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)

FAPESP

Workshop BIOEN de Pesquisa

06 e 07 de Novembro de 2012 - FAPESP - São Paulo

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

Germano Siqueira, Anikó Várnai, André Ferraz, Adriane M.F. Milagres. ENHANCEMENT OF CELLULOSE HYDROLYSIS IN SUGARCANE BAGASSE BY THE SELECTIVE REMOVAL OF LIGNIN WITH SODIUM CHLORITE. **Applied Energy** (2012) in press.

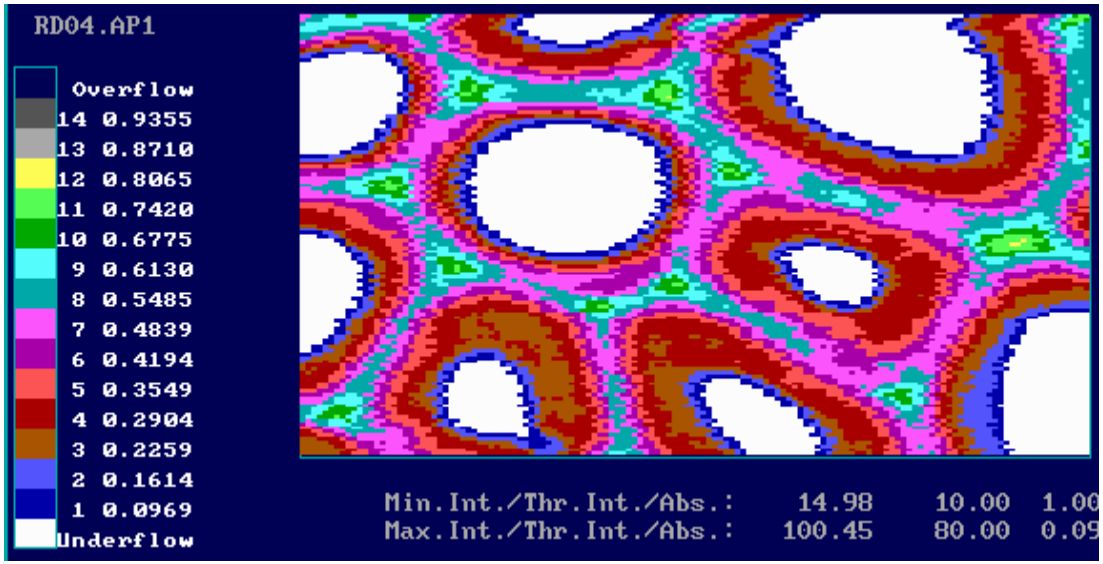
Michel Brienzo, Joseana R Monte, Adriane MF Milagres. INDUCTION OF CELLULASE AND HEMICELLULASE ACTIVITIES OF *Thermoascus aurantiacus* BY XYLAN HYDROLYZED PRODUCTS. **World Journal of Microbiology & Biotechnology,** v. 28, p. 113-119, 2012.

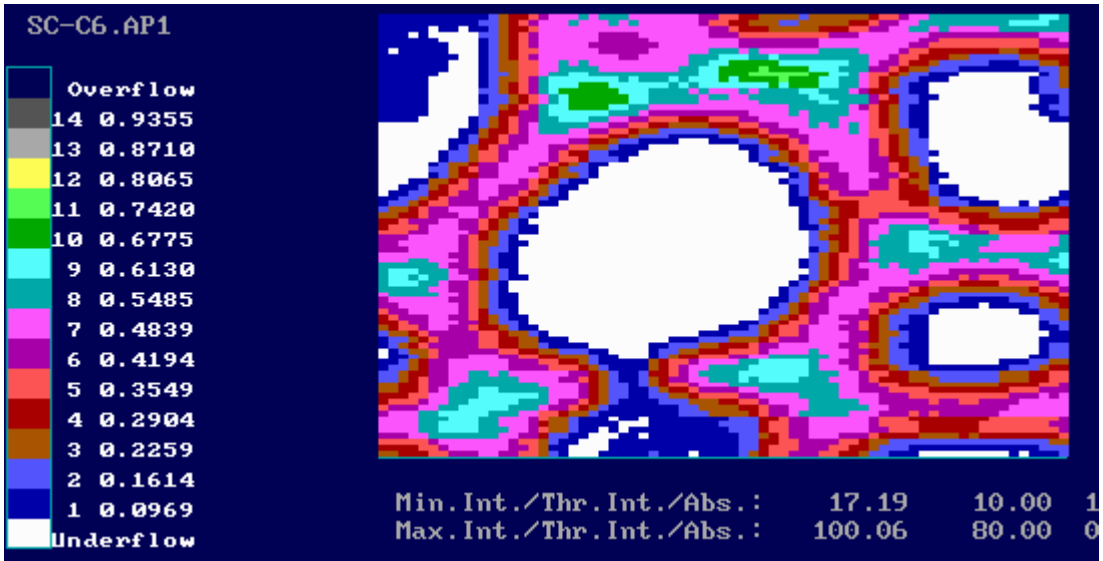
Germano Siqueira, Adriane MF Milagres, Walter Carvalho, Gerald Koch, André Ferraz. TOPOCHEMICAL DISTRIBUTION OF LIGNIN AND HYDROXYCINNAMIC ACIDS IN SUGAR-CANE CELL WALLS AND ITS CORRELATION WITH THE ENZYMATIC HYDROLYSIS OF POLYSACCHARIDES. **Biotechnology for Biofuels**, v. 4, p. 7, 2011.

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.

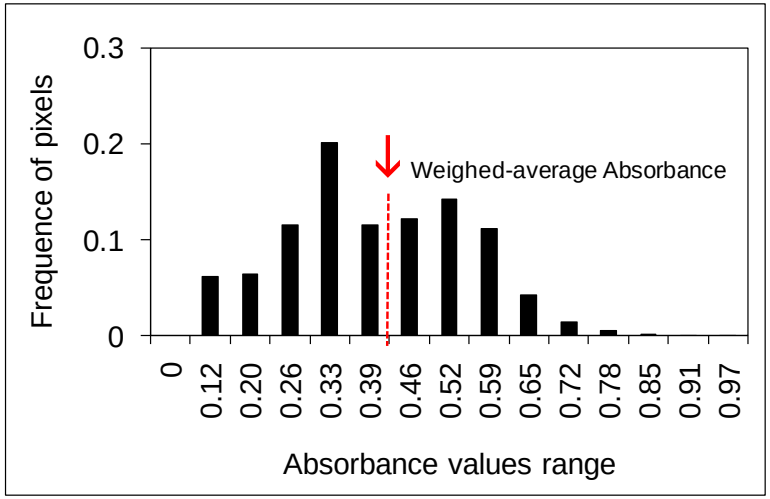
Fernando Masarin, Daniela B Gurpilhares, David CF Baffa, Márcio HP Barbosa, Walter Carvalho, André Ferraz, Adriane MF Milagres. CHEMICAL COMPOSITION AND ENZYMATIC DIGESTIBILITY OF SUGARCANE CLONES SELECTED FOR VARIED LIGNIN CONTENT. **Biotechnology for Biofuels**, v. 4, p. 55, 2011.

Topochemical distribution of lignin





Frequency histograms



Rind fibers

$Abs_{280\ nm}$=0.40

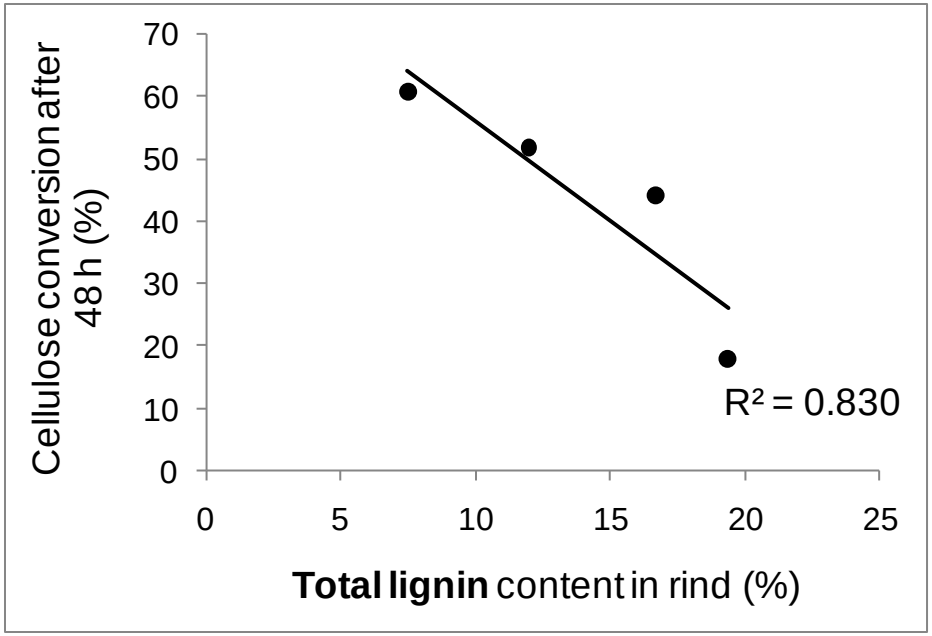
Pith fibers

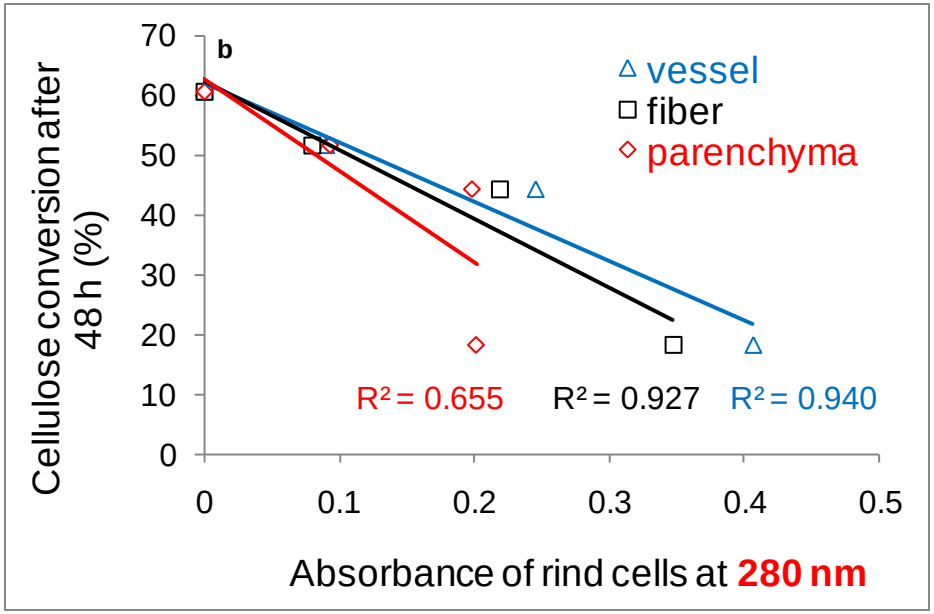
Abs_{280nm}=0.39

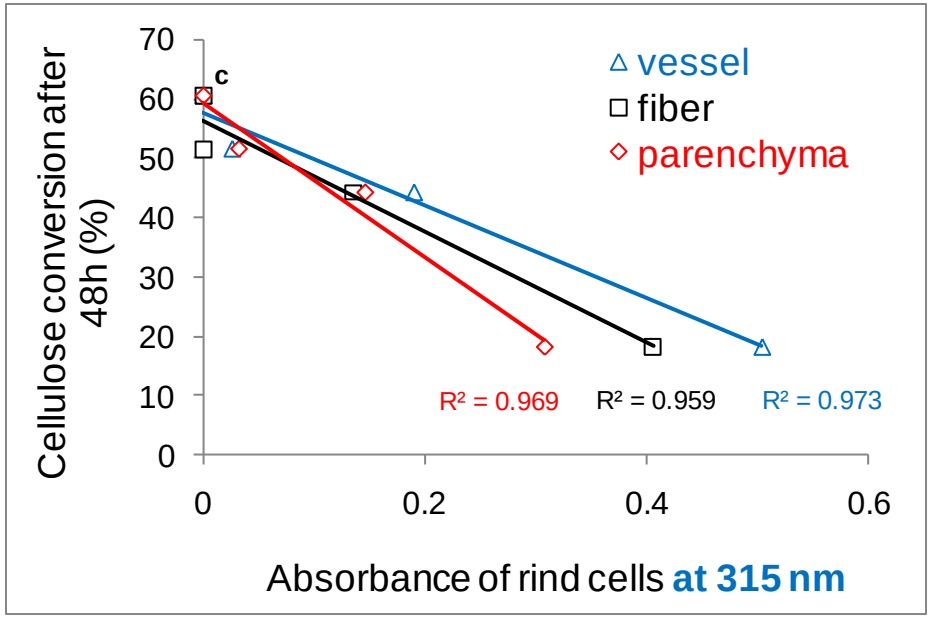
Similar lignin contents

Why different cells of sugar cane present varied recalcitrance?

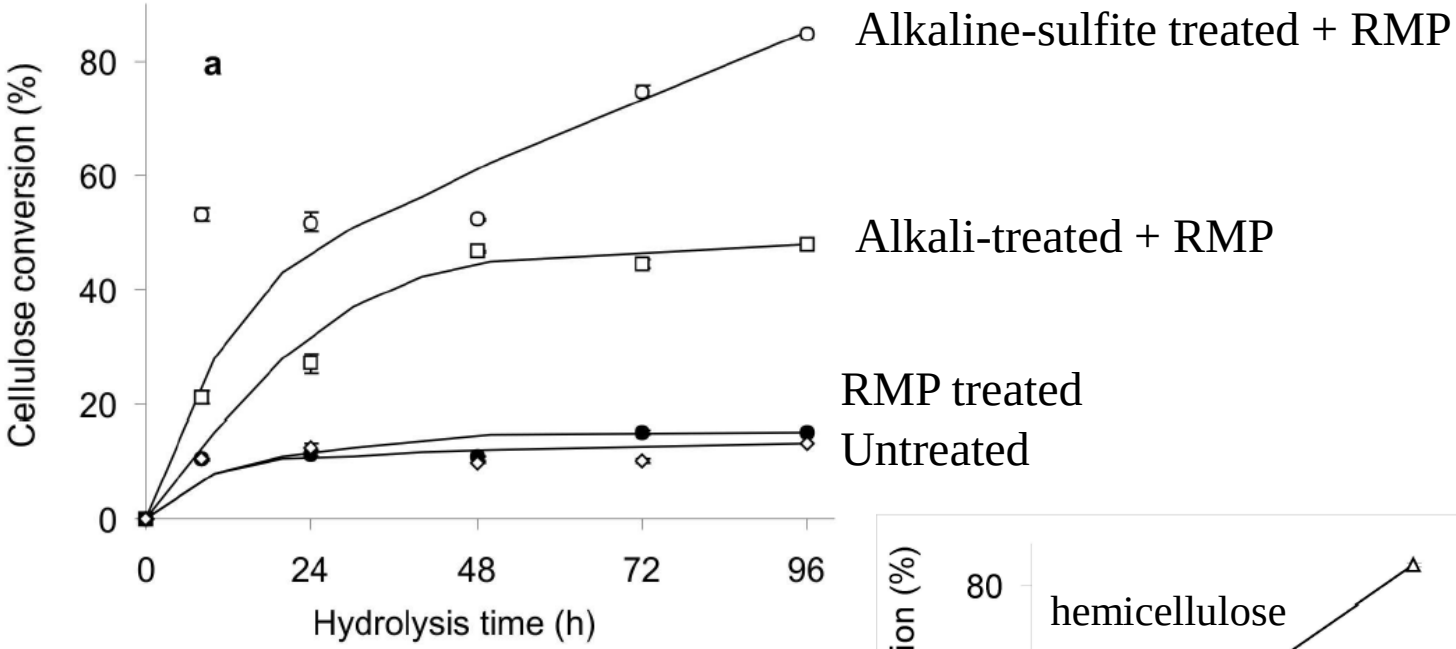
Correlation of enzymatic hydrolysis efficiency of the rind with the lignin contents and UV-microspectrophotometric data

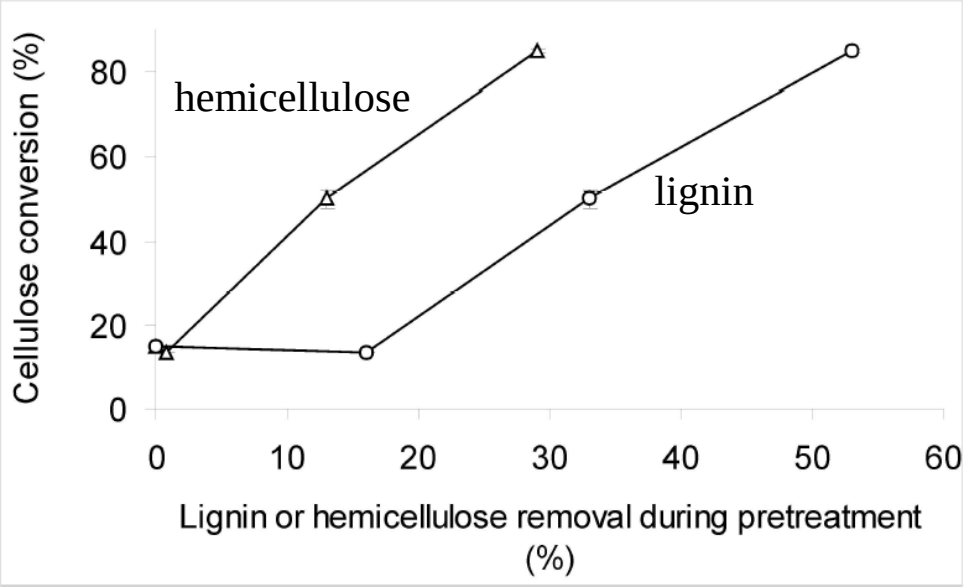




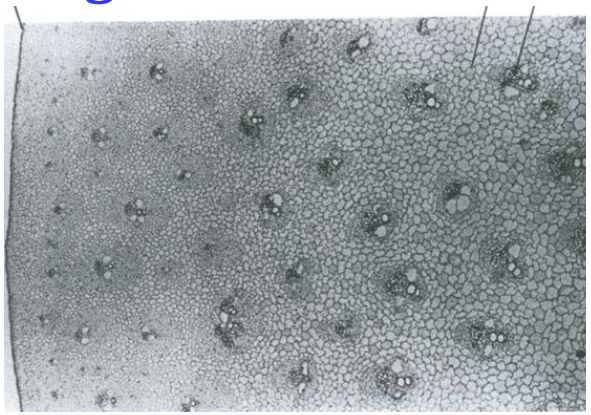


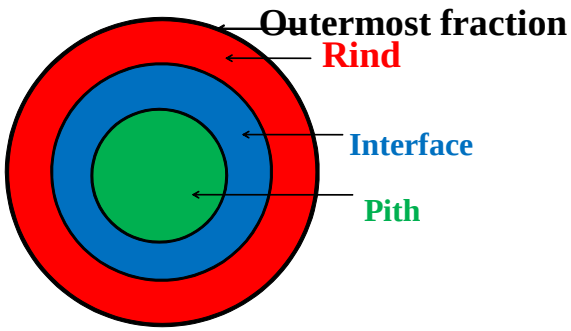
Enzymatic hydrolysis of alkaline-sulfite pretreated sugar cane bagasse

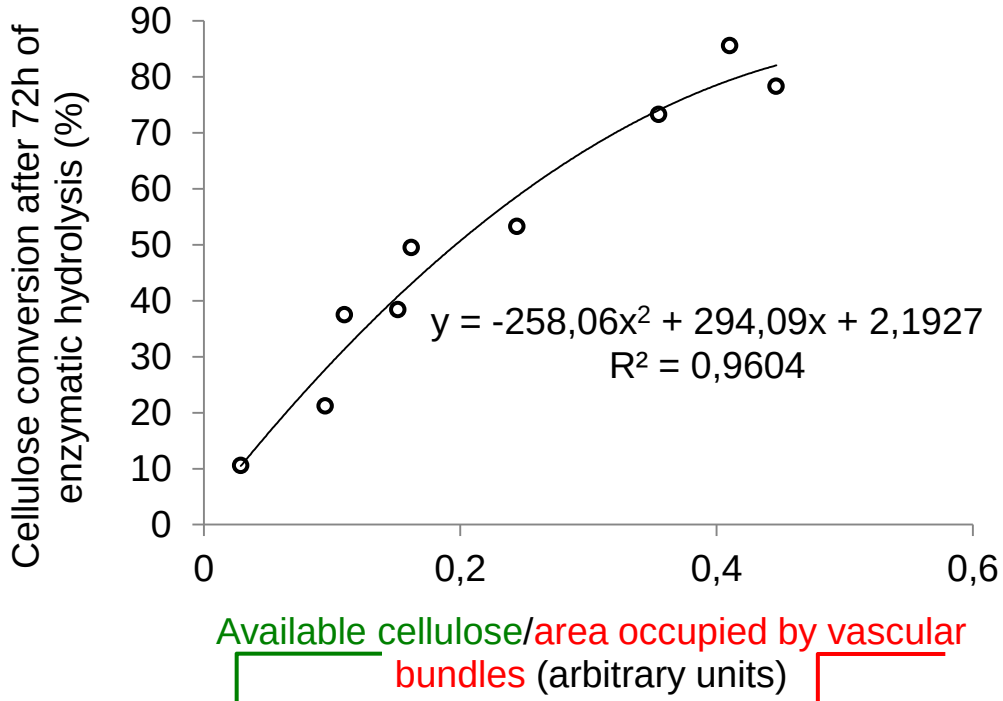




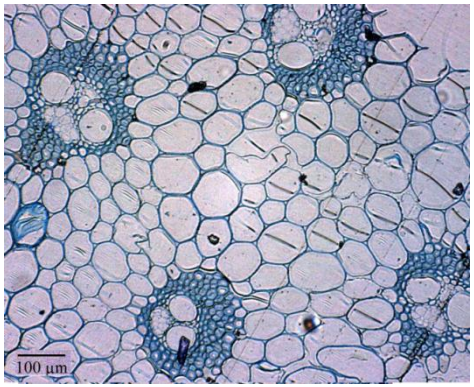
Enzymatic hydrolysis of different regions dissected from the sugar cane internodes from hybrids with varied lignin contents

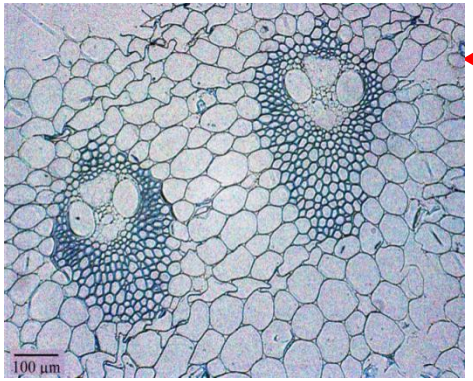






= cellulose / (lignin + hemicellulose)





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

Gurgel, L.V.A. et al. CHARACTERIZATION OF DEPOLYMERIZED RESIDUES FROM EXTREMELY LOW ACID HYDROLYSIS (ELA) OF SUGARCANE BAGASSE CELLULOSE: EFFECTS OF DEGREE OF POLYMERIZATION, CRYSTALLINITY AND CRYSTALLITE SIZE ON THERMAL DECOMPOSITION. INDUSTRIAL CROPS AND PRODUCTS. **Industrial Crops and Products**, 36, 560-571 (2012).

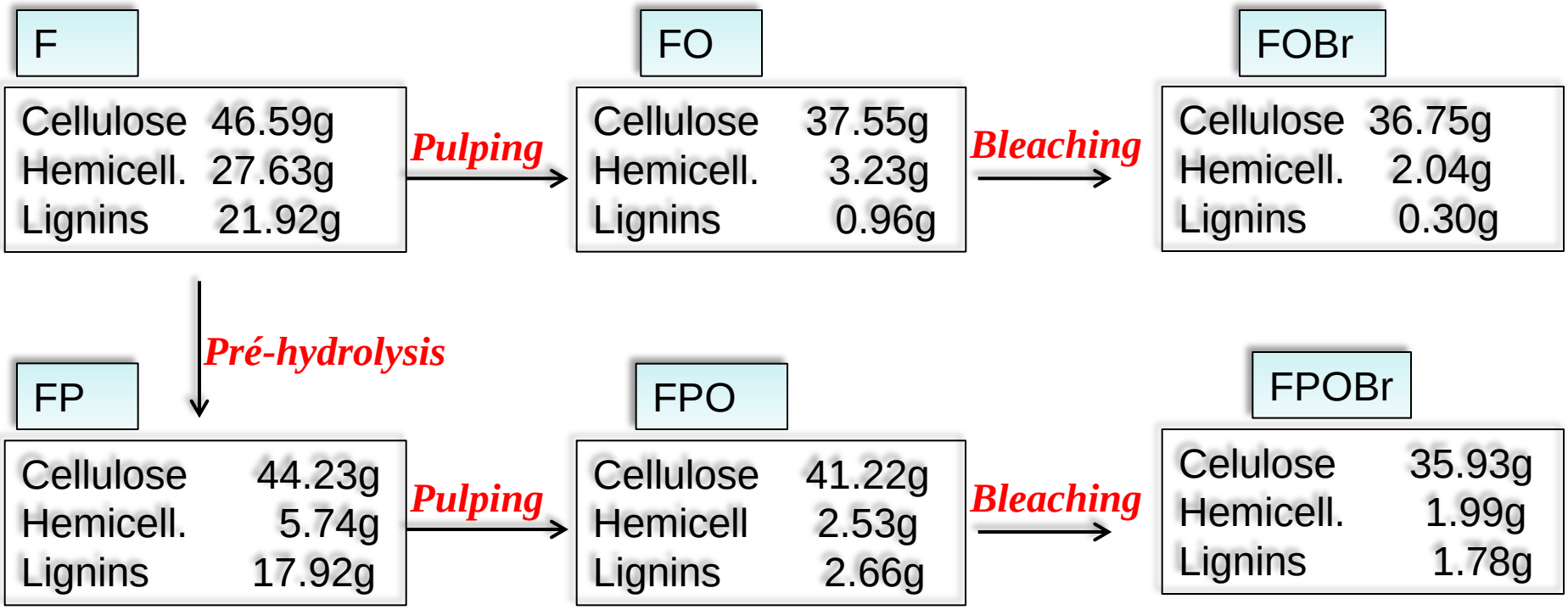
Gurgel, L.V.A. et al. DILUTE ACID HYDROLYSIS OF SUGAR CANE BAGASSE AT HIGH TEMPERATURES: A KINETIC STUDY OF CELLULOSE SACCHARIFICATION AND GLUCOSE DECOMPOSITION. PART I: SULFURIC ACID AS THE CATALYST. **Ind. & Eng. Chem. Res**., 51 (3), 1173-1185 (2012).

Vallejos, M.E. et al. LOW LIQUID SOLID RATIO (LSR) HOT WATER PRETREATMENT OF SUGARCANE BAGASSE. **Green Chemistry**, 14(7), 1982-1989 (2012).

Vallejos, M.E. et al. CHEMCIAL AND PHYSICO-CHEMICAL CHARACTERIZATION OF LIGNINS OBTAINED FROM ETHANOL-WATER FRACTIONATION OF BAGASSE. **Bioresources**, 6(2), 1158-1171 (2011).

Novo, L.P. et al. DELIGNIFICATION OF SUGARCANE BAGASSE USING GLYCEROL WATER MIXTURES TO PRODUCE PULPS FOR SACCHARIFICATION. **Bioresource Technology**, 102, 10.040 – 10.046 (2011).

Mass balances for the integrated organosolv processing of sugar cane bagasse



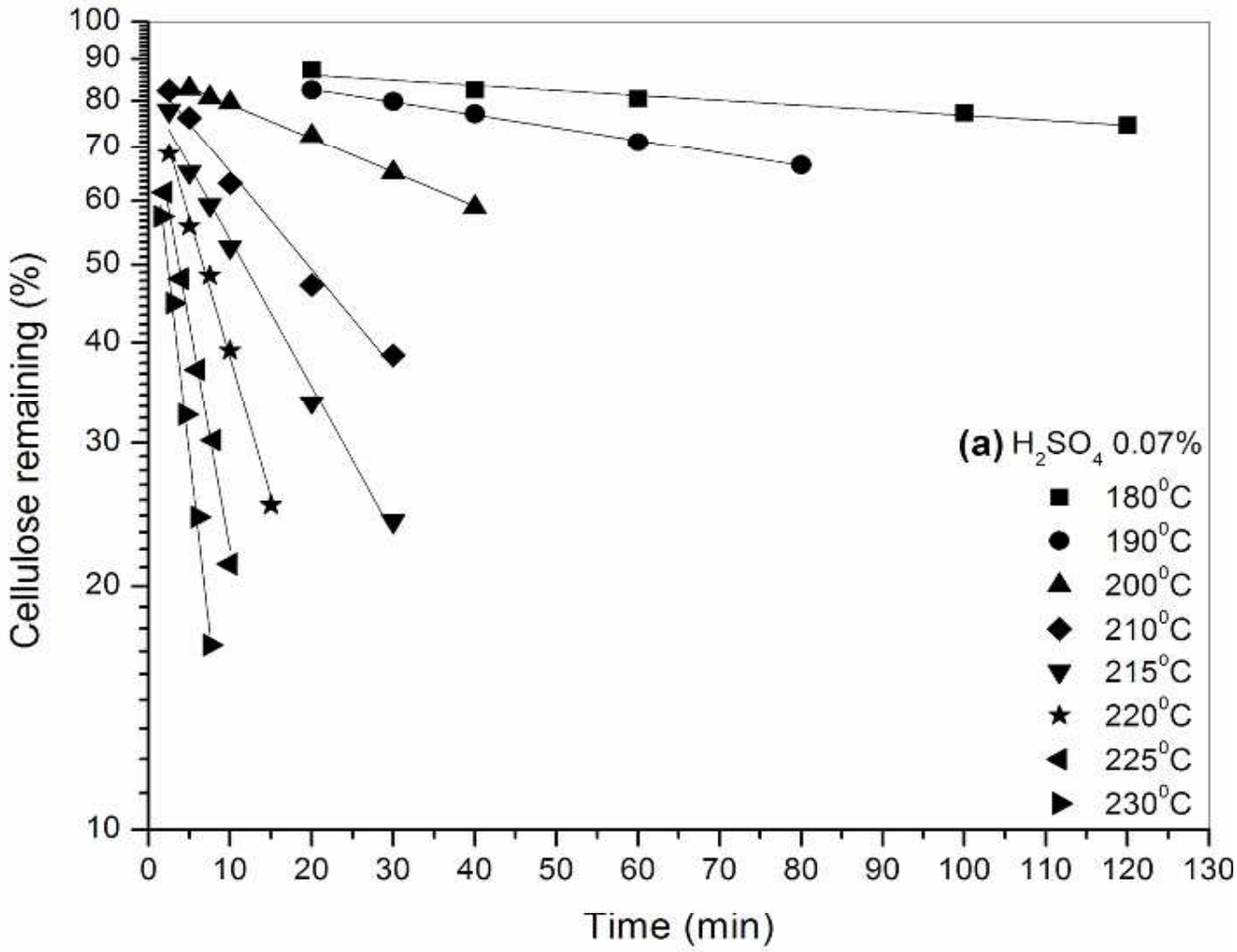
Efficient solubilization of the hemicellulosic fraction using mineral acids and supercritical fluids

Table 5 Concentrations of xylose (*X*), xylan (Xyn) and total xylan (TXyn) in the spent liquor and composition (wt%) of spent liquor[a]

T (°C)	*t* (min)	LSR (g g^{-1})	X (g L^{-1})	Xyn	TXyn	G (wt%)	X	A	AAc	Gln	Xyn	Ara	HMF	F	Xyn*[b]
150	30	3	0.42	4.97	5.34	0.12	0.10	0.53	0.20	0.59	1.22	0.27	n.d.	n.d.	5.60
170	30	3	2.98	31.13	33.76	0.30	0.75	0.92	0.60	0.58	7.85	0.31	0.01	0.10	37.55
150	60	3	2.22	10.34	12.30	0.24	0.54	0.71	0.19	0.57	2.78	0.37	n.d.	0.02	13.29
170	60	3	13.76	36.18	48.29	0.30	3.44	1.25	1.42	0.91	9.64	0.13	0.10	0.29	56.96
150	30	9	0.20	1.27	1.44	0.43	0.16	0.55	0.21	0.29	1.10	0.28	n.d.	n.d.	4.54
170	30	9	0.93	8.49	9.31	0.60	0.76	1.26	0.52	0.65	7.93	0.34	0.02	0.56	32.73
150	60	9	0.29	3.50	3.76	0.42	0.24	0.83	0.49	0.59	3.20	0.39	n.d.	0.02	11.85
170	60	9	2.14	10.28	12.17	0.36	1.78	1.39	1.10	0.82	8.55	0.32	0.02	0.31	40.81
143	45	6	0.24	2.07	2.29	0.12	0.12	0.50	0.13	0.39	1.01	0.27	n.d.	n.d.	5.09
177	45	6	7.01	13.29	19.46	0.31	3.72	1.36	1.52	0.76	8.01	0.14	n.d.	0.05	43.47
160	20	6	0.22	1.83	2.02	0.24	0.11	0.43	0.20	0.20	1.03	0.29	n.d.	n.d.	4.21
160	70	6	1.20	12.40	13.46	0.26	0.61	1.10	0.66	0.63	6.70	0.39	n.d.	0.08	29.01
160	45	1	3.02	40.36	43.02	0.08	0.14	0.44	0.20	0.42	2.01	0.17	n.d.	n.d.	14.87
160	45	11	0.78	5.29	5.97	0.31	0.76	1.24	0.41	0.80	5.58	0.42	n.d.	n.d.	23.13
160	45	6	1.02	8.54	9.44	0.24	0.52	0.99	0.22	1.05	5.28	0.82	n.d.	0.01	19.98
160	45	6	0.87	9.92	10.68	0.24	0.44	1.04	0.42	0.69	5.21	0.36	n.d.	n.d.	22.42

[a] 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) [b] 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 occuring 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

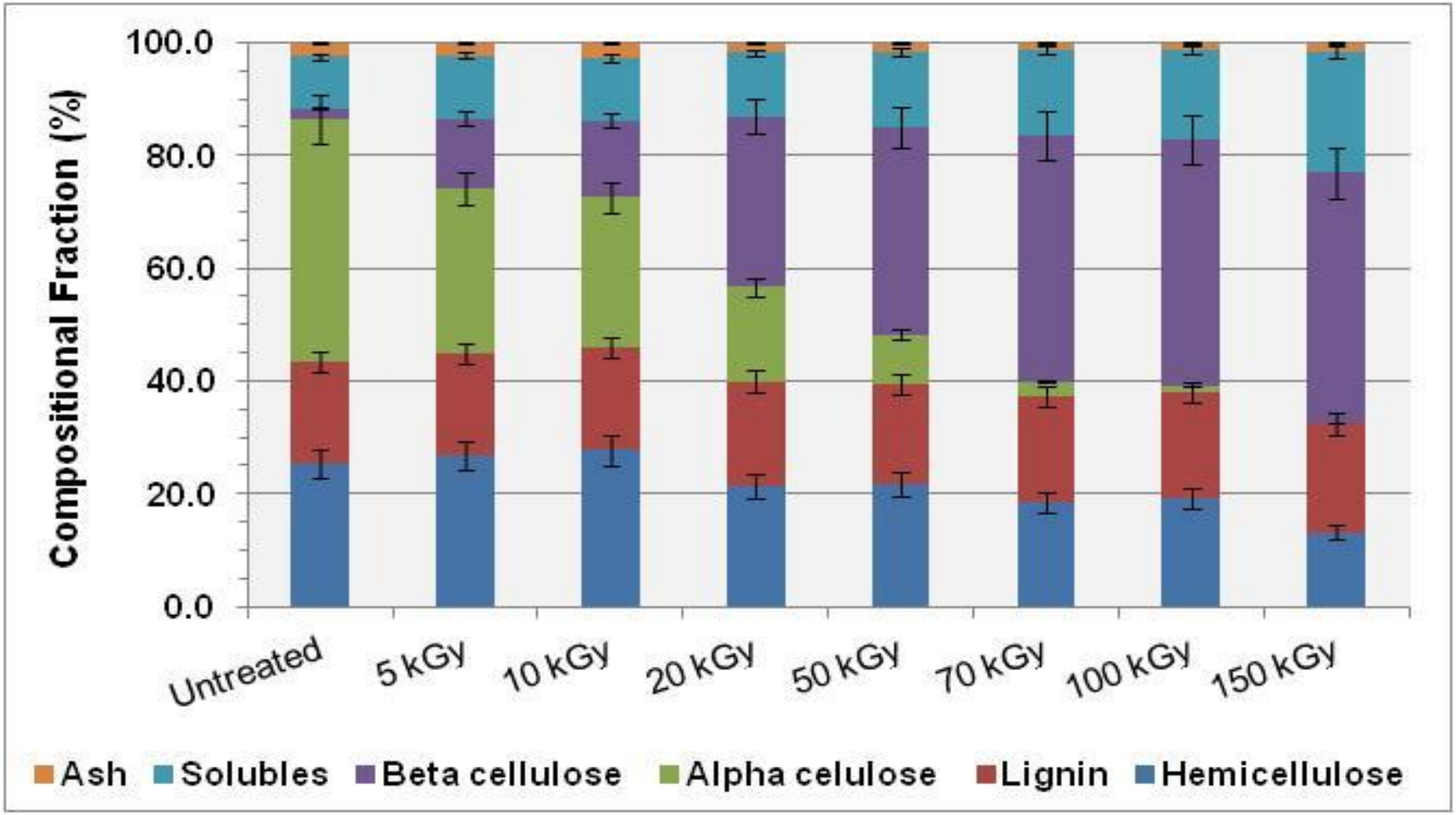
Publications based on the project results

Duarte,CL. Ribeiro, MA, Oikawa H, Mori, MN, Napolitano, CM, Galvão, CA. ELECTRON BEAM COMBINED WITH HYDROTHERMAL TREATMENT FOR ENHANCING THE ENZYMATIC CONVERTIBILITY OF SUGARCANE BAGASSE. ***Radiat. Phys. Chem***. 81 (8), 1008-1011, 2012.

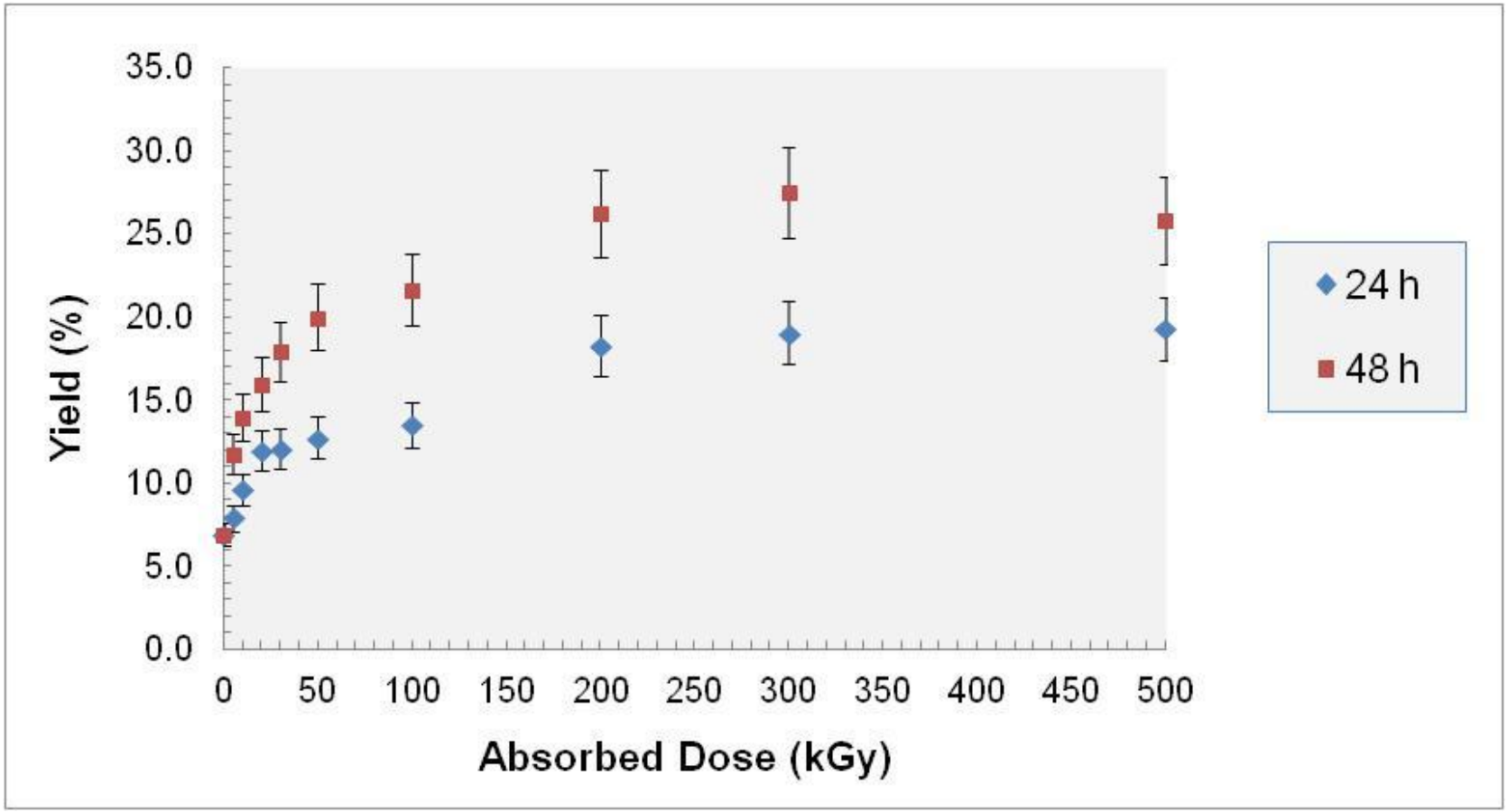
Duarte, C.L., et al., STUDY OF THERMAL TREATMENT COMBINED WITH RADIATION ON THE DECOMPOSITION OF POLYSACCHARIDES IN SUGARCANE BAGASSE. **Radiat. Phys. Chem.** (2012), http://dx.doi.org/10.1016/j.radphyschem.2012.06.019

Ribeiro, M.A., et al., DEGRADATION MECHANISM OF POLYSACCHARIDES ON IRRADIATED SUGARCANE BAGASSE. **Radiat. Phys. Chem.** (2012), http://dx.doi.org/10.1016/j.radphyschem.2012.06.034

Effect of irradiation intensity on the chemical composition of sugar cane bagasse components

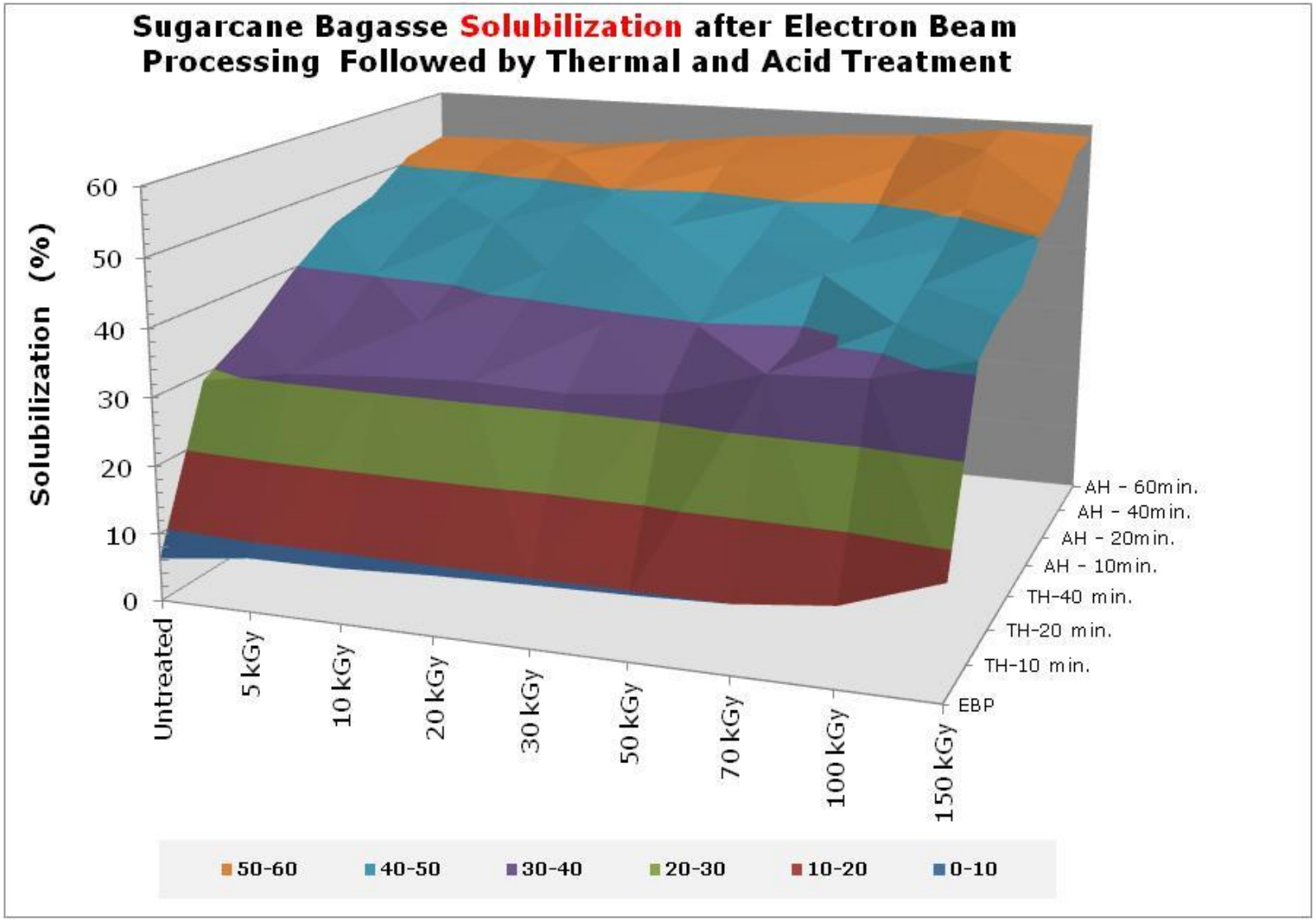


Enzymatic conversion of the pretreated materials



Enzyme complex of *Trichoderma* <u>*reesei*</u>, 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

J. A. Souza-Corrêa, M. T. B. Pimenta, G. J. M. Rocha, E. O. Gomez, F. M. Squina, A. A. S. Curvelo, J. Amorim. HYDROTHERMAL AND ATMOSPHERIC PRESSURE MICROPLASMA PRETREATMENT PROCESSES OF SUGARCANE BAGASSE. In**: Plasma for environmental issues, pp 68-76, Sofia, 2010.**

Loureiro and J. Amorim, **Phys. Rev. E**, 82, 035401 (R) (2010).

L. Reis Jr., J. Amorim, and A. Dal Pino JR., **Phys. Rev. E**, 83, 017401 (2011).

Márcio H.F. Bettega, Romarly F. da Costa, and Marco A. P. Lima, LOW-ENERGY ELECTRON COLLISIONS WITH ETHANE, ***Braz. J. Phys.*** *39, 69-73 (2009).*

T. C. Freitas, Marco A. P. Lima, Sylvio Canuto, and Márcio H. F. Bettega, ELECTRON COLLISIONS WITH THE CH2O-H2O COMPLEX, **Phys. Rev. A** 80, 62710-3 (2009)**.**

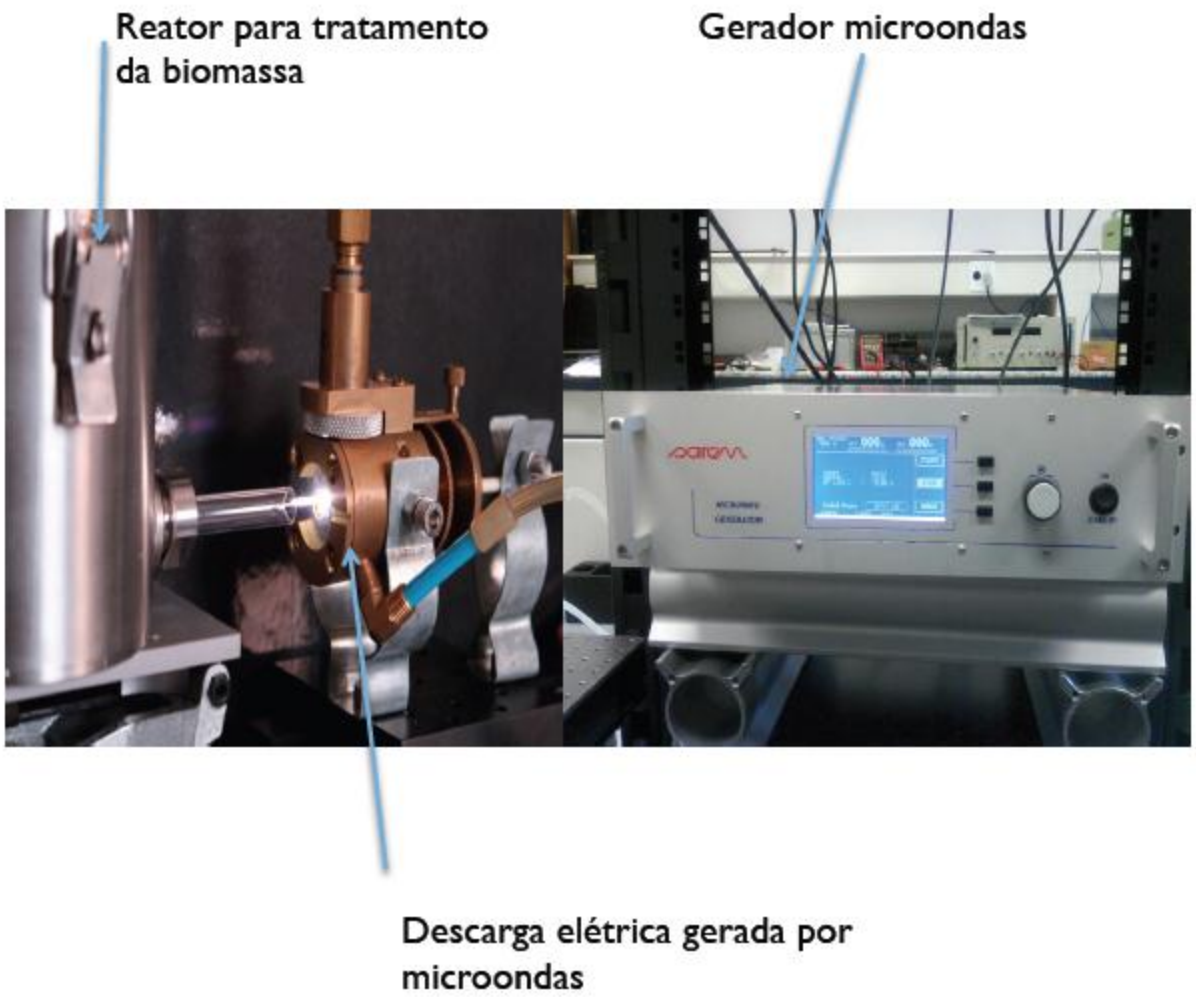
Romarly F. da Costa, Márcio H. F. Bettega, Márcio T. do N. Varella, and Marco A. P. Lima, ELECTRON COLLISIONS WITH A-GLUCOSE AND B-GLUCOSE MONOMERS, ***J. Chem. Phys.*** 132, 124309 (2010).

Eliane M. de Oliveira, Marco A. P. Lima, Márcio H. F. Bettega, Sergio d'A. Sanchez, Romarly F. da Costa, and Márcio T. do N. Varella, LOW-ENERGY ELECTRON COLLISIONS WITH PIRROLE, ***J. Chem. Phys***. *132, 204301 (2010).*

M. A. Khakoo, J. Muse, K. Ralphs, Romarly F. da Costa, Márcio H. F. Bettega and Marco A. P. Lima, LOW ENERGY ELASTIC ELECTRON SCATTERING FROM FURAN, ***Phys. Rev. A*** 81, 062716 (2010).

Romarly F. da Costa, Márcio H. F. Bettega, Marco A. P. Lima, Maria C. A. Lopes, Leigh R. Hargreaves, Gabriela Serna, and Murtadha A. Khakoo, ELECTRONIC EXCITATION OF GAS-PHASE FURAN MOLECULES BY ELECTRON IMPACT, ***Phys. Rev. A*** *85*, 062706-11 (2012).

Eliane M. de Oliveira, Sergio d'A. Sanchez, Márcio H. F. Bettega, Alexandra P. P. Natalense, Marco A. P. Lima, and Márcio T. do N. Varella, SHAPE RESONANCES IN LOW- ENERGY ELECTRON SCATTERING BY LIGNIN SUBUNITS, ***Phys. Rev. A (Rapid* Communication)** 6, 020701(R)(2012)



- The plasma-micro-wave equipemt 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

RO Moutta, AK Chandel, RCLB Rodrigues, MB Silva, GJM Rocha, SS Silva. STATISTICAL OPTIMIZATION OF SUGARCANE LEAVES HYDROLYSIS INTO SIMPLE SUGARS BY DILUTE SULFURIC ACID CATALYZED PROCESS, **Sugar Tech**, 14: 53-60, 2011.

AK Chandel, G Chandrasekhar, MB Silva, S Silvério da Silva THE REALM OF CELLULASES IN BIOREFINERY DEVELOPMENT, **Critical Reviews in Biotechnology** 32 (3), 187-202, 2012

RM Cadete, MA Melo, KJ Dussán, RCLB Rodrigues, SS Silva, JE Zilli, MJS Vital. DIVERSITY AND PHYSIOLOGICAL CHARACTERIZATION OF D-XYLOSE-FERMENTING YEASTS ISOLATED FROM THE BRAZILIAN AMAZONIAN FOREST, **PloS one** 7(8), e43135, 2012

AK Chandel, SS da Silva, OV Singh DETOXIFICATION OF LIGNOCELLULOSE HYDROLYSATES: BIOCHEMICAL AND METABOLIC ENGINEERING TOWARD WHITE BIOTECHNOLOGY, **BioEnergy Research** (in press)

Giese EC, Pierozzi M, Dussán KJ, Chandel AK, Silva SS. ENZYMATIC SACCHARIFICATION OF ACID-ALKALI PRETREATED SUGARCANE BAGASSE USING COMMERCIAL ENZYMATIC PREPARATIONS. **J. Chem. Technol. Biotechnol.** (in press)

Strain screening and use of selected strains for xylose utilization

224 yeast strains were isolated

32 yeast strains were selected

Table 3. Ethanol yield [$Y_{p/s}^{et}$ (g/g)], xylitol yield [$Y_{p/s}^{xy}$ (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.

Yeast species	Yeast strains	Sugars consumption (%)[1]	Cells (g/L)	$Y_{p/s}^{et}$ (g/g)[2]	Qp (g/L•h)[3]	H (%)[4]	Ethanol concentration (g/L)	$Y_{p/s}^{xy}$ (g/g)[5]	Xylitol concentration (g/L)	Fermentation time (h)[6]
Candida lignosa	CBS 4705	55.3	7.8	0.16	0.05	31.6	4.6	–	–	96
Scheffersomyces stipitis	NRRL Y-7124	34.9	3.4	0.25	0.10	49.3	4.9	–	–	48
S. stipitis	UFMG-XMD-15.2	80.9	12.7	0.34	0.20	65.7	14.1	–	–	72
Spathaspora passalidarum	UFMG-HMD-1.1	84.9	13.4	0.20	0.09	40.0	8.8	–	–	96
S. passalidarum	UFMG-HMD-14.1	91.0	13.1	0.18	0.10	36.0	9.5	–	–	96
Spathaspora sp. 1	UFMG-XMD-16.2	72.9	7.9	0.23	0.10	46.0	9.3	0.57	18.2	96
Spathaspora sp. 2	UFMG-XMD-23.2	68.6	6.4	0.22	0.08	42.4	7.2	0.61	17.1	96

[1] Sugar consumption (%) – percentage of the initial D-xylose and glucose consumed.
[2] $Y_{p/s}^{et}$ (g/g) – ethanol yield: correlation between ethanol ($\Delta P_{ethanol}$) produced and D-xylose and glucose (ΔS_{sugars}) consumed.
[3] $Y_{p/s}^{xy}$ (g/g) – xylitol yield: correlation between xylitol ($\Delta P_{xylitol}$) produced and D-xylose (ΔS_{xylose}) consumed.
[4] Qp (g/L·h) – ethanol productivity: ratio of ethanol concentration (g/L) and fermentation time (h).
[5] η (%) – fermentation efficiency: percentage of the maximum theoretical ethanol yield (0.51 g ethanol/g D-xylose and glucose).
[6] Time when the maximum ethanol production (g/L) value was attained or time of the end of the fermentation experiment.

Time course of xylose consumption and ethanol production by *Scheffersomyces stipitis* selected strain

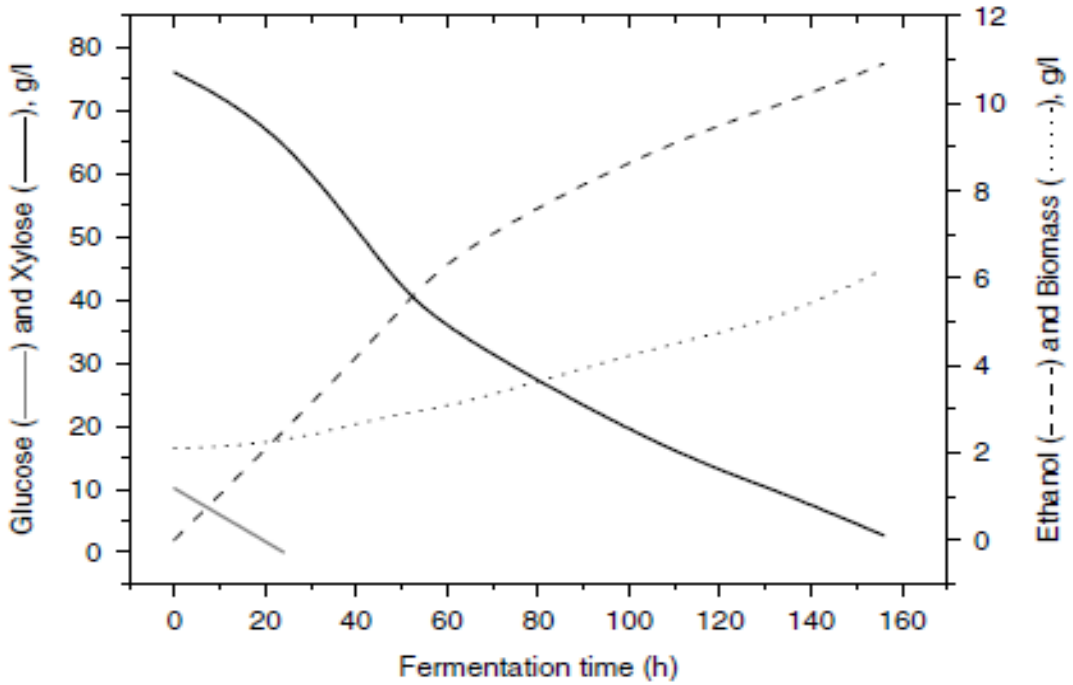


Figure 1. Time evolution of xylose and glucose consumption and biomass and ethanol production by *Scheffersomyces stipitis* UFMG-IMH 43.2 when cultivated in sugarcane bagasse hemicellulosic hydrolysate (conditions of assay 8; Table 1)

André Ferraz

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)