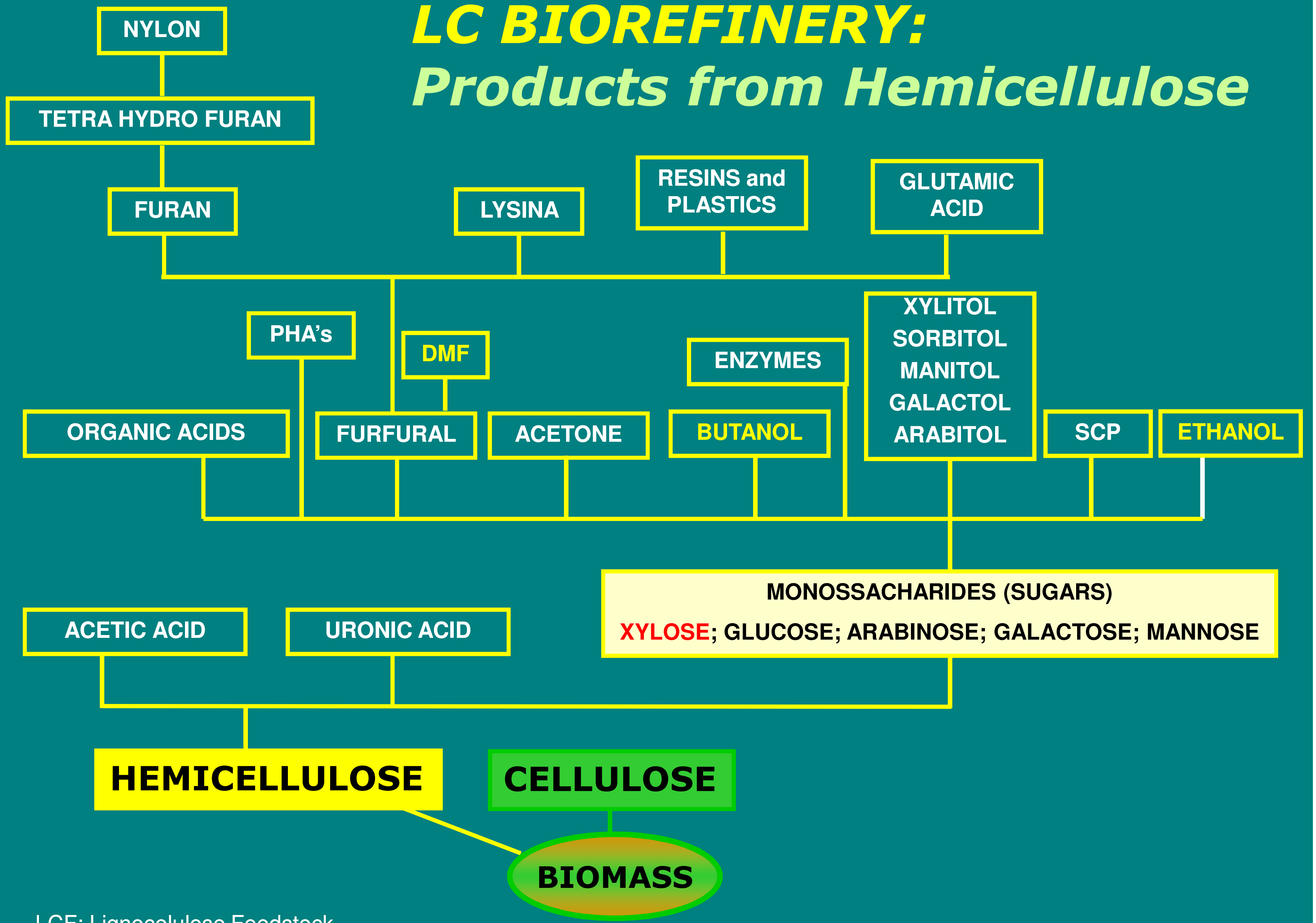


LC BIOREFINERY: Products from Celulose



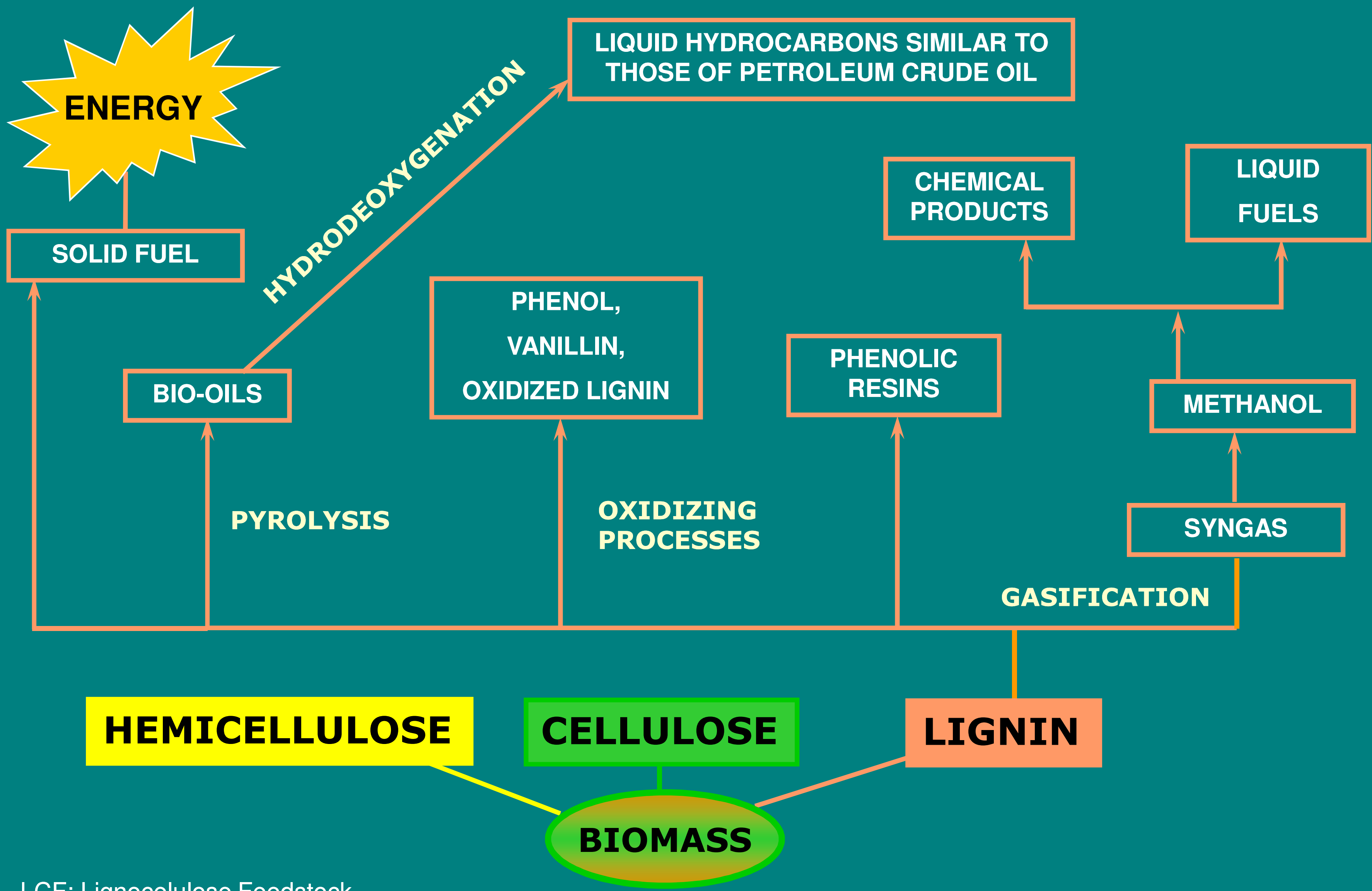
LCF: Lignocelulose Feedstock

LC BIOREFINERY: Products from Hemicellulose



LCF: Lignocelulose Feedstock

LC BIOREFINERY: Products from Lignin



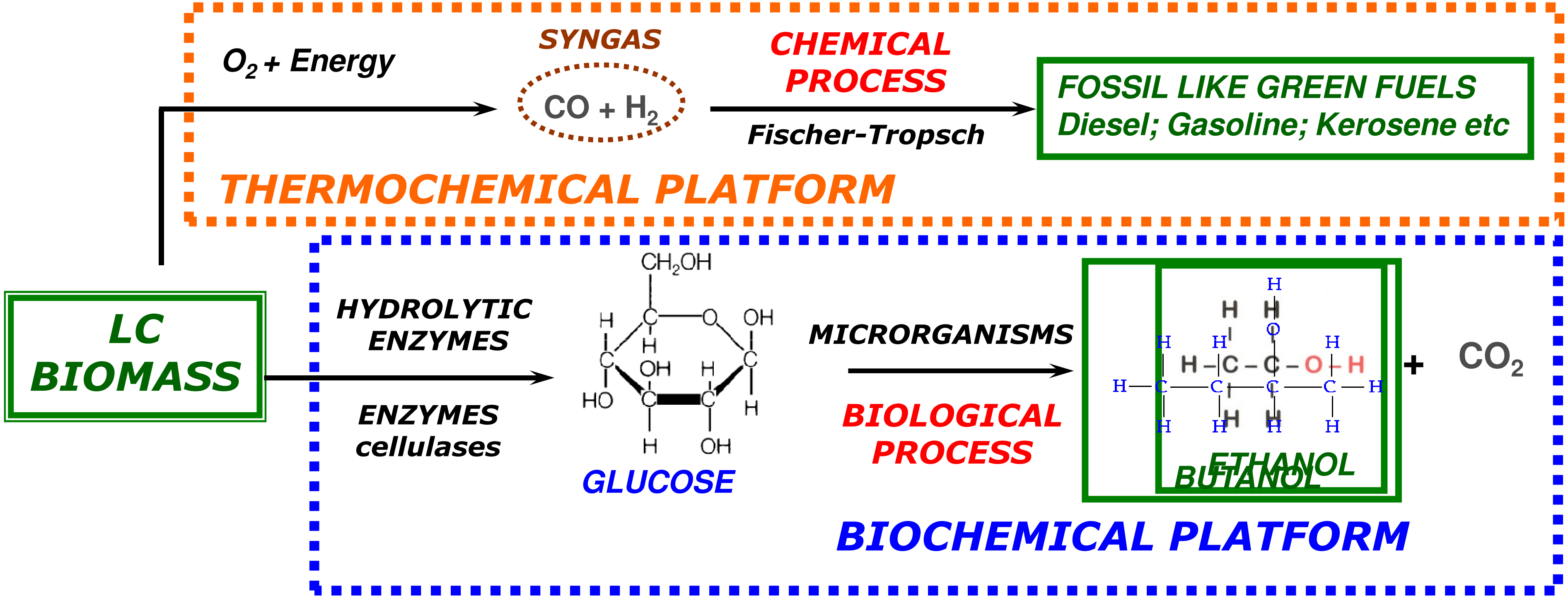
LCF: Lignocelulose Feedstock

LIQUID BIOFUELS FROM LIGNOCELLULOSIC BIOMASS

POTENTIAL CANDIDATES

BIOFUELS	Process	Technological maturity
BIOETHANOL	Biochemical	Emerging
BIOBUTANOL	Biochemical	Embrionary
DMF	Hybrid	Embrionary
FOSSIL LIKE GREEN FUELS	Themochemical	Growing

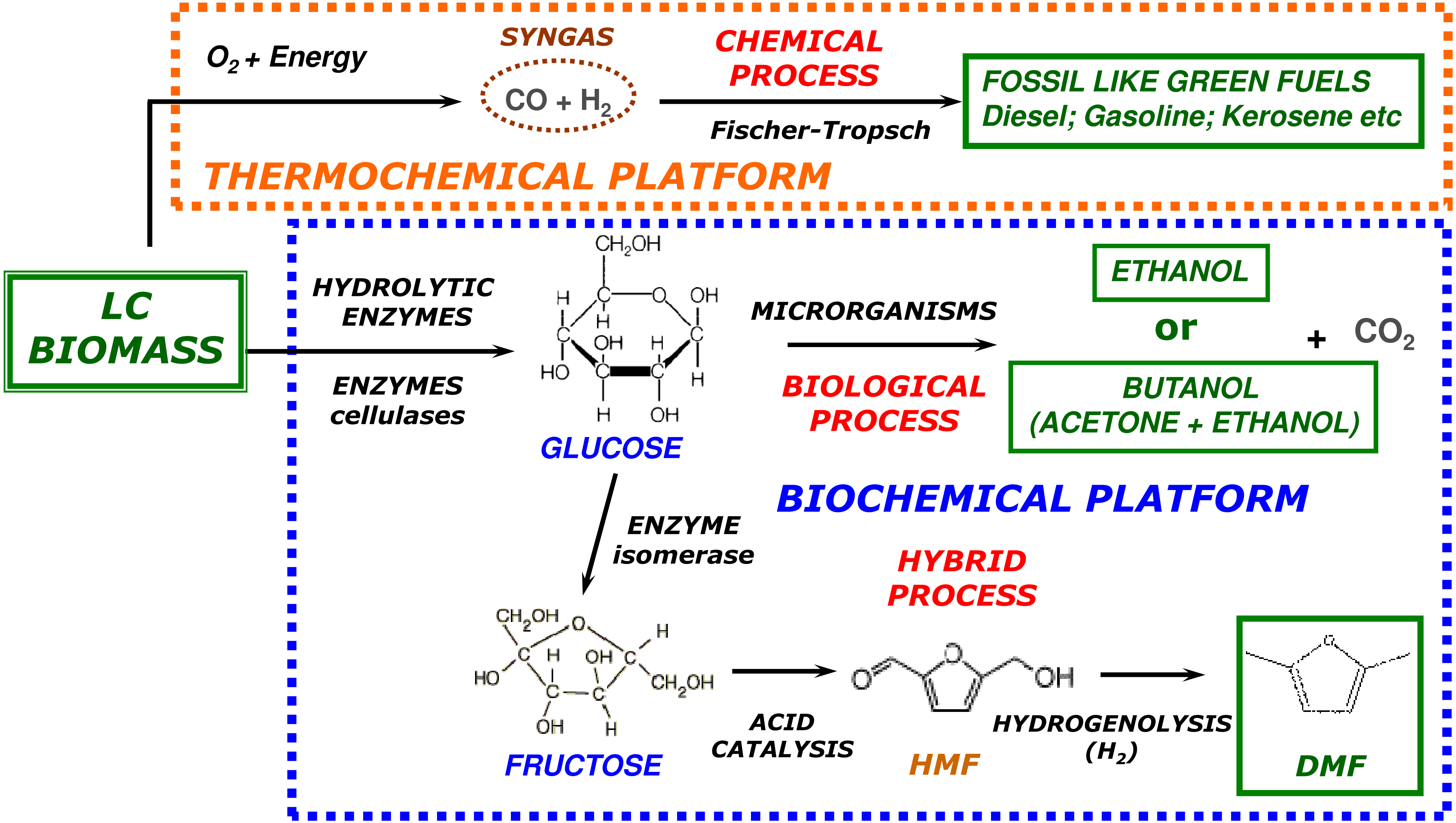
PRODUCTION OF BIOFUELS FROM LIGNOCELLULOSIC BIOMASS



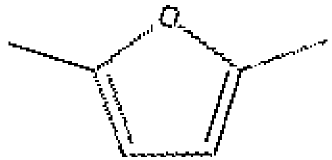
Fischer-Tropsch (1920) is a catalyzed chemical reaction in which carbon monoxide and hydrogen (syngas) are converted into liquid hydrocarbons of various forms (**Diesel**, **Gasoline**, **Kerosene** e **Lubricants**).

$$(2n+1)H_2 + nCO \rightarrow C_nH_{(2n+2)} + n\,H_2O$$

PRODUCTION OF BIOFUELS FROM LC BIOMASS



DMF: 2,5 dimethyl furan



☺ **Energy density higher than ethanol and close to gasoline;**
☺ **Low water solubility (2.3 g/L);**
☺ **Boiling point (~92ºC) close to conventional fuels;**
☺ **Octane Number close to ethanol (DMF ≅120 e Ethanol ≅130);**
☺ **Production by hybrid process (enzymatic and chemical), with chemical steps completely dominated by the industrial sector.**

FUELS' ENERGY DENSITY

Fuel	**ENERGY DENSITY** ***per volume (KJ/cm³)***
Liquid Hydrogen	**10.1**
Gas Hydrogen compressed (700 bar)	**4.7**
Gasoline	**32.0-34.6**
Diesel	**38.7**
Gasohol (10% etanol-90%gasolina)	**28.1**
Biodiesel	**30.5**
Buthanol	**29.2**
Methanol	**15.6**
Ethanol	**24.0**
Dimethyl Furan (DMF)	**30.0**

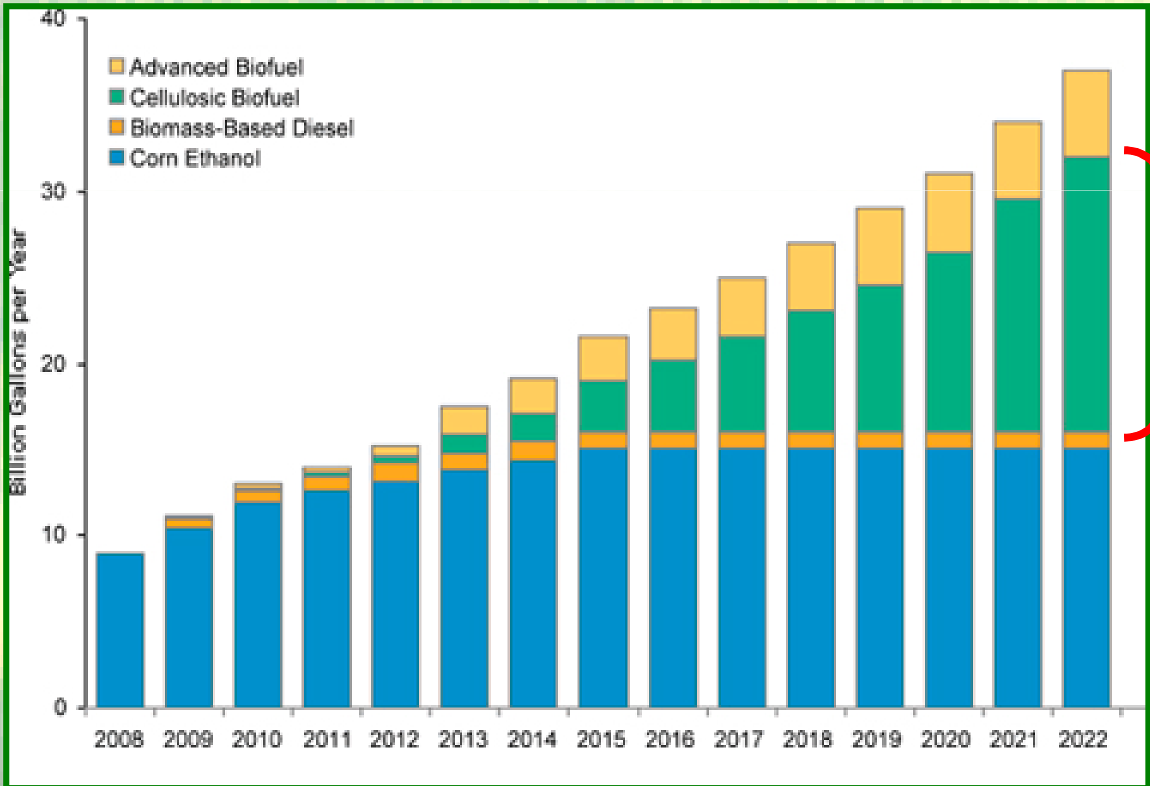
Source: Román-Leshkov *et al.* (2007). Nature, 447, 982-985.

Recently-awarded Projects (Small Biorefineries) (2009)

APPLICANT	TOTAL COST	DOE SHARE	ANNUAL PRODUCTION CAPACITY	PROJECT LOCATION	FEEDSTOCK	Technology
VERENIUM	91,347,330	76,000,000 (granted 2007)	1,500,000	Jennings, LA	Bagasse, energy crops, agricultural wastes, wood residues	**Biochemical**
FLAMBEAU LLC	84,000,000	30,000,000	6,000,000	Park Falls, WI	Forest residue	**BTL**
ICM	86,030,900	30,000,000	1,500,000	St. Joseph, MO	Switchgrass, forage sorghum, stover	**Biochemical**
LIGNOL INNOVATIONS	88,015,481	30,000,000	2,500,000	Commerce City, CO	Woody biomass, agricultural residue	**Biochemical** Organosolve
PACIFIC ETHANOL	73,040,000	24,340,000	2,700,000	Boardman, OR	Wheat straw, stover, poplar residuals	**Biogasol** (ETOH, biogas, solid fuels)
NEW PAGE	83,653,212	30,000,000	5,500,000	Wisconsin, WI	Wood biomass – mill residues	**BTL**
RSE PULP	90,000,000	30,000,000	2,200,000	Old Town, Maine	Wood chips (mixed hardwood)	**Biochemical**
ECOFIN, LLC	77,000,000	30,000,000	1,300,000	Washington Country, KY	Corn cobs	**Biochemical** (Solid State Fermentation)
MASCOMA	135,000,000	25,000,000	2,000,000	Monroe, TN	Swichgrass and hardwoods	**Biochemical**
TOTAL	**808,086,923**	**305,340,000**	**25.2 M gallons = 95.4 M liters**			

US Government Mandatory Biofuel Supply Volume

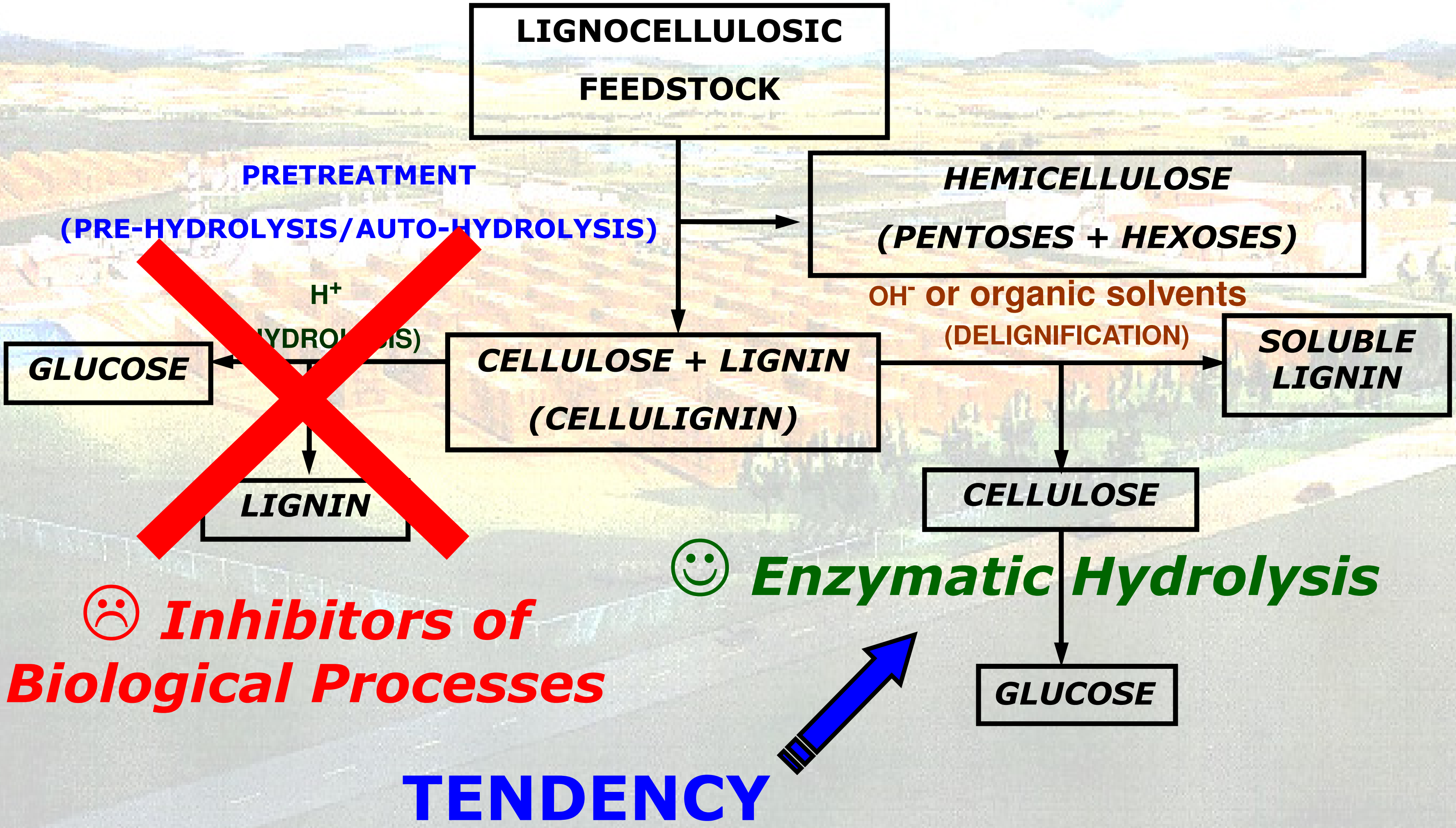
- **9 billion gallons (≈34 billion liters) of renewable fuels in 2008.**
- **36 billion gallons (≈136 billion liters) in 2022.**



16 billion gallons (≈ 60 billion liters) MUST BE CELLULOSIC BIOFUELS

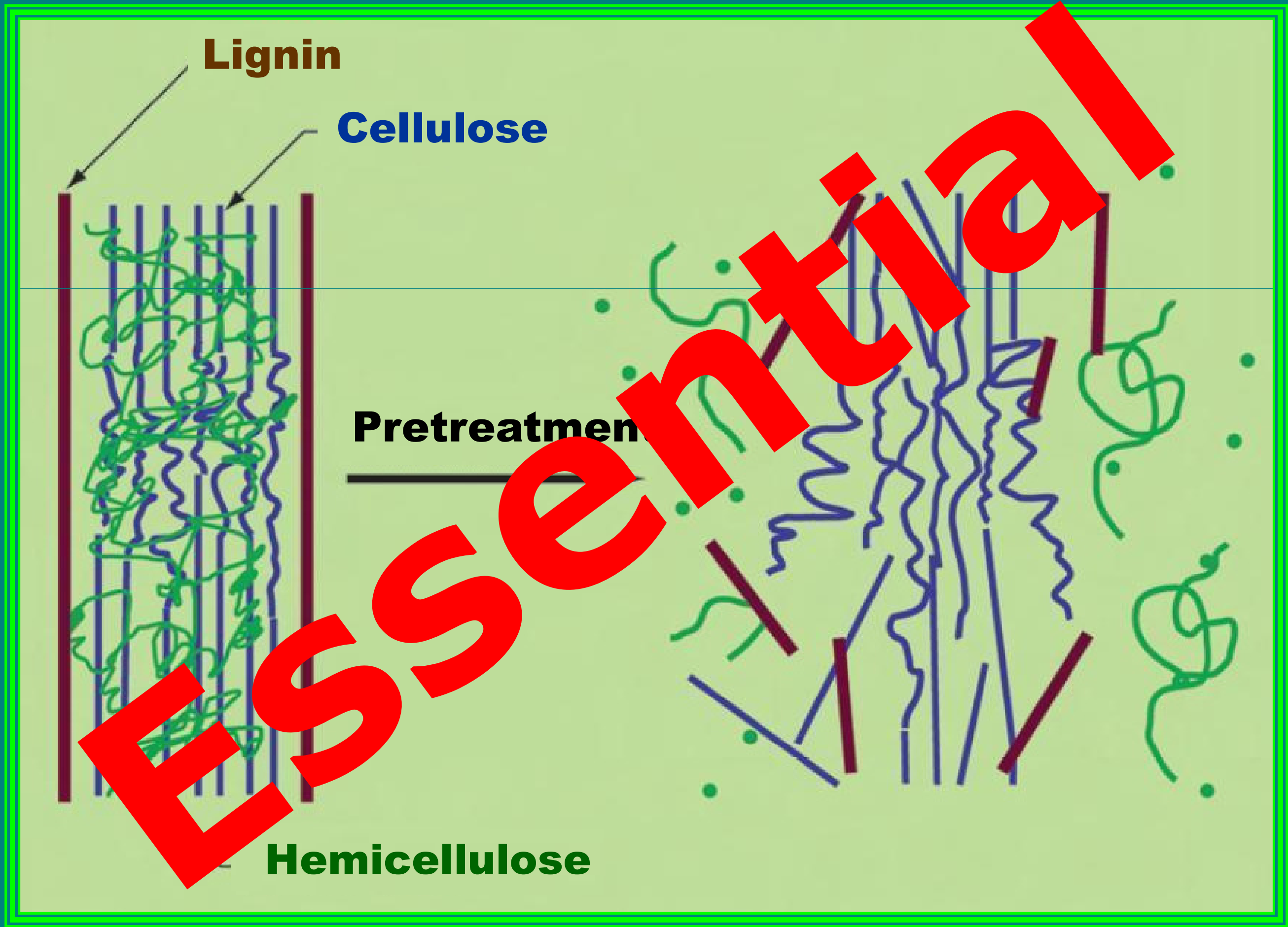
Fonte: http://www.verenium.com

FRACTIONING LIGNOCELLULOSIC BIOMASS FOR BIOCHEMICAL PLATFORM



Pretreatment of Lignocellulosic Feedstock

Aims at disorganizing the lignocellulosic complex, resulting in an increase of CELLULOSE DIGESTABILITY (enzymes accessibility to cellulose molecules)



Characteristics of the Main Pretreatment Technologies for LC Feedstsocks (Biochemical Platform)

Characteristics	Pretreatment Technology				
	SE	CSE	DAH	TH (LHW)	AFEX
Typical operational conditions	Batch or Continuous 2 to 10 min	Batch or Continuous 2 to 10 min	Batch or Continuous 5 to 30 min	Batch 5 to 60 min	Batch 5-15% amônia 10-30 min
Temperature	190-270ºC	160-200ºC	150-180ºC	170-230ºC	100-180ºC
Consumption of chemical inputs	No	Yes	Yes	No	Yes
Removal of hemicelullose	Yes	Yes	Yes	Yes	No, only after washing
Xylose yield	10% Xylose; 90% Xylosaccharides	70-90%	85-95%	50% Hemicellulose derived sugars	60% HDS after washing and removal of lignin
Removal of Lignin	Minor effect	Moderate effect	Moderate effect	Minor effect	Major effect
Formation of inhibitors	Yes, under severe conditions	Yes, under severe conditions	Yes, under severe conditions	Few	Yes, under severe conditions
Reduction of the required particle size	Medium	Medium	High	Medium	High
Efficiency of the Cellulose Enz. Hydrol.	80-90%	80-85 %	80-85 %	80-90%	50-90 %
Waste Generation	Less significant	Moderate	Significant	Less significant	Significant
Corrosivity of the medium	Low	Low to moderate	Moderate to high	Low	Low to moderate
Simplicity of the process (potential)	High	Moderate to high	Moderate	Not evaluated	Moderate
State of arte	Pilot plants	Pilot plants	Pilot and demo plants	Bench scale	Pilot and demo plants

SE: Steam-explosion; **CSE**: Catalysed Steam-explosion; **DAH**: Diluted Acid hydrolysis; **TH**: Thermohydrolysis (liquid hot water pretreatment); **AFEX**: Ammonia fiber explosion. Sources: Adapted from LYND (1996); OGIER *et al.* (1999) and Balat *et al.* (2008); Pérez *et. Al.* (2007)

Diluted Acid / Acid Catalysed Steam Explosion Pretreatments of Lignocellulosic Feedstocks

VARIABLES

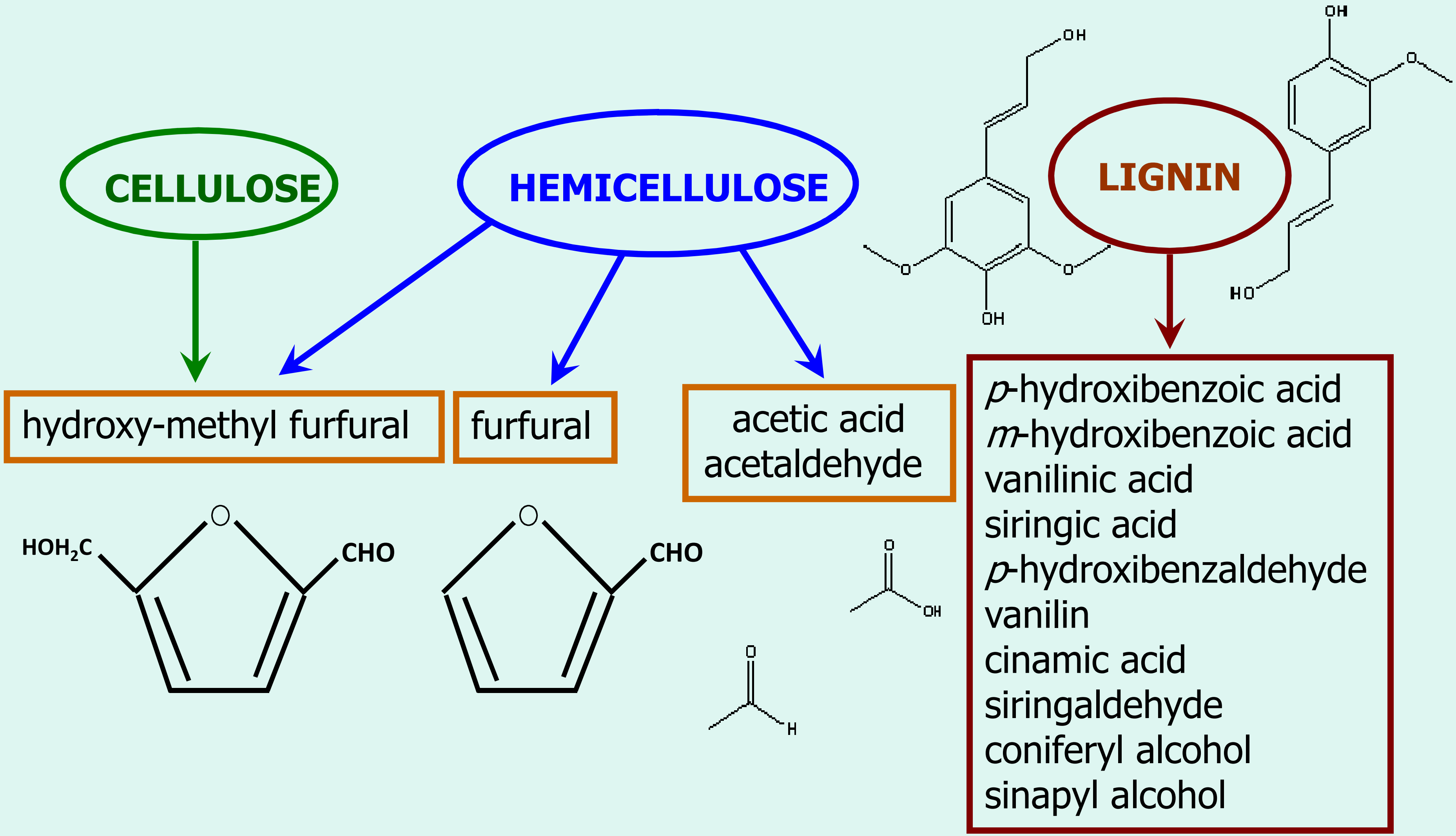
- **Acid Concentration**: $0.5\% \leq [acid] \leq 10\%$
- **Type of Acid**: H_2SO_4; HCl; HNO_3 e H_3PO_4
- **Temperature**: $98^o \leq T \leq 260^oC$
- **Time of exposition**: $sec < \theta < hs$
- **Sol:Liq Ratio**: $1{:}20 \leq S{:}L \leq 1{:}4$

Hemicellulose hydrolysis efficiency ↗ | **Inhibitors generation ↘**

Hemicellulose derived sugar concentration ↗

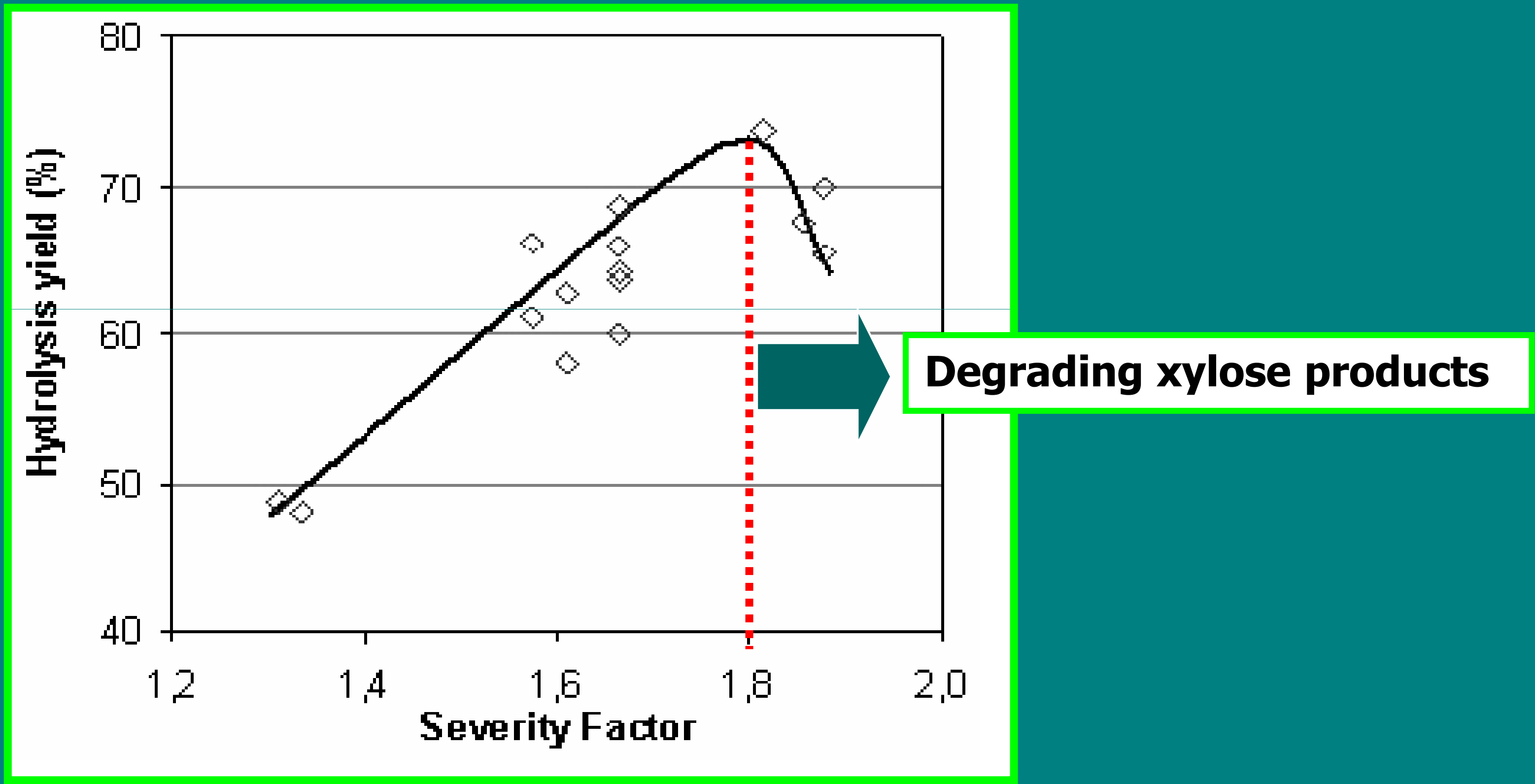
$$DS = \log \{\theta \cdot \exp [(T-100)/14.75]\} - pH$$

Substances that are commonly reported as inhibitors of the metabolic activity, originated from the acid hydrolysis/pretreatment of lignocelulosic materials



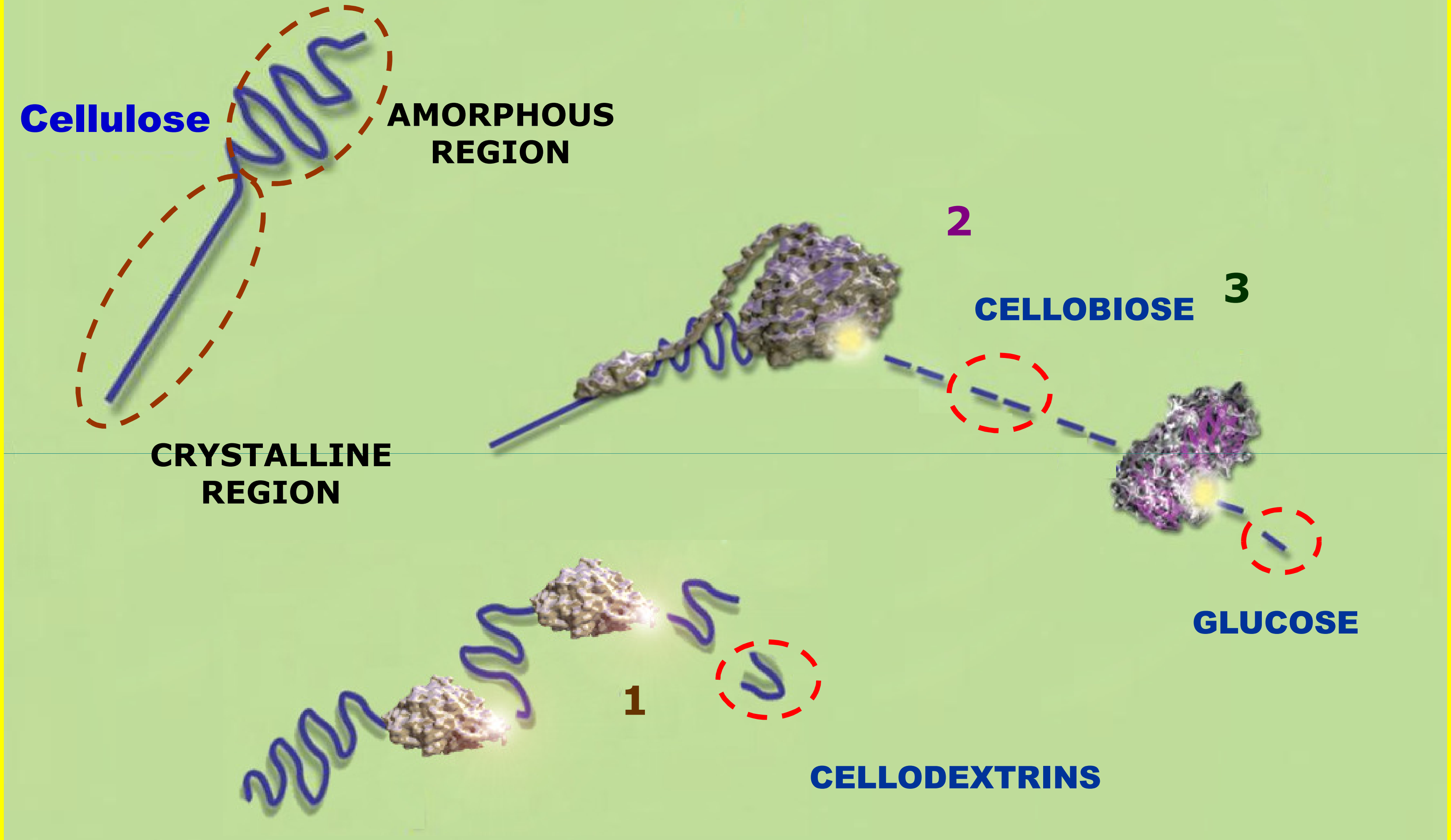
Parajó *et al.* (1998)

EFFECT OF THE SEVERITY FACTOR OVER HEMICELLULOSE HYDROLYSIS YIELD DURING DILUTED ACID PRETREATMENT



Fonte: Betancur & Pereira Jr. (2009).

Enzymatic Hydrolysis of Cellulose



1: ENDOGLUCANASE   2: EXOGLUCANASE   3: β-GLUCOSIDASE

☞ **CELLULASES ARE INHIBITED BY CELLOBIOSE E GLUCOSE**

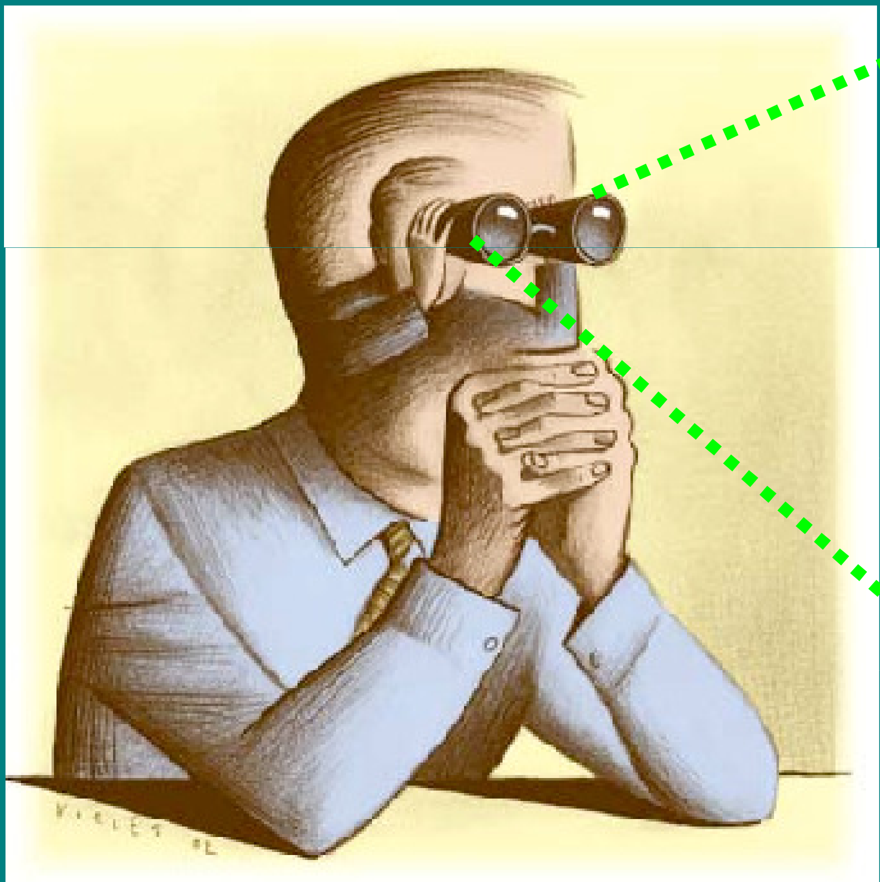
Why hydrolyse cellulose enzymatically?

- ***Milder conditions of pressure, temperature and pH;***
- ***High specificity;***
- ***Elimination of hydroxymethyl furfural, amongst other toxic substances (lignin derivatives);***
- ***Low energy consumption;***
- ***Low material costs with construction of equipments, differently of those processes which utilize acid hydrolysis.***

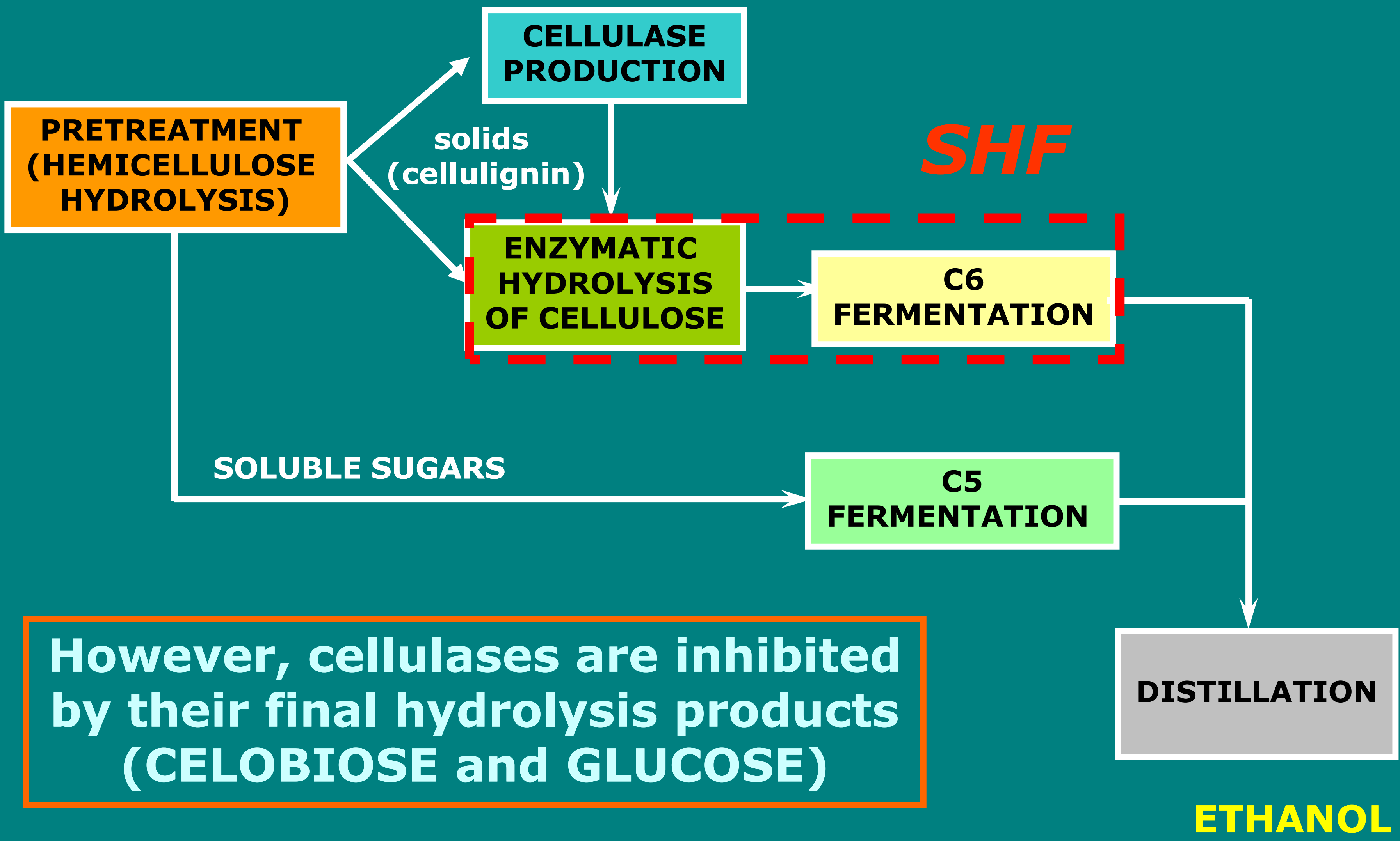
However,

- ***High production costs;*** ← **R & D & I**

Strategies for Ethanol Production from Lignocelulosics Following the Biochemical Platform

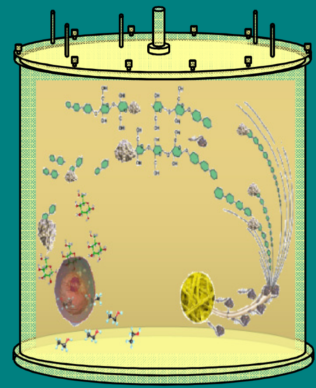


SEPARATE HYDROLYSIS AND FERMENTATION



However, cellulases are inhibited by their final hydrolysis products (CELOBIOSE and GLUCOSE)

SIMULTANEOUS SACCHARIFICATION AND FERMENTATION



PRETREATMENT (HEMICELLULOSE HYDROLYSIS)

CELLULASE PRODUCTION

solids (cellulignin)

SSF

ENZYMATIC HYDROLYSIS OF CELLULOSE & C6 FERMENTATION

SOLUBLE SUGARS

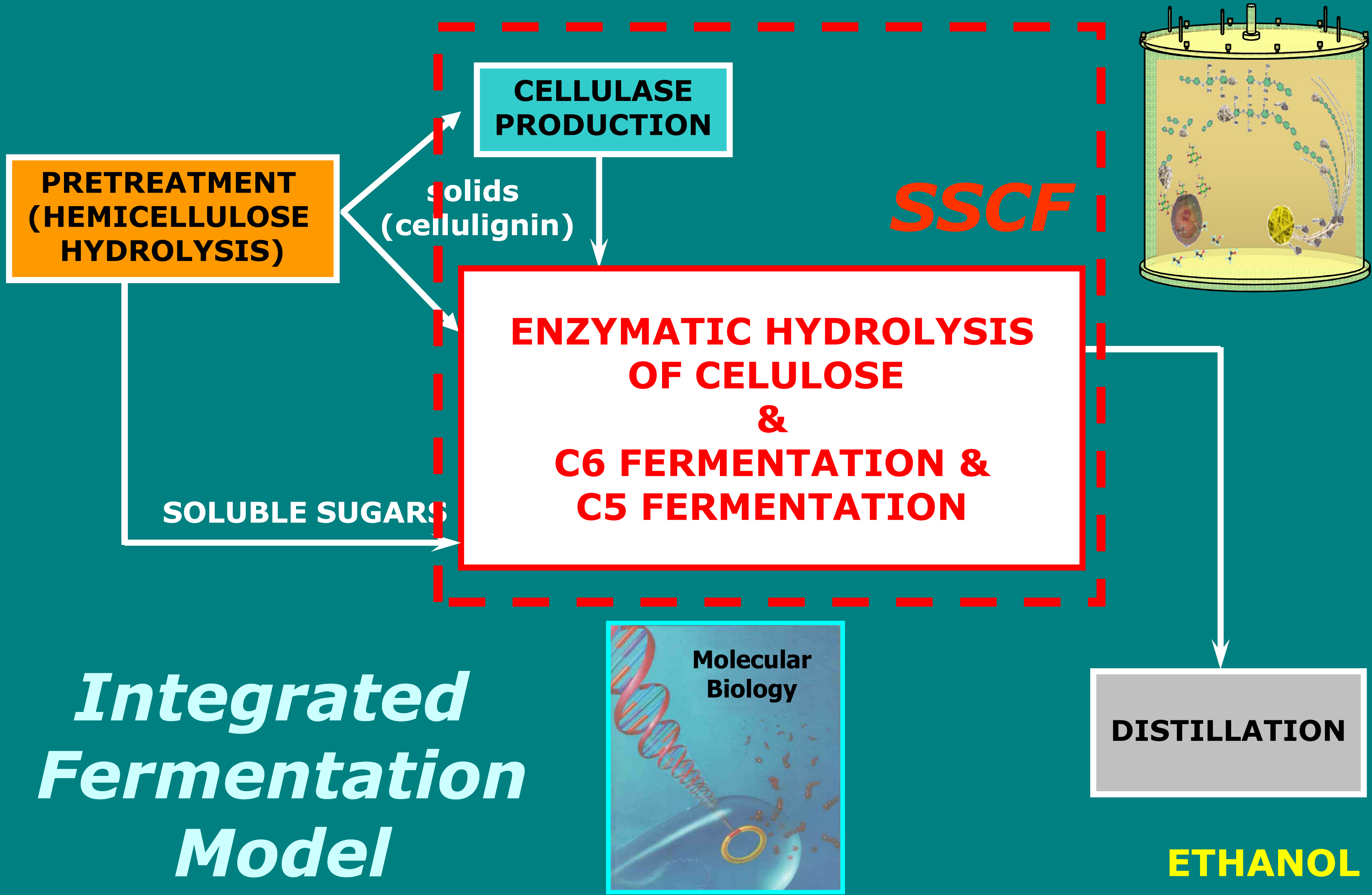
C5 FERMENTATION

DISTILLATION

ETHANOL

Two Stream Fermentation Model

SIMULTANEOUS SACCHARIFICATION AND COFERMENTATION



Integrated Fermentation Model

CONSOLIDATED BIOPROCESSING

CBP

PREATRETMENT (HEMICELLULOSE HYDROLYSIS)

solids (cellulignin)

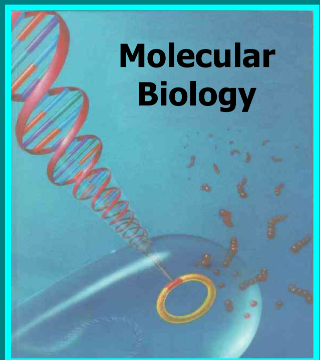
SOLUBLE SUGARS

CELLULASE PRODUCTION
&
CELLULOSE HYDROLYSIS
&
C6 FERMENTATION
&
C5 FERMENTATION

DISTILLATION

ETHANOL

Consolidated Bioprocess Model



Ethanol Production Plant from Corn Processing Residues (‘corn stover’ e ‘corncob’) in USA
Simultaneous Saccharification and Co-Fermentation Process
NREL-DOE/Golden-CO/USA

04 bioreactors of 9.000 L; 02 of 1.450 L and 02 of 160 L (ethanol concentration = 30-40 g/L)
Processing Capacity: 2 ton of lignocellulosic material/day
Ethanol Production Capacity = 750 L/day

Ethanol Production Plant from Lignocellulosic Residues arising from Corn Industry (*corn stover* e *corncobs*) in Canada

Partnership: IOGEN/Petro-Canada/Shell

(Investments of US$ 110 million)

Processing capacity: 12,000-15,000 ton/year
Production capacity: 4 . 10^6 liters/year

Press Release 21 April, 2004
CELLULOSE ETHANOL IS READY TO GO
Iogen producing world's first cellulose ethanol fuel

Prime Minister Paul Martin congratulates Iogen on its success in the cellulose ethanol technology at an event at the Iogen demonstration plant April 21, 2004.

Prime Minister Paul Martin and Iogen President Brian Foody launch the world's first shipment of cellulose ethanol to the commercial fuel market April 21, 2004.

- **Owns a demo plant with a producing capacity of 5.2 million liters/year cellulosic ethanol of non-food based sources;**

- **Produces its own enzyme *cocktail*;**

- **Explore the biochemical platform, through the two stream model technology (C5 fermentation separately of C6 fermentation)**

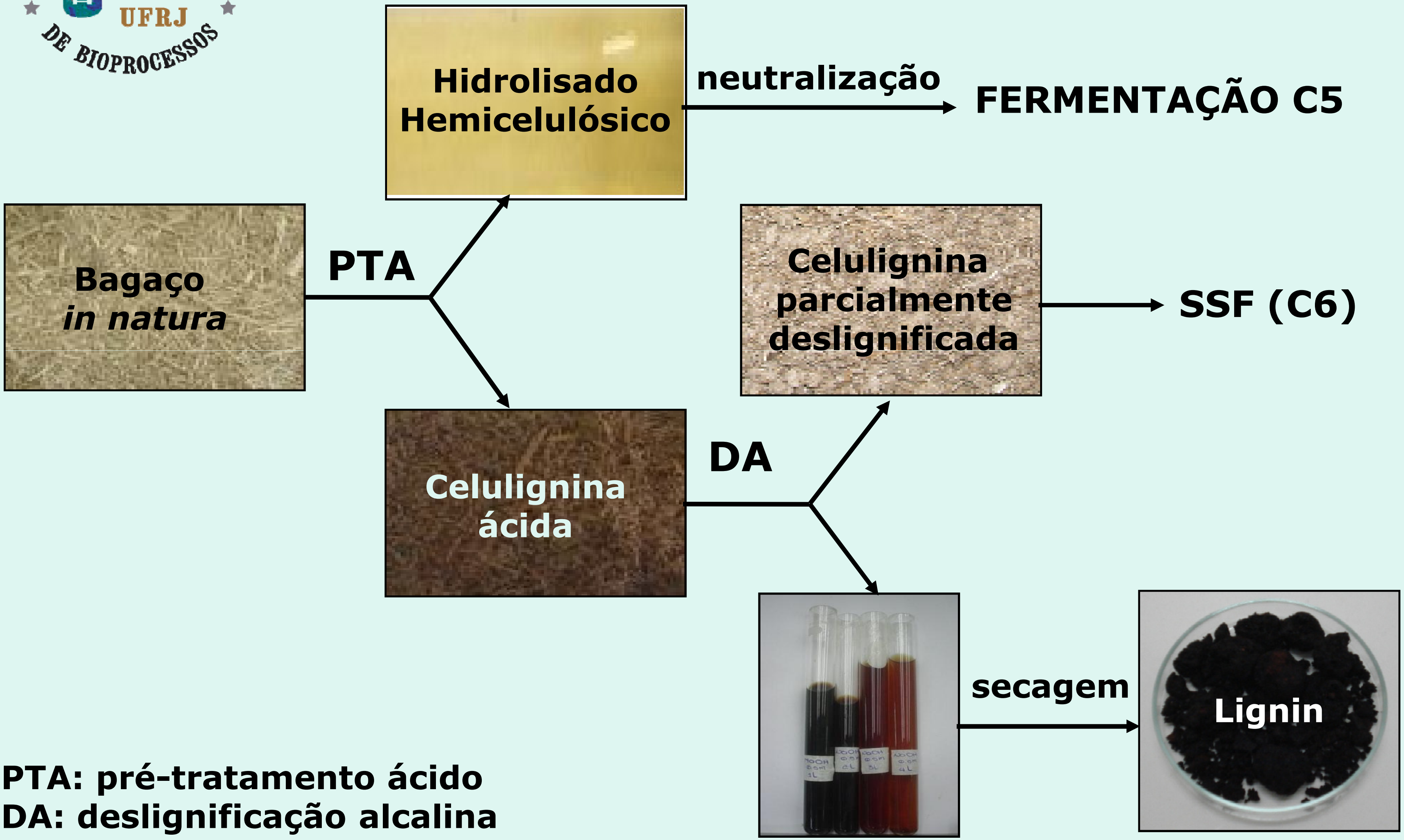
- **In July 2008, the company was selected for an award under a US$240 million Federal Program, operated by the DOE, to support the development of up to nine small-scale Biorefineries in the United States**

Verenium's state-of-the-art demonstration facility in Jennings, Louisiana, dedicated in May 2008.

Empresas estrangeiras que operam unidades de produção de etanol de segunda geração (Biorrefinaria)

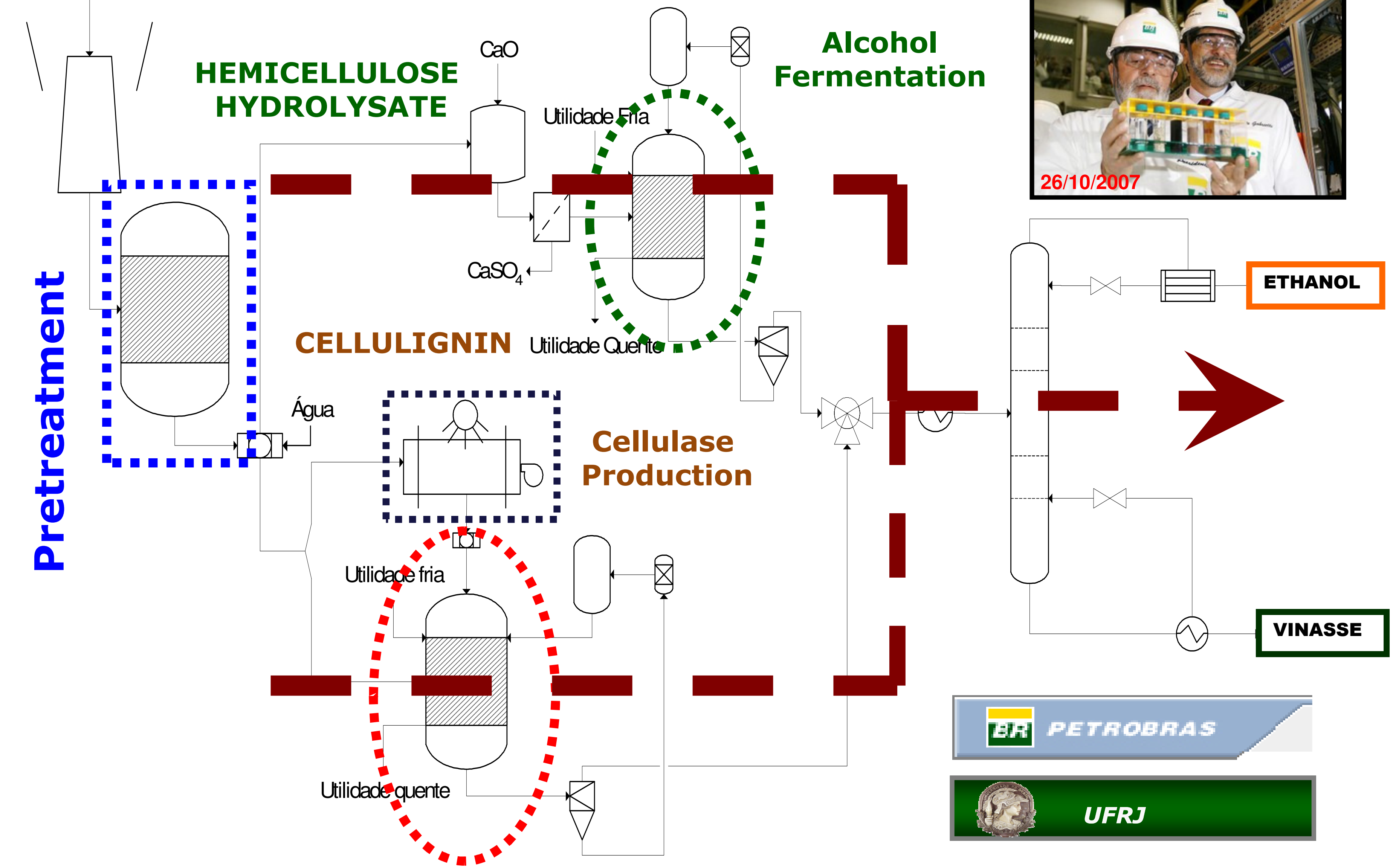
EMPRESA	PAÍS DE ORIGEM	CARACTERÍSTICA DE PROCESSO	LOCALIZAÇÃO	CAPACIDADE (m^3/ano)
AE Biofuels	EUA	Hidrólise enzimática	Montana	567
Blue Fire Ethanol	EUA/JAPÃO	Hidrólise c/ácido concentrado	Califórnia Izumi	12.110 n.d.
Chempolis Ou	FINLÂNDIA	Hidrólise c/ácido diluído	Oulu	n.d.
Iogen	CANADÁ	Hidrólise enzimática	Ontario	4.000
KL Energy	EUA	Hidrólise enzimática	Wyoming	5.680
Lignol Energy	CANADÁ	Pré-tratamento *Organosolv* - HEnz	Vancouver	2.500
Mascoma	EUA	n.d.	Nova Iorque	1.890
Poet	EUA	n.d.	Dakota do Sul	75
Sekab	SUÉCIA	Hidrólise enzimática	n.d.	n.d.
ST1	FINLÂNDIA	n.d.	Lappeenranta Hamina Närpiö	1.000 1.000 1.000
St. Petersburgo State Forest-Technical Academy	RÚSSIA	Hidrólise c/ácido diluído	13 unidades no país	n.d
Sun Opta	CANADÁ	Hidrólise enzimática	China	n.d.
Universidade da Flórida	EUA	Hidrólise enzimática com *E. coli* recombinantes (modelo integrado)	Flórida	7.570
Verenium	EUA	Hidrólise enzimática (modelo de duas correntes)	Louisiana Japão	5.300 4.920

Estratégia LADEBIO para a Produção de Etanol de 2ª Geração



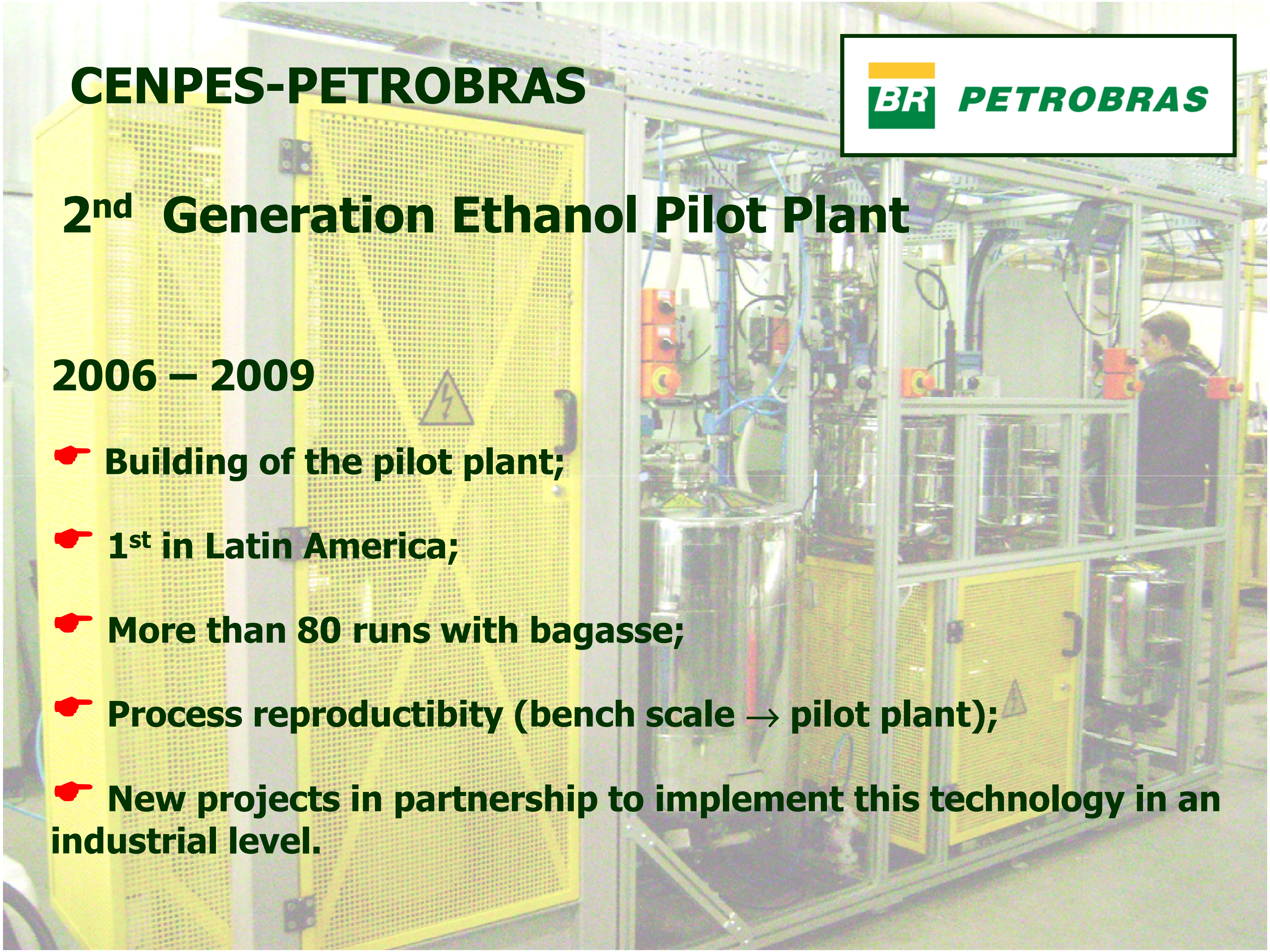
PTA: pré-tratamento ácido
DA: deslignificação alcalina
SSF: *simultaneous saccharification and fermentation*

PETROBRAS PILOT PLANT



SSF (Simultaneous Saccharification and Fermentation))

Patent PI0505299-8 (11/2005)
Patent PI0605017-4 (12/2006) (PATENT nb. 1000)
Patent PI0200801-58 (12/2008)



CENPES-PETROBRAS

2nd Generation Ethanol Pilot Plant

2006 – 2009

- **Building of the pilot plant;**
- **1st in Latin America;**
- **More than 80 runs with bagasse;**
- **Process reproductibity (bench scale → pilot plant);**
- **New projects in partnership to implement this technology in an industrial level.**

UFRJ

PETROBRAS

anp
Agência Nacional do Petróleo, Gás Natural e Biocombustíveis

Biofuels Center (RJ)

4-stored Building
Area: 2000 m^2

- **Pilot Plant (850 m^2)**
- **Biomass Storage Chamber**
- **Supporting Research Labs (8):**
  - ✓ **Biomass Characterization**
  - ✓ **Analytical Center**
  - ✓ **Microbiology**
  - ✓ **Molecular Biology**
  - ✓ **Fermentation Technology**
  - ✓ **Product Engineering**
  - ✓ **Unconventional Biofuels**
  - ✓ **Thermochemical Processes**
- **Utilities**
- **Workshop Room**

Biotechnological Valorization of Agro and Forestry Residues

Ongoing Projects

Residues:

- **SUGAR CANE BAGASSE**
- **SUGAR CANE STRAW**
- **CORN COBS**
- **CASTOR BEAN CAKE**
- **CELLULOSE INDUSTRY WASTES**
- **MOLASSES**
- **STILLAGE (VINASSE)**

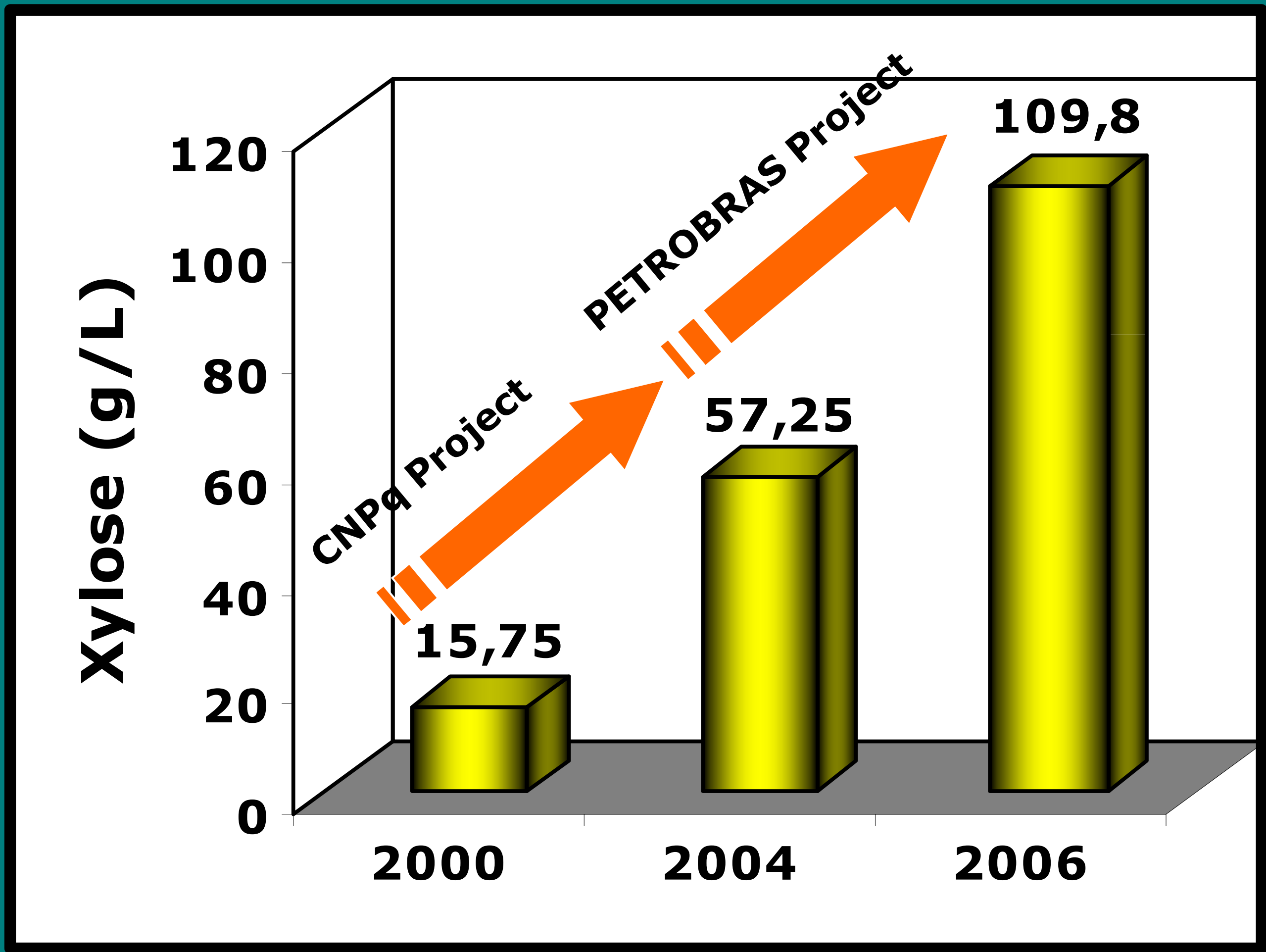
Target-Products:

- **ETHANOL**
- **BUTANEDIOL**
- **XYLITOL**
- **SUCCINIC ACID**
- **PROPIONIC ACID**
- **XYLANASES**
- **CELLULASES**

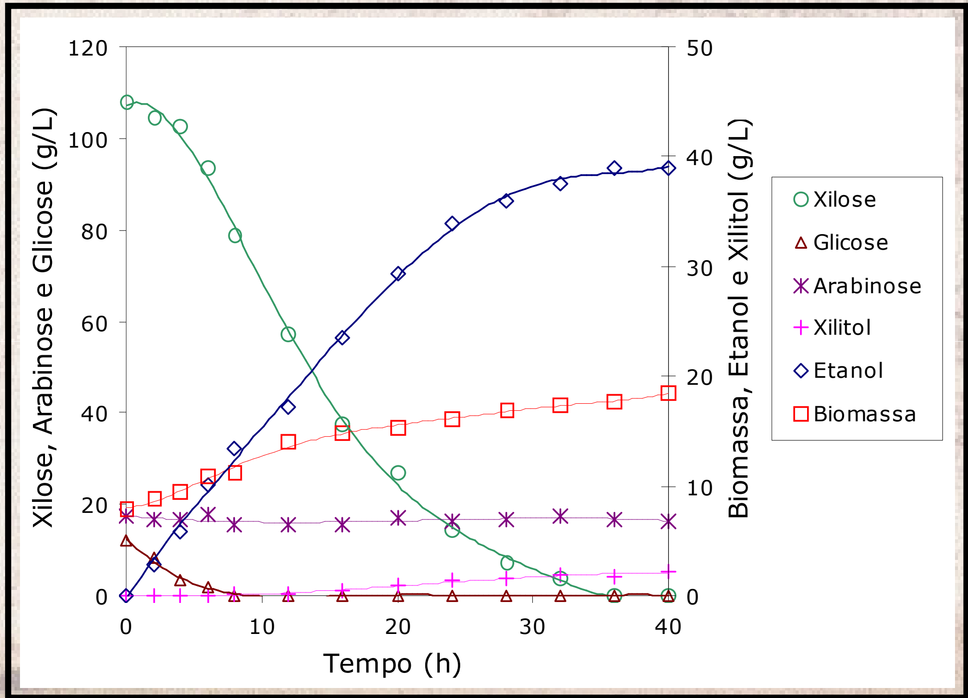
35 *MSc* Dissertations; 12 *PhD* Theses and 03 Post-doctorate Projects

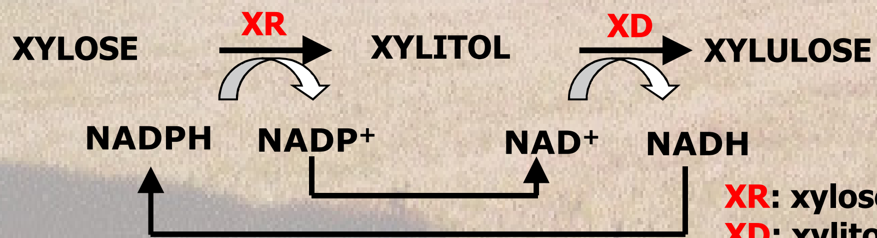
Partners/Financial Supporters: PETROBRAS; FAPERJ; PRONEX (CNPq); ICB/UFRJ; DBMol/UNB; CAM/UFAM; LC/USP; IQ/UFRJ; IM/UFRJ; ARACRUZ, NREL-DOE/USA and INETI/PT.

Progresses in Acid Pretreatment of Sugar Cane Bagasse (Hemicellulose Hydrolysis)



Ethanol Fermentation of Sugar Cane Bagasse HEMICELLULOSE Hydrolysate by *Pichia stipitis*

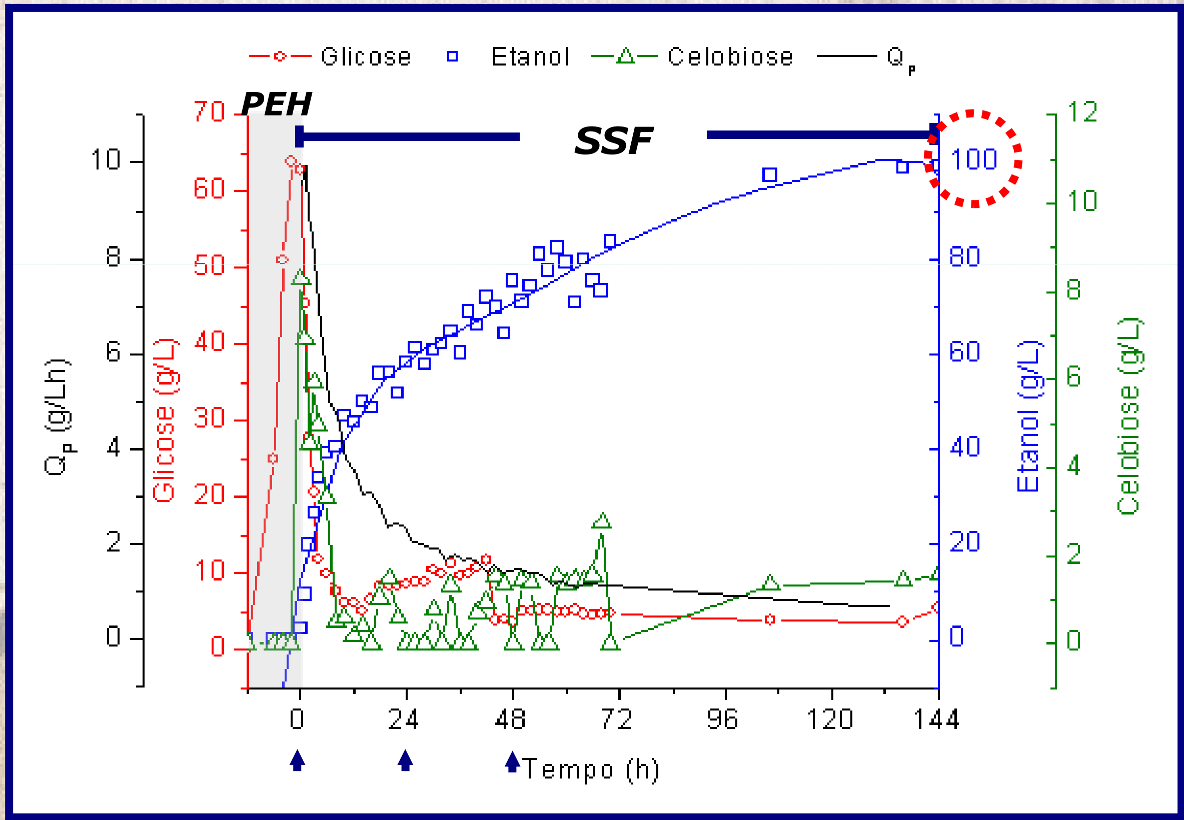




XR: xylose reductase;
XD: xylitol dehydrogenase

Simultaneous Saccharification and Fermentation of CELLULOSE

Kinetics of ethanol production from sugarcane bagasse cellulignin with solid load by SSF process using a homemade enzymatic preparation obtained by a selected strain of *Penicillium* sp.

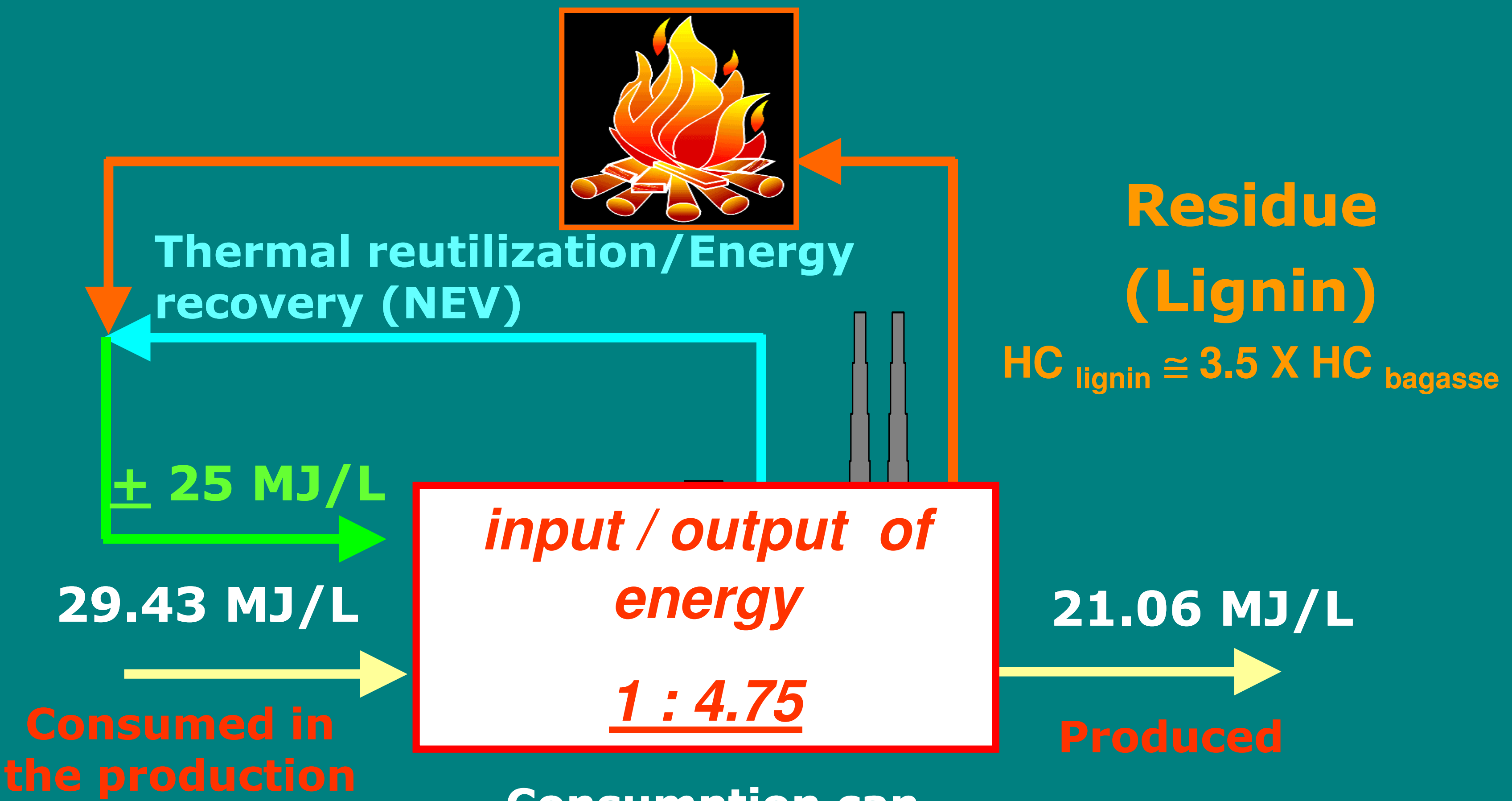


Arrows indicate the time in which the reactor was fed with cellulignin. The grey range depicts the pre-enzymatic (PEH)

Bioprocess Analysis: Energy Balance

1,000 ton of Bagasse: 220,000 L

SuperPro Design
(Inteligen Co.)



Consumption can reach values of only 5 MJ/L EtOH

Mapa tecnológico do tema "Biorrefinarias: Rota Bioquímica" no Mundo e no Brasil (2010 – 2030)

Estágios	Desenvolvimento do tema "biorrefinarias: rota bioquímica" no mundo		
	2010 - 2015	2016 - 2025	2026 - 2030
Comercialização	T1b T1 T1d T1e	T1c	
Produção/ processo	T1 T1e T1b T1d	T1c	
Inovação/ implantação	T1a d T1b	T1c	
Scale-up	T1 T	T1c	
Fase demonstração	T1d T1a T1c		
Fase piloto	T1 T1c a		
Pesquisa em bancada	1c		

Estágios	Desenvolvimento do tema "biorrefinarias: rota bioquímica" no Brasil		
	2010 - 2015	2016 - 2025	2026 - 2030
Comercialização	T1e	T1d T1a	T1b T1c
Produção/ processo	T1e	d T1a	1b T1c
Inovação/ implantação		T1 T1d T1b T1c	
Scale-up		T1d T1c T1b T1a	
Fase demonstração	T1d a T1b	T1c	
Fase piloto	T1d b T1c T1a		
Pesquisa em bancada	T1d 1b a T1c		

Notação: T1a – Pré-tratamento; **T1b –** Produção de celulases; **T1c –** Biologia molecular; **T1d** – Produção de biocombustíveis de segunda geração e de outras moléculas; **T1e –** Produção de energia e Integração energética de processo.

Challenges for efficient ethanol production from lignocellulosic biomass

- ☞ **Genetic Modification of Plant Cell Walls for Enhanced Biomass Production and Utilization;**
- ☞ **Cost-effective pretreatment technology, with minimum generation of toxic substances (fermentation inhibitors);**
- ☞ **Dedicated (*in plant*) cellulase production;**
- ☞ **Enzyme engineering for efficient biomass hydrolysis;**
- ☞ **Genetically modified microorganisms for efficient fermentation of C5 and C6;**
- ☞ **Process energy integration (valorization of lignin).**

Appl Biochem Biotechnol (2010) 161:411–422
DOI 10.1007/s12010-009-8875-6

Nitrogen Source Optimization for Cellulase Production by *Penicillium funiculosum*, using a Sequential Experimental Design Methodology and the Desirability Function

Roberto Nobuyuki Maeda ·
Mariana Mello Pereira da Silva ·
Lídia Maria Melo Santa Anna · Nei Pereira Jr.

J Ind Microbiol Biotechnol (2010) 37:151–158
DOI 10.1007/s10295-009-0656-2

ORIGINAL PAPER

Cellulases from *Penicillium funiculosum*: production, properties and application to cellulose hydrolysis

Aline Machado de Castro · Marcelle Lins de Albuquerque de Carvalho ·
Selma Gomes Ferreira Leite · Nei Pereira Jr.

Appl Biochem Biotechnol
DOI 10.1007/s12010-010-8986-0

Trichoderma harzianum IOC-4038: A Promising Strain for the Production of a Cellulolytic Complex with Significant β-Glucosidase Activity from Sugarcane Bagasse Cellulignin

Aline Machado de Castro · Kelly Cristina Nascimento Rodrigues Pedro ·
Juliana Cunha da Cruz · Marcela Costa Ferreira · Selma Gomes Ferreira Leite ·
Nei Pereira Jr

SAGE-Hindawi Access to Research
Enzyme Research
Volume 2010, Article ID 854526, 8 pages
doi:10.4061/2010/854526

Research Article

High-Yield Endoglucanase Production by *Trichoderma harzianum* IOC-3844 Cultivated in Pretreated Sugarcane Mill Byproduct

Aline Machado de Castro,[1] Marcela Costa Ferreira,[2] Juliana Cunha da Cruz,[2]
Kelly Cristina Nascimento Rodrigues Pedro,[2] Daniele Fernandes Carvalho,[2]
Selma Gomes Ferreira Leite,[2] and Nei Pereira Jr.[2]

Acta Cryst. (2010). F66, 1041-1044 [doi:10.1107/S1744309110026886]

Purification, crystallization and preliminary crystallographic analysis of the catalytic domain of the extracellular cellulase CBHI from *Trichoderma harzianum*

F. Colussi, L. C. Textor, V. Serpa, R. N. Maeda, N. Pereira Jr and I. Polikarpov

Abstract: The filamentous fungus *Trichoderma harzianum* has a considerable cellulolytic activity that is mediated by a complex of enzymes which are essential for the hydrolysis of microcrystalline cellulose. These enzymes were produced by the induction of *T. harzianum* with microcrystalline cellulose (Avicel) under submerged fermentation in a bioreactor. The catalytic core domain (CCD) of cellobiohydrolase I (CBHI) was purified from the extracellular extracts and submitted to robotic crystallization. Diffraction-quality CBHI CCD crystals were grown and an X-ray diffraction data set was collected under cryogenic conditions using a synchrotron-radiation source.

Keywords: cellobiohydrolases; *Trichoderma harzianum*; cellulases.

Química Nova
versão impressa ISSN 0100-4042
Quím. Nova vol.33 no.1 São Paulo 2010
doi: 10.1590/S0100-40422010000100031

Review

Production, properties and application of cellulases in the hydrolysis of agroindustrial residues

Aline Machado de Castro[I, *]; Nei Pereira Jr.[II]

[I]Centro de Pesquisas e Desenvolvimento Leopoldo Américo Miguez de Mello, PETRÓLEO BRASILEIRO S.A., Av. Horácio Macedo, 950, Ilha do Fundão, 21941-920 Rio de Janeiro - RJ, Brasil
[II]Escola de Química, Universidade Federal do Rio de Janeiro, 21945-970 Rio de Janeiro - RJ, Brasil

Electronic Journal of Biotechnology ISSN: 0717-3458 Vol.13 No.3, Issue of May 15, 2010
Received July 2, 2009 / Accepted November 10, 2009

DOI: 10.2225/vol13-issue3-fulltext-3

Sugar cane bagasse as feedstock for second generation ethanol production. Part I: Diluted acid pretreatment optimization

Gabriel J. Vargas Betancur
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Escola de Química, Centro de Tecnologia
Universidade Federal do Rio de Janeiro, Brasil

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Website: www.ladebio.org.br

Financial support: Brazilian Council for Research (CNPq) and Brazilian Oil Company Research Center (PETROBRAS).

Keywords: hemicellulose hydrolysis, severity factor, xylose.

Electronic Journal of Biotechnology ISSN: 0717-3458
http://www.ejbiotechnology.info
DOI: 10.2225/vol13-issue5-fulltext-8

Sugar cane bagasse as feedstock for second generation ethanol production. Part II: Hemicellulose hydrolysate fermentability

Gabriel J. Vargas Betancur[1#] **· Nei Pereira Jr.**[1]

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Corresponding author: nei@eq.ufrj.br
Received October 18, 2009 / Accepted June 1, 2010
Published online: September 15, 2010

Appl Biochem Biotechnol
DOI 10.1007/s12010-010-9096-8

Ethanol Production from Residual Wood Chips of Cellulose Industry: Acid Pretreatment Investigation, Hemicellulosic Hydrolysate Fermentation, and Remaining Solid Fraction Fermentation by SSF Process

Neumara Luci Conceição Silva · Gabriel Jaime Vargas Betancur · Mariana Peñuela Vasquez · Edélvio Barros · Nei Pereira Jr.

Received: 19 May 2009 / Accepted: 20 September 2010

Electronic Journal of Biotechnology ISSN: 0717-3458 Vol.13 No.2, Issue of March 15, 2010
Received May 11, 2009 / Accepted October 14, 2009

DOI: 10.2225/vol13-issue2-fulltext-1
RESEARCH ARTICLE

Simultaneous saccharification and fermentation process of different cellulosic substrates using a recombinant *Saccharomyces cerevisiae* harbouring the β-glucosidase gene

Verônica Ferreira **Mariana de Oliveira Faber** **Sabrina da Silva Mesquita**

Nei Pereira Jr.*
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First Page

Appl Biochem Biotechnol (2010) 161:93–105
DOI 10.1007/s12010-009-8810-x

Ethanol Production from Sugarcane Bagasse by *Zymomonas mobilis* Using Simultaneous Saccharification and Fermentation (SSF) Process

Danielle da Silveira dos Santos · Anna Carolina Camelo · Kelly Cristina Pedro Rodrigues · Luís Cláudio Carlos · Nei Pereira Jr.

Renewable and Sustainable Energy Reviews 14 (2010) 3041–3049

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

journal homepage: www.elsevier.com/locate/rser

ELSEVIER

The situation of biofuels in Brazil: New generation technologies

Antonio Carlos Augusto da Costa[a,*], Nei Pereira Junior[b,1], Donato Alexandre Gomes Aranda[c,2]

[a] Laboratório de Bioprocessos, Departamento de Tecnologia de Processos Bioquímicos, Instituto de Química, Universidade do Estado do Rio de Janeiro, Brasil, R. S. Francisco Xavier 524, Pav. Haroldo Lisboa da Cunha, Room 427, Rio de Janeiro 20550-013, Brazil
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[c] Laboratório de Tecnologias Verdes - Greentec, Departamento de Engenharia Química, Escola de Química, Universidade Federal do Rio de Janeiro, Brasil, Av. Horácio Macedo 2030, Bloco E, Brazil

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 July 2010 (08.07.2010)

PCT

(10) International Publication Number
WO 2010/076552 A1

PROCESS FOR PRODUCTION OF AN ENZYMATIC PREPARATION FOR HYDROLYSIS OF CELLULOSE FROM LIGNOCELLULOSIC RESIDUES AND APPLICATION THEREOF IN THE PRODUCTION OF ETHANOL

Inventors/Applicants *(for US only)*: **DE CASTRO, Aline Machado** [BR/BR]; Rua Dias da Cruz N°827, Ap. 404 Méier, CEP-20720-011 Rio de Janeiro, RJ (BR). **SANT'ANNA, Lídia Maria Melo** [BR/BR]; Rua Condominio Vale de Itaipu, Rua 03, Casa 332, Itaipu, CEP-24340-240 Niterói, RJ (BR). **JUNIOR, Nei Pereira** [BR/BR]; Rua Humberto de Campos, N°410, Ap 503, Leblon, CEP-22430-190 Rio de Janeiro, RJ (BR). **GOMES, Absai da Conceição** [BR/BR]; Rua Himalaia N°396, Anchietta, CEP-21655-330 Rio de Janeiro, RJ (BR). **MENEZES, Emerson Pires** [BR/BR]; Rua Juquerí, N°185-F, Irajá, CEP-21371-021 Rio de Janeiro, RJ (BR). **SILVEIRA, Claudia Julia Groposo** [UY/BR]; Rua Nononha Torrezao N°181, Ap. 1307, Bl.03, CEP-24240-185 Santa Rosa Niterói, RJ (BR). **MOYSES, Danuza Nogueira** [BR/BR]; Av. Sete de Setembro N°85, Ap. 1601 Icaraí, CEP-24230-250 Niterói, RJ (BR). **BANDEIRA, Luiz Fernando Martins** [BR/BR]; Rua Afonso Pena N°132, Ap. 401 Tijuca, CEP-20270 245 Rio de Janeiro, RJ (BR). **MAEDA, Roberto Nobuyuki** [BR/BR]; Av. Nossa Senhora de Copacabana N° 245, Ap. 1008 Copacabana, CEP-20270 245 Rio de Janeiro, RJ (BR).

República Federativa do Brasil
Ministério do Desenvolvimento, Indústria
e do Comércio Exterior
Instituto Nacional da Propriedade Industrial.

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BRPI0805560A2

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(43) Data da Publicação: 08/09/2010
(RPI 2070)

(51) Int.Cl.:
C12N 9/42
C12R 1/80
C13K 1/02

(54) Título: **PROCESSO DE PRODUÇÃO DE UM PREPARADO ENZIMÁTICO PARA HIDRÓLISE DE CELULOSE DE RESÍDUOS LIGNOCELULÓSICOS E SUA APLICAÇÃO NA PRODUÇÃO DE ETANOL**

(73) Titular(es): Petroleo Brasileiro S.A. - PETROBRAS

(72) Inventor(es): Absai da Conceição Gomes, Aline Machado de Castro, Claudia Julia Groposo Silveira, Danuza Nogueira Moyses, Emerson Pires Menezes, Luiz Fernando Martins Bandeira, Lídia Maria Melo Santa Anna, Nei Pereira Junior, Roberto Nobuyuki Maeda

(57) Resumo: PROCESSO DE PRODUÇÃO DE UM PREPARADO ENZIMÁTICO PARA HIDRÓLISE DE CELULOSE DE RESÍDUOS LIGNOCELULÓSICOS E SUA APLICAÇÃO NA PRODUÇÃO DE ETANOL O processo da presente invenção baseia-se na produção microbiana de enzimas realizada a partir do crescimento do fungo Penicilllum funiculosum em meio de cultivo adaptado com substrato celulósico. O processo da invenção compreende especialmente o tratamento fermentativo de um substrato lignocelulósico por meio de um fungo especialmente adaptado, visando à obtenção de um preparado enzimático capaz de hidrolisar a celulose e a hemicelulose para a produção de etanol.

Thanks to my "Army", and Thanks for you attention !