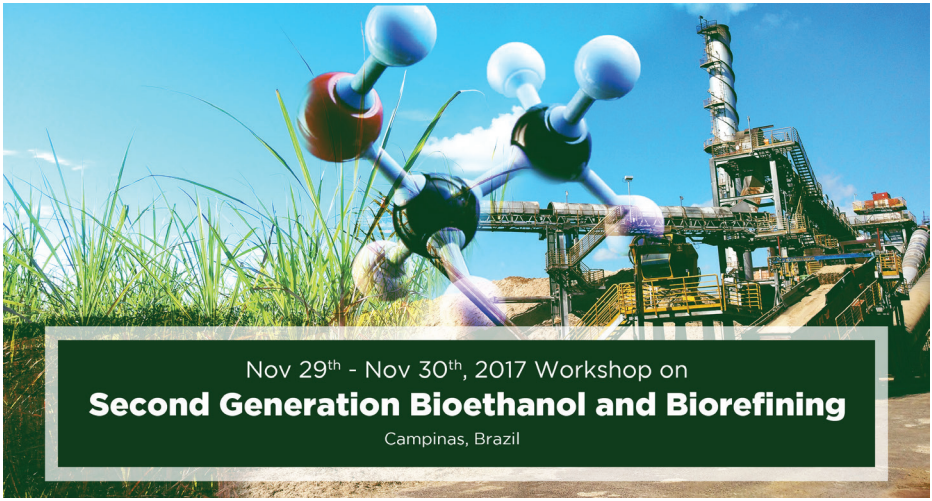
Nov 29th - Nov 30th, 2017 Workshop on

Second Generation Bioethanol and Biorefining

Campinas, Brazil

Abstract book

CTBE CNPEM MINISTÉRIO DA CIÊNCIA, TECNOLOGIA, INOVAÇÕES E COMUNICAÇÕES BRASIL GOVERNO FEDERAL



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Content

Program

29th November

Time	Session	Confirmed Speakers
8:00-9:00	Registration	–
9:00-9:20	Opening	Opening session
9:20-11:00	Biomass	**Managing crop residues for productivity, ecosystem services, and the bioeconomy** | **SLIDES** Douglas Karlen *United States Department of Agriculture – USDA, USA* **Sucre Project – lessons learned so far to recovery sugarcane straw for energy purposes** | **SLIDES** João Luís Nunes Carvalho *CTBE/CNPEM, Brazil* **Biomass Processing and 2G Ethanol Production – CTC Technology Demonstration Facilities** Suleiman Hassuani *CTC, Brazil* **Energy cane as main biomass for second generation biofuels** | **SLIDES** José Bressiani *GranBio, Brazil*
Coffee break		
11:20-13:00	Pretreatment	**Novel DMR processing of corn stover achieves high monomeric sugar concentrations from enzymatic hydrolysis (230 g/L) and high ethanol concentration (10% v/v) during fermentation** Xiaowen Chen *National Renewable Energy Laboratory – NREL, USA* **Factors involving the development of glucan accessibility in pretreated cellulosic materials** Luiz Pereira Ramos *Universidade Federal do Paraná – UFPR, Brazil* **Pretreatment interactions with the multiscale architecture of sugarcane bagasse** | **SLIDES** Carlos Driemeier *CTBE/CNPEM, Brazil* **Biomass pre-hydrolysis: a key step to sustainable biofuels** Marcelo Hamaguchi *Valmet, Brazil*
Lunch		
14:00-15:40	Hydrolysis	**Biomass Deconstruction by Thermophiles: What Have We Learned and Can We Improve Their Cellulolytic Activity?** Yannick Bomble *NREL, USA* **Engineering Trichoderma reesei for on-site cellulase production** Simo Ellilä *VTT, Finland* **What can we learn from *Xanthomonas* phytopathogens for plant cell wall depolymerization?** Mario Murakami *CTBE/CNPEM, Brazil*
16:00-17:20	Alcoholic Fermentation	**Novozymes Cellic enzymes – Tailored solutions for first-of-a kind commercial biorefineries** Sarah Teter *Novozymes, USA*
Coffee break		
		Cloning new sugar transporters and enzymes for second-generation bioethanol production by recombinant S. cerevisiae Boris Ugarte Stambuk *Universidade Federal de Santa Catarina – UFSC, Brazil* **Development of a genomic atlas for second-generation ethanol production** Leandro Vieira dos Santos *CTBE/CNPEM, Brazil* **Advanced Yeast Biocatalysts for Brazilian Ethanol Production** John McBride *Lallemand, USA*
17:20-19:00	Posters	Presentation of the accepted posters.

Program

30th November

Time	Session	Confirmed Speakers
9:00-10:20	Biomaterials	**Development of advanced lignocellulosic bioproducts** Orlando Rojas *Aalto University, Finland* **Enzymatic processing of plant-based cellulose for production of cellulose nanocrystals** Valdeir Arantes *Escola de Engenharia de Lorena, USP, Brazil* **Cellulose nanofibers extracted from sugarcane bagasse as a platform for nanostructured materials** Juliana da Silva Bernardes *LNNano/CNPEM, Brazil*
Coffee break		
10:40-12:40	Green Chemistry	**Innovative approaches to make the n-butanol fermentation economically viable** Carolina Grassi *CTBE/CNPEM, Brazil* **Industrial Biotech: Amyris' Case** | **SLIDES** João Paulo Cherubim *Amyris, Brazil* **Challenges of the industrial biotechnology for the innovative chemical companies** Gabriel Gorescu *Solvay, Brazil* **Innovation in renewable technologies** José Geraldo Pradella
		Innovation in renewable technologies José Geraldo Pradella *Braskem, Brazil* **Advancing Functional Materials: is 'Green' an Advanced Function?** Luuk van der Wielen *University of Limerick, Ireland; TU Delft, Netherlands; BE-Basic Foundation, Netherlands*
Lunch		
14:00-16:00	2G Biorefineries	**Technological Opportunities in the Sugarcane Mill** Daniel Atala *CTBE/CNPEM, Brazil* **Axens' innovative solutions for processing multiple biomass-derived feedstocks: an overview of Futurol Technologies™** | **SLIDES** Jorge Martinez Gacio *Axens, France* **Bringing Cellulosic Ethanol to Scale** | **SLIDES** Martin Mitchell *Clariant, USA* **Progress and lessons learnt in commercializing cellulosic biofuels** Diego Cardoso *DSM, Brazil* **Production of 2G ethanol at Raizen** Antônio Stuchi *Raizen, Brazil*
Coffee break		
16:20-18:00	Round Table: Accelerating the Bioeconomy	Igor Ferreira Bueno *FINEP, Brazil* Artur Yabe Milanez | **SLIDES** *BNDES, Brazil* Bernardo Silva *ABBI, Brazil* Rubens Maciel Filho *FAPESP, Brazil* Raquel Coutinho | **SLIDES** *Petrobras, Brazil*

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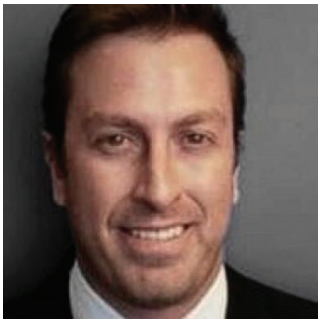
Abstracts

Antônio Stuchi

Graduated Chemical Engineer by the University of Campinas. MBA in business management. Works in the sugarcane sector for more than 30 years, occupying the positions of process engineer, industrial manager and industrial director. Currently works with New Technologies at the Raizen Group.

Production of 2G ethanol at Raizen

Artur Yabe Milanez



Bernardo Silva

Boris Ugarte Stambuk

Dr. Boris U. Stambuk is Full Professor of Biochemistry at the Federal University of Santa Catarina (UFSC), in Florianópolis, SC. He earned a M.S. in Microbiology and Immunology from Federal University of São Paulo (1991), and a Ph.D. in Biochemistry from University of São Paulo in 1997. He was senior research associate at the National Renewable Energy Laboratory in Golden, CO (2001-2002), and visiting professor at the Department of Genetics, Stanford University School of Medicine, CA (2006-2007). Dr. Stambuk is professor at the Graduate Program in Biotechnology and Biosciences, and Graduate Program in Biochemistry, both at UFSC. His Yeast Molecular Biology and Biotechnology Laboratory focusses on sugar transport and fermentation by yeasts, both for the brewing and biofuels industries, having published over 60 articles in the field. His research focusses in the genomic analysis of fermentative and industrial yeasts for further optimization, metabolic (and evolutionary) engineering, and discovery of new species, genes and proteins/enzymes useful for second-generation bioethanol production.

Cloning new sugar transporters and enzymes for second-generation bioethanol production by recombinant S. cerevisiae

Lignocellulosic biomass is an attractive raw material for bioethanol production, and abundant research has been devoted to improve xylose and cellobiose utilization by recombinant S. cerevisiae strains. We have cloned and expressed in this yeast novel xylose reductases (accepting both NADH and NADPH co-substrates) and xylitol dehydrogenases from Spathaspora yeasts isolated in Brazil. Since limited uptake is one of the bottlenecks for xylose fermentation, we also aimed to identify novel sugar transporters from the xylose-fermenting yeasts for expression in an hxt-null S. cerevisiae strain, lacking the major hexose transporters (hxt1D-hxt7D and gal2D) but having high xylose reductase, xylitol dehydrogenase and xylulokinase activities. Five genes allowed xylose and glucose consumption by the recombinant strain: the SUT1 permease from S. passalidarum, the SUT2 permease from S. arborariae, and three genes from S. stipitis (XUT1, HXT2.6 and QUP2). Other genes from S. arborariae or S. passalidarum failed to consume all sugars tested. Thus, we undertook a novel approach cloning the transporters but removing sequences from the N- or C-terminal intracellular domains. The truncated version of the transporters allowed glucose and xylose fermentation. Finally, novel cellobiose transporters and intracellular β glucosidases cloned from S. passalidarum allowed efficient cellobiose fermentation by recombinant S. cerevisiae cells. We will also address the development of recombinant industrial yeasts used in Brazil for second-generation bioethanol production from sugarcane.

Financial support: CNPq, CAPES, FINEP and FAPESC.

Carlos Driemeier

Carlos Driemeier is a research scientist at the Brazilian Bioethanol Science and Technology Laboratory (CTBE), which integrates the Brazilian Center for Research in Energy and Materials (CNPEM). Concluded bachelor (2004) and doctorate (2008) degrees in Physics from Federal University of Rio Grande do Sul, with a research intern period at the University of Texas. Held a post-doctorate (2009) in photovoltaic systems at the Institute of Energy and Environment from the University of São Paulo. Works mainly with Condensed Matter Physics, specifically with surfaces, interfaces, physical chemistry of water, crystallography, and image analysis. Recent research has focused on the multiscale architecture of lignocellulosic biomass and its influence in biomass processing. Has a broad interest in data-intensive analyses, biomass valorization technologies, renewable energies, and the transformation of the global energy system.

Pretreatment interactions with the multiscale architecture of sugarcane bagasse

Lignocellulosic biomass such as sugarcane bagasse have multiscale architecture, ranging from coarse particles (centimeters) down to sugar units (sub-nanometer). In the production of cellulosic ethanol, relevant phenomena occur across all these length scales. In this work, we present our understanding of the following critical phenomena: (1) trapping of mineral particles in the biomass structure; (2) nanoscale changes promoted by the high-temperature pretreatments; (3) role of mechanical action on the biomass; (4) nanostructural differences between bagasse treated in hydrothermal or in mild alkaline deacetylation conditions. Against this background of results, we discuss the prospects for pretreatments.

Carolina Grassi

Carolina Grassi is a researcher and associate coordinator of the Molecular and Agricultural Division at Brazilian Bioethanol Science and Technology Laboratory (CTBE). She holds a degree in Biological Science and a PhD in Genetics and Molecular Biology from the State University of Campinas, UNICAMP. She has worked as a researcher in large companies such as Braskem, GranBio and SGBio Renováveis (Joint venture between GranBio and Rhodia) in production of second generation biochemicals and biofuels. She is also a collaborating researcher at the Laboratory of Genomics and Expression of UNICAMP, author of important articles in biotechnology area and inventor of patent applications filled in several countries.

Innovative approaches to make the n-butanol fermentation economically viable

Daniel Atala

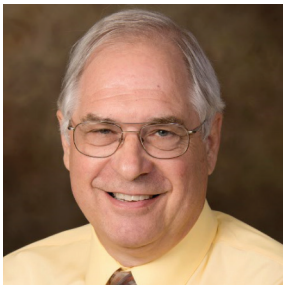
Daniel Atala is the Manager of the Industrial Division and also Head of Pilot Plant for Process Development (PPDP) at the Brazilian Bioethanol Science and Technology Laboratory (CTBE). Atala holds a degree in Food Engineering (1997) at Federal University of Rio Grande, Master in Food Engineering (2000), PhD in Food Engineering (2004) and Postdoctoral in Chemical Engineering (2004-2006), both at Unicamp. Atala has over 10 years of experience in operational and management positions in Research, Development and Innovation in large companies such as BP (British Petroleum), CTC (Sugarcane Technology Center) and Raízen. Atala also has experience in management of technology portfolio for improving production and performance, processes intensification, debottlenecking process, total management of R&D area, experience in technologies development from design to industrial scale, through feasibility assessment and testing in pilot plant.

Technological Opportunities in the Sugarcane Mill

The talk will address technological aspects and opportunities in the sugarcane mill that can contribute to increase the industrial performance operation as well as its configuration in the short, medium and long term. Selected topics will be discussed such as biodigestion, Sugar Mill 4.0 (industry 4.0), Sugar mill 365 and others.

Diego Cardoso

Diego is a Chemical Engineer and is currently the Business Development Manager at DSM Bio-based Products and Services.



Douglas Karlen

B.S., 1973, University of Wisconsin Madison, Soil Science; M.S., 1975, Michigan State University, Soil Science; Ph.D., 1978, Kansas State University, Agronomy. Author or co-author for 230 refereed journal publications and 154 peer-reviewed book chapter or proceedings papers. Co-editor for "Sustainable Agriculture Systems," "Agricultural Utilization of Urban and Industrial By-Products," "Sustainable Alternative Fuel Feedstock Opportunities, Challenges and Roadmaps for Six U.S. Regions" and editor for "Cellulosic Cropping Systems." His currently researches are focused in using soil quality/health assessment to quantify physical, chemical, and biological effects of various soil and crop management practices including sustainable tillage, crop rotation, nutrient management, manure management and cellulosic feedstock harvest for second-generation bioenergy and bio-products.

Managing Crop Residues for Productivity, Ecosystem Services, and the Bioeconomy

Balancing soil carbon (C) is essential for sustainable production of cellulosic, second-generation bioenergy and bio-product feedstock supplies that can be derived from a variety of plant materials including crop residues, perennial forages, dedicated energy crops, woody species, urban lawn and garden waste, unused construction materials, or co-products such as sugarcane (Saccharum sp.) bagasse. During the past two decades, substantial progress has been made globally to identify, collect, store, transport, and develop optimum uses for these materials. This has required close collaboration among chemical, agricultural, and biosystems engineers, as well as agronomists, geneticists, animal nutrition specialists, and soil scientists. Knowledge, skill and abilities from each discipline is essential because C, captured through photosynthesis, not only provides the energy associated with biofuels, but also nutrition for animals, food for soil microorganisms, binding materials for enhanced soil structure, and surface protection against wind and water erosion. Key advances by public and private sector groups have and continue to be made regarding the science and technology needed to efficiently capture and utilize the solar energy to meet ever-increasing food, feed, fiber, and food requirements of an expanding population.

This presentation will focus on the soil C balance, because it provides the connecting link among the multitude of ecosystem services associated with production, collection, storage, and transport of cellulosic feedstocks. Challenges such as how to: (1) manage variable land areas, (2) balance crop residue management to sustain soil resources without creating emergence, stand establishment, growth or yield reductions in subsequent crops, (3) determine the amount of crop residue or plant material that can be sustainably harvested, (4) adjust for additional nutrient removal, (5) harvest without degrading soil physical quality (i.e., compaction, destruction of soil aggregates, crusting, or erosion), and (6) address policy questions such as the effects of feedstock harvest on carbon intensity (CI) estimates will be explored using corn (Zea mays L.) and other field research data in conjunction with the concepts of soil quality or health. Economics of these complex cellulosic management practices will be explored using sub-field return on investment (ROI) concepts and calculations.

Gabriel Gorescu

João Nunes Carvalho

João Luís Nunes Carvalho, PhD. Agronomist at Federal University of Lavras (UFLA) in 2004, Master and PhD in Soil Science at ESALQ/USP (2006 and 2010) and Post doc at University of Illinois Urbana-Champaign (2014). Since 2011, he is a researcher at Brazilian Bioethanol Science and Technology Laboratory (CNPEM/CTBE). His research has an emphasis on best agricultural practices to improve Biomass Production and Environmental Impacts of biomass production. Currently, he is involved in following projects: i) Impacts of crop rotation and no-tillage on sugarcane production; ii) Implications of sugarcane straw removal to bioenergy production on soil quality indicators and biomass production; iii) Agronomic and environmental impacts of energy cane production.

Sucre Project – lessons learned so far to recovery sugarcane straw for energy purposes

The Project BRA/10/G31 – Sugarcane Renewable Electricity (SUCRE) seeks to launch a commercial and environmental success history with sugarcane straw biomass (trash) electricity generation in Brazil, with significant impact on other sugarcane growing countries. The overall objective of the project is to catalyze the establishment of a commercial market for sugarcane straw-based electricity supply to the Brazilian grid, to displace fossil-fuel electricity that otherwise need to be generated to meet the growing electricity demand in Brazil.

To maximize the potential for electricity generation from sugarcane, the project will launch the widespread use of sugarcane straw (or “trash”): the tops and leaves of the sugarcane plant that historically have been burned on the cane field as a waste product and now, with unburned cane harvesting, left in the field to be decomposed. By adding straw to bagasse, biomass resource used as fuel increases significantly. This offers the opportunity for large amounts of renewable electricity to be exported from sugarcane mills to the grid, since all of the additional biomass harvested will be solely utilized for additional electricity generation. In spite of the project is still going, during the Ethanol 2G workshop the main lesson learned so far will be presented, mainly those issues related to the impacts of straw removal in the environment and straw quality for energy purposes.

João Paulo Cherubim

Reponsible for Process Development using as base the strain engeneering from US. Oversees all site operation in the first farnecene facility with a breakthrough technology, using GMM (genetically modified organism). Define Operations Management by reviewing processes, key performance indicators – as well as their deployment – establishing results oriented meetings and organizing a team work environment. Broad experience in managing all manufacturing areas: production, quality, inbound and outbound logistics, procurement, storaging, engineering, maintenance and S&OP. Strong interface with commercial and financial areas. Solid experience in expansion and start up activities on a manufacturing plant.

Industrial Biotech: Amyris' Case

Amyris' case will present the current challenges for Industrial Biothec Industry, exploring its difficulties, constant learning, strategical thinking and adjustment and accomplishments. It´s expected to be possible to elaborate, after exploring this case, a list of competences necessary to succeed on building such brand new, exciting and promising industry.

O case Amyris apresentará os desafios atuais da Biotecnologia Industrial através de suas dificuldades, constante aprendizado, ajustes e realizações. Espera-se ao final da análise do case, ser possível a elaboração de uma lista das competências essenciais termos sucesso na construção de uma novo e promissor setor.

John McBride

John McBride is a Sr. Director of R&D at Mascoma LLC, a subsidiary of Lallemand Inc., leading the companies' technology development and deployment for new products in the Brazilian ethanol production market, and for cellulosic ethanol production. In particular, John has led the efforts to bring the first genetically modified strain of yeast appropriate for cell recycle and acid washing process to the Brazilian ethanol market, a commercial product called SucraMax™. He has also overseen the efforts to bring a commercial product called C5Fuel™, a robust yeast strain that can ferment both C5 and C6 sugars, to the market for cellulosic ethanol fermentations. John obtained his B.A. Mathematics and Biology from St. Olaf College in Northfield, Minnesota and his Ph.D. in Biochemical Engineering from the Thayer School of Engineering at Dartmouth College in New Hampshire. His Ph.D. work focused on expressing cellulolytic enzymes in Saccharomyces cerevisiae. Since joining Mascoma in 2007, John has worked as a scientist and technical manager developing yeast biocatalysts, associated enzyme systems and metabolic pathways, and optimized fermentation processes. He has been named an inventor on 6 granted patents, more than 10 additional patent applications, and authored or co-authored more than 10 publications.

Advanced Yeast Biocatalysts for Brazilian Ethanol Production

Mascoma LLC, a subsidiary of Lallemand Inc., has developed and deployed the first commercial genetically modified (GM) yeast to be widely used for fuel ethanol production. To date, we have released several generations of products for corn ethanol which reduce enzyme requirements and increase ethanol yields.

In the last several years Mascoma has been adapting our core technologies for application to the Brazilian sugar ethanol sector. We have developed and deployed the first GM yeast strain for the cell recycle fermentation process, called SucraMax™ which increases ethanol yields and decreases glycerol production. Mascoma has also developed a suite of technologies for the processing of lignocellulosic derived sugars. In particular, we have used our experience in process modeling and design of cellulosic ethanol facilities, coupled with our knowledge of advanced yeast biocatalysts to propose an integrated cellulosic biorefinery concept for the Brazilian market. This concept involves the solubilization and co-extraction of C5 sugars along with sucrose by incorporating a pretreatment reactor with the traditional milling process. An advanced, C5 utilizing yeast strain is then used to co-ferment the sucrose and C5 sugars in the typical cell recycle and acid washing process configuration. We find that this configuration would produce by far the lowest cost 2G ethanol available, with estimated minimum ethanol selling price (MESP) as low as R$0.57/L to R$0.97/L at a 10% return. The focus of this talk will be to describe our progress with respect to bringing advanced biotechnology to improve Brazilian ethanol production, both through our first commercial product (SucraMax™), and our vision for future advances that can access and incorporate 2G sugars.

Jorge Martinez Gacio

Jorge Martinez Gacio is a Chemical Engineer and Technologist in the Clean Fuels and Biotechnology business line of the Process Licensing Division at Axens. He joined Axens in 2006 and he is currently involved in the commercialization of processes for clean and renewable fuels and particularly the FuturolTM technology for second generation cellulosic ethanol.

José Bressiani

Agronomist, PhD in Genetics and Plant Breeding at Sao Paulo University, plant breeder in sugarcane at CTC for 15 years, breeding manager at CanaVialis / Monsanto for 5 years and since November 2011, agricultural technology director at GranBio, company that built the first cellulosic ethanol plant in the southern hemisphere, in which has the responsibility of to bring the biomass solution for 2G ethanol and/or biochemical plants of the group, especially the development of energy cane.

Energy cane as main biomass for second generation biofuels

The last century was the scene of an extraordinary social and economic development of mankind. This development had the fossil energy as one of its pillars. The discovery of petrol led the society to shape a development model highly dependent on this source of energy, which has finite resources and also promotes a big increase on the greenhouse gases, with unforeseeable consequences for the human beings as well as the entire life. It is imperative that we change the pillars of energy from fossil to renewables that will be more sustainable and less aggressive to the environment. One of the sources of this new energy platform, probably the best, is biomass. Fibrous plants bring several advantages and fit well within the requirements deemed important to be elected as producers of biomass. Among these characteristics, we have the high processing capacity of solar energy into biomass, fast growth, long-term canopy, possibility of large-scale production. Despite of that this plants are adapted to suboptimal environments that allows its production not compete with food production because it requires less energy input, bringing marginal lands into production with its all social-benefit consequences. Among the fibrous plants, SUGARCANE or, better, ENERGYCANE has one of the biggest potential for biomass productions. Breeding development of energycane varieties has been showings the biggest potential to produce highest yields of cellulosic sugars in comparison with sugarcane and other biomass dedicated crops like sorghum, elephant grass and eucalyptus. Also, these types of varieties are much more adapted to poor and dry soils, allowing it's cultivation degraded area like bad used pastures and nom competing in land with food. Results from Granbio breeding program will be presented.

José Geraldo Pradella

PhD in Chemical Engineering. Currently, Pradella is specialist in Bioprocesses in the Braskem Research Center for Renewable Chemicals.

Juliana da Silva Bernardes

Researcher at the National Nanotechnology Laboratory (LNNano). Her actual research interests involve the preparation of high performance materials based on cellulose nanoparticles to be used as: rheology modifier for complex fluids, drug carrier and substrate/ink for sensors and devices. She is also responsible for X- ray photoelectron spectrometer, an open facility from LNNano. Graduated in Chemistry (bachelor's degree from 2003 to 2007) and Ph.D. in Chemistry (2008) from UNICAMP with internship in the Lund University, Sweden (2007). She studied the phase behavior of cationic surfactant and polyanions in different systems by Small Angle X-ray Scattering (SAXS). In 2017 held guest research position at Stockholm University in the area cellulose nanoparticles for biomedical applications.

Cellulose nanofibers extracted from sugarcane bagasse as a platform for nanostructured materials

Cellulose nanofibrils (CNF) have been gaining a lot of attention due to their distinctive properties, like production of high viscous dispersions at low solid contents. Besides, there is a growing demand for the development of green and renewable products, and cellulose, the most abundant biopolymer on Earth, fulfills these requirements. During my presentation, I will show our recent activities on using cellulose nanofibers from sugarcane bagasse as rheology modifier for complex fluids, drug carrier and substrate/ink for sensors and devices.

Igor Ferreira Bueno

Leandro Vieira dos Santos

Leandro Vieira is a research scientist at CTBE (Brazilian Bioethanol Science and Technology Laboratory). Bachelor in Biological Sciences (2007), Master (2009) in Microbiology at Federal University of Viçosa (UFV) and Ph.D. (2017) in Genetics and Molecular Biology at State University of Campinas (UNICAMP). He is also a collaborator researcher at UNICAMP. He was researcher at GranBio/BioCelere, being responsible for the development of C5-yeasts for use in 2G industry. The yeast developed was approved by the CTNBio and is currently being used in the industry. Recent research includes synthetic biology, metabolic and evolutionary engineering of yeasts to metabolize five-carbon (C5) sugars and for tolerance to multiple inhibitors from lignocellulosic biomass feedstocks, molecular basis for xylose adaptation, engineering of xylose transporters, thermotolerance and prospection of microorganisms and genes of industrial interest.

Development of a genomic atlas for second-generation ethanol production

The development of biocatalysts capable of fermenting xylose, an abundant five-carbon sugar in lignocellulosic biomass, is a key step to achieve a viable production of second-generation (2G) ethanol. Despite the engineering of Saccharomyces cerevisiae strains for 2G ethanol production dates from decades ago, the identification of the genetic basis emerged during the adaptive evolution processes, responsible for xylose assimilation and inhibitor tolerance, is still in its infancy and few information are available. Our research group have modified a robust industrial strain of S. cerevisiae by the addition of essential genes for pentose metabolism. Subsequently, after cycles of adaptive evolution with selection for optimal xylose utilization, strains could efficiently convert xylose to ethanol. Though evolved independently, strains shared genomic mutations which improved the ability to metabolize xylose without adaptive evolution, suggesting some key players in a complex signaling regulatory network for xylose fermentation. Our group is now focused on the development of a genomic atlas to identify the molecular basis involved in the metabolism and regulation of xylose consumption and tolerance to inhibitors present in hydrolysates from lignocellulosic materials. Our results will provide promising new targets for metabolic engineering of C5-yeasts to improve second-generation ethanol production.

Luiz Pereira Ramos

Full Professor in Analytical Organic Chemistry at the Federal University of Paraná, with his Ph.D. received from the Ottawa-Carleton Institute of Biology, University of Ottawa, Canada in 1992. Associate Editor of the ACS journal Energy & Fuels and leader of many research activities in the areas of renewable resources and biofuels, with 125 indexed publications for an H factor of 32 and a total of 3333 citations in the SCOPUS database until August 10, 2017. Permanent staff in the UFPR's graduate programs in Chemistry and Bioenergy.

Factors involving the development of glucan accessibility in pretreated cellulosic materials

Luana Marcele Chiarello, Douglas Henrique Fockink, Giselli Torres da Silva, Luiz Pereira Ramos Research Center in Applied Chemistry (CEPESQ), Department of Chemistry, Federal University of Paraná (UFPR).

One of the biggest obstacles for the industrial production of cellulosic ethanol is the degree of association of the main macromolecular components of the plant cell wall. This association involving cellulose, hemicellulose and lignin hinders the access of enzymes to plant polysaccharides, reducing the overall ethanol yields at the later stage of fermentation. Several pretreatment methods have already been used to deconstruct the plant cell wall with the aim to increase the susceptibility of the resulting lignocellulosic materials to enzymatic hydrolysis at high yields. In this work, the chemistry involved in acid-catalysed pretreatment of cane bagasse and eucalypt harvest residues will be addressed with regard to changes in their main macromolecular components and these data will be tentatively correlated with changes in pretreament severity. Results about enzymatic hydrolysis at high total solids using relatively low enzyme loadings of Cellic CTec3 will be presented as well, together with the fermentability of substrate hydrolysates using industrial strains of Saccharomyces cerevisiae.

Support: CNPq, Fundação Araucária, Novozymes Latin America, European Union (FP7 and RISE).

Luuk van der Wielen

Prof.dr.ir. Luuk A.M. van der Wielen (Amsterdam, 16-06-1964) holds a MSc degree in Chemical Engineering from Twente University (Netherlands), and a PhD degree (with honours) from Delft University of Technology (TUD). Since February 2017, he is Director of the Bernal Institute at the University of Limerick, Ireland, (http://www.bernalinstitute.com/) and Bernal Professor for Biosystems Design and Engineering, while continuing as Distinguished Professor for Biobased Economy of TUD. He is Full Professor at it's Dept. of Biotechnology at (www.bt.tudelft.nl), where he headed the Bioprocess Engineering Section effectively since 1998. The activities of the section were ranked as excellent by consecutive national research evaluations and have resulted in several spin-off companies. His research interests include thermodynamics for bioprocesses, bioseparation/-conversion technologies, multifunctional bioreactors, miniaturized ('on-chip'), high-throughput technology for rapid process development, analysis and development of biorenewables production systems, and their societal impacts. He is since 2004 director of BE-BASIC (www.be-basic.org), the globally operating private-public research organisation for Biobased Sustainable Industrial Chemistry & Energy, which is based in The Netherlands with hubs in South East Asia and Brazil, and a cumulative budget exceeding 250 M€. BE-BASIC executes a R&D, training and innovation program in the field of industrial and environmental biotechnology, via a consortium of 50 academia and industries. He initiated the multi purpose pilot facility (www.bpf.eu, ~ M€ 80). In 2012, he coordinated the Netherlands' Bioenergy and Biochemicals Innovation plan under the new Dutch Topsector Policy (budget exceeding 1 billion euro), and was appointed in the 1st Board of Directors of Foundation TKI-BBE. In 2007, he joined (part-time) Royal Dutch Shell as Principal Scientist Biotechnology. He was Visiting Professor at the Univ. San Carlos, the Philippines until 2008; and 2009-'13 at Univ. of Technology Malaysia. The last Google Scholar count shows over 280 publications and patents as of july 2016 (H-index 32; RG 42.83). Luuk van der Wielen is/was member of editorial and advisory boards of several leading international scientific journals, and chaired several scientific conferences (a.o. ESBES4, BPP2005, RRB4, ECOBIO2016, BBEST 2017). He is/was member/chair of national and European committees: AgroPolo (agro/forestry re-industralisation initiative Sao Paulo, BR), coordinator Bioenergy and Biochemicals RD&I programming in NL Topsector Policy (2011-12), Supervisory Board of Dutch Separation Technology Institute, of NL Platform Renewable Feedstocks, Sustainable Energy Cie of the Royal NL Academy of Sciences (KNAW), Steering Group of the EU Technology Platform Suschem/ Industrial Biotechnology, Steering Committee BBE (Min LNV) and BioPort of Rotterdam, Taskforce Bioenergy Systems (EU Fed. for Biotechnology), Advisory Boards of US-EU Taskforce on Biotechnology Research, KP Sinha Bioenergy Center (IIT Kharagpur, India), of CLIB2021 (Germany), of BIO4EU (EU Commission), Oversight Board Global Sustainable Bioenergy Project and advisor to several European and international industries. He is in the Boards of Commisioners of Dutch Greentech Fund and SHIFT Invest, Bioprocess Pilot Facility BV and chairs BioPort Holland1(aviation industry group).

He is one of the initiators of the successful academic program on Life Science & Technology (www.lst.tudelft.nl) of Leiden University and TU Delft, and director of the postgraduate program Bioprocess Design (www.bodl.bt.tudelft.nl). Luuk van der Wielen is married, has 3 children, and has an active and passive interest in jazz music.

Advancing Functional Materials: is 'Green' an advanced function?

For many good reasons, chemicals, fuels and energy industries transfer to shift their product portfolio's to more sustainable, 'green' products. 'Green' is a loosely defined quality, and mostly refers to a product that has the same ('drop-in') or comparable ('substitute') functional performance as the conventional, usually fossil, product. In many cases, this is quantified through one of the versions of Life Cycle Analysis; in a very crude simplification this reduces to lower carbon emission profile. Given the still fairly low volumes of 'green' products in a fossil-dominated market, simple scaling rules will dictate higher (processing) prices and, when the volume grows, increasing price levels of limited 'sustainable' feedstocks like agro and forestry residues. With respect to the latter, the economic reality already kicked in since the price of UCO (used cooking oil) is approaching that of virgin palm oil (S600-700/ton). The same happens with the potentially much larger lignocellulosic residues market which is practically absent a few years ago and usina's had inefficient power plants to get rid of bagasse , and now there is a serious price for bagasse that approaches and soon exceeds that of coal.

For advancing a 'green' materials (and chemicals, fuels and energy) industry, we need to understand what the function of 'green' really is. Because if it is better than conventional, a higher price or premium is justified. If it is more rare then conventional, a higher price is justified as well. Those are simple economic principles. If that is the case, then investors will become increasingly motivated, and insurers will calculate lower risks when they insure those investments (and thus lower prices). This is a fundamentally different and more positive model then the current negative schemes of penalties (carbon and other emission taxations), or environmental subsidies which destroy economic value.

So we have to look back into what 'green' really means. If green implies reduced emission profile, there is a different group of technical innovations necessary, then when 'green' implies -for instance- higher oxygen content in the 'green' building blocks and thereby lower flammability in the resulting materials. The latter is a key advanced functionality for construction and airplane materials. This contribution targets to look into a number of examples based on aviation sector where 'greenification' should come from a broad portfolio of more advanced airplanes, more advanced (bio)materials and more advanced (bio)fuels.

Marcelo Hamaguchi

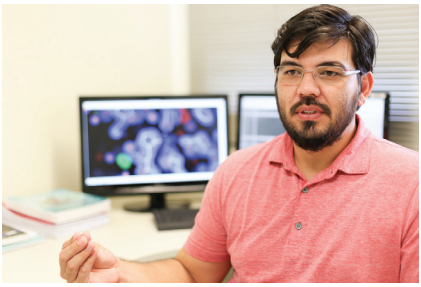
Chemical engineer, São Paulo University; Master's degree in Process Control and Simulation, São Paulo University; Doctoral degree in Sustainable Energy Systems, Lappeenranta University of Technology, Finland. Twelve years of experience in the Pulp and Paper industry and working with biorefining technologies since 2008. Currently responsible for R&D projects for Valmet in South America.

Biomass pre-hydrolysis: a key step to sustainable biofuels

Producing first generation ethanol from sugarcane juice is quite straightforward because it contains simple sugars that yeast can easily digest. On the other hand, the production of cellulosic ethanol requires additional steps to handle more complex carbohydrates prior to the fermentation process. A route that has been mostly explored so far consists of: pretreatment (pre-hydrolysis) of biomass waste, enzymatic hydrolysis, followed by fermentation of the resulting sugars.

Based on the knowledge gained in the pulp and paper industry, Valmet has been supplying industrial scale equipment for biomass pretreatment. The main objective is to make the cellulose more accessible to enzymes and to dissolve hemicellulose sugars, mainly C5 sugars, as oligo or monosaccharides. One important feature of a well-designed pretreatment is the capability in achieving reliable and uniform biomass feeding flow at high pressure conditions.

Valmet pre-treatment process can be performed with steam, hot water or with addition of dilute acid. The reactor temperature is usually in the range of 170-220°C, meaning that the system can be flexible in terms of both raw material and process. Feeding operation at high pressure can lead to frequent failures, especially when raw-materials such as bagasse and straw are used. One example is the frictional effect of abrasive materials present in biomass that can lead to equipment erosion. Technology suppliers such as Valmet are continuously working to minimize the negative impacts caused by these impurities. The objective of this presentation is to show how Valmet has been contributing to develop reliable pre-hydrolysis systems, which includes e.g. biomass pre-compression to accomplish a steady feed into the reactor while improving safety and availability.



Mario Murakami

Mario Murakami is Principal Investigator at CNPEM (LNLS/LNBio) since 2008 and was the coordinator of the X-ray Crystallography Village from 2008 to 2016. Currently, he is the coordinator of the Molecular Division at CTBE/CNPEM. He is graduated in Engineering at UNESP and doctorate in Molecular Biophysics at UNESP with sandwich period at University of Hamburg and DESY. PostDocs in Macromolecular Crystallography and NMR at UNESP and Rutgers University, respectively. He received his "Habilitation" (Dr rer. Nat. habil.) in Biotechnology at UNICAMP in 2013. He has broad experience in enzyme structure, function and engineering, using a multidisciplinary approach (in silico, in vitro and in vivo), acting on the understanding of molecular mechanisms associated with plant cell wall degradation and modification. Murakami has published over 125 peer-reviewed publications and has more than 1500 citations in the last 5 years.

What can we learn from Xanthomonas phytopathogens for plant cell wall depolymerization?

Xanthomonas plant pathogens attack a broad range of economically-relevant agricultural crops such as cruciferous vegetables, sugarcane, rice and citrus. Most of the diseases caused by these bacteria remain poorly understood at the molecular level and there are no effective treatments available so far, highlighting the importance of studies with such phytopathogens. Interestingly, some Xanthomonas species, like X. citri, contain a plethora of Carbohydrate-Active Enzymes (CAZymes), which is comparable to the repertoire of filamentous fungi specialized in biomass degradation, in terms of diversity and abundance. However, these enzymes are yet unexplored regarding their potential industrial use and how they could mediate or contribute to infection and virulence. In this way, we have been extensively characterizing the entire CAZome of X. citri and, as envisaged, novel activities and molecular mechanisms were unveiled, broadening our current understanding of the nature´s strategies for plant cell depolymerization and revealing a superb biotechnological potential of Xanthomonas CAZymes in biomass degradation.

Financial support: FAPESP, CNPq and CAPES.

Martin Mitchell

Martin Mitchell is a Business Development Manager for the Biofuels & Derivatives Division at Clariant which is based in Munich, Germany. Martin leads the business development efforts to commercialize the sunliquid® process technology in North America, South America and Asia based out of Des Moines, Iowa – USA.
Prior to Martin's work at Clariant, he was an International Project Manager at the State of Iowa Department of Economic Development where he managed a portfolio of projects in the biotechnology, biofuels and biorenewable chemical sector for seven years and prior to that he was a Global Market Analyst for Kemin Industries where he worked in the nutraceutical, cosmeceutical and functional food ingredient sector to develop the global market for nutritional products such as Lutein.
Martin holds a B.A. in Public Relations & Communication from The University of Northern Iowa and an M.B.A. in International Business from Iowa State University including an international study at Universidade Federal de Minas Gerais in Belo Horizonte, Brazil. Martin is a native Iowan from Mount Pleasant and currently resides in Des Moines, Iowa with his family.

Bringing Cellulosic Ethanol to Scale

Recent years have seen a lot of progress both on the biotechnology as well as on the process development side of cellulosic ethanol technology. Making cellulosic ethanol a reality is all about the value chain. First, let's look at the feedstock. Lignocelluloses show a huge potential as a new feedstock for the production of advanced biofuels and biobased chemicals globally. To ensure supply of biomass at an affordable cost, plants should be designed at a scale that corresponds with a realistic sourcing radius in an area with ample feedstock potential. In addition a thorough planning and management of feedstock procurement and logistics needs to be in place. Second, the technology has to show best in class, validated commercial performance. Clariant's sunliquid® technology achieves this performance through a thorough and entirely integrated process design and innovative technology features offering a one-stop shop solution flexible to be used to convert different feedstock and adopt to various plant concepts, combining high process yields with low OPEX and CAPEX. The production cost can compete with those of first-generation bioethanol and the greenhouse gas savings of the sunliquid® ethanol are 95% compared to fossil fuels. Since July 2012 Clariant has successfully been operating a pre-commercial plant in Straubing, Germany. Performance runs with wheat straw, corn stover and sugarcane bagasse have shown excellent results and validated the technology further confirming that sunliquid® can be implemented worldwide. Comprehensive tests on over 40 containers of sugarcane bagasse and tops & leaves from Brazil have been performed at pilot and pre-commercial facilities in Straubing, Germany to receive in-depth technical and economic validation of the sunliquid® technology. The performance runs at the pre-commercial plant in Straubing were conducted on multiple variations in composition (bulk and bale) and different qualities of sugarcane bagasse and straw were processed.

A yield of up to 300 Liters of ethanol per ton of dry bagasse was achieved and validated during extended performance runs. These tests constitute an important milestone for the realization of a commercial-scale project with sugarcane residues. Third, you need a simple, harmonized and easy to implement process design. The sunliquid® Process Design Package delivers a technological blueprint for commercial facilities between 50 and 150 kt (20 – 60 million gallons) of ethanol per year. The process uses only established equipment that has proven performance in other industries for many years, thus mitigating process risks. The modular design offers a flexibility that makes it easy to adjust for project and site specific boundary conditions like i.e. different energy production scenarios. The high level of detail means short engineering phase and fast implementation. The high quality and sustainability of the produced cellulosic ethanol has already been proven in several applications. In collaboration with Mercedes-Benz and Haltermann, Clariant has successfully tested a fuel of the future, sunliquid®20 –a premium-grade E20 blend that contains 20% cellulosic ethanol, in a fleet test with Mercedes-Benz series vehicles. In Brazil at Clariant's Suzano plant, ethanol-fueled Scania trucks are used to transport chemical products. The Ecotrucks, as they are known, started using second-generation ethanol produced from sugarcane bagasse using Clariant's sunliquid® technology.

Orlando Rojas

Dr. Rojas is Professor of Bio-based Materials in Aalto University (Finland). Previously, he was Professor in the departments of Chemical and Biomolecular Engineering and Forest Biomaterials of North Carolina State University (USA). Earlier in his career he was a senior scientist appointed by the Royal Swedish Academy of Sciences in the Royal Institute of Technology (KTH), a postdoctoral fellow in the Institute for Surface Chemistry, Sweden and research assistant in Auburn University. He was appointed as Finland Distinguish Professor (2009-2014) and was Chair of the "Division of Cellulose and Renewable Materials" of the American Chemical Society (2009-2011). He was elected with the distinction of Fellow of the American Chemical Society (2013) for his scientific and professional contributions. He is the recipient of the 2015 Nanotechnology Division Technical Award and IMERYS Prize for outstanding contributions that have advanced the industry's technology. He was appointed as a "2013-2017 Faculty Scholar" of NCSU and ACS Division Award of "Cellulose and Renewable Materials". He received the Fibrenamics Award (University of Minho, Portugal, 2016) in recognition for his scientific work and impact in the field of advanced materials from lignocellulose. Recently he was selected as front-runner for the Academy Professor of Finland. Dr. Rojas work is centered on the utilization of lignocellulosic materials in novel, high performance applications and the interfacial and the adsorption behaviors of surfactants and biopolymers at solid/liquid interfaces. He has published over 260 peer-reviewed papers related to these topics. He and his students have given +300 conference presentations and has been invited numerous times (+190) as a speaker in conferences, universities and research centers worldwide. The recent efforts of his group, "Bio-based Colloids and Materials", deal with the development of nanostructures from the fiber cell wall and cellulose derivatives, the dynamics of enzymatic reactions and the design of stimuli-responsive materials and multiphase systems.

Development of Advanced Lignocellulosic Bioproducts

In this seminar I will introduce our work related to the application of surface and colloid science in the development of lignocellulose bioproducts. These efforts take advantage of the inherent ability of biomolecules to assemble into fibers and other highly hierarchical and multidimensional structures. Lignin, cellulose nanofibrils (CNF) and cellulose nanocrystals (CNC) will be presented as the main building blocks that we use in our research. An underlying aspect is the possibility to control non-specific forces to assemble these materials at the air-liquid-solid interfaces. In turn, dispersions, hydrogels, aerogels, foams and emulsions are produced to enable effective processing into 1D, 2D and 3D structures. From these, we will highlight some examples in our quest to develop functional properties and to address current and future applications. Lignin fibers, nano and microparticles, super strong filaments and fibril networks will be shown as examples of passive or active components in a number of advanced functional materials.

Raquel Coutinho

Rubens Maciel Filho

Professor Rubens Maciel Filho. B.S., Chemical Engineer- São Carlos Federal University-1981, Nuclear Engineer- São Carlos Federal University, M.Sc. Chemical Engineering- State University of Campinas-1985,. Ph.D.,Chemical Engineering, University of Leeds, UK-1989. Top Productivity (1A) of National Research Council (CNPQ) since 2000 and Member of Science Academy of State of São Paulo since October 2015 Full Professor at Chemical Engineering School- Department of Process and Product Development and Coordinator of the Laboratory of Optimization, Design and Advanced Process Control (LOPCA) since 1989, from 2007, Head of the Laboratory of Innovation in Biofuels- UNICAMP (LIB), and from 2010, Coordinator of the Brazilian Institute of Biofrabication (BIOFABRIS). From april 2012 is also Coordinator of the Laboratory of Petroleum Valuation (VALPET). The main research areas covers Modeling of Chemical and Biochemical Process: Computer Aided Design, operation and control and off/on line optimization, with special focus on Green Process Development and Biorefinery, specifically with bio-ethanol and byproducts from fermentation as feedstock. Consideration is also given for the use of CO2 and bioethanol as raw material for chemicals. He served as Head of Chem. Process Development, Director for Under Graduate Studies, Dean of Chemical Engineering School and Pro-Rector at State University of Campinas (UNICAMP). He was also a member of the Higher Scientific Research Council of Brazil Research Council (CA of CNPq.). His professional experience includes teaching and research positions at State University of Campinas since 1983, invited Professor from UIS (Universidad Industrial de Santander- Colombia) and several worldwide collaboration in the Chemical Engineering area. He is permanent member of Brazilian Chemical Engineering Association and He was Head of Brazilian Chemical Engineering Association and Inter-American Association of Chemical Engineering. He is coordinator of the Engineering at Bioenergy Program of FAPESP (BIOEN/FAPESP).

Sarah A. Teter

Dr. Sarah A. Teter is Global Manager of Biomass R&D at Novozymes. Teter's R&D teams have had a significant impact in reducing costs for production of cellulosic ethanol. Since 2001, when Novozymes initiated focused work on delivering enzymes for biomass conversion, Teter has been a key part of innovative research programs developing improved cellulases, hemicellulases, and auxillary enzymes. Teter has extensive experience in coordinating multidisciplinary research teams, as well as customer-facing application engineering projects. Teter's technical training includes post-doctorate studies at University of Michigan (1999-2001) and Max-Planck Institute for Biochemistry (1997-1999), a Ph.D. from UC Davis (1997), and a B.A. from Swarthmore College (1992).

Novoymes Cellic enzymes – Tailored solutions for first-of-a kind commercial biorefineries

In recent years, several industry front-runners have ramped up cellulosic ethanol production in commercial scale biorefineries. Novozymes Cellic® enzyme products are used in most of these plants, and new producers entering the market space look to Novozymes to provide biocatalysts to enable expansion of this growing industry. A diverse set of biomass feedstocks and fundamentally unique technologies in the marketplace have led Novozymes to deploy tailored enzyme cocktails, with biocatalysts designed to meet specific customer needs. Close collaboration with leaders in the biomass biorefining industry brings opportunities to further reduce biofuel production costs.

Simo Ellila

Simo Ellilä is a molecular biologist working as a Research Scientist in the Bioprocess engineering team at VTT Technical Research Centre of Finland, where he joined in 2011. He has been involved in several EU and industry projects aiming at converting lignocellulosic biomass into ethanol or organic acids. His work has focused mainly on the discovery, production and characterization of hydrolytic enzymes, biomass hydrolysis and bioprocess development. He spent more than three years (2013-2016) in Brazil working in the FINEP-funded PAISS program, where he was engaged in developing low-cost cellulase production processes for the local sugarcane industry.

Engineering Trichoderma reesei for on-site cellulase production

Cellulase enzymes can represent up to 40% of the operational costs of a cellulosic biorefinery. Currently, these enzymes are primarily delivered to biorefineries in large volumes by specialty enzyme manufacturers. The operational cost of cellulases could be decreased by producing the enzymes on-site at the biorefinery using low-cost local residues and simplified processes. The mesophilic ascomycete fungus Trichoderma reesei is the most studied organism for the production of cellulolytic enzymes. However, the use of standardT. reesei strains for on-site enzyme production faces some notable technical hurdles. VTT has been engineering T. reesei for more than three decades, and has elucidated many of the key genes and enzymes involved hydrolytic enzyme production. In this talk I will summarize some of our recent efforts to engineer this fungus to enable economically viable on-site cellulase production processes.

Suleiman José Hassuani

Mechanical Engineer by the Aeronautics Institute of Technology – ITA
Engineering Manager at the CTC – Centro de Tecnologia Canavieira
More than 25 years developing technology for the sugarcane industry, including harvesting, processing of residues, transport of sugarcane and sugar, biomass recovery, processing and storage (bagasse and straw), industrial processes, biomass energy generation, cellulosic ethanol, electrical efficiency and emissions control.
Some projects conducted
• Implementation of a Cellulosic Ethanol Demo plant using bagasse.
• Bagasse pelletization.
• Energy generation for electricity.
• Sugarcane Dry Cleaning Station for the separation of trash and mineral impurities (soil).
• Trash baling and processing.
• Biomass storage.
• Technologies to reduce boiler particulate emissions
• Reduction of water use in the industry.
• Vinasse concentration.
* CTC – Centro de Tecnologia Canaviera (www.ctc.com.br) – A Brazilian sugarcane private research organization dedicated to the development of new technologies, technology transfer and services to the sugarcane industry.

Biomass Processing and 2G Ethanol Production – CTC Technology Demonstration Facilities

The presentation will show two different technologies related to biomass that have been developed by CTC which were already implemented at demonstration scale. In the first case, the processing of sugarcane straw bales as a means of increasing the available biomass that could be used for Energy or E2G production. The second, an E2G small scale facility for process and equipment improvement and technology demonstration.

Valdeir Arantes

Valdeir Arantes has a B.Eng in Industrial Chemical Engineering (with emphasis in Biotechnology) and a Ph.D. in Industrial Biotechnology from the University of São Paulo with research stays at Purdue University and University of Maine in the USA. Following a Postdoctorate in Industrial Biotechnology for Renewable Products at the University of British Columbia in Canada, he was appointed as a Research Associate and team leader of the Enzymatic Processing of Biomass for Biorefinery Group at the same University, when for six years he also coordinated multi-international projects in Biochemical Conversion of Lignocellulose involving academic, government, and private institutions from across Canada, China, and the USA. Appointed as an Assistant Professor at the University of São Paulo, he returned to Brazil where at the Lorena School of Engineering he is the Coordinator of the Undergraduate Program in Biochemical Engineering and Vice-Head of the Department of Biotechnology, implemented and coordinates the Biocatalysis and Bioproducts Laboratory with focus on the development of bioprocessing technologies for converting biomass into nanocelluloses and industrial sugars. Valdeir is also a Research Productivity Fellow in Chemical Engineering/Bioprocesses for the National Council for Scientific and Technological Development's (CNPq), a member of the editorial board of the international journals Bioethanol Journal, BioMed Research International Biotechnology, and Frontiers in Bioengineering and Biotechnology and Energy Research, and have also served as a consultant to several international research funding agencies such as the US Department of Agriculture's Small Business Innovation Research, Kentucky Science & Engineering Foundation, Estonian Research Council, and to the Research Executive Agency of the European Commission.

Enzymatic processing of plant-based cellulose for production of cellulose nanocrystals

Cellulose nanocrystals have unique properties, innumerous possible applications and have been mainly produced by acid hydrolysis of cellulosic pulp. Alternatively, cellulose nanocrystals can also be isolated from cellulose materials by enzymatic hydrolysis, which offers many advantages as well as some drawbacks over the traditional acid hydrolysis. This presentation will first give an overview on the state-of-the-art of nanocellulose production assisted by enzymes. Then, we will show and discuss the research progress achieved in the NANOCEL project, a multi-year project aimed at developing/identifying enzyme cocktails for nanocellulose production. In this context, we have comprehensively characterized several enzyme preparations, with an apparent potential to be used for nanocellulose production, regarding their reaction kinetics and enzyme activities during enzyme-assisted production of CNC. Results for the major properties of the CNCs produced like crystallinity, particle size and size distribution, stability of the CNC suspensions, as well as their thermal stability, have revealed that the type and ratio of the enzymes are just important and influential as the reaction conditions (i.e. time and enzyme dosage). Examples with commercially available enzymes will also be given to illustrate how one can tailor some of the CNC properties by adjusting the reaction conditions and key enzyme components.

Xiaowen Chen

Dr. Xiaowen Chen received his master's and Ph.D's degree in chemical engineering from University of Maine. He is now a chemical engineer at the National Renewable Energy Lab. His research interest is in process development and biochemical engineering in cellulosic ethanol.

Novel DMR processing of corn stover achieves high monomeric sugar concentrations from enzymatic hydrolysis (230 g/L) and high ethanol concentration (10% v/v) during fermentation

Distilling and purifying ethanol, butanol, and other products from second and later generation lignocellulosic biorefineries adds significant capital and operating cost for biofuels production. The energy costs associated with distillation affects plant gate and life cycle analysis costs. Lower titers in fermentation due to lower sugar concentrations from pretreatment increase both energy and production costs. In addition, higher titers decrease the volumes required for enzymatic hydrolysis and fermentation vessels. Therefore, increasing biofuels titers has been a research focus in renewable biofuels production for several decades. In this work, we achieved over 200 g/L of monomeric sugars after high solids enzymatic hydrolysis using the novel deacetylation and disc refining (DDR) process on corn stover. The high sugar concentrations and low chemical inhibitor concentrations from the DDR process allowed ethanol titers as high as 82 g/L in 22 hours, which translates into approximately 10 vol% ethanol. To our knowledge, this is the first time that 10 vol% ethanol in fermentation derived from corn stover without any sugar concentration or purification steps has been reported. Techno-economic analysis shows the higher titer ethanol achieved from the DDR process could significantly reduce the minimum ethanol selling price from cellulosic biomass.

Yannick Bomble

Yannick J. Bomble is a senior research scientist and project lead at the National Renewable Energy Laboratory in Golden, Colorado. His team is multidisciplinary and focuses on the characterization/engineering of biomass degrading and metabolic enzymes as well as metabolic engineering/modeling of microorganisms to improve the production of biofuels and bioproducts. He received his Ph.D in Chemical Physics from the University of Texas at Austin (2006) and conducted his postdoctoral work in Computational Biology at the Scripps Research Institute in La Jolla, California (2007). Prior to this he obtained a B.S in Physics and Chemistry from the Universite de Lille (France) (2001) after two years of Mathématiques Superieures and Mathématiques Speciales at the Lycée Faidherbe in Lille (France).

Biomass Deconstruction by Thermophiles: What Have We Learned and Can We Improve Their Cellulolytic Activity?

Microorganisms have evolved different and yet complementary mechanisms to deconstruct biomass in the biosphere. The chemical biology of lignocellulose deconstruction is a complex and intricate process that appears to vary in response to specific ecosystems. These microorganisms rely on simple to complex arrangements of glycoside hydrolases to conduct most of these polysaccharide depolymerization reactions. It is now clear that these deconstruction mechanisms are often more efficient in the presence of the microorganisms. In general, a major fraction of the total plant biomass deconstruction in the biosphere results from the action of various microorganisms, primarily aerobic bacteria and fungi, but also, a variety of anaerobic bacteria. Understanding the interplay between these organisms (and their biomass degrading enzymes) within or across ecosystems is crucial to further our grasp of chemical recycling in the biosphere, which we believe will further enable optimization of enzymes and microorganisms used in the burgeoning plant-based bioeconomy. Here we focus on biomass deconstruction by promising thermophilic cellulolytic anaerobes being considered for consolidated bioprocessing. We describe in details their deconstruction mechanisms and show how these microorganisms can be engineered for increased cellulolytic activity.

Nov 29th - Nov 30th, 2017 Workshop on

Second Generation Bioethanol and Biorefining

Campinas, Brazil

Posters

Data mining and metrics standard for selection, comparison and improvement of lignocellulosic biomass convertion

1 Goulart, A. K.; 1 Seidl, P. R.; 1 Leite, L. F.; 2 Orleans, L. F.
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Different pretreatments are being tested under a wide range of operating conditions to meet the different characteristics of plant species and the production of a range of derived chemicals. Continuous analysis of technical and economic processes evolution is essential to identify improvements and which technology is most appropriate to be applied in a commercial plant. However, a consistent comparison of conversion efficiency is currently expensive, complex and inaccurate, since it is required to perform a repetitive series of bench treatments and its final yields are influenced by the alteration of a large number of parameters. The standardized and organized laboratory and pilot scale process data collection, published in articles and patents, in a single data storage structure is crucial for monitoring the history of the evolution of the lignocellulosic biomasses conversion. Also, an interface software will expedite comparison of optimum operating conditions, including economical, energy and environmental aspects of the project. Such a routine contributes to improve the decision-making and reducing the risks of investment in PD&I and marketing.

Multi-omic analysis of the fungus *Laetiporus sulphureus* on the deconstruction of sugarcane bagasse

1,2 Oliveira, A.C.P.; 1 Gonçalves, T.A.; 2 Persinoti, G.F.; 3 Squina, F.
1 Institute of Biology (IB), UNICAMP; 2 Brazilian Bioethanol Science and Technology Laboratory (CTBE), CNPEM; 3 University of Sorocaba, SP, Brazil

Multi-omic approaches such as genomic, transcriptomic and proteomic data can allow the identification of genes, transcripts and proteins involved in the degradation of lignocellulosic biomass. In the present study, the genome of the brown rot fungus *Laetiporus sulphureus* was analyzed, as well as its transcriptome in response to sugarcane bagasse induction. Additionally, studies of the microorganism secretory were carried out to identify proteins involved on plant biomass deconstruction. Analysis of the *L. sulphureus* genome identified 363 carbohydrate active enzymes ("Cazymes") of which 231 were expressed and 71 were overexpressed in response to growth on bagasse. The secretomic identified enzymes interesting for the breakdown of the vegetal cell wall such as xilanase, cellobiohidrolase and auxiliary (redox) activities. The present study supported the initial understanding of the functional role of genes and proteins involved in the degradation of lignocellulosic material by the fungus *L. sulphureus,* which may contribute to commercial enzymatic cocktails improvement.

Sex, alcohol and Darwin: evolution of ethanol tolerance in fission yeast

1 Jacobus AP; 1 Gross J
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Two different evolution protocols were applied to challenge the fission yeast Schizosaccharomyces japonicus with prohibitive ethanol levels. In a first one, we propagated six populations with increasing amounts of ethanol (up to 10% v/v)) for about 2700 generations. Whole genome sequencing (WGS) revealed 239 point mutations, with 16 genes being hit more than once across the six populations. Their functional analysis suggests that control over the cytosolic ATP/PPi pools, amino acid homeostasis, and trehalose metabolism were selected during the experiment. In a second protocol, six populations were submitted to 80-120 cycles of ethanol treatment (32°C for 2 hrs.) with increasing ethanol content (17% v/v upward). Remarkably, three sexual lineages displayed a fast rise in adaptation up to 63% (v/v) ethanol, thanks to the emergence of a constitutive mating/sporulation mutant phenotype. Surprisingly, two clonal populations also developed a constitutive sporulation phenotype, similarly displaying a rapid increase in ethanol tolerance up to 34-40% v/v. WGS uncovered 46 mutations revealing that most populations have mutations in a key gene presumably controlling trehalose degradation.

Potential targets for epigenetic control of Eucalyptus cell wall

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Second generation biofuels are gaining prominence as alternative renewable energy sources. Brazil, the world's largest Eucalyptus short fiber cellulose producer, has the potential to expand second generation cellulosic ethanol production considerably. The main challenge for using Eucalyptus biomass is the lignocellulose complexity which hinders cellulose accessibility to enzymes. Supplementation of Eucalyptus with flavonoids affects expression of cell wall formation and lignification metabolism genes, reducing lignin content and altering its composition and also improving sugars content and saccharification in juvenile plants, as showed by transcriptomics analysis of xylem samples. Furthermore, it was showed that these effects were maintained in adult plants for three years after the suspension of flavonoids supplementation, suggesting that there are epigenetic mechanisms possibly involved. Adult plants xylem samples were submitted to transcriptomics and metabolomics analysis, and here we present our first results and insights regarding epigenetic control of the cell wall of Eucalyptus.

Expression, purification and characterization of cellobiose dehydrogenase of the thermophilic fungus *T. Thermophila*

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Cellobiose dehydrogenase (CDH), is produced by several biomass-degrading fungi and it is known that when generating electrons by the cleavage cellobiose, these electrons can be donated to the oxidative enzymes LPMOs and activate oxygen molecules, helping and potentiating the degradation of the crystalline regions of the cellulose (2). Analysis by mass spectrometer showed 47% coverage against the *T. thermophila* genome. The optimal reaction conditions were determined as pH 5.5 and temperature of 65°C. The thermal stability remained at about 60% for 120 min at temperatures of 50 and 55°C. Stability at various pH remained satisfactory above 60 min of incubation. CDH activity was increased with addition of NaMoO4 and MgCl2. In summary, the enzyme showed to be very active in cellobiose and with a rapid reaction of decolorizing of the electron acceptor DCIP. The optimum temperature confirmed its activity at high temperatures and its thermostability is satisfactory if incubated at 50°C. In addition, studies of the addition of CDH in commercial cellulase cocktails will be done to evaluate the potential for increased degradation of pretreated and in natura biomass by CDH.

Enzymatic hydrolysis of paper to hydrogen production

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The increment in waste generation has become a challenging problem in developing and developed countries, among them, waste paper represents a substantial share of municipal solid waste, necessitating innovative waste management solutions. Biological processes such as the fermentation of carbohydrates from waste paper for the production of hydrogen seems highly attractive, because H2 is a clean fuel and its burning generates only water. The hydrolysis of filter paper (FP) at 15% (w/v) final concentration was estimated at 45°C and 200 rpm, in 100 mM acetate buffer, pH 5.5. The use of Celluclast® (10 FPU/g paper) supplemented (1) or not (2) with β-glucosidase resulted in maximal reducing sugars yields of 65.4% (1) and 11.8% (2), after 48 h. C. beijerinckii was used as an H2-producing microorganism. The initial pH was adjusted to 7 and incubated at 35°C during the tests. After 40h of assay, the maximum production of H2 found was 4.50 mL and 5.16 mL for tests (1) and (2) respectively. C. beijerinckii was able to grow and produce H2 in paper hydrolyzate, making it promising for future investigations on the production of fermentative H2 with this residue.

Mineral particle identification in sugarcane bagasse by image analysis of X-ray microtomography data

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Sugarcane bagasse is a lignocellulosic biomass with potential to be used as raw material for applications involving renewable fuel production, more specifically cellulosic ethanol. A challenge for these applications is the presence of mineral particles in the biomass, which causes problems such as corrosion, sintering, and vitrification in boilers, gasifiers, and combustors because of the presence of inorganic constituents. On the other hand, X-ray microtomography is a non-invasive technique able to capture three-dimensional images of solid samples with a resolution up to ~1 µm. At this scale, it is possible to visualize the cellular structure of the sugarcane bagasse, allowing quantification and morphological analysis of its different cells and mineral particles. In this work, image processing and analysis were employed to quantify 3D morphological features of the mineral particles trapped in the sugarcane bagasse. The 3D images of the bagasse were obtained by x-ray computed microtomography at the LNLS IMX beamline of the Brazilian Synchrotron Light Laboratory (LNLS). Results show the sizes, shapes and preferred location of mineral particles within the bagasse structure and demons.

Innovation Ecosystems in the Bioeconomy

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The Bioeconomy can be defined as the production of renewable biological resources and the conversion of these resources and waste streams into value added products, such as food, feed, bio-based products and bioenergy. To meet the requirements of this set of dynamic and innovative industries, it is necessary to use innovation theories that capture the specificities of these new processes and products. Seeking to understand the dynamism of industries at birth, the concept of Innovation Ecosystems arises. An Innovation Ecosystem (I.E.) is a set of collaborative and competitive arrangements through which firms combine their individual offerings into a coherent, customer-facing solution. Biofuels are among the main products of the Bioeconomy, having as an important representative the 2G ethanol. Thus, this paper aims to present and analyze the structure of the 2G ethanol innovation ecosystem in order to verify its implications in the elaboration of business strategies and in the formulation of incentive policies. The main results are that the I.E. of 2G ethanol differ largely from the 1G ethanol I.E., with different leaders, challenges and structures.

Transcriptional analysis of *trichoderma harzianum* CBMAI-0179 during cellulose and glucose degradation

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The filamentous fungus *Trichoderma harzianum* has a great biotechnological importance. It is a well-known biocontrol agent and it can also secret many biomass-degrading enzymes which includes cellulases and hemicellulases. The goal is to identify and analyze the global transcriptional profile under cellulose and glucose growth conditions by the fungus *T. harzianum* CBMAI-0179, aiming on the Carbohydrate-Active Enzymes (CAZymes) genes and then compare with some other fungi of the same genus (T. harzianum IOC-3844 and T. atroviride CBMAI-0020) with high potential of biomass biodegradation. The RNA-seq was performed and the reads were mapped and aligned using the *T. harzianum* T6776 public genome as reference. The data analysis was performed on the software CLC Genomics WB 9.0. PCA charts, Cluster analysis, Hierarchical cluster, differential expression and the upregulated genes were determined. Venn diagrams were generated to compare the transcriptome profile among the strains. Thus, through the generated data and results, CAZy genes were classified and in-depth studies were conducted identifying some specific CAZy families expressed by CBMAI-0179 and then compared with other species.

Why are the first commercial 2g ethanol plants almost experimental?

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The first E2G commercial plants were inaugurated after 2013. Today, there are 7 plants in operation, but these pioneer units still face operational issues and have had difficulties to operate with stability. These problems, considering the long development period of these projects, raise important questions about the future of advanced biofuels and the bioeconomy. This article proposes to analyze and discuss the nature of the innovation process involved, searching to shed light on the particularities that lead the flagship plants to be still at a stage of facing unanticipated technological challenges. This stage, giving the nature of the problems faced, tends to demand complementary development programs that are often company-specific. The article uses the principles of economics of innovation and explores the notion of technology readiness level (TRL) to compare the pioneer plants, identify their technological problems and solution strategies.

Composition and Structure of the Genomic Regions Related to Plant Biomass Degradation in *Trichoderma harzianum*

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Understanding the organization of fungal genomes has fundamental importance because of their potential biotechnology use. *Trichoderma harzianum* is a filamentous fungus capable of hydrolyzing the plant-derived biomass. Herein, we performed an in-depth study of the major genes in T. harzianum IOC3844 classified by CAZy database (Carbohydrate-Active Enzymes Database). Crossing transcriptome data, 60 BACs (Bacterial Artificial Chromosome) clones containing genes of interest were selected and sequenced through of the PacBio SMRT sequencing platform. A total of 1289 genes were annotated in these genomic regions Among these, 235 were identify and annotated as CAZymesin *T. harzianum* IOC3844, including 130 glycoside hydrolases (GHs), 59 glycosyl transferases (GTs), 25 carbohydrate esterases (CEs), 21 auxiliary activities (AAs) and 28 carbohydrate-binding modules (CBMs). The GH families were the CAZyme class with the highest gene number, including GH18 (9 genes), GH3 (9 genes), GH5 (6 genes) GH16 (4 genes) and GH2 (3 genes). Bio-prospecting the main proteins used by *T. harzianum* IOC3844 for biomass degradation can ensure new advances in the biofuel production field.

Adaptive evolution of *Saccharomyces cerevisiae* PE-2 to treatments of high ethanol content

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One important trait sought in industrial yeasts is the tolerance to high ethanol titers. To select for ethanol resistant strains, we conducted an ethanol survival experiment in which four Saccharomyces cerevisiae PE-2 populations were submitted to harsh ethanol treatments for two hours at 32 °C, followed by a recovery period in ethanol-free medium (2-4 days). Cycles of shock/recovery were reinterred with increasing ethanol content, from initial 19% (v/v) to 28-30% (v/v) after about 70-80 cycles. Competition assays between the evolved populations and the ancestor show a pattern of antagonistic pleiotropy, in which the evolved strains achieved higher fitness than the progenitor to tolerate ethanol shocks, but are largely outcompeted by the ancestor when grown under normal conditions or at 8% (v/v) ethanol. Whole genome sequencing recovered 67 point mutations across the four final populations. Functional analysis of the affected genes suggests a prominent role of trehalose accumulation and inhibition of the RAS/PKA pathway in improving survival rates to ethanol shocks. Molecular genetic analysis of key mutations is underway and will allow fine understanding of the evolution process.

Production and characterization of cellulases from a novel hypercellulolytic mutant of *Trichoderma reesei*

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This work reports a new strain of *Trichoderma reesei* with a high cellulase production, obtained by exposing of spores from Trichoderma reesei QM9414 in ultraviolet light followed by selecting colonies fast-growth on plates. The mutant named T. reesei RP98 showed shorter time of cultivation and some biochemical characteristics improved. At submerged fermentation, the production of FPase, CMCase and Avicelase was increased up to 5, 8 and 3-folds, respectively, compared with strain QM9414. The highest levels of cellulase were obtained with Avicel, cellobiose and sugarcane bagasse as carbon source. The best time of cultivation was 72h at 27°C. The temperature and pH optima for FPase, CMCase and Avicelase were all about 60ºC and 5.0, respectively. The cellulase activity was not affected by glucose addition on the reaction and the saccharification of cellulosic substrates was higher than the wild type strain. When tested in mixtures with addition of β-glucosidase, there was a large increase in glucose release, confirming the low inhibition by the final product accumulation. All these features indicate the use of this mutant in biotechnological applications.

Identification of sugarcane glycosyl hydrolases possibly related to enhanced biomass production

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In this work we focus on GHs identified from plant cell wall proteomes of sugarcane and Brachypodium distachyon. To predicting the function of these proteins, an alignment was made between homologous plants and microorganisms sequences to find functional domains and phylogenic relationships. All the plant sequences have been retrieved from Phytozome v12.1 whereas the microorganism sequences originate from the ncbi website. Forty nine cell wall GHs were identified in sugarcane and 114 in B. distachyon. Predicted functional and structural domains in newly identified CWPs using the PredictProtein bioinformatic software and group them in families. The MEGA6 software has been used to generate the tree . Thus, GH1, some GH3 and GH17 were predicted to have a β-glucosidase activity. Others GH3 had possible β-xylosidase and AFase activities. The GH27 and GH35 families were predited to have α- and β-galactosidase activity, respectively. Therefore, this work has contributed to provide target proteins that could possibly be used in future research to facilitate cheaper E2G production, besides allowing a more detailed analysis of the cell wall proteomes of the grasses.

Ethanol Production by *Spathaspora passalidarum* in the presence of inhibitors: Effect of the initial cells concentration

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The use of raw materials for ethanol production, such as the reuse of the biomass generated (bagasse and sugarcane straw) can result in a raise of up to 40% in your production. The presence of inhibitory compounds in lignocellulosic hydrolysates is the major challenge for fermentation step. The use of high cell density during fermentation processes may be a suitable action to collaborate for E2G production viability, increasing the yield of these fermentations. In this work, the performance of *Spathaspora passalidarum* was compared using three different initial cells concentrations in fermentations containing acetic acid, furfural, 5-HMF and vanillin, the major inhibitors of C5 fraction, in concentrations around the values found on sugarcane bagasse liquor derived from acid pretreatment (Santoro et al, 2015). The results showed an improvement in yield and productivity when comparing different levels of initial cell concentrations. The kinetic parameters demonstrate that high cell density can be used as a strategy to enable the production of ethanol in hemicellulosic hydrolysates containing inhibitors.

New expansin-like proteins and LPMOs and their validation as additive in biomass saccharification process

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Lytic polysaccharides monooxygenases (LPMOs) and expansin-like proteins have the potential for increasing the efficiency of the lignocellulosic biomass conversion. However, a small number of these proteins have been characterized. In this work, new LPMOs (10 genes) from fungi and bacteria, and expansin-like proteins from microorganisms (2 genes) and from sugarcane plants (4 genes) were selected using an evolutionary approach and were expressed in Komagataella (Pichia) pastoris cells. Model proteins were used for comparison in activity assays. In addition, the production of the expansin-like proteins was performed in a benchtop bioreactor. Functional analysis was performed for some LPMOs with cellulosic substrate using mass spectrometry analysis. The results allowed the visualization of several ions that confirm oxidative cleavage. This work obtained a libray of characterized accessory proteins, which have relevant characteristics for the customization of enzymatic cocktails and decrease of the amounts of cellulases that must be added in the hydrolysis of lignocellulosic biomasses.

***Penicillium echinulatum* 9A02S1 enzymatic extract is efficient in the saccharification of cellulose fraction of biomass**

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Commercially-available cellulase complexes generally contain small amounts of B-glycosidase leading to incomplete hydrolysis of cellulose, releasing cellobiose in the culture medium, which cannot be metabolized by industrial yeast strains, thus affecting the ethanol conversion. In this work, the hydrolysis of cellulose fraction of the mixture of oat and soybean hulls was evaluated by the application of cellulase produced by mutant strain 9A02S1 of Penicillium echinulatum. Three concentrations of enzyme (10 FPU g -1, 15 FPU g-1 and 20 FPU g-1 of substrate) in solid/liquid ratio of 1:20, in sodium citrate buffer, were tested over shell oats (1:1) and soybean hull pretreated with 1 % diluted sulfuric. The experiment was conducted at 50 °C and 120 rpm for 120 h.The maximum glucose release was 9 g L-1 and there were no difference between the enzyme concentrations of 15 and 20 FPU g -1. The extract of P. echinulatum 9A02S1 showed promising results as an alternative for the hydrolysis of the cellulose fraction of lignocellulosic biomass. Future tests using saccharification and fermentation on this hydrolysate will be carried out using industrial yeasts.

Directed evolution of a GH1 β-glucosidase from *Humicola insolens* stimulated by glucose and xylose

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Inhibition of β-glucosidases by glucose limits the efficiency of cellulose hydrolysis, impacting the economic viability of bioethanol production. Thus, there is great interest in tolerant or glucose-stimulated enzymes, as well as in the identification of the structural determinants of this tolerance / stimulation. The bglhi gene encoding a glucose-xylose-stimulated β-glucosidase from Humicola insolens was used as template for error-prone PCR (epPCR) methodology. A library of gene diversity aiming at the selection of enzymes with different percentages of glucose stimulation was generated. The best condition for epPCR, generating 2-3 mutations per template, corresponded to standard PCR protocol conditions, but employing 7 mM MgCl2 and 0.1 mM MnCl2. Of the 4435 clones analyzed, 418 expressed enzymes with detectable catalytic activity in solid media. Among them, glucose (100 mM) on pNP-glucosidase activity, using liquid screening, revealed 3 distinct groups, according to the percentage of stimulation (FE) of the enzymes by glucose: 16 with FE< 30%, 2 with FE> 100% and the others with 30%>FE>100%.

Evaluation of the adsorption of *Chrysoporthe cubensis* enzymes under hydrolytic conditions

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The hydrolysis efficiency of the lignocellulosic biomass by the enzymes presents some limitations, among them the non-specific adsorption of the enzymes in lignin. The aim of this work was to evaluate the adsorption profile of proteins and enzymes from phytopathogenic fungus *Chrysoporthe cubensis* in pretreated sugarcane bagasse with different lignin contents, under hydrolytic condition. In order to obtain the enzymatic extract, the fungus was grown under semi-solid state fermentation with wheat bran. The sugarcane bagasse was pretreated with NaOH (BAL) and H2SO4 (BAC), in order to obtain 8.1% and 33.8% of lignin, respectively. After hydrolysis, BAC was able to adsorb greater amounts of proteins (50%), compared to BAL (35%). The enzymes were adsorbed on the BAC and BAL at different extends and β-glycosidase was the enzyme less adsorbed on both substrates. These results demonstrated the negative effect of the lignin present in complex biomasses, which promoted a greater adsorption of proteins and some enzymes of the *C. cubensis.*

Evaluation of removal of inhibitors in hydrolysate of sisal fiber by vacuum evaporation

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There are several factors that may influence the yield of fermentation, such as the presence of inhibitory compounds, which may cause inhibition of microorganism metabolism, leading to microbial death or reduction in alcohol productivity. The main inhibitors are acetic acid, 5-hydroxymethylfurfural (hmf) and furfural. Detoxification is the process most commonly used to minimize the negative effect of inhibitory compounds. Among the most commonly used types of detoxification of lignocellulosic hydrolysates is evaporation under vacuum. The objective of this work was to evaluate the efficiency of vacuum evaporation in the removal of the inhibitors hmf, furfural and acetic acid in the hydrolysate of sisal fiber, obtained by acid treatment. The acid treatment was carried out in a stainless steel reactor using 2.5% sulfuric acid. The hydrolysate was concentrated 1.5 times in the evaporator. The quantification of sugars (xylose and glucose) and inhibitors (hmf, furfural and acetic acid) was carried out through High-Performance Liquid Chromatography. Vacuum evaporation was efficient in completely removing of furfural and hmf, and reduced the concentration of acetic acid.

Comparison through exergetic analysis of two alternatives for the energy exploitation of vinasse

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The production of sugar and ethanol has an important role in the Brazilian agribusiness. The ethanol and sugar industry has been developing in search of a sustainable energy source, and, even though the technology of this industry has developed, one of its residues, the vinasse, still represents a problem. The vinasse, which is the effluent generated in the distilation step of the ethanol production process, has polluting characteristics, and is generated in large amounts, making its disposition problematic and costly. This work addresses the vinasse problem by way of a comparison between two alternatives to use it: i) concentration and subsequent incineration, and ii) biodigestion. The comparison is carried out through energetic and exergetic analysis of the concentration-incineration system for the first case, and the biodigestion system for the second. The main results indicate the concentration-incineration case as the best alternative, with an exergetic efficiency of 13%, which was higher than the efficiency of the biodigestion case. Nevertheless, it is worth mentioning that case i requires and additional vapor consumption, while case ii does not.

Second generation ethanol production from solid residues through a hybrid technology

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The National Solid Waste Politics (PNRS) proposes changes relating to environmentally adequate management and disposal of solid waste. In Brazil, 50% of municipalities direct the solid waste to open-air dumps. The utilization of lignocelllosic-containing solid residues can be accomplished by two routes: the biochemical platform, based on hydrolysis and fermentation processes, and the thermochemical platform, based on burning the biomass through pyrolysis; gasification or combustion. In this context, an important alternative for the disposal of solid urban waste seeking the exploitation of its energy, would be the thermal process of pyrolysis and subsequent valorization of the syngas through its transformation into an energy product. Therefore, a hybrid technology is proposed combining a termochemical technology for the production of syngas, and biochemical rout that consists in the transformation of this gas in biofuels. Hollow fibers membrane bioreactors will be used with strains of Clostridium to ferment the produced syngas. Clostridium carboxidivorans presented great potential for ethanol production between three Clostridium strains, with a faster growth and higher productivity.

Eco-Efficiency evaluation of first and second generation of ethanol production processes via computational simulation

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The main objective of this work is to simulate and evaluate the whole Ethanol Production using the sugarcane juice and bagasse, considering environmental and production parameters. For the process simulation it was used the software UniSim R 390. In order to evaluate the process considering environmental and production aspects, it was adopted the Eco-Indicator approach, consisting of a ratio of an environmental variable and an economic variable. Five different eco-indicators were chosen to evaluate the ethanol process (Electric Energy Consumption, Water Consumption, CO2 emission, Waste Production and Effluent Production). All the eco-indicators were evaluated considering two scenarios. The first scenario is a comparison between the Ethanol production (1st Generation) without cogeneration against the integrated production (1st and 2nd Generation). The second scenario was considered the Ethanol production (1st Generation) with cogeneration against the Ethanol integrated production (1st and 2nd Generation). The first scenario was 41,5% more advantageous for Ethanol 1G without cogeneration. The second scenario the difference was even higher, being 59,7% in favor of 1st Generation.

Transcriptomic analysis of *Kalmanozyma brasiliensis* GHG001 grown on xylan and its pentose monomer xylose

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The production of Second Generation (2G) Bioethanol is strongly dependent on pretreatment of plant biomass (e.g. sugarcane bagasse) followed by fermentation of monomers present in the cell wall polymers. In this work, we explore transcriptomics of the xylanolytic yeast-like fungus *Kalmanozyma brasiliensis* GHG001 grown on xylan, a main component of hemicellulose, and its pentose monomer xylose. RNAseq experiments were performed to study the metabolism of these sugars through differential gene expression (DGE) analysis comparatively with growth in glucose. DGE revealed that at least two previously characterized enzymes were identified as highly expressed in xylose and xylan: an extracellular GH11 endoxylanase involved in xylan hydrolysis and a xylitol dehydrogenase, involved in xylose metabolism. The identification of other enzymes involved in cell wall deconstruction and in internal sugar metabolism that follows the same expression profiles may indicate a synergistic action on hemicellulose deconstruction and xylose metabolism; additionally, identified transcription associated proteins indicate their possible roles on the regulation of genes involved in biomass deconstruction.

Enzymatic detoxification of sugar cane bagasse hemicellulosic hydrolysate for pentose fermentation

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The liquid stream coming the sugar cane bagasse hydrothermal pretreatment followed by acid post hydrolysis is characterized as the hemicellulosic hydrolysate or C5 liquor and contains about 65% of hemicellulose sugars from this biomass. Although, C5 liquor is not fermentable, due high phenolics content arising from lignin (8 g L-1) and furans (2 g L-1). In this work, we developed an in-situ enzymatic process for C5 liquor detoxification aiming to improve fermentation by the yeast Scheffersomyces stipitis and the anaerobic bacteria Clostridium saccharoperbutylacetonicum. Statistical experimental designs between the phenolic degradation/modification enzymes (in patent analysis) were correlated to the microorganisms' fermentation rates after the detoxification step. Three of four enzymes used in this study had significant synergism in both, lignin modification and fermentation improvement. The maximum phenolic removal observed was of 65%, and the main modification observed was lignin polymerization. Besides that, both microorganism could only ferment the detoxified C5 liquor. In conclusion, such enzymes may become a promising enzymatic cocktail for C5 liquor detoxification.

Integrated genomic and transcriptomic analysis for an evolutionary engineering experiment to xylose consumption

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Second-generation technology has been standing out in ethanol production in yeast by reducing costs and increasing productivity, it is based on hydrolyzed biomass which requires a deconstruction process to release fermentable sugars (mainly glucose) and non-fermentable sugars (mainly xylose). To increase the yield of fermentation process, it is necessary perform genetic modifications to allow a xylose-consumption by insertion of endogenous xylose pathway genes combining with an evolutionary engineering step. In this work, genetically modified industrial yeast was submitted for three rounds of evolution in a selective pressure medium (just xylose as carbon source) and has been selected a total of five evolved strains with different fermentative performances, which had their genome sequenced. Moreover, the transcriptomic analysis of three strains (parental and two evolved) were performed during glucose and xylose co-fermentation in three different time points. These data have been analyzed through bioinformatics tools for detecting genome variations (copy number/mutations) and gene expression profiles that can be used to give new insights for xylose consumption in the evolved strains.

A low-cost and highly efficient cocktail for waste paper saccharification

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The bioconversion of cellulosic wastes into high-value bio-products by saccharification and fermentation processes is an significant step that can reduce the environmental pollution caused for example by waste paper. In this study, enzymatic saccharification of different kinds of untreated waste papers by β-glucosidase from Humicola insolens (BglHi2), in purified or crude form, in synergy with two different preparations of cellulases for the hydrolysis of was investigated. Mixtures of pure BglHi2 or H. insolens crude extract (CE) with crude extracts from Trichoderma reesei fully hydrolyzed Whatman no. 1 paper. Mixtures of H. insolens CE with T. reesei CE or Celluclast 1.5 L fully hydrolyzed untreated printed office paper, napkin, and magazine papers after 24–48 h, and untreated cardboard was hydrolyzed by a H. insolens CE / T. reesei CE mixture with 100% glucose yield. Data revealed the good potential of BglHi2 for the hydrolysis of waste papers, promising feedstocks for cellulosic ethanol production.

Medium components minimization for 1,3-PDO production from crude glycerol by *Clostridium butyricum*

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The growing demand for polyesters production such as polytrimethylene terephthalate (PTT) has been stimulating 1,3-propanediol (1,3-PDO) production. 1,3-PDO is a monomer used in PTT synthesis and also is well suited for cosmetics, liquid detergents and industrial applications like anti-freeze. Bioprocess directly using crude glycerol as substrate is interesting and particularly attractive for biorefinery concept. Glycerol can be biologically converted into different value-added products, such as 1,3-PDO. Some Clostridia species are the best 1,3-PDO producers such as *C.butyricum*. The aim of this study was to improve 1,3-PDO production from crude glycerol by *C.butyricum* NCIMB 8082 through medium components minimization. Statistical experimental designs of medium components were performed in anaerobic flasks containing 12.5 g.L-1 of crude glycerol as carbon source and incubated at 37°C and 150 rpm during 24 hours. Samples were taken at 0 and 24 h for analysis of glycerol and 1,3-PDO. Results allowed to reduce culture medium from 9 components to 3 components and 1,3-PDO production was improved to 6,01 g.L-1 after 24 hours of fermentation, 28,7% higher than before medium minimization.

Improvement of industrial yeast strains by breeding and adaptive evolution for second-generation etanol

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Lignocellulosic residues, such as sugarcane bagasse, are considered a promising feedstock for second generation ethanol. The nutritional deficiencies of sugarcane bagasse hydrolysate could be circumvented by molasses supplementation, which might function as a nutrient source for the fermenting organism and also as an ethanol booster for distillation. Addition of nutrient rich molasses is expected to allow yeast cells to maintain high viability, which is of paramount importance in a cell recycling process. In this scenario, yeast tolerance toward inhibitors coming from both substrates is therefore essential for the deployment of this process. The objective of the present work was to generate multi-tolerant yeast strains by adaptive evolution of hybrids coming from industrial strains. For this purpose, Saccharomyces cerevisiae strains widely used in Brazilian sugarcane mills (PE-2, CAT-1 and SA-1) were sporulated and the tetrads of these strains were dissected resulting in ca. 200 haploids cultures. These haploids were used for poly-crossings by mixing 7 different pools of all haploids.

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