



BBEST 2020/21



bio ENERGY +
ECONOMY
innovation - policies - markets

november 30th to december 2nd 2020

VIRTUAL

may 24th to 26th 2021

VIRTUAL

proceedings

BBEST 2020-21/BIOFUTURE SUMMIT II

The Brazilian Bioenergy Science and Technology Conference and the 20-country Biofuture Platform multilateral initiative have joined forces to bring together the world's very best in policies, innovation, science and market outlook in the bioenergy and bioeconomy sectors, in a landmark event which will take place from May 24-26th, 2021.

This unique event will combine two different conferences in one: the second Biofuture Platform policy conference (**BIOFUTURE SUMMIT II**), and the fourth edition of the Brazilian Bioenergy Science and Technology Conference (**BBEST**).

BBEST is a triannual international conference on bioenergy now in its fourth edition, after the very positive outcome from the previous versions in 2011, 2014 and 2017, attended by hundreds of researchers, entrepreneurs and government officials from Brazil and abroad. This time, besides covering relevant aspects of bioenergy, from biomass production to conversion and final use, the 2020-2021 edition will highlight innovation.

The first **BIOFUTURE SUMMIT** (São Paulo, 2017) was highly successful, gathering more than 47 speakers and 320 select delegates from 28 countries, including policy makers from the 20 Biofuture member governments, international organizations, private sector representatives, and experts in different fields, discussing how to put together policy, innovation and finance to create an enabling environment for the sustainable low carbon bioeconomy.

In line with this, **BBEST 2020-21/BIOFUTURE SUMMIT II** event will devote to discuss policy and the role of the low carbon bioeconomy in a sustainable world and the ways to reach it, including key debates related to finance, transport policy, demand and carbon internalization policies, and sustainability governance, that are important to foster bioenergy innovation in the context of sustainable development, considering the perspectives of all significant stakeholders. The event will have live webinars, plenary sessions, round tables and technical sessions with networking opportunities made possible through a virtual conferencing platform. The program will include highlights on technologies for agronomy, biotech and biomass growing; biofuel production and advanced biorefining; aviation and transport applications; sustainability and carbon cycle analysis, among others.

We intend to showcase state-of-the-art processes and products, an urgent policy debate, and new trends in using the bioeconomy to create value and actual environmental and social benefits. Modern bioenergy vectors, such as bioelectricity and liquid biofuels, are competitive and readily available options for the urgent energy transition towards renewable energy, as imposed by global and local environmental concerns, as well as an improver of rural sector economy, social development and national energy security, in industrialized and developing countries.

Glaucia Mendes Souza (USP, BIOEN)

Luiz Augusto Horta Nogueira (NIPE/UNICAMP, BIOEN)

Renato Domith Godinho (Biofuture)

Conference Chairs

Committees

Glaucia M. Souza, Universidade de São Paulo, Brazil

Luiz Augusto Horta Nogueira, Universidade Estadual de Campinas, Brazil

Renato D. Godinho, Ministry of Foreign Affairs, Biofuture Platform Chairperson, Brazil

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Luiziana Ferreira da Silva, University of São Paulo, Brazil

Clarissa Forecchi, Ministry of Foreign Affairs, Brazil

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Heitor Cantarella, Instituto Agrônômico de Campinas, Brazil

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Universidade de São Paulo, Brazil

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Carolina Grassi	Paulo Selegim	Sarita Cândida Rabelo	Thiago Olitta Basso
Diego Mauricio Riaño-Pachón	Raffaella Rossetto	Simone Pereira de Souza	

**PROCEEDINGS OF THE BRAZILIAN BIOENERGY SCIENCE AND TECHNOLOGY CONFERENCE and
BIOFUTURE SUMMIT II**

Program Committee

Carlos E. Pellegrino Cerri	Igor Polikarpov	Patricia Osseweijer	Rubens Maciel Filho
David Chiaramonti	Jack Saddler	Paulo Arruda	Solange Mussatto
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Hugo Bruno Correa Molinari	Nick Carpita		

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Bernardo Silva	Helena Chum	Marcos Landell	Ricardo Tomczyk
Bruce Dale	Herman Hoffman	Mateus Schreiner Lopes	Sergio Trindade – “In Memoriam”
Carlos Henrique de Brito Cruz	Jim McMillan	Marie-Anne Van Sluys	
Décio Oddone	Jim Spaeth	Oscar Chamberlain	

BBEST Awards

Among the posters presented during BBEST2020/21, 12 were chosen to receive the "BBEST Award" in the categories: Undergraduation, Master Student, PhD Student and Post Doctor. The winners received a certificate.

Undergraduate

1st Place

Jonathan Cardoso Climaco Vieira

Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Brazil

Evaluation of cell wall components of contrasting sugarcane progenies for biomass production [A-25]

2nd Place

Manuela Temtemples de Carvalho

Laboratório de Desenvolvimento de Bioprocessos, Escola de Química, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brazil)

Evaluation of Methylocystis hirsuta growth in stirred tank and bubble column bioreactors with variation of culture medium and gas phase recycle [C-21]

3rd Place

Tainá Fagotti Ferreira

Instituto de Ciência e Tecnologia, Universidade Federal de Alfenas (Minas Gerais, Brasil)

Biogas generation from hybrid anaerobic treatment of dairy industry using lipolytic fungal cells and anaerobic sludge [E-17]

Master Student

1st Place

Flávia da Silva Barbosa

Engenharia de Alimentos, Universidade de São Paulo (São Paulo, Brasil)

Soybean oil extraction using ethanol as solvent: effect of moisture of oleaginous solid and temperature of extraction [B-27]

2nd Place

Lucca Bonjy Kikuti Mancilio

Departamento de Química Fundamental, Instituto de Química, Universidade de São Paulo, Brasil

Using byproducts from the hydrolysis of lignocellulosic substrates to generate energy in a microbial fuel cell [B-54]

3rd Place

Franz Wagner Laurett Veras

Laboratory of Bioproducts, Microbiology Department, University of São Paulo (São Paulo, Brazil)

Production of the copolyester P3HB-co-3HV by Burkholderia sacchari prp mutants using xylose and propionate and evaluation of the modulation of its monomeric composition [C-06]

PhD Student

1st Place

Nathália Vilela

Departamento de Bioquímica e Biologia Tecidual, Universidade Estadual de Campinas (SP, Brazil)

Multi-omic characterization of a novel red yeast strain with biotechnological potential for lignin valorization strategies [C-14]

2nd Place

Igor Severo Gonçalves

Faculty of Food Engineering, University of Campinas (São Paulo, Brazil)

Increasing sugarcane straw pre-treatment efficiency using ionic liquid mixtures at low temperature [B-28]

3rd Place

Fernanda Silva Martinelli

Center for Development Research, University of Bonn (Bonn, Germany)

Are greener policies able to make biofuels sustainable? An experts opinion analysis using the SDG Framework [E-13]

Post Doctor

1st Place

Matheus Aparecido Pereira Cipriano

Microbiologia do Solo, Instituto Agronômico (São Paulo, Brazil)

Effect of beneficial microorganisms on sugarcane plant growth, metabolism, biological control and soil bacterial community [A-30]

2nd Place

Julianne de Castro Oliveira

School of Agricultural Engineering (FEAGRI), University of Campinas (São Paulo, Brazil)

Assessment of pastureland changes on the net primary production-based exergy in São Paulo State, Brazil [A-32]

3rd Place

Lívia Beatriz Brenelli

Physico-Chemical Processes, Brazilian Biorenewables National Laboratory, Brazilian Center for Research in Energy and Materials (São Paulo, Brazil)

An integrated approach to obtain xylo-oligosaccharides from sugarcane straw for bioethanol production [C-05]

Program

Opening Ceremony

Monday – May 24th – 8:30 to 9:00 (UTC -3)

Minister Bento Albuquerque, Ministry of Mines and Energy, Brazil
Fatih Birol, Executive Director, International Energy Agency (IEA)
Marco Antonio Zago, President of FAPESP, Brazil
Marcos Penido, Secretary of Infrastructure and Environment of the State of São Paulo, Brazil
Sarquis Sarquis, Secretary for Foreign Trade and Economic Affairs, Ministry of Foreign Affairs,
Brazil
Augusto Pestana, President, APEX-Brazil
David M. Turk, Deputy Secretary of the U.S. Department of Energy

Keynotes: The place of the low carbon bioeconomy in the new dynamics of global energy

Monday – May 24th – 9:00 to 11:00 (UTC -3)

Introduction from Conference Chairs:

Glaucia Souza (USP, BIOEN-FAPESP, Brazil)
Renato Godinho (Ministry of Foreign Affairs, Biofuture Platform Chairperson, Brazil)

Keynotes

Luiz Augusto Horta Nogueira (UNICAMP, BIOEN-FAPESP, Brazil)
Carlos Henrique de Brito Cruz, Unicamp, Brazil
Paolo Frankl, Head of Renewable Energy, International Energy Agency (IEA)
Jeremy Shears, Shell Research Limited
Thelma Krug, Vice-Chair, Intergovernmental Panel on Climate Change (IPCC)

LIVE DEBATE SESSIONS

Monday – May 24th – 11:00 to 13:00

Webinar Live 1 – Biomass quantification and Sustainability Governance: How to achieve consensus

Chair: Renato D. Godinho, Biofuture Platform Chairperson, Ministry of Foreign Affairs, Brazil

Moderator: Clarissa Forecchi, Ministry of Foreign Affairs, Brazil

Glaucia Mendes Souza, University of São Paulo and FAPESP Bioenergy Program
Keith Kline, Oak Ridge National Laboratory, USA
M. Michela Morese, Global Bioenergy Partnership (GBEP)
Inge Stupak, University of Copenhagen
Gerard Ostheimer, Biofuture Workshop
Mariam Kenza Ali, Climate Program Officer, Packard Foundation

PROCEEDINGS OF THE BRAZILIAN BIOENERGY SCIENCE AND TECHNOLOGY CONFERENCE and
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Tuesday – May 25th – 10:30 to 12:30

Webinar Live 2 – The Biofuture Policy Blueprint: Results and next steps

Chair: Paolo Frankl, Head of Renewable Energy Division, International Energy Agency (IEA), Renewable Energy Division

Moderator: Heitor Cantarella, Agronomic Institute of Campinas & FAPESP BIOEN

Heloisa Esteves, Agencia Nacional de Petroleo, Gás e Biocombustiveis, Empresa de Pesquisa Energética EPE

Michael Wang, Argonne National Laboratory

Kees Kwant, Netherlands Enterprise Agency (RVO), Biofuture Platform Core Group

Adam Brown, Biofuture Facilitation Team

Wednesday – May 26th – 10:30 to 12:30

Webinar Live 3 – Biofuture Summit Wrap-Up: A Roadmap for the Bioeconomy acceleration

Chair: Renato D. Godinho, Biofuture Platform Chairperson, Ministry of Foreign Affairs, Brazil

Moderator: Clarissa Forecchi, Ministry of Foreign Affairs, Brazil

James J. Spaeth, US DOE Bioenergy Technologies Office System Development & Integration Program Manager, U.S. Department of Energy

Devin O'Grady, Natural Resources Canada (NRCan)

Sangita Kasture, MOST, Mission Inovation

Paolo Frankl, IEA

Kees Kwant, Biofuture Platform, IEA Bioenergy

Renato D. Godinho, MRE, Biofuture Platform

Monday – May 24th – 14:00 to 16:00

Webinar Live 4 – Emerging Markets and New Frontiers

Chair: Luiz Augusto Horta Nogueira, Unicamp, BIOEN-FAPESP

Moderator: Marcelo Menossi, University Of Campinas

Stephen Karekezi, African Energy Policy Research, Kenya

Aida Lorenzo, Asociacion Combustibles Renovables, Guatemala

Rocio Diaz Chavez, Stockholm Environment Institute, Energy and Climate Change Programme Leader (SEI-Africa)

Damião Namuera, Head of Renewable Energy Dept at Ministry of Mineral Resource and Energy, Maputo, Mozambique

Brian D. Healy, USA Grains Council

Tuesday – May 25th – 14:00 to 16:00

Webinar Live 5 – Is there a climate benefit in the temporary absorption of biogenic carbon in products?

Chair: Annie Levasseur, École de Technologie Supérieure

Moderator: Karla Censi, Braskem

Marcelo Morandi, Embrapa, Brazil

Harald Käß, Narocon Innovation Consulting, Germany

Yuki Kabe, Braskem

Marcelo Moreira, Agroicone

Hugo Maria Schally, ENV.F3 – Multilateral Environmental Cooperation

PLENARIES PANELS AND ROUND TABLES

Plenary Panel 3: Bioenergy in transportation and the electrification debate

Chairs: Kees Kwant, Netherlands Enterprise Agency (RVO), the Netherlands, Biofuture Platform Core Group and Luiz Augusto Horta Nogueira, UNICAMP, BIOEN-FAPESP, Brazil

Plinio Nastari, National Council on Energy Policy (CNPE), Brazil

Martin Rothbart as Product Manager for Energy and Sustainability

Ricardo Abe, Senior Engineering Manager, Nissan, Brazil

Dina Bacovsky, BST, Austria

David Chiaramonti, ISAF

Plenary Panel 4: Financing the Bioeconomy: the role of green bonds and financial institutions

Chairs: Lynn Cote, Export Development Canada – CleanTech Lead, and Ambassador Sarquis Sarquis, Secretary of Foreign Trade and Economic Affairs

Mafalda Duarte, World Bank, USA

Leisa Souza, Climate Bonds Initiative (CBI)

Xian Zhu, New Development Bank, China

Petrônio Cançado, Director, National Brazilian Development Bank (BNDES), Brazil

Patricia Genelhu, Sustainable & Impact Investing at BTG Pactual

Don Roberts, Nawitka Capital Advisors

Plenary Panel 5: Technology push policies and supporting the bioeconomy innovation

Chairs: Paul Durrant, IRENA, and

Luis Fernando Cassinelli, BIOEN-FAPESP, Brazil

Eduardo Couto, Director, National Laboratory on Biorenewables (LNBR), Brazil

James Spaeth, Bioenergy Technologies Office (BETO), US Department of Energy, Biofuture Core Group, USA

Shilpi Gupta, Department of Biotechnology, Ministry of Science and Technology, India

Peter Coleman, Head of Land Use and Bioenergy Science in BEIS, UK

Dou Kejun, China National Renewable Energy Centre

Round Table 1: Aviation Biofuels Takeoff

Chair: Gerard Ostheimer, Biofuture Workshop

Jane Hupe, International Civil Aviation Organization (ICAO)
Landon Loomis, VP da Boeing
Maria Carolina Grassi, Roundtable on Sustainable Biomaterials (RSB)
Pedro Mancuello Pérez, Vice Minister for Industry and Trade, Paraguay
Sami Jauhiainen, Vice-President, Business Development, Renewable Aviation Fuels of Neste

Round Table 2: The Biobased Future and Biorefining

Chair: Jim Spaeth, US Department of Energy, Biofuture Core Group and IEA Bioenergy Chair, USA

Lasse Rosendahl, Aalborg University, Denmark
Luuk Van Der Wielen, Univ. of Limerick, Ireland
Paolo Corvo, Clariant, Germany
Jorge Soto, Braskem
Sarah Gallo, BIO, USA

Round Table 3: Investing in the Biobased Future in Brazil

Chair: Amaury Pekelman, President, National Bioenergy Union (UDOP), Brazil

Carlos Henrique de Brito Cruz, Unicamp, Brazil
Raphaella Gomes, Diretora de Transição Energética, Raízen
Rainer Janssen, BIOFIT Project, WIP Renewable Energies, Germany
Artur Milanez, Brazilian Development Bank (BNDES)
Thiago Falda, Executive President, Brazilian Bioinnovation Association (ABBI), Brazil

Round Table 4 – Industrial Implementation of Energy Cane and Advanced Biofuels

Chair: Glaucia M. Souza, IQ-USP, Brazil

José Antonio Bressiani, Granbio, Brazil
Marisa Coral, Raízen, Brazil
Walter Maccheroni, São Martinho, Brazil
Hermann Hoffmann, RIDESA, Brazil
Dario Giordano, Versalis, Italy
Lee Lynd, A2G Lab, Brazil and USA

TECHNICAL SESSIONS

TS-01. Research in Small Business: Biomass, BIOFUELS and BIOREFINERIES

Chair: Luis Cassinelli, FAPESP, Brazil

Energycane Vertix: The Most Competitive Biomass for Second Generation Industry

José Antonio Bressiani, Granbio, Brazil

OleoLev® Production of biodiesel and defoamers from vinasse

Mario Lúcio Lopes, Fermentec, Brazil

Sugarcane Precision Monitoring by a 3-Band Drone-borne Radar

Hugo Enrique Hernandez Figueroa, FEE, Unicamp

Alcoholic content and increased industrial productivity in fermentations for ethanol production

Marcel Salmeron Lorenzi, Fermentec, Brazil

TS-02. Deconstructing Biomass

Chair: Marcos Buckeridge, USP, Brazil

Redesigning the plant cell wall for fuels, chemicals, and materials

Nick Carpita, National Renewable Energy Laboratory, Golden (CO), USA

C-CBP: An Alternativa Cellulosic Biomass Deconstruction Paradigm

Lee Lynd, Dartmouth College, USA

Reducing Transportation Carbon through Low-Costa Consolidated Alcohol Deoxygenation and Oligomerization (CADO) to Gasoline, Diesel, and Jet Fuel Blendstocks and Higher Value Chemicals (BTEX)

John Hannon, Vertimass

A new strategy for successful expression of heterologous D-xylose transporters in

Saccharomyces cerevisiae

Boris J.C.U. Stambuk, UFSC, Brazil

Deletion of N-glycosylation pathway genes in Aspergillus Nidulans and effects on enzyme production

André Damasio, Unicamp, Brazil

TS-03. Task 45 Governing a Sustainable Bioeconomy: Assessment and Monitoring (Experience and Perspective)

Chair: Uwe Fritsche, Germany

Argentina's Bioeconomy and the Meaning of Sustainability

Maria Rosa Murmis, Miniserion de Agricultura, Argentina

Driving Bioeconomy Sustainable Development in Brazil

Marcelo Poppe, CGEE, Brazil

Governance of The Bioeconomy – Common visions in the Colombian context

Nella Canales, SEI, Colombia

The measurement of the GBEP Sustainability Indicators in Paraguay

Guillermo Parra Romero, Oil & Gas, Paraguay

Recommendation of the Council on Assessing the Sustainability of Bio-based Products

Jim Philp (OECD)

TS-04 – Biodiversity Nexus

Chair: Luciano Verdade, USP, Brazil

Biodiversity and the fascinating universe of natural products

Vanderlan Bolzani, UNESP, Brasil

Biofuels and wildlife management in multifunctional agricultural landscapes

Luciano M. Verdade, USP, Brazil

The ecology of sugarcane fields

Luis C. Schiesari, USP, Brazil

The role of Silviculture of Native Species and R&D in a New Forestry Economy in Brazil

Miguel Calmon, WRI Brazil

TS-05. Crop Biotech

Chair: Marcelo Menossi, UNICAMP, Brazil

Engineering of bioenergy crops for reduced recalcitrance and accumulation of bioproducts

Henrik Vibe Scheller, UCLA, USA

New Biotechnology trades for sugarcane

Hugo Bruno Correa Molinari, Embrapa, Brazil

Dynamic control of secondary metabolism in plants

Maurício Antunes, University of North Texas, USA

Plant Transformation Facility and Genetic Engineering in Sugarcane breeding

Paulo De Lucca, Pangeia, Brazil

TS- 06. BIOEN Biofuels

Chair: Rubens Maciel, UNICAMP, Brazil

Challenges and perspectives in biomass gasification aiming at biofuel production in BtL plants
Ingrid Motta, UNICAMP, Brazil

Multi-scale observations of mineral impurities in sugarcane bagasse and straw
Carlos Eduardo Driemeier, LNBR, Brazil

Adaptive Evolution, Genomics and Synthetic Biology for Improving Yeast Tolerance to Fermentation Stresses
Ana Paula Jacobus, UNESP, Brazil

Chemical and structural assessment of the acid hydrolysis of cassava (Manihot esculenta) bagasse: power ultrasound versus mechanical agitation
Tiago Carregari Polachini, UNESP, Brazil

TS-07. SAE Biofuels in the Mobility Strategy

Chair: Erwin Franieck, SAE

Brazil's alternatives for sustainable mobility
Ricardo Abreu, Unicamp-FEA

Water injection enabling high efficiency flex fuel engines focused on ethanol
Gustavo Lopes, Bosch PS-PC

P8 Technology feasibility (EuroVI) with +B15
Eduardo Nogueira Dias, BASF

Biodiesel future challenges for Proconve P8
Christian Wahnfriend, Bosch PS-CV

Renewables fuels for transportation
Mike Lu, Curcas Diesel

TS-08. Biodiesel, Biokerosene, Bio-oils

Chair: Luuk van der Wielen, Univ. of Limerick, Ireland

Biodiesel, Green Diesel, SAF and What is coming up next?
Donato Aranda, União Brasileira do Biodiesel e Bioquerosene (UBRABIO)

Catalytic Pyrolysis of Saccharum Munja in a Fixed Bed Reactor
Khurram Shahzad, University of the Punjab, Pakistan

On the way to obtain Renewable Jet Fuel (Kerosene), Diesel and Hydrocarbons
Rubens Maciel, Unicamp, Brazil

Physiology and lipidomics of Saccharomyces cerevisiae at extremely low O₂ availability in the absence of ergosterol and unsaturated fatty acids
Thiago Olitta Basso, USP, Brazil

South America Debuts on Advanced Biofuels
Erasmio Carlos Battistela, ECB Group, Brazil

TS-09. SynBio

Chair: Marie-Ann Van Sluys, USP, Brazil

Engineering approaches for diverting lignin biosynthesis towards the production of platform chemicals in plants

Aymerick Eudes, Laurence Berkeley Laboratory, USA

InovaUSP: The new front-end of innovation at USP

Luiz Henrique Catalani, InovaUSP, Brazil

Genomic resources for crop engineering for energy and bioproducts

Glauca Mendes Souza, USP, Brazil

Sugarcane Rhythms in the field

Carlos Hotta, USP, Brazil

Lignin metabolism: A systems view

Igor Cesarino, USP, Brazil

TS-10. BIOEN Sustainability

Chair: Patricia Osseweijer, TU Delft, The Netherlands

Sugarcane Bioenergy Research in Brazil – Bibliometric Evaluation of the Bioen Research Program

José Maria Ferreira Jardim da Silveira, UNICAMP, Brazil

Dynamics and Resilience of soil microbiome under organic (vinasse) and inorganic disturbances in sugarcane fields

Késia Silva Lourenço, IAC, Brazil

Evaluating Hydrological trade-offs due to different land covers and land uses as fundamental input for assessment for ecosystem services

Edson Cezar Wendland, USP, Brazil

2nd crop ethanol in the context of advanced fuels

Marcelo Moreira, Agroicone, Brazil

The Social economic impacts of RenovaBio policy in the Brazilian economy described by a computable general equilibrium model

Marcelo P. Cunha, Unicamp

TS-11. Biofuels application in Engines and Fuel Cells

Chair: Francisco Nigro, USP, Brazil

The opportunity for advanced biofuels from sugarcane residues: Overview of the potential to utilise sugar cane residues to help decarbonise Marine emissions

Stephen Rogers, Licella, Australia

Solutions for hydrogen production & purification

Daniel Gabriel Lopes, Hytron, Brazil

Niobium Enhances Electrocatalytic Pd Activity in Alkaline Direct Ethanol and Glycerol Fuel Cells

Mauro Santos, UFABC, Brazil

The most recent R&D Projects in ICE using renewable fuels Developed by CTM – EEUFMG

José Guilherme Coelho Baeta, UFMG

Electrification by Ethanol

Gonçalo Pereira, UNICAMP, Brazil

TS-12. Bioproducts from Biomass

Chair: Solange I. Mussatto, Technical University of Denmark

Total utilization of biomass and making smart materials from lignin to enable the circular economy

Mahdi Abu-Omar, University of California, Santa Barbara, USA

Biopolymers and other bacterial bioproducts from biomass

Luiziana Ferreira, USP, Brazil

Innovation in Renewable Chemicals

Mateus Schreiner Garcez Lopes, Braskem, Brazil

Valorization of the cellulosic and hemicellulosic fractions of wheat straw through microbial productions of lipids and carotenoids

Giuliano Dragone, Technology University of Denmark

Towards smart biomanufacturing: Strategies to get maximum value from biomass

Solange I. Mussatto, Technical University of Denmark

TS-13. BIOEN Biomass

Chair: Raffaella Rossetto – IAC, Brazil

Biotechnological approaches to increase sugarcane biomass utilization

Marcelo Menossi, UNICAMP, Brazil

*Identification of novel regulators of lipids biosynthesis in the microalgae *Chlamydomonas reinhardtii* through multi-OMICS approach*

Flavia Vischi Winck, USP, Brazil

Using endogenous molecular and biochemical mechanisms for biomass pretreatment

Marcos Buckeridge, IB-USP, Brazil

Different Nitrogen formulations can enhance sugarcane fields

Raffaella Rossetto – IAC, Brazil

Evaluation of the antagonistic effect of volatile organic compounds on sugarcane pathogens

Juliana Velasco de Castro Oliveira, LNBR/CNPEN, Brazil

Gene expression profiling in sugarcane

Gabriel Margarido, USP-Esalq

TS 14/17. IEA Bioenergy Task 39 – Commercialization of Biofuels

Chairs: Jack Saddler, University of British Columbia, and Jim McMillan, NREL, USA

Commercializing Conventional and Advanced Transport Biofuels from Biomass and Other Renewable Feedstocks

Jack Saddler, University of British Columbia

Biofuel LCA Issues

Don O'Connor, S&T Squared Consultants Inc, Canada

Life-Cycle Greenhouse Gas Emission Reductions of Ethanol with the GREET model

Michael Wang, ANL, USA

Comparison of Biofuel Life Cycle Analysis Tools: Biochemical 2G ethanol production and distribution

Mateus F. Chagas, LNBR, CNPEM, Brazil

The Role of Renewable Fuels in Decarbonizing Road Transport

Dina Bacovsky, BST, Austria

Synergies of biobased and electricity-based process chains – the example of a PTG HEFA refinery

Franziska Mueller-Langer, DBFZ Germany

TS-16. Land Use for Biomass Production

Chair: Carlos Eduardo Pellegrino Cerri, Esalq-USP, Brazil

Attractive Systems for bioenergy feedstock production in sustainably managed landscapes

Luc Pelkmans, IEA Bioenergy

Payback Time for soil carbon and sugarcane ethanol

Carlos Eduardo Pellegrino Cerri, Esalq-USP, Brazil

Estimating eucalyptus and sugarcane biomass production in Sao Paulo State, Brazil, using a single and integrated process-base model – ECOSMOS AGROIBIS

Michel Anderson Almeida Colmanetti, Unicamp, Brazil

TS 18 – BIOEN Biorefinery

Chair: Flavia Winck, USP-Brazil

Anaerobic NADH-driven polyhydroxybutyrate accumulation in Escherichia coli

Karel Olavarria Gamez, TU DELFT, The Netherlands

Routes for production of sugarcane-based tires: efficient conversion of sugars to 1,3-butadiene precursors techno-economic environmental assessment

Adriano Mariano, UNICAMP, Brazil

A Competitive Platform for enzyme production – Rationally improving RUT-C30 for CAzyme secretion

Mario Tyago Murakami, LNBR-CNPEM, Brazil

Green and sustainable manufacture of nanocellulose materials in integrated biorefineries

Cristiane Sanchez Farinas, EMBRAPA, Brazil

TS 19 – PIPG

Chair: Igor Polikarpov, IFSC-USP, Brazil

CAZymes and their applications in lignocellulosic biomass hydrolysis

Igor Polikarpov, IFSC-USP, Brazil

New technological opportunities of feedstock to improve the sugar and ethanol industry

Pedro Oliva Neto, Unesp-Assis, Brazil

Biochemical characterization of new CAZymes and their roles in biomasses breakdown

Taisa Dinamarco, FFCLRP-USP, Brazil

Understanding the pros and cons of crop residues removal for bioenergy production on soil greenhouse gas emissions

João Luís Nunes Carvalho, LNBR/CNPEM, Brazil

TS 20 – Innovation Office

Chair: Eduardo Giacomazzi, Fiesp, Brazil

University of Sao Paulo Innovation Agency

Marcos N. Martins, NIT USP, Brazil

Inova Unicamp Innovation Agency

Newton Frateschi, NIT, UNICAMP, Brazil

NIT UNESP – AUIN

Saulo Guerra, NIT, Unesp, Brasil

UFSCar Technology Transfer Office

Rafael Aroca, NIT UFSCAR

TS 21 – Lignin, Wood Products and Solid Biofuels

Chair: Saulo Guerra, Unesp

Impact to Dilute Acid Pretreatment Conditions on Corn Stover Lignin Properties and Their Suitability as a Phenol Replacement in Phenol Formaldehyde Resins

David Hodge, University of Michigan, USA

From Renewables to Value-Added Products: Applying Discovery-based Omics of Natural Lignocellulolytic Systems

Fabio Squina, UNISO, Brazil

Raw cassava rhizome waste as a binder in sugarcane bagasse and straw briquettes

Andrea Cressoni De Conti, UNESP, Brazil

Lignins converted to valuable materials in biorefinery concept

Adilson Roberto Gonçalves, Unesp-Rio Claro

TS 22 – Task 45 Innovative Landscape Approaches for Sustainable Bioenergy

Chair: Floor Van Der Hilst, Utrecht University, The Netherlands

Beneficial Land Use Change: Expanded use of perennial crops for bioenergy can reduce land-use impacts in Europe

Goran Berndes, Chalmers University of Technology, Sweden
Oskar Englund, Mid-Sweden University

Supply potential and greenhouse gas footprint of lignocellulosic energy crops and advanced biofuels

Ivan Vera Concha, Utrecht University, The Netherlands

Contribution of sugarcane fertilization and byproduct management to GHG emissions in the context of the Renovabio legislation

Heitor Cantarella, IAC, Brazil

Designing biofuel landscapes for sustainable outcomes

Lorenzo di Lucia, Imperial College, UK

TS 23 – Biogas

Chair: José Valverde, Secretaria de Infraestrutura e Meio Ambiente do Estado de São Paulo

Biogas in Europe and Brazil 2030: Sustainable biogas production. Biogas full integration for Transportation and Industrial utilization including CO₂ for P2X

Jens Bo Holm-Nielsen, Alsborg University, Denmark

Atlas of Bioenergy of São Paulo State: biomass residues potential

Suani Teixeira Coelho, USP, Brazil

Biogas production in sustainable biorefineries: the hub of the bioeconomy

Bruna Moraes, Unicamp

Brazilian Biogas Association

Alessandro Gardemann, Abiogás, Brazil

TS 24 – Climate Effects

Chair: Adriano Bonotto, Advisor, Division for Energy Progress, Ministry of Foreign Affairs, Brazil.

Energy for a sustainable world

Paulo Artaxo, USP, Brazil

Global bioenergy potentials from abandoned cropland

Jan Sandstad Næss, Norwegian University of Science and Technology

Removing CO₂ from atmosphere through biofuels

Joaquim E.A. Seabra, UNICAMP, Brazil

Climate effects of forest-based bioenergy

Annette Cowie, University of New England, Australia

TS 25 – Horizon 2020, BECOOL, BioValue

Chair: Antonio Bonomi, LNBR, Brazil

Biomass production and feedstock diversification for advanced biofuels

Myrsini Christou, CRES, Greece

Design and assessment of optimal logistic chains

Mateus F. Chagas, CNPEM, Brazil

Fast Pyrolysis in Becool

Bert van de Beld, BTG, The Netherlands

Thermochemical routes for advanced biofuels (jet and green diesel) production

Ricardo Reis Soares, UFU, Brazil

Closing session

General Secretaries: Luiziana Ferreira, USP, Brazil

Marcelo Menossi, UNICAMP, Brazil

Clarissa Maria Forecchi Gloria, MRE, Brazil

Luiz Eugênio Mello, FAPESP, Brazil

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Round Table 2 - The Biobased Future and Biorefining

RT-02.01 - Hydrothermal Liquefaction – a key enabling technology for circular valorization of organic residues from agriculture and urban activity to drop-in biofuels

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In recent years, Hydrothermal Liquefaction (HTL) has developed from small scale laboratory research to demonstration scale implementation, due to the very promising conversion effectiveness and sustainability potential shown in continuous pilot plants. For both lignocellulosics (eg sugar cane bagasse) and other types of organic materials, HTL converts ~80 % or more of the incoming energy into a biocrude, which is refinable into synthetic versions of common hydrocarbon fuels: gasoline, diesel, jet fuel and heavy bunker fuel. As a thermochemical process, HTL destroys pathogens and harmful substances present in such residues as for example sewage sludge, and allows the inorganic fraction to be fully isolated. In such cases as the residues contain valuable fertilizer compounds such as phosphates (eg sewage sludge, organic fraction of MSW), this in turn enables full recycling of these to agriculture. Finally, HTL offers an attractive opportunity for carbon negative production (Bio-Energy CCS – BECCS), as a major byproduct is almost pure CO₂ which can be separated and sequestered. Alternatively, it can be used for additional fuel production in a hybrid HTL/Power-to-X implementation. This presentation will discuss state-of-the-art of HTL in the context of process implementation, refining pathways to sustainable drop-in fuels and sectoral integration, using data and examples from current European and Danish research projects.

Keywords: Circular bioeconomy, Drop-in biofuels, Hydrothermal Liquefaction

Supported by: Horizon 2020, Innovation Fund Denmark, Danish Energy Development and Demonstration Program (EUDP)

RT-02.02 - Next Gen (Bio)refinery development for a profitable, low carbon economy.

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Demand for carbon based fuels and materials is expected to remain high and growing for sectors that have limited or no alternatives such as energy carriers for aviation and long haul transport, as well as for commodity materials such as plastics and chemicals. Important sustainability challenges are drastic lowering the nett carbon footprint and reduction of other emissions by circular concepts and process intensification, as well as improvement of the energy efficiency in manufacture and recycling of those. Voluntary commitments (eg ICAO CORSIA agreement for aviation emissions reductions) as well as legal actions (URGENDA induced court orders in The Netherlands and others) increasing guide integral national systems to develop climate agendas, that comply with those but also seek the creation of 'green jobs', impact investments and promote economic growth. Scalable solutions are critical which are tuned to local context of rural/distributed or industrial/metropolitan economies. Biobased solutions can contribute positively to both scenarios, but need to be seen in a regional context of other renewables' generation, storage and recycling: sustainable carbon feedstocks and renewable, mostly electrical, energy (wind and solar). Technology development is key in both including use of Big Data and IoT developments as well as smart and distributed manufacturing. Scalable solutions don't automatically imply large installations, connected precision technology is transforming agriculture, and stem cell technology is disrupting (medical as well as) the food sector. Of specific importance is the recovery, reuse and possible replacement of essential and rare elements: phosphor in agriculture and rare metals in electronics and power storage. A last aspect are systems constraints: sustained biodiversity and soil carbon, as well as societal and community structures, culture and values. Not all techno-economically feasible solutions are acceptable under all conditions. In our contribution, we focus on scalable solutions that appear to still benefit from large scale, integrated manufacturing installations for energy carriers and materials; in our nomenclature 'refineries'. Where the original refinery concept stems from the petrochemical industry, similar thinking applies to hub-structured processing of biobased feedstocks into pulp & paper, sugar and ethanol, potato and corn starch, as well as ores into steel and aluminium or manufacturing construction materials such as cement. In all cases, energy in the form of power, liquid fuels and heat are important (co)products, usually with an excess relative to the refinery's own needs. We consider the different cases in context: (1) nett exporter, rural/distributed regions where the production potential is larger than expected regional consumption. This includes locations such as Limerick in MidWest of Ireland, Delfzijl in the North of The Netherlands, and the Brazilian Center South region . Those are home to specific regional energy intensive industries such as aluminium and cement, with regional energy industries with mixed fossil/renewable feedstocks such as wind, hydrodam and biobased energy. All are (relatively) adjacent to unique ecological zones (Shannon estuary, Waddensee, Mata Atlantica) and a range of agri-forestry industries. (2) The above are (remotely) linked to nett importer, metropolitan/industrial regions such as Randstad (Amsterdam-Rotterdam-The Hague-Utrecht – 10m inhabitants), Dublin (3m inhabitants), or Sao Paulo/Campinas (20m inhabitants). Domestic, transport and (chemical/materials) industry related energy consumption are large in all cases, with significant and growing demand from air travel, long haul and shipping. The sustainable energy, materials and income generation potential of the exporter regions is significantly larger than currently exploited, whereas the future challenges of the importer regions are significant. Both sides will benefit from cross-sectorial plans focused on the specifics of the regions, especially exploring sustainable rural development, job creation potential, impact investment opportunities, and broader environmental and social impacts. This contribution is related to a recently initiated project in The Netherlands by part of authors centred around refinery development for sustainable aviation fuels (SAFs) with ambitions to link to comparable projects in the other jurisdictions.

Keywords: Next Gen Biorefinery, sustainable energy, low carbon economy

Round Table 4 - Industrial Implementation of Energy Cane and Advanced Biofuels

RT-04.03 - Industrial Implementation of Energy Cane

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The first commercial varieties of sugarcane were made through conventional breeding by crossing several species of *Saccharum*. During more than 100 years breeders in different parts of the world selected, from the seedlings coming out of the crosses, plants with high sugar content and low fiber content. More recently, issues concerning global warming brought to great attention biomass (fiber) as the feedstock for energy production due to its renewable feature. Since the early 70's, breeder have been recreating the original steps towards sugarcane variety production only that this time selection was bias towards fiber instead of sugar. Varieties from this line of selection were named as "Energycanes". In Brazil, sugarcane mills are conceived to crush varieties with low fiber content and are optimized to extract much sugar as possible from the fibrous biomass. To process Energycanes, a different industry will be required. At the same extend, agricultural practices will have to be modified to take into consideration new diseases and pests, planting and harvesting machinery, plant nutrition protocols and so forth. As the value of biomass is, in most of the time and situations, directly related to the prices of other conventional energy sources (hydroelectricity, solar, petroleum, etc.), new business models built on biomass feedstock must concentrate efforts in two major concepts, i) high value energy markets and ii) highly valuable products from biomass.

Keywords: sugarcane, biomass, sustainability

Technical Session 01 - Research in Small Business: Biomass, Biofuels and Biorefineries

TS-01.04 - Alcoholic content and increased industrial productivity in fermentations for ethanol production

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The average alcoholic content of industrial fermentations in the country for ethanol production is around 8.0% (v/v) which represents a production of 12.4 litres of vinasse for each liter of ethanol produced. Increasing the alcohol content reduces the volume of vinasse, saves steam in the distillery and reduces water consumption per liter of ethanol produced. However, the increase in productivity in litres of ethanol per week is not always evident due to other factors that affect the industry such as the overall utilization time. In addition, alcohol content may affect other industrial indicators, such as fermentation time and residual sugar in raw wine. On the other hand, the benefits of increasing alcoholic content can be as significant as the savings generated by reducing, transporting and applying vinasse in the field. The objective of this work was to evaluate the relationship between alcohol content and increase in industrial productivity and its impact on other indicators. The evaluation was based on the prospecting and data analysis of Brazilian mills in the last five harvesting seasons and the monitoring of the harvest of mills that increased the alcohol content of fermentations. The results show a significant increase in the alcohol content of fermentations in recent years, which correspond to a significant reduction in vinasse volumes. In one case study, for every 1% more alcohol, the mill produced 950,000 litres more ethanol per week, kept fermentation time below 11 hours and no significant residual sugars in wine (below 0.08% w/v) throughout the harvesting season. These results demonstrate the benefits that can be obtained with fermentations with alcohol contents between 11 and 12% (v/v) when well conducted.

Keywords: fermentation, alcohol content, productivity

Technical Session 02 - Deconstructing Biomass

TS-02.02 - Second generation ethanol: looking beyond the current paradigm

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Research advances are described pertaining to consolidated bioprocessing of lignocellulose using engineered thermophilic anaerobes and milling during fermentation, cotreatment. This approach avoids the two factors most responsible for the high cost of current processing – thermochemical pretreatment and added cellulase – and has documented potential for disruptive cost reductions. Realizing of this potential, however, requires further research-driven advances. A substantial database has been developed documenting the comparative effectiveness of cellulosic biomass deconstruction by various biocatalysts under controlled conditions. *Clostridium thermocellum*, a thermophilic, anaerobic bacterium, is found to be substantially more effective than several other pure cultures as well as a commercial cellulase preparation, and – to our great surprise – as effective in terms of solubilization yield and more effective in terms of rate compared to mixed enrichments. Imitating the cow, cotreatment is explored as an alternative to thermochemical pretreatment in order to enhance the accessibility of cellulosic feedstocks to biological attack. As a proof of concept, fermentation by *C. thermocellum* was carried out in the presence of continuous ball milling. Total carbohydrate solubilization on the order of 90% was achieved for corn stover, switchgrass, and *Populus*. Results will also be presented for cotreatment using disc milling, which is thought to be more practical for industrial application. Commercially viable ethanol yield has been engineered into both C6- and C5- utilizing thermophiles; commercially viable ethanol titer has been engineered into C5-utilizing thermophiles but not C6-utilizing thermophiles. Work will be presented aimed at understanding and ameliorating limitations of current strains relative to two key aspects of robustness: production of high ethanol titers by *C. thermocellum* and fermentation at high solids loading.

Keywords: ethanol, lignocellulose, consolidated bioprocessing

TS-02.04 - A new strategy for successful expression of heterologous D-xylose transporters in *Saccharomyces cerevisiae*

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The major fermentable sugars in lignocellulosic biomass are D-glucose and D xylose, and abundant research has been devoted to improve D-xylose utilization by recombinant *Saccharomyces cerevisiae*. Since limited uptake is one of the bottlenecks for D-xylose fermentation, we aimed to identify novel sugar transporters from D-xylose-fermenting yeasts. An *hxt*-null *S. cerevisiae* strain, lacking the major hexose transporters (*hxt1Δ-hxt7Δ* and *gal2Δ*) but having high xylose reductase, xylitol dehydrogenase and xylulokinase activities, was transformed with plasmids containing a genomic library from *Scheffersomyces stipitis*, or alternatively, putative sugar transporters present in the published genomes of *Spathaspora passalidarum* or *Spathaspora arborariae* were also amplified by PCR and cloned. From the three genes isolated from *S. stipitis* (*XUT1*, *HXT2.6* and *QUP2*), the *Ss-XUT1* permease allowed the best fermentation performance with D-xylose when expressed in *S. cerevisiae*. Curiously, the genomes of *S. passalidarum* and *S. arborariae* had genes (*Sp-XUT1* and *Sa-XUT1*, respectively) highly homologous (>78% identity) to the *Ss-XUT1* gene, but when these genes were expressed in the *hxt*-null strain, the yeast cells could not grow in any of the carbon sources tested, although the cells expressing the *Sp-XUT1* permease clearly had kinetics of ¹⁴C-glucose and ¹⁴C-xylose transport similar to those seen with the *Ss-XUT1* transporter. We explored the possibility that these transporters could be removed from the plasma membrane by ubiquitylation and endocytosis. When we cloned truncated versions of the transporters, removing lysin residues in the C-terminal domain of the protein, the truncated *Sp-XUT1* permease allowed growth and fermentation of D-glucose and D xylose. A *hxt*-null strain deleted in the α -arrestin encoded by the *ROG3* (but not the *ROD1*) gene also allowed growth and sugar consumption by the cloned permeases. Novel sugar transporters are promising target genes for improving lignocellulosic ethanol production by yeasts, and our results indicate that permease stability at the membrane is also important.

Keywords: endocytosis, transporter, xylose

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TS-02.05 - Deletion of N-glycosylation pathway genes in *Aspergillus nidulans* and effects on enzyme production

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The genus *Aspergillus* includes microorganisms that naturally degrade lignocellulosic biomass, secreting large amounts of carbohydrate-active enzymes (CAZymes), which characterizes their saprophytic lifestyle. Among other CAZymes, β -xylosidases are glycosyl hydrolases (GHs) that aid in the degradation of plant biomass by releasing xylose from xylooligosaccharides in the complete degradation of hemicellulose. *Aspergillus* is capable of performing post-translational modifications (PTMs) such as proteolytic cleavage, disulfide bond formation and glycosylation of proteins, providing an additional advantage for the use of such organisms as a host for large-scale protein production. Asparagine-linked protein N-glycosylation is a prevalent PTM in eukaryotic systems. N-glycosylation of proteins is essential for a range of cellular processes such as immune responses, cellular communication, intracellular trafficking, stability, secretion, folding and protein activity. In a previous work *A. nidulans* β -xylosidase AN8401 (BxIB) was used as a model enzyme to study the effect of N-glycosylation on enzyme secretion and functional properties. Seven BxIB N-glycomutants were designed and kinetic analysis showed that completely removing N-glycosylation sites increased the affinity enzyme-substrate, but in a partially N-glycosylated mutant the V_{max} was higher than the wild-type and the completely non-glycosylated mutant. Based on preliminary data, our hypothesis is that the deletion of genes involved in N-glycan assembly in *A. nidulans* can modulate the secretion of a target protein or proteins in general. Thus, genes associated with the N-glycosylation pathway will be deleted in *A. nidulans* recombinant strain overexpressing BxIB. The influence of single-gene deletions will be evaluated regarding fungal growth, total protein secretion, and BxIB secretion.

Keywords: fungal cell factories, enzymes production, N-glycosylation

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Technical Session 04 - Biodiversity Nexus

TS-04.02 - Biodiversity and biomass in agricultural landscapes dominated by biofuels' production

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Biomass is divided into two major components in agricultural landscapes including those dominated by biofuels production: plant biomass produced at the landscape matrix and biodiversity biomass interspersed mostly in nearby conservation areas but also in the landscape matrix. The former is the main reason for the current land use, whereas the later uses agricultural landscapes to live in complement of conservation units like national parks and ecological reserves. The former depends on the wilderness (including their own wild ancestors) to respond by genetic improvement to environmental changes, or they get extinct, whereas the later adapt by natural selection to anthropic pressures, or also get extinct. The former depends on the wilderness (including biodiversity) to be sustainable, whereas the later depends on agricultural landscapes to survive. Although traditionally treated as a conflict, their relationship is, therefore, based on interdependency. Such concept imposes technological and societal adaptation including the implementation of land-sharing multifunctional agricultural landscapes and wildlife friendly agricultural techniques. Conceptually, the main challenge for it is the inevitable discrepancy between value and costs concerning biofuels and biodiversity biomass. The former has local yearly costs whereas the later has a global millennial value.

Keywords: Biodiversity , biomass , biofuels

TS-04.03 - The ecology of sugarcane fields

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The debate regarding the environmental benefits of a large-scale adoption of biofuels has heavily emphasized a reduction in GHG emissions, neglecting other relevant forms of environmental change such as biodiversity loss. Our research programme aims at understanding the ecological consequences of land use intensification by combining sampling surveys of algae, predatory insects, fish and amphibians in freshwater ecosystems in cerrados, pastures and sugarcane fields with experimentation in multiple scales ranging from lab to mesocosm to experimental plots to field. Biodiversity in sugarcane fields is periodically subject to both physical disturbances (soil preparation, planting, and harvesting) and to physical (harsher microclimates) and chemical stressors (fertilizers, vinasse and pesticides). Not surprisingly, then, sugarcane fields are impoverished in biodiversity. Nearly half of the species of amphibians found in native habitats were deleted from sugarcane landscapes; importantly, however, amphibian and insect faunas did not differ between sugarcane fields and pastures. Indeed, sugarcane fields appear to function as habitat for a fraction of sturdy, generalized species. Measures of freshwater primary production, morphometry of field-caught individuals, and the experimental manipulation of detritus as a resource indicate that generalized amphibian larvae may have positive performance in sugarcane fields. At the same time, vinasse and some pesticides, even when used according to technical recommendations, may elicit adverse, lethal and sublethal effects. The recovery of disturbed communities can be fast, depending on proximity to other waterbodies that can function as sources of colonists in the landscape. Whereas better land management practices can improve the quality of sugarcane fields as habitat for generalized species and as matrix for the dispersal of more sensitive species, biodiversity conservation in sugarcane dominated landscapes necessarily depends on the protection, restoration and management of nearby native habitats. Only then can sugarcane fulfill its claim as a promoter of the environmental sustainability of biofuels for transportation. Keywords: land management, biodiversity, conservation Funding: FAPESP

Keywords: land management, biodiversity, conservation

Supported by: FAPESP

Technical Session 05 - Crop Biotech

TS-05.01 - Engineering bioenergy crops for improved conversion and environmental resilience

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Biomass consists largely of complex polysaccharides and lignin of plant cell walls. Lignin is the major cause of biomass recalcitrance and is difficult to convert into valuable intermediates. The most abundant hemicellulose in biomass is xylan, which is composed of xylose, arabinose, glucuronic acid and acetate esters as the main constituents. Pentoses such as xylose and arabinose are more difficult to convert into biofuels and bioproducts in a cost-efficient manner than hexoses such as the glucose originating from cellulose. Therefore, we have devised strategies to develop plants with a higher ratio of C6 to C5 sugars, and a low content of lignin in their cell walls. An efficient way of reducing xylan relies on dominant negative mutations in the xylan synthase that results in inhibition of a xylan synthase complex. Because the inhibition is dominant, it can be easily transferred to a variety of bioenergy crops. Likewise, we have used several methods based on dominant gene constructs to reduce or alter lignin composition. One of the most successful approaches has been the expression of QsuB, a dehydroshikimate dehydratase from *Corynebacterium glutamicum*, which results in decreased lignin and altered lignin composition. The resulting plants are indistinguishable from the wild type under normal growth conditions. However, Arabidopsis plant expressing QsuB have been shown to exhibit substantial drought tolerance. The Joint BioEnergy Institute focuses on switchgrass (*Panicum virgatum* L.), sorghum and poplar as potential bioenergy feedstocks. We have transferred several low lignin and improved saccharification traits to these three crop species, and conducted field trials with switchgrass and sorghum. The plants grown in the field showed changes in biomass composition in general agreement with previous results. Surprisingly, the plants expressing QsuB had higher biomass yield than control plants.

Keywords: biomass, cell walls, biotechnology

Supported by: US Department of Energy

TS-05.02 - New biotechnology traits for sugarcane

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The barrier established by interspecific reproductive isolations has always been limited to the available genetic basis for plant breeding. Only the natural allelic variations within the gene pool and the random variations induced by radiation or chemical mutagenesis have been available to plant breeders for the development of more productive and commercial varieties adapted for different environments. However, rapid advances in the field of genetics have enabled the sequencing of entire genomes within hours, allowing for the exploration of the entire genetic diversity of a given species and the identification of new metabolic routes and genes in species never before studied for agricultural, medicinal, and industrial use. The advent of genetic engineering opened the possibility to overcome sexual incompatibility of living organisms, even across different kingdoms. More recently, new breeding techniques (NBTs), such as CRISPR systems, are emerging rapidly from advances in genomic research, for application in crop improvement. They enable accurate, targeted and reliable changes in the genome (and therefore, are different from genetically modified organisms (GMOs), previously produced) and have significant potential for sustainable intensification of agriculture and food security when used as part of the deployment of all available approaches and building on existing good agronomic practice. Thus, the development of these new techniques to use at the farm level is progressing and the use of these approaches to the development of improved sugarcane varieties is greatly desired. Embrapa Agroenergy has several GM sugarcane events being tested under field conditions for drought tolerance, biomass accumulation, improved sucrose content, insect-pests and herbicide tolerance. However, in 2018, Embrapa, to promote the use of NBTs in crops, launched a competitive call which our group, in partnership with other Embrapa Centers, approved a big project to develop soybean, sugarcane, maize and common bean cultivars with edited genome via Non Homologous Ending Joining (NHEJ) and Homologous Direct Repair (HDR), depending of the strategy used for each specific target gene/crop. Strategically, it can represent a great opportunity to Embrapa develop more effectively superior varieties/cultivars with high productivity and added value to the market.

Keywords: *Saccharum* spp., NBTs, genetic engineering

TS-05.03 - Dynamic control of secondary metabolism in plants

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Plants and other photosynthetic organisms provide a means towards sustainable life on Earth when used as bio-based production systems for food, materials, energy and more. To realize their full potential, efficient plant-based production systems will require engineering of novel traits, including the design and introduction of new biosynthetic and metabolic pathways that are capable of dynamic responses to changes in their environment and/or endogenous conditions. These novel traits are made possible by the introduction of complex synthetic biological circuits that, much like their electronic counterparts, enable decision-making and production of specific responses in the organism's cells and tissues. Biological circuits with predictable and robust function are built from quantitatively defined genetic components and the aid of computational tools. We are developing genetic circuits with a variety of functions, including sensing, information processing and actuation. Sensing can be engineered from naturally occurring transcription factors whose activity is modulated by a ligand, or from synthetic ligand-binding proteins. Information processing circuits and modules use Boolean logic gates, which can be constructed from synthetic protein-protein interaction domains. We have also developed on-off genetic "toggle" switches and feedback loops to precisely control bio-production. We are applying these circuits to rewire secondary metabolism in plants, specifically the production of useful compounds derived from the phenylpropanoid pathway. The next generation of transgenic crops will contain synthetic gene networks with decision-making properties and the ability to reshape patterns of plant metabolism and growth, for applications that range from food production to security and bio-manufacturing.

Keywords: genetic circuits, biosensors, secondary metabolism, synthetic biology

Supported by: DARPA – U.S. Dept. of Defense, UNT

TS-05.04 - Development of energy cane varieties as biomass source for second generation industry

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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. Unfortunately, this fossil energy source promoted 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. Among the biomass crops, energy cane has one the biggest yield potential and additionally requires less energy input, bringing marginal lands into production with its all social-benefit consequences. In order to obtain commercial energy cane varieties, Granbio initiated in 2012 a conventional breeding program at Alagoas State, that received an addition of a transgenic pipeline in 2015. The purpose was to obtain high yield varieties adapted to poor and/or dry environments and with low production costs, for being used as biomass source for second generation industry in the country. Seven years after, the breeding program obtained nine commercial varieties, a population of promising clones under final evaluation and a group transgenic events under selection in the field, with potential to increase yield and/or saccharification and, also, conferring resistance to pests and herbicides. Commercial energy cane varieties are producing 2,0 times more yield than conventional sugarcane, 20% more sugars in juice and 3,5 times more fiber, with is two times more fiber yield than eucalyptus, for instance. These first varieties had validated the yield potential of energy cane and production costs are showing that it is possible to produce biomass with prices lower than USD\$ 25,00 per dry tone, with is imperative for the viability of second generation industry.

Keywords: biomass, biofuels, biochemicals

Technical Session 06 - Bioen Biofuels

TS-06.02 - Multi-scale observations of mineral impurities in sugarcane bagasse and straw

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Sugarcane bagasse and straw are vast, inexpensive renewable feedstocks for bioenergy and biorefining. However, these feedstocks have high contents of mineral impurities that cause problems such as severe wear out of industrial equipment, impeding the utilization of the full potential of the biomass. In this work we provide a multiscale vision of the nature of the mineral impurities associated with sugarcane biomass. We will discuss the experience of biomass cleaning for pilot-scale operations as well as micro-scale observations of mineral particles impregnated into the biomass structure. The micro-scale observations used a combination of synchrotron X-ray microtomography and X-ray microfluorescence to provide new insights on the nature of the mineral found in bagasse and straw. We will discuss (1) the extreme case of macroscopic rocks and metal pieces observed in pilot plant operations; (2) the size of mineral particles (circa 5-27 μm) observed to have higher affinity with the biomass; (3) the contribution of water to keep larger mineral particles adhered to the biomass; (4) the presence of soil aggregates at the surface of sugarcane straw, with no equivalent found in bagasse; and (5) the relations between biomass contamination and soil mineralogy. With this multiscale vision, we provide a comprehensive framework to rationalize the high variability of mineral impurities in agroindustrial residues such as sugarcane bagasse and straw.

Keywords: micro-tomography, mineral impurity, sugarcane

Supported by: FAPESP, CNPq and CAPES

TS-06.03 - Chemical and structural assessment of the acid hydrolysis of cassava (*Manihot esculenta*) bagasse: power ultrasound versus mechanical agitation

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Cassava processing leads to the generation of high amounts of wastes in Brazil, such as the wet bagasse. Cassava bagasse (CB) has been considered as a potential raw material for bioethanol production mainly due to its high content of residual starch and fibrous matter. The pretreatment with phosphoric acid is highlighted by being a mild and effective method to enhance the posterior conversion of both starch and lignocellulose. In fact, this work aimed at studying the acid hydrolysis of CB with the assistance of emerging technologies, as high-intensity ultrasound (US), or conventional methods, as mechanical agitation (AG). The kinetics of reducing and total sugar release were differentiated according to the sugar dissolution in aqueous medium and actual acid hydrolysis to be, then, mathematically modeled. The acoustic field produced by US and the viscous properties of the suspensions were measured at the beginning and after 60 min of acid hydrolysis. Additionally, microscopic analyses (bright field, fluorescence and polarized light) were performed to evaluate changes produced by the treatments. Both AG and US-assisted treatments were able to hydrolyze cassava bagasse. However, US-assisted ones seemed to reach the equilibrium in the reducing sugar release process earlier with slight higher values for total sugars released. The Naik model fitted the experimental data with good accuracy and the fitting parameters were in agreement with the observations. A greater loss in the birefringence of the starch granules and the degradation of lignocellulosic matter was also observed in US-assisted hydrolysis. The actual acoustic power applied was reduced by 50% after hydrolysis, probably due to the increase of 48% in the apparent viscosity of the resulting sonicated suspensions. Therefore, the US application may be used in place of conventional technologies to enhance the accessibility to starch and hemicellulose/cellulose from CB for further enzymatic hydrolysis and sugar fermentation.

Keywords: pretreatment, acoustic field, birefringence

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Technical Session 08 - Biodiesel, Biokerosene, Bio-oils

TS-08.02 - Catalytic Pyrolysis of Saccharum Munja(Biomass) in a Fixed Bed Reactor

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Introduction: Saccharum Munja Roxb. has different synonyms and is locally called Munja, Sara, sarkanda and kana. It is a tall perennial grass growing in compact culms with a solid pith. The flowering season of this plant is from October to December in Pakistan, Afghanistan and India where it grows profusely, chiefly in the regions of Jammu and Kashmir, and Punjab. It is mostly found on roadsides, rivers and canal banks. In this unique study, saccharum Munja (a biomass) is converted to bio-oil through catalytic pyrolysis in a fixed bed reactor. Objectives: Focus of this research work was to check the outcome of catalyst to biomass ratio on the yield of bio-oil, to study the effect of feed particle size and temperature on bio oil yield. Materials and Methods: Saccharum Munja in a fixed Bed Reactor is pyrolyzed & nitrogen gas is used to create inert atmosphere. The effects of particle size (mesh sizes 40, 50, 60, 70, 80), temperature (460°C, 485°C, 510°C) & catalyst to Biomass ratio are analysed. Copper impregnated on HZSM-5 (Si/Al = 300, 370m²/g, Nankai catalyst Organization, Tianjin, China) are examined by XRD. 7 g of feed is used with different particle sizes and pyrolysis reactor is operated at Nitrogen flow rate of 0.25 liter/min for different reaction temperatures, water temperature (coolant) at 12°C & reaction time 30 min respectively. Results, Discussion and Conclusions: The optimum reaction conditions investigated for the mass ratio of CuSO₄/HZSM-5 to be 0.4 and the catalyst/biomass mass ratio to be 0.3. Bio-oil Yields is optimized with 40 mesh size at 460°C. An elaborated study of wide range of particle sizes indicated that the percentage yield increases with decrease in particle sizes (Percentage yield was maximum for 40 mesh size at 460°C). Similarly, for different catalysts to Biomass Ratio (10% to 50%) percentage yield was found to be optimum for 30% ratio at 460°C. Cu is impregnated on ZSM-5 Catalyst (ZEOLITE based catalyst) and confirmed by XRD analysis. GCMS of Bio-Oil is carried out as well, which revealed that bio-Oil contained aromatic hydrocarbon, aliphatic hydrocarbon along with other compounds like phenolic, alcoholic etc. Significantly phenol, 2-methoxy- (12.40%), cis-9-hexadecenal (8.62%), Cyclopentanone (6.30%), Phenol, 4-ethyl-2-methoxy-(3.96%), n-hexadecanoic acid (3.63%), etc. It is inferred that it is feasible to acquire furfuryl liquor from a sustainable source. Various unit processes can be utilized to produce targeted chemicals from these bio-based chemicals.

Keywords: Pyrolysis, Catalyst, Bio-based Chemicals

Supported by: University of the Punjab

TS-08.04 - Physiology and lipidomics of *Saccharomyces cerevisiae* at extremely low oxygen availability in the absence of ergosterol and unsaturated fatty acids

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We sought to investigate how far the growth of *Saccharomyces cerevisiae* under full anaerobiosis is dependent on the widely used anaerobic growth factors (AGF) ergosterol and oleic acid. A continuous cultivation setup was employed and, even forcing ultrapure N₂ gas through an O₂ trap upstream of the bioreactor, neither cells from *S. cerevisiae* CEN.PK113-7D (a lab strain) nor from PE-2 (an industrial strain) washed out after an aerobic-to-anaerobic switch in the absence of AGF. *S. cerevisiae* PE-2 seemed to cope better than the laboratory strain with this extremely low O₂ availability, since it presented higher biomass yield, lower specific rates of glucose consumption and CO₂ formation, and higher survival at low pH. Lipid (fatty acid and sterol) composition dramatically altered when cells were grown anaerobically without AGF: saturated fatty acid, squalene and lanosterol contents increased, when compared to either cells grown aerobically or anaerobically with AGF. We have also investigated how the anaerobic growth factors Tween 80[®] and ergosterol, and anaerobicity itself, influence yeast lipidomics. Results showed that during aerobic steady-state cultures the most abundant lipid classes were the phospholipids phosphatidylcholine and phosphatidylinositol (PC and PI), and the hydrolysates lysophosphatidylcholine and lysophosphatidylethanolamine (lysoPC and lysoPE). On the other hand, in cells collected from steady-state cultures under anaerobiosis +AGF, ceramides and cardiolipins (Cer and CL) were the most abundant compounds. In addition, in cells cultivated under the anaerobiosis -AGF condition, ceramide was the most abundant class, but cardiolipin was hardly detected. In cells collected from the three conditions tested, PI was detected in similar amounts. This study, to our knowledge, is the first one exploring yeast lipidomics in different conditions of oxygen availability, in continuous cultivations. We concluded that these lipid alterations negatively affect cell viability during exposure to low pH or high ethanol titers.

Keywords: Yeast, Lipids, Ethanol

Supported by: FAPESP and CAPES

Technical Session 09 - Synbio for biomass, biofuels and bioproducts

TS-09.01 - Production of Platform Chemicals in Bioenergy Crops: Stacking Low-Recalcitrance Traits with Co-Products

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Muconic acid (MA) is used for the production of important chemicals such as adipic acid, terephthalic acid, and caprolactam. 2-Pyrone-4,6-dicarboxylic acid (PDC) is a promising building block chemical used to make diverse biodegradable polyesters with novel functionalities. There is no chemical synthesis method currently available for manufacturing PDC, whereas synthesis of MA utilizes petroleum-derived chemicals. Therefore, the development of alternative strategies for bio-based production of MA and PDC has garnered significant interest. Plants represent advantageous hosts for engineered metabolic pathways towards the production of chemicals. We demonstrate that plants can be used for the bio-manufacturing of MA and PDC by re-routing intermediates of the shikimate pathway within chloroplasts. In particular, expression of bacterial 3-dehydroshikimate dehydratase (QsuB) in plastids results in concomitant reductions of lignin and accumulation of protocatechuate (PCA) in biomass. Additional engineering strategies are currently designed to enhance PCA titers and enable its conversion into MA and PDC in-planta. Specifically, bacterial feedback-insensitive 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase was overexpressed to increase carbon flux through the shikimate pathway, co-expression of PCA decarboxylase with catechol 1,2-dioxygenase allowed MA production, and co-expression of PCA 4,5-dioxygenase with 4-carboxy-2-hydroxymuconate-6-semialdehyde dehydrogenase enabled PDC synthesis. The implementation in bioenergy crops (switchgrass, poplar, and sorghum) of MA and PDC biosynthetic routes that divert phenylpropanoid pathway intermediates away from lignin biosynthesis will be presented. These engineering approaches combine in plant biomass the production of value-added chemicals with low-recalcitrance traits towards sustainable development of biorefineries.

Keywords: biomass, co-products, lignin

Supported by: U.S. Department of Energy

TS-09.02 - Post genomics resources for sugarcane molecular breeding

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Among bioenergy crops, sugarcane stands out as the world's leading biomass crop with vast potential to mitigate climate change without affecting food security. Opportunities to accelerate breeding progress and enrich the knowledge of the fundamental biology of this important crop drive efforts to explore and dissect its complex genome using different genomic tools and to develop a high-quality reference genome. After over a decade of multiple parallel genome sequencing initiatives, multiple references are now available: (i) a mosaic monoploid genome reference of the modern cultivar R570, consisted of 382 Mb of high-quality sequence in 3,965 contigs, organized as a single tiling path, representing the single copy sugarcane gene space, and includes 25,316 predicted protein-coding gene models; (ii) an allele-defined genome reference of a tetraploid *S. spontaneum* genotype AP85-441, including 32 pseudo-chromosomes (2.9 Gbp) comprising 8 homologous groups of 4 members each, bearing 35,525 genes; and (vi) the gene space of the Brazilian modern cultivar SP80-3280, which includes the complete sequence of 373,869 genes and their upstream regions. This is the largest genomic data set available for the sugarcane researchers' community and includes putative homo(eo)logs (mostly 2-5 copies). These resources will facilitate the determination of biological processes, such as carbon partitioning, and support translation of research findings into improved and sustainable agricultural technologies, towards closing the gap between current (~84 ton/ha) and potential (~381 ton/ha) yield. Taking advantage of multiple reference genomes, comparative genomic approaches and transcriptomic data is imperative to unravel the transcriptional landscape of sugarcane. We developed a database and datamining tools for this end. Current efforts include integrating physiology, regulatory elements, genomic, transcriptomic and metabolomic data from sugarcane towards a systems biology approach. Keywords: Reference genome, Gene space, Gene models, Funding: FAPESP Proc. 2014/50921-8

Keywords: Genome, Transcriptome, Metabolome

Supported by: FAPESP

TS-09.03 - Identification of novel regulators of lipids biosynthesis in the microalgae *Chlamydomonas reinhardtii* through multi-OMICS approach

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The current scenario of climate change and exceeding levels of atmospheric CO₂ can be circumvented using renewable bioenergy sources. The microalgae biomass and bioproducts may be introduced in substitution of fossil-derived products and it has been proposed as one possible solution for current environmental problems related to climate change. However, the optimization of biomass and lipids production is necessary. Many unicellular microalgae species are capable to synthesize Triacylglycerols under environmental stress conditions, such as nutrient deprivation and excess salt concentrations. However, the underlying mechanisms that control such phenotype are not fully understood. The analysis of different layers of biological information through OMICS approaches can contribute to a better description and elucidation of the intricate set of molecules and mechanisms that coordinate such complex phenotype. The OMICS data analysis can reveal the identity of molecular targets and characteristics of the cellular responses, particularly those related to the control and regulation of carbon and lipids accumulation. Therefore, we performed a multi-OMICS, time-resolved approach, to investigate the different aspects of the cellular responses in the microalgae *Chlamydomonas reinhardtii* that contribute to the enhancement of Triacylglycerols biosynthesis. The multi-OMICS data of cells under nitrogen deprivation revealed the identity of novel candidate genes, proteins and metabolites that may modulate the TAGs accumulation in *C. reinhardtii*. Our results indicate that glycolytic pathway and glutathione, porphyrin and chlorophyll metabolism are affected, indicating increased respiration and a potential redox unbalance in the cells during nitrogen deprivation. The metabolic stimulus of lipids accumulation by selected amino acids has also revealed novel regulators of the lipid's biosynthesis pathway. Moreover, we developed a multi-OMICS data platform which contains data mining and visualization options. The platform is available through our recently released platform Phycomine, that can be accessed through the URL <https://phycomine.iq.usp.br/>.

Keywords: amino acids, microalgae, lipids

Supported by: FAPESP

TS-09.04 - Sugarcane Rhythms in the Field

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Introduction Sugarcane is a high-density crop in which individuals shade each other as they develop, which generates distinct microenvironments in different parts of the field. **Objectives** We hypothesized that microenvironment rhythms due to shading evoke changes in metabolic and transcriptional rhythms. **Materials and Methods** Sugarcane leaves were harvested every 2 h for 26 h in 4-months and 9-months old plants. Transcriptional rhythms were measured by using Agilent custom oligo arrays or RT-qPCR. Metabolic rhythms were measured using a Agilent 7890 gas chromatograph coupled to a Leco Pegasus 2 time-of-flight mass spectrometer. **Results and Discussion** Transcription levels of *ScLHY*, a circadian clock gene, peak near dawn in 4-months old sugarcane. However, *ScLHY* peak is later in 9-months old plants. These phase changes can also be seen in metabolite rhythms and in the transcriptional levels of other genes. We hypothesized that these phase changes are evoked by microenvironment changes due to shading. We tested this hypothesis by building a wooden wall in the field and testing the phase of circadian clock genes. **Conclusions** We suggest that the microenvironments caused by shading lead the plant circadian clock to detect dawn hours later than the sunrise.

Key Words: sugarcane, rhythms, circadian clock

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TS-09.05 - Employing systems biology to dig deep into secondary cell walls in C4 grasses

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In the context of the bioeconomy, grasses emerge as prominent lignocellulosic feedstocks due to their high yield potential for biomass production. However, grasses differ considerably from eudicots in vascular patterning and cell wall composition, suggesting the presence of many grass-specific molecular and biochemical mechanisms that are not found in eudicots and whose knowledge cannot be extrapolated from data obtained with eudicot model plants. Here, we report on the establishment and characterization of two model systems to facilitate the study on SCW deposition in C4 grasses. First, cell wall analyses, phenolic profiling and RNAseq were performed along an elongating sorghum internode, which represents a powerful system to study SCW deposition because it is formed by different developmental zones with increasing amounts of SCW. Along the elongating internode, lignin and cellulose amounts increased from bottom to top and major metabolic shifts in the phenolic metabolism were observed. In addition, RNAseq revealed clusters of genes with similar expression patterns along the developmental gradient, allowing the identification of genes with so far unknown function that are co-expressed with lignin biosynthetic genes. These genes are top candidates to also play a role in lignification in grasses. Second, a xylogenic culture was developed for sugarcane, in which suspension cells transdifferentiate into tracheary elements *in vitro*. Confocal microscopy analyses revealed two patterns of SCW deposition in the induced tracheary elements, pitted and reticulate, which are also naturally found during sugarcane stem development. The amount of SCW components increased significantly within a period of 4 weeks in treated cells compared to the control, revealing an active process of transdifferentiation. In order to identify global changes in gene expression during tracheary elements formation, RNAseq analyses were performed after 0h, 12h, 48h and 1 week of treatment, whereas the same time points were used for non-induced cells as control. Differentially expressed genes were clustered according to their expression patterns and gene ontology analysis was performed to identify the enriched functional categories in each cluster. Genes involved in vascular development, secondary cell wall deposition and programmed cell death were differentially expressed upon treatment. Co-expression analysis using Weighted Correlation Network Analysis method further allowed the identification of candidate genes potentially involved in these target processes. The obtained knowledge might be very helpful for future biotechnological strategies to reduce biomass recalcitrance in bioenergy crops, such as sugarcane.

Keywords: secondary cell walls, lignin, C4 grasses, systems biology, metabolomics, transcriptomics

Supported by: FAPESP

Technical Session 10 - BIOEN Sustainability

TS-10.01 - Analysis of Scientific Cooperation in BIOEN Program with Bipartite Networks Approach

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Analysis of Scientific Cooperation in BIOEN Program with Bipartite Networks Approach Silveira, José Maria F.J. .1; Junior, Jaim da Silva.2; Souza, Roney F.3Julio César de Camargo Miranda4
1Institute of Economics, University of Campinas, São Paulo, Brazil; 2 Agribusiness Department, University of Brasilia, DF, Brazil;3 Department of Economics, Federal University of Mato Grosso, Mato Grosso, Brazil; 4Julio Cesar de Camargo Miranda, Federal Univeristy of Mato Grosso, Brazil. Bioenergy is a science-based sector showing an increasing intertwining of scientific areas of knowledge (Bueno et Al. 2018; Salles Filho et Al. 2018). Cooperation is one of the critical factors for technology development and innovation. Although Souza et Al. (2018) have shown that in bioenergy only 3.6 years are required to double the number of scientific publications, some commercial setbacks like those occurred in second-generation ethanol, contributes to even undermine research efforts, generating discontinuities that can jeopardize the future of relevant problems of the economy, from waste treatment to the unfolding of technological frontiers. Scientometric studies carried by Souza et al. (2015) and Bueno et al (2018) highlighted the prominent position of Brazilian Research in Sugarcane in Networks based on WOS data. The present paper investigates the contribution of the BIOEN program (2008-2018) to consolidate the Brazilian position in bioenergy international scenery. Bipartite Networks of scientific combined with appropriate blockmodeling methodologies showed three main results: the central role of Bioen institutions in sugarcane bioenergy field, the existence of a persistent international cooperation network comprising 22 countries, and of a regional network also guided by Bioen organizations scientific priorities. Summing up, Bioen program has shown its important role to guarantee the continuity of sugarcane bioenergy research, contributing to spurs technology solutions that are becoming innovation to energy sector in Brazil. |Keywords: sugarcane, scientific cooperation, bipartite networks, blockmodeling Funding: FAPESP.

Keywords: Sugarcane, Bipartite Networks, Blockmodeling

Supported by: FAPESP

TS-10.02 - Dynamics and resilience of soil mycobiome under organic (vinasse) and inorganic disturbances in sugarcane fields

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Disturbances in soil can cause short-term soil changes, consequently changes in microbial community what may result in long-lasting ecological effects. Here, we evaluate how multiple pulse disturbances effect the dynamics and resilience of fungal community, and the co-occurrence of fungal and bacterial communities in a 389 days field experiment. We used soil under sugarcane cultivation as soil ecosystem model, and organic (Vinasse - byproduct of the sugarcane biofuel industry) and inorganic amendments (organic residue applied 30 days before or together with mineral N fertilizer) as disturbances. Application of organic residue alone as a single disturbance or 30 days prior to a second disturbance with mineral nitrogen resulted in similar changes in the fungal community. The simultaneous application of organic and mineral N as a single pulse disturbance had the greatest impact on the fungal community. Organic amendment increased the abundance of saprotrophs, fungal species capable of denitrification, and fungi with copiotrophic and oligotrophic lifestyles. Furthermore, the changes in the fungal community were not correlated with the changes in the bacterial community. The fungal community was neither resistant nor resilient to organic and inorganic disturbances over the one-year sampling period. Our findings provide insights on the immediate and delayed responses of the fungal community over one year to disturbance by organic and inorganic amendments.

Keywords: Fungal ecology, Sustainability, Microbial community

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TS-10.03 - Evaluating hydrological trade-offs due to different land covers and land uses as fundamental input for the payment for ecosystem services

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Farmland expansion in the Brazilian Cerrado, considered one of the largest agricultural frontiers in the world, has the potential to alter water fluxes on different spatial scales. Despite some large-scale studies being developed, there are still few investigations in experimental sites in this region. Here, we investigate the water balance components in experimental plots and the groundwater table fluctuation in different land covers: wooded Cerrado, sugarcane, pasture and bare soil. Furthermore, we identify possible water balance trade-offs due to the different land covers. Thus, we provide informations to the assessment of land covers in the context of the payment for ecosystem services. This study is being developed since 2012 in the central region of the state of São Paulo in southern Brazil. Hydrometeorological variables, groundwater table, surface runoff and other water balance components were monitored inside experimental plots containing different land covers; the datasets were analyzed using statistical parameters; and the water balance components uncertainties were computed. Replacing wooded Cerrado by pastureland and sugarcane shifts the overland flow (up to 42 mm yr⁻¹) and the water balance residual (up to 504 mm yr⁻¹) and may affect groundwater table behavior. This fact suggests significant changes in the water partitioning in a transient land cover and land use (LCLU) system, as the evapotranspiration is lower (up to 719 mm yr⁻¹) in agricultural land covers than in the undisturbed Cerrado. We recommend long-term observations for continuing the evaluations initiated in this study, mainly because there are few basic studies on tropical environments at the hillslope scale and more assessments are needed for a better understanding of the real field conditions. Such efforts should be made to reduce uncertainties, validate the water balance hypothesis and catch the variability of hydrological processes. A detailed understanding of the physical processes associated to the intensive use of land for meeting the water-energy-food nexus demand is of paramount importance in the context of sustainability. Farmers, stakeholders, and decision-makers need to understand the value and importance of watershed services through a straightforward cost-effective analysis of conserving and/or protecting them. Economic feasibility affects the volunteer enrolment in payment for ecosystem services (PES) programmes for adopting soil conservation practices in rural areas. In this context, it is fundamental understanding how uncertainties in the input data, economic models structure, and parameters estimation are integrated in a consistent way in hydro-economic models. By this, we will assess different hydro-economic scenarios of water availability to understand uncertainties and hydrological trade-offs.

Keywords: Cerrado, experimental plots, runoff

Technical Session 11 - Biofuels application in Engines and Fuel Cells

TS-11.01 - Sugar Cane residues to Bio-Crude

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The Burdekin region harvests around 8.5 million tonnes of cane each year. Agronomic issues require that around 1.65 million tonnes of cane tops and trash is burnt prior to harvesting. Biocrude equivalent, if all the tops and trash in the Burdekin was processed, is around 350 megalitres per year. Issues that are being addressed include: - End-of-field separation of tops and trash from mechanically harvested sugar cane is a key step forward to beneficially utilise tops and trash whilst retaining the existing logistics supply chain for the delivery of billets to the sugar mill. - The reduction of contaminants such as chlorine and potash in tops and trash to acceptable levels is necessary to produce a commercial cellulosic product such as pellets or biocrude. - To maximise biocrude production, the handling of bulky tops and trash into a suitable feedstock utilising Licella's Hydro Thermal CatHTR technology. Licella is proposing to install a Demonstration Plant producing 20,000 tonnes per year of biocrude (125,000 barrels per year) from 95,000 field tonnes of tops and trash. A program to assess and address these issues is underway.

Keywords: biomass, biocrude, agri-residue

Supported by: Queensland Government, Burdekin Renewable Fuels, Licella Holdings

TS-11.03 - Niobium Enhances Electrocatalytic Pd Activity in Alkaline Direct Ethanol and Glycerol Fuel Cells

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PdxNby/C binary electrocatalysts supported on Printex 6L carbon black were prepared. The electrocatalysts were characterized by using SEM, TEM, XRD, EDS, ICP-MS, water contact angle and Raman spectroscopy. The best performance for the alkaline direct glycerol fuel cell (ADGFC) was obtained by using Pd1Nb1/C, which yielded a maximum power density of 27 mW cm⁻² at 70 °C while the best performance for the alkaline direct ethanol fuel cell (ADEFEC) was obtained with a using Pd1Nb1/C with a maximum power density of 27 mW cm⁻² at 60 °C. Residues of products formed from the glycerol and ethanol oxidation were analyzed by using FT-Raman and high-performance liquid chromatography (HPLC). The Pd1Nb1/C electrocatalyst exhibited a more negative onset potential for the CO oxidation reaction and a higher conversion rate of both glycerol to carbonate and ethanol to carbonate, respectively. We suggest that the higher hydrophilicity, higher degree of disorder, and bifunctional effects enhanced the electrocatalytic activity of Pd, as Nb facilitated the oxidation of adsorbed CO in Pd and increased the transfer rate of electron from Pd to carbon black support. For the reasons pointed out above, Nb is a promising co-catalyst for Pd-based electrocatalysts for ADGFCs and ADEFECs.

Keywords: biomass, energy, alcohol fuel cells

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Technical Session 12 - Bioproducts from Biomass

TS-12.04 - Valorization of the cellulosic and hemicellulosic fractions of wheat straw through microbial production of lipids and carotenoids

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The production of lipids by oleaginous yeasts is expected to become a sustainable and significant source of biodiesel in the future. In addition, some oleaginous yeasts can accumulate carotenoids simultaneously with lipids. Carotenoids are valuable natural pigments, and normally act as vitamin A precursors. In this study, six oleaginous yeasts, including *Rhodotorula glutinis*, *Rhodospiridium toruloides*, *Saitoella coloradoensis*, *Lipomyces starkeyi* and 2 different strains of *Yarrowia lipolytica* were tested in glucose or xylose media with carbon to nitrogen ratios ranging from 25 to 100 in order to identify the best lipid and carotenoid producers. *R. toruloides* NRRL Y-1091 and *L. starkeyi* NRRL Y-1389 produced the highest amount of carotenoids and lipids, respectively. These yeasts were then cultivated in cellulosic and hemicellulosic hydrolysates obtained from wheat straw and their performances were compared. The highest concentration of lipids, 3.99 ± 0.35 g/L, was obtained by cultivating *L. starkeyi* in washed cellulosic hydrolysate; while the highest production of carotenoids, 24.58 ± 1.88 mg/L, was obtained by cultivating *R. toruloides* in decolorized cellulosic hydrolysate. An efficient utilization of both cellulosic and hemicellulosic hydrolysates was achieved when they were submitted to detoxification with activated charcoal, revealing that inhibitors have an important influence in the bioconversion process. It was demonstrated that lowering inhibitory levels improves the sustainable processing of biomass for lipids and carotenoids production. In this sense, the choice of the detoxification method is a relevant aspect to take into account in order to avoid extra costs for the overall process.

Keywords: fermentation, lignocellulosic biomass, oleaginous yeast

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TS-12.05 - Investigating the potential of non-conventional yeasts for application in lignocellulosic biorefineries

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Non-conventional yeasts or non-Saccharomyces yeasts have interesting characteristics that could be useful for application in lignocellulosic biorefineries, such as ability to metabolize complex substrates as well as to support high osmolarity, toxic compounds, and high temperature. In addition, such yeasts are able to produce others compounds besides ethanol, including value-added chemicals like sugar alcohols, organic acids and esters, which can be interesting options for incorporation in biorefineries. Even though some commercial-scale biorefineries have been implemented worldwide, technical improvements are still required to maximize the sugar conversion yields and profitability. In this study, two wild-type non-conventional yeasts, namely *Hansenula polymorpha* CBS 4732 and *Debaryomyces hansenii* CBS 767, were cultivated in lignocellulosic hydrolysates with the aim of exploiting their potential for application in biorefineries. Briefly, the yeasts were cultivated in wheat straw cellulosic and hemicellulosic hydrolysates and their sugar conversion performance and inhibitor resistance were evaluated. The results revealed that both yeasts present characteristics of interest for application in the fermentation of lignocellulosic hydrolysates. *H. polymorpha* was able to growth at 37 °C, showed highlighted resistance to toxic compounds found in hemicellulosic hydrolysates, and could convert xylose into ethanol and/or xylitol as main products. On the other hand, *D. hansenii* presented good performance in cellulosic hydrolysate and was able to produce high amount of arabitol from glucose. Fermentation variables, especially aeration and substrate concentration, are key points to be optimized for both yeasts in order to intensify the product formation with the minimum necessary cell growth. These results open up new opportunities for the development of new biorefinery models based on the use of non-conventional yeasts for bioprocesses.

Keywords: bioprocess, biorefinery, non-conventional yeasts

Supported by: Novo Nordisk Foundation (Denmark, grant number: NNF10CC1016517)

Technical Session 13 - Bioen Biomass

TS-13.03 - Papaya sugarcane: using endogenous molecular and biochemical mechanisms to “soften” biomass for bioethanol production

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Besides being a source of sugar, sugarcane is one of the primary sources of ethanol. The expansion of sugarcane for biofuel production is possible without effects on food production or biodiversity. To achieve this goal, we need to use not only the free sugars but also the cell wall (CW) carbohydrates. A large number of hydrolases from microorganisms are now available. Combined into enzyme cocktails, they are used in industry but still represent an expensive step in the process. Another limitation is the process of biomass pretreatment, which is used to make CWs available to hydrolysis. To bypass these barriers, we have been trying to find ways to endogenously “soften” the CWs. After the determination of the chemical composition of sugarcane CWs, we found that the development of the gas spaces in sugarcane roots (aerenchyma) includes an endogenous CW modification system that controls wall degradation. We think that it may be possible to “install” parts of this system in the whole plant to ease pretreatment and gain control of hydrolysis. We assembled a collection of CW-related genes of sugarcane belonging to the classes of transcription factors, miRNAs, and hydrolases. Together, these mechanisms became part of a strategy to turn sugarcane CWs more amenable to hydrolysis. We are now using integrative biology to reengineer a sugarcane variety capable of softening its own CWs in a similar fashion as fruits such as papaya do during ripening. We nicknamed it “papaya sugarcane project.” <https://www.botany.one/2019/07/how-to-break-down-cell-walls-in-sugarcane/>

Keywords: sugarcane, hydrolysis, cell walls

TS-13.04 - Post genomics resources for sugarcane molecular breeding

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In the tropical region, nitrogen plays an important role to increase sugarcane yield. As the cultivation cycle is long, the crop goes through periods of intense rainfall and periods of drought. Nitrogen fertilization can have reduced efficiency due to losses in soils before the plants take it up. Ammonia (NH₃) volatilization is one important N loss mechanism mainly when urea, which is the main nitrogen fertilizer in Brazil, is employed. The thick layer of harvest residue (straw) usually increases NH₃ volatilization losses; therefore, the search for ways to reduce such losses is important. Field experiments were conducted during the rainy season, in traditional sugarcane cultivation areas in Sao Paulo state, Brazil, to compare NH₃ losses when using conventional urea, urea treated with a urease inhibitor (NBPT: thiophosphate of N-n-butyl triamide) or urea plus superphosphate and potassium chloride coated with micronutrient powder. NBPT-coated urea reduced N losses due to volatilization by 6% compared to non-coated urea. Urea plus P and K coated with micronutrients also caused a reduction of NH₃ losses. N losses from conventional urea were 18 kg/ha (12% of N added in fertilization) but dropped to 12 kg/ha and 9 kg/ha, respectively, with urea coated with NBPT and urea coated with micronutrient powder. NPK fertilization increased sugarcane yield, as expected. Highest yields were found for the NBPT-coated urea treatment (135,5 ton/ha) compared to the control treatment (96,6 ton/ha and the conventional urea (116,5 ton/ha). The economic return was highest with urea coated with NBPT (30% higher than that of the unfertilized control – without NPK – and 15% higher than that of the conventional urea). Straw biomass was mineralized very quickly within the 1-year season: The straw also contributed some N to the sugarcane. Total straw biomass was reduced by 85 to 95% whereas 16% to 30% of N in the straw was mineralized in this period, depending on the amount of straw that remained over the soil surface. It can be concluded that fertilizer formulations that reduce NH₃ losses are viable options to increase sugarcane yields.

Keywords: biomass, N volatilization, urease inhibitor

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TS-13.05 - Evaluation of the antagonistic effect of volatile organic compounds on sugarcane pathogens

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Sugarcane is an important agricultural crop for Brazil due to its use in bioenergy production and, more recently, it has become the foremost candidate for biomaterial and bio-based chemical productions. However, its productivity may be affected by several diseases caused by microorganisms, such as the fungi *Sporisorium scitamineum* and *Ceratocystis paradoxa* which cause smut and pineapple diseases in sugarcane, respectively. To treat these diseases, we need to use fungicides, but these agrochemicals can cause several negative impacts on the environment. As a sustainable alternative, some microorganisms can produce volatile organic compounds (VOCs), small signaling molecules that inhibit phytopathogen growth and can protect crops against diseases. The use of biocontrol based on microbial VOCs for sugarcane diseases is promising, since it does not depend on the direct contact and plant colonization, making them ideal molecules for mediating short- and long-distance organism interactions. Thus, our main objective is the identification and use of bacterial VOCs to inhibit the growth of these sugarcane pathogens. An additional aim is to understand how these volatiles can inhibit these phytopathogens. Using co-cultivation assays, we identified 2 bacterial strains able to inhibit *S. scitamineum* growth by 100%. These strains also inhibited two types of haploid sporidia (yeast-like cells) that are formed in the *S. scitamineum* cycle life. We also identified 3 bacterial strains able to inhibit *C. paradoxa* by almost 95%. The genera of all strains were identified by 16S rRNA sequencing (these results will not be shown). The volatilomes of these strains were analyzed using a Headspace-Solid-Phase Microextraction and Gas Chromatography-Mass Spectrometry platform and more than 30 VOCs were identified. We evaluated some VOCs using synthetic compounds in different concentrations (1 M to 10 nM) and the pentadecane was confirmed as a small molecule able to inhibit the growth of both phytopathogens. To expand our knowledge about the molecular mechanisms behind fungal growth inhibition by VOCs, we performed RNA-seq experiments and the data are being processed (this analysis will be concluded until January). This is the first study with VOCs and sugarcane pathogens, and it can have positive impacts on the Brazilian sugar-energy sector, increasing sugarcane health and productivity, reducing production costs and protecting the environment.

Keywords: Volatile organic compound, Sugarcane, Biological control

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Technical Session 14 - IEA Bioenergy Task 39 – Commercialization of Biofuels

TS-14.03 - Life Cycle Analysis of Biofuel Production Pathways with the GREET Model

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Since 1994, Argonne has been developing the GREET model for life-cycle analysis (LCA) of vehicle technologies and energy systems. At present, there are more than 40,000 registered GREET users worldwide including government agencies, automotive companies, energy companies, universities, and other institutions. Biofuel production pathways are an important group of the energy systems in the GREET model. Argonne has been expanding GREET to include new biofuel pathways and to examine emerging LCA issues. LCA of a typical biofuel pathway in GREET includes production of farming inputs (e.g., fertilizers, diesel fuels, etc.), farming of biofuel feedstocks, feedstock processing and transportation, biofuel production, biofuel transportation, and biofuel combustion. Critical LCA issues addressed in GREET include LCA system boundary, co-product methodologies, indirect effects such as land use change, among others. For individual biofuel pathways, GREET generates LCA results for energy and environmental impacts (e.g., petroleum use, greenhouse gas emissions, criteria air pollutant emissions, and water consumption). This presentation will cover energy and GHG results of key biofuel pathways such as corn ethanol, sugarcane ethanol, and cellulosic ethanol; and how key LCA issues affect biofuel LCA results.

Keywords: biofuels, life cycle analysis, greenhouse gases

Technical Session 16- Land Use for Biomass Production

TS-16.04 - Estimating eucalyptus and sugarcane biomass production in São Paulo State, Brazil, using a single and integrated process-based model - AGROIBIS

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Sugarcane and eucalyptus plantations are the most representative crops in the state of São Paulo, Brazil. They cover approximately 8 million hectares in the wealthiest Brazilian state, playing an important role in the biomass production, energy supply and above and belowground carbon stock. Understanding of the factors driving sugarcane and eucalyptus plantations productivity across São Paulo state can support agricultural planning by public agencies and guide the investments conducted by private corporations. AGRO-IBIS is an integrated process-based model that has a specific submodels dedicated to sugarcane and eucalyptus that are able to estimate their productivity from small to large scales. This study included three sequential steps: (1) model parameterization, calibration and validation using data from intensively monitored sites or inventories datasets; (2) model validation at regional scale using sets of parameters which were considered to be constant in space and/or time based on data from local experiments, as well as other sets of parameters which could vary spatially; and (3) mapping the productivity of both crops in São Paulo state, for contrasting climates and soil properties. Carbon fluxes of both crop plantations were simulated at the regional scale, and their inter-annual and spatial variability was analyzed. Biomass production showed the importance of the environmental variability, i.e., climate and soil, on plant growth and production over successive years. The previous good performances of the model in the validation steps for sugarcane and recently for eucalyptus ($R^2 > 0.8$ and $RMSE < 30$) allows running the model for mapping purposes. The information provided by the AGRO-IBIS model on the multiannual quantification of carbon fluxes and biomass production at large scale improved our understanding of the performance of these crops in São Paulo state and will be useful to predict biomass productions in response to climate change.

Keywords: biomass, eucalyptus, sugarcane

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Technical Session 18- BIOEN Biorefinary

TS-18.01 - Anaerobic NADH-driven polyhydroxybutyrate accumulation in *Escherichia coli*

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Introduction: Many current bio-based processes rely on aerobic cultivations, implying significant energy input as well as loss of reducing power. In the case of polyhydroxybutyrate (PHB) accumulation, the use of the classical NADPH-consuming pathway implies a mismatch between the NADH produced in the glycolytic pathways and the NADPH required for the PHB synthesis. Moreover, biomass formation and product pathway compete for NADPH. **Objectives:** To solve the problem of the competition between biomass and product formation, we looked for a NADH-preferring acetoacetyl-CoA reductase (AAR) and verify its engagement in PHB formation. **Methods:** Here we isolate and characterize the AAR from *Candidatus Accumulibacter phosphatis*. Moreover, an artificial operon joining the *phaCA* genes from *Cupriavidus necator* with the *phaB* gene encoding for this AAR was constructed, establishing a complete PHB synthesis pathway. **Results and Discussion:** The purified enzyme showed the highest NADH preference so far reported among the studied homologues. Based on the comparison of the amino acid sequence of this enzyme and other homologues, we hypothesized that the negatively charged residues E37 and D39 should be the structural base for the discrimination against NADPH in this enzyme. Accumulation of polyhydroxybutyrate (PHB) under oxygen limiting conditions was observed after the heterologous expression of the constructed operon in *Escherichia coli*, indicating the engagement of this AAR in PHB synthesis. Finally, the PHB accumulation was increased after the suppression of potentially competing pathways and the reduction of dissolved oxygen in the culture. **Conclusions:** Taken together, these results show that it is possible to accumulate PHB in *E. coli* using a NADH-driven anaerobic process, opening opportunities for further metabolic pathway engineering aiming higher PHB titers.

Keywords: Anaerobic fermentation, Polyhydroxybutyrate, *Escherichia coli*

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TS-18.02 - Routes for production of sugarcane-based tires: efficient conversion of sugars to 1,3-butadiene precursors and techno-economic-environmental assessment

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Changes in the petrochemical industry have resulted in decreasing production of four-carbon olefins and, consequently, it has affected the supply of 1,3-butadiene to the rubber tires industry. For this reason, chemical companies have been searching for renewable sources of butadiene precursors, thereby creating a potential market for sugarcane companies. However, it is not obvious which technological route can give sugarcane and chemical companies the best win-win solution. The route based on the conversion of first-generation ethanol (1G) to butadiene can accelerate the penetration of sugarcane in the tires value chain because this option does not require investments from sugarcane companies. On the other hand, for sugarcane companies planning to produce second-generation ethanol (2G) from sugarcane bagasse, the conversion of hemicelluloses from the bagasse into n-butanol or 2,3-butanediol (other possible routes) may be more interesting. The bagasse-based routes can boost the economic feasibility of 2G ethanol biofuel while offering chemical companies better buying price and yields. Nevertheless, fermentative conversion of the hemicellulose hydrolysate faces technical challenges. The hydrolysate is dilute (~20 g/L xylose) and biomass pretreatment-derived microbial inhibitors prevent the efficient conversion of xylose. Therefore, our research aims at increasing the conversion efficiency of sugarcane hemicellulose hydrolysate (non-detoxified) into n-butanol and 2,3-butanediol. We are developing a fermentation strategy that features three aspects: mixing with molasses, fed-batch operation, and cell immobilization using the sugarcane bagasse as cell carrier. Moreover, we are assessing the techno-economic and environmental (carbon footprint) potential of producing 1,3-butadiene from butanol, butanediol, and 1G ethanol. At BBEST-Biofuture 2020 we will be presenting the advances in our research, which ultimately aims to accelerate the penetration of sugarcane in the tires value chain in a way that is economic and environmentally attractive for sugarcane and chemical companies.

Keywords: Biorefinery, sugarcane bagasse, rubber tire

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TS-18.04 - Green and sustainable manufacture of nanocellulose materials in integrated biorefineries

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The sustainable use of renewable resources for the development of a low carbon economy involving the production of bioenergy, chemicals, materials and other bio-based products stands as the foundation of the so-called emerging bioeconomy. However, in order to meet the challenges and demands of this bio-based economy it is critical to focus on the development and innovation of the industrial processes involved in these production chains. For that, the use of lignocellulosic biomass as feedstock in biorefineries is being considered as the most attractive option in the transition from a fossil-based to a bio-based economy. A sustainable biorefinery industry will require markets that combine large volumes of commodities with high value-added products, such as nanocellulose materials. Nanocellulose in the form of cellulose nanofibrils (CNF) or cellulose nanocrystals (CNC) presents remarkable properties and a wide range of applications in the medical, electronic, packaging, and agricultural sectors. The production of nanocellulose has already been demonstrated on an industrial scale, but the conventional processes use the chemical route via acid hydrolysis. Therefore, the development of processes using more environmentally friendly routes, such as enzymatic hydrolysis, is necessary. Here, we report on the feasibility of using biomass degrading enzymes to obtain nanocellulose materials using sugarcane lignocellulosic biomass as feedstock. The cellulose nanomaterials obtained presented high crystallinity index (~70%), thermal stability (degradation onset temperatures higher than 300 °C) and dimensions in the ranges expected for this type of material. These findings indicate the potential of using the enzymatic route as a platform to obtain nanocellulose as a value-added product from sugarcane lignocellulosic biomass. In addition to being eco-friendly, this process also releases a stream rich in soluble sugars that can be used to produce biofuels or other bioproduct, thus expanding the portfolio of products and markets for the biorefinery.

Keywords: nanocellulose, enzymes, biorefinery

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Technical Session 19- PIPG

TS-19.01 - CAZymes and their applications in lignocellulosic biomass hydrolysis

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Currently Brazil is the second biggest ethanol producer in the world. The country has well-developed agriculture and generates large amounts of agroindustrial residues that can be used and are being used for second generation bioethanol (2GE) production. Lignocellulosic biomass conversion into 2GE, green chemicals and renewable materials holds promises of more sustainable and environmentally friendly economy. Modern 2GE technologies involve several technological steps, including pretreatment, enzymatic hydrolysis, fermentation and distillation. Pretreatments that modify physical structure and chemical composition of biomass are essential for enzymatic hydrolysis of plant biomass, whereas optimized composition of activities in industrial enzymatic cocktails are crucial for efficient transformation of the pretreated biomass in 2GE and nanocellulose, having significant impacts on cost-effectiveness of the whole process. Our current understanding of synthesis and enzymatic degradation of complex carbohydrates are at least incomplete. Less than 3.8% of enzymes and auxiliary proteins involved in synthesis and degradation of carbohydrates are currently characterized biochemically and less than 0.42% of them are characterized structurally (Lombard et al., Nucl. Acids Res. (2014) 42: D490 - D495). Therefore, systematic structural and functional study of the enzymes with potential in depolymerization and, also synthesis of polysaccharides, aiming to understand their specific mechanisms of action is crucial [1-4]. Here we will present some of our results on CAZymes structural and biochemical analysis, investigations of changes in lignocellulosic biomass structure and composition in the process of pretreatment and discuss available infrastructure and know-how. References: 1. Mello, B.L. et al. "Targeted metatranscriptomics of compost-derived consortia reveals a GH11 exerting an unusual exo-1,4- β -xylanase activity" *Biotechnology for Biofuels* (2017) 10:254 (doi: 10.1186/s13068-017-0944-4) 2. Alessi, A. et al. "Revealing the insoluble metasecretome of lignocellulose-degrading microbial communities" *Scientific Reports* (2017) 7: 2356 (doi:10.1038/s41598-017-02506-5) 3. Kadowaki, M.A.S. et al. "Biochemical and structural insights into a thermostable cellobiohydrolase from *Myceliophthora thermophila*" *FEBS J.* (2018) 285 (3): 559–579 (doi: 10.1111/febs.14356) 4. Godoy, A.S. et al. "Structure, computational and biochemical analysis of PcCel45A endoglucanase from *Phanerochaete chrysosporium* and catalytic mechanisms of GH45 subfamily C members" *Scientific Reports* (2018) 8:3678 (doi:10.1038/s41598-018-21798-9)

Keywords: biomass, enzymes, biofuels

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Technical Session 21 - Lignin, Wood Products and Solid Biofuels

TS-21.01 - Impact of Dilute Acid Pretreatment Conditions on Corn Stover Lignin Properties and their Suitability as a Phenol Replacement in Phenol Formaldehyde Resins

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Lignin derived from a biorefinery utilizing dilute acid pretreatment of corn stover was recently demonstrated to be suitable as a 100% replacement for phenol in phenol formaldehyde (PF) resins used as an adhesive in engineered wood products applications. In this work, we will build on these findings and investigate the how processing during pretreatment and recovery/purification of lignin from the hydrolysis residue (“lignin cake”) impact the suitability of these lignins as a phenol replacement. As the lignin cakes may be comprised of

Keywords: Pretreatment, Lignin, Wood adhesive

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TS-21.04 - Lignins Converted to Valuable Materials in a Biorefinery Concept

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Lignin is a byproduct from the pulping of wood and is also obtained in high amounts from the processing of lignocellulosics to obtain advanced biofuels. These lignins can be converted in high value added products in the concept of biorefinery. Kraft eucalyptus and sugarcane bagasse lignins were oxidized in mild conditions, evaluating the kinetics of the process. The obtained materials were applied to formulations with citric acid as a model for controlled release of herbicides, showing a rate dependence on the nature of the lignin. Oxidized lignins were tested as heavy metal scavengers using copper ions. As a third technical application, lignin was mixed with pigments to verify the photodecay reduction in paint formulations.

Keywords: lignin biorefinery, lignocellulosic chemistry, sugarcane bagasse

Supported by: FAPESP

Technical Session 22 - Task 45 Innovative Landscape Approaches for Sustainable Bioenergy

TS-22.03 - Contribution of sugarcane fertilization and byproduct management to GHG emissions in the context of the Renovabio legislation

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Nitrogen (N) fertilizers are relevant components of greenhouse gases (GHG) emissions for ethanol production so that the sustainability of sugarcane biofuels may be strongly affected by N use. Data of 13 studies (89 observations) in which GHG emissions were measured in the field as affected by N fertilizer types, use of vinasse and filter cake, as well as nitrification inhibitors were analyzed to derive regional specific N₂O emission factors for sugarcane production. How these factors affect the total GHG of a typical biorefinery producing 338.8 ML ethanol per year was modeled using life cycle analysis in the Virtual Sugarcane Biorefinery framework to calculate options to decrease the ethanol GHG emissions. Using the IPCC Tier 1 default values, the estimated total GHG emission of the biorefinery was 188 Gg CO₂ eq. When the regional emission factors based on local data replaced the IPCC default values, the GHG emissions were 155 Gg CO₂ eq. Emissions further decreased to 152 and 150 Gg CO₂ eq when nitrification inhibitors were added to the N fertilizers and when the time of vinasse application was adjusted to avoid its interaction with N fertilizers, respectively. These fertilization options permitted to reduce GHG emissions by 33 to 38 Gg CO₂ eq per year for a typical biorefinery. According to the new Brazilian Renovabio legislation, one Decarbonization Certificate (CBIO) will be granted per Mg CO₂ eq avoided emission. Therefore, the adoption of Tier 2 N₂O emission factors for N fertilizers, nitrification inhibitors, and better vinasse management options would generate GHG savings equivalent to between 33,000 and 38,000 CBIO more than those obtained when using Tier 1 default values. The price of the CBIO will determine whether changes in management practices will pay off.

Keywords: Greenhouse gases, Renovabio, Fertilizer

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TS-22.04 - Designing biofuel landscapes for sustainable outcomes

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The design of socio-ecological landscapes has become a key concept in current sustainable development thinking to advance multifunctionality and sustainable outcomes. However, its application in the practice of landscape management has proved difficult. In our work we combined ideas of landscape ecology with methods for collaborative decision-making to develop an approach for the practical application of landscape design to bioenergy projects. The approach consists of five components: (a) system definition, with special attention to boundaries and scales, (b) knowledge co-development, (c) stakeholder analysis and participation throughout the process, (d) collaborative decision-making and (e) monitoring. Here we illustrate the application of the approach employing a case study of a commercial project for large-scale production of cellulosic ethanol from dedicated crops in Sardinia, Italy. The analysis revealed an overall positive effect of the biofuel project in the biomass catchment area, with only marginal negative impacts outside the area. Positive synergies include a reduction of climate change emissions, improved biodiversity in terms of habitat quality and connectivity, and generation of employment and additional income for farmers. Potential trade-offs affect, primarily, food and animal feed production and water availability. Overall, the results suggest that the landscape design approach has the potential to guide the planning of complex biofuel projects advancing sustainable outcomes. In view of negative perception and local opposition to biofuel production, this research confirms that successful implementation of biofuel projects could be facilitated if pre-emptive landscape design processes were employed to guide the planning of projects.

Keywords: land use, decision making, ecosystem services

Technical Session 23 - Biogas

TS-23.01 - Biogas in the European context. Sustainable production, potentials, technologies. Biogas full integration for Transportation fuels. Denmark as case example for Biorefinery platforms.

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Biomass of any kind for biogas and bioenergy purposes is biodegradable products, wastes and residues of biological origin from agriculture, forestry - Terrestrial and Aquatic biomasses. Future concerns are to evaluate what sources are sustainable biomasses for the energy and transport sectors. Biomass as an integrated and important part of renewable energy has the advantage that it can easily be stored, transported and utilized with a flexible load and multiple applications at the place and time of energy and products needed. This makes the biomass unique among renewable energy sources. Biogas can without harming the nature and food/feed sectors grow from today's 18-20 mill. TOE (2019) to 3-4 time's higher production in 2030 in EU. Biogas from sustainable lignocellulosic and manure based biomasses need pre-treatment. This has to be low cost and robust processes. Case examples of integration of low cost biomasses from the agricultural, farming and food sectors will be documented and results gained from R&D&D will be highlighted. The utilization of large-scale production of biogas has to be flexible and dynamic integrated in the rapid growing Renewable Energy sectors in Northern Europe. Biogas is creating the backbone for the transfer towards full supply of the societies by wind, solar and bioenergy. The storage and flexible utilization of biogas integrated in the Natural gas grid is outstanding. This contains a key role in the transition and disruption situation shifting from fossils towards 100 pct. renewables. Biogas utilization in CHP-Combined Heat & Power or biogas upgraded for biomethan to natural gas quality and transported to metropole areas for bus and/or truck transportation fuel will be documented and demonstrated; finally new projects for utilizing the CO₂ in the context of BECCU will be highlighted. Biography: 30 Years of experience in the field of Biomass Feedstock production, Biorefinery concepts and Biogas pro-duction. Board member of R, D & D committees of cross-governmental body of biogas developments 1993-2009, Denmark. Secretary and/or chair of NGO biogas and bioenergy organizations. Experience of a variety of EU projects, Organizer of international conferences, workshops and training programs in EU, Canada, China, Brazil, India, Iran, Russia, Ukraine among others. Research: Managing research, development and demonstration programs in Integrated agriculture, environment and energy systems. Fulfilled biomass and bioenergy R & D projects. Main focus in biofuels, biogas and biomass Resources. EDU and Supervising M.Sc. and Ph.D. students in these research fields. Training programmes: International courses, training programmes and supervision for Ph.D. students and academic staff, governmental bodies and experts in bioenergy systems. Full biography can be found at www.aau.dk – profile search Jens Bo Holm-Nielsen

Keywords: Biomass resource potential, Biogas Technology, Lignocellulosic biomass

TS-23.02 - Biogas and bio-methane potential in São Paulo State

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This study presents the potential of biogas and bio-methane production in the state of São Paulo, from different sources (urban waste, animals and the sugar and alcohol sector), for each municipality, through a georeferenced and interactive map. The results obtained and their potential contribution to a more sustainable energy matrix in the state are presented, as well as a preliminary economic analysis of bio-methane insertion in the grid. The environmental benefits to the state are also evaluated, with the potential reduction of GHG emissions. The results are significant considering the 2017 base year: (i) The annual bio-methane potential could exceed in 3.87 billion Nm³ of the annual volume of natural gas traded; or (ii) The annual volume of bio-methane produced could replace 72.4% of diesel commercialized; (iii) The potential for electricity generated annually from biogas, 36,197 GWh, corresponds to 93% of residential consumption in the state. In addition, the results obtained for the economic analysis indicate an important feasibility for injection into the NG network. However, the most relevant aspects refer to the positive environmental and social impacts. In addition to the possibility of replacing fossil fuels, there is important synergy with respect to basic sanitation. Energy utilization of waste contributes to prevent inappropriate disposal, providing better health conditions and inclusion, especially in low-income populations without adequate sanitation conditions. Another social aspect contemplated would be the creation of jobs and income for the communities around the biogas and bio-methane. This research, developed by GBIO / IEE / USP, is part of Project 27 - “The prospects for bio-methane's contribution to increasing natural gas supply” at the Research Center for Gas Innovation (RCGI) from USP, financed by FAPESP and SHELL.

Keywords: biogas, bio-methane, energy

Supported by: FAPESP and SHELL

Technical Session 25- Horizon 2020, BECOOL, Bio Value

TS-25.01 - Lignocellulosic low-ILUC biomass feedstock options for advanced biofuels: Recent research developments

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This paper will report work on the biomass production and diversification strategies applied in the BECOOL project “Brazil-EU Cooperation for Development of Advanced Lignocellulosic Biofuels” (<https://www.becoolproject.eu/>). The main objective of the BECOOL (EU) and BioVALUE (Brazil) projects is to strengthen EU-Brazil cooperation on advanced lignocellulosic biofuels. As far as biomass is concerned, the BECOOL project aim to increase the lignocellulosic biomass production and feedstock diversification without reducing food crop land for the production of advanced biofuels. Lignocellulosic biomass feedstock options refer to i) agricultural/forest residues, ii) annual lignocellulosic crops grown as intermediates in crop rotation schemes and ii) growing perennial lignocellulosic crops. Selected feedstocks will be processed through gasification and pyrolysis technologies for the production of advanced biofuels. Biomass assessment includes information on biomass quantities, availability, relevant analytical characteristics, existing harvesting and logistic options. The inventory covers Southern EU regions, whereas relative work from Brazil is also included. In addition to field residues, dedicated annual lignocellulosic crops (sunn hemp, fibre sorghum, kenaf and hemp) are being grown in innovative cropping systems with traditional food crops (wheat, maize) in South Europe. Results so far indicate that wheat grain yields were not affected by the precedent crops in all rotations and environments. On the contrary, biomass yields increased when these energy/industrial crops were included in the rotation scheme. The rotation maize-biomass sorghum-wheat-sunn hemp lead to the highest biomass yields in Italy and Greece, whereas in Spain the most productive one was the rotation maize-kenaf-wheat-sunn hemp. The lowest biomass yields were recorded in the conventional maize –fallow –wheat rotation in all environments. Dedicated perennial lignocellulosic herbaceous crops, such as miscanthus, switchgrass, giant reed and tall wheatgrass are also being cultivated in existing stands of the crops under marginal conditions. Historical and new data from the annual harvests are collected so as to estimate long term yields, which will be reported and discussed.

Keywords: energy crops, crop rotations, perennial grasses

TS-25.03 - The development of biobased products from fast pyrolysis oil

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Biomass is a valuable, sustainable feedstock for the production of chemicals and materials, and will play an important role in the transition towards a Sustainable Process Industry. Bio-based products – products wholly or partly derived from materials of biological origin – can make the society more sustainable and lower its dependence on fossil fuels. For the optimal utilization of bio-resources, fractionation on the basis of functionalities is required. Most commonly, biomass is separated into its main constituents lignin, cellulose and hemi-cellulose by steam or acid treatment. Subsequently, each of the fractions are then further processed for specific applications. An alternative biorefinery approach combines a short thermal treatment at elevated temperature (fast pyrolysis) followed by a low temperature fractionation of the mineral free, liquid product (FPBO) that keeps the key chemical functionalities intact in separate, liquid, depolymerized fractions ('pyrolytic fractions'). These fractions consist of components derived from the de-polymerization of cellulose, hemicellulose and lignin. Both the fractionation of FPBO and the use of the fractions in bio-based products is further developed in the EU-funded project called Bio4Products. The project will demonstrate how a variety of biomasses can be exploited, creating renewable and natural alternatives to petrochemical products. The application of the pyrolytic fractions will be demonstrated in a number of end products such as modified wood, moulding & foundry resins, and insulation foams. Exploratory research on both the fractionation and the applications has been carried on bench-scale using pyrolysis oil derived from different types of biomass. Results were very positive and larger quantities of pyrolytic fractions are required for further product development & demonstration. A dedicated fractionation unit with a throughput capacity of 3 ton FPBO/day has been designed, constructed and commissioned. The presentation will show this development of the fast pyrolysis based bio-refinery with a special emphasis on the realisation and operation of the fractionation pilot-plant. Furthermore, the use of the pyrolytic fractions in some selected applications will be discussed.

Keywords: fast pyrolysis, fractionation, biobased products

Supported by: H2020 - Europe

Webinar Live 1: Biomass quantification and Sustainability Governance: How to achieve consensus

WL-01.3 - Sustainability indicators of the Global Bioenergy Partnership (GBEP) to monitor and enhance bioenergy sustainability while contributing to climate targets

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Modern bioenergy production and use can have a number of positive effects, e.g. in terms of agricultural and rural development, increased energy diversity and climate change mitigation. However, bioenergy sustainability depends on the local context and on the types of production and use practices. In order to assess the environmental, social and economic sustainability of bioenergy, the Global Bioenergy Partnership (GBEP) has developed a set of 24 relevant, practical, science-based, voluntary sustainability indicators for bioenergy, which have been implemented in many countries. These indicators and the respective methodologies, which address the production and use of all forms of bioenergy, are intended to guide any analysis of bioenergy undertaken at the domestic level with a view to inform decision making and facilitate the sustainable development of bioenergy. The uniqueness of these indicators lies in the fact that they are the result of an international consensus process amongst approximately 80 governments and international organizations and in the fact that the emphasis is on providing measurements useful for informing national-level policy analysis and development. Measured over time, the indicators will show progress towards or away from a sustainable development path as determined nationally. The Partnership has recently finalized an Implementation Guide on the use of the GBEP Sustainability Indicators for Bioenergy, to further improve their practicality and related guidance for users.

Keywords: biomass, biofuels, sustainability, indicators, international initiative

Webinar Live 4- Emerging Markets and New Frontiers

WL-04.01 – Development of Bio-Energy and Other Sustainable Energy Options in Agro-industries of Sub-Saharan Africa

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This paper reviews the potential of agro-industries to make a substantial contribution to sub-Saharan Africa's sustainable energy supply as well as widen access to clean energy in the rural areas.. Agro-industries already represent a major source of rural employment and constitute a significant contributor to the economy of many sub-Saharan African countries as well as provide a major source of income for millions of small scale farming out-growers. The paper argues that agro-industries have the financial and technical capabilities to overcome many of the common problems that bedevil new small and medium scale rural clean energy enterprises. Sugar, tea and horticulture agro-industries already utilise bio-energy and other clean energy options for their processing, and in some cases, supply clean energy for crop processing, rural clinics and schools for their employees within their estates. While some agro-industries already sell energy products including bio-electricity and ethanol to national markets, agro-industries in sub-Saharan Africa could also provide cleaner energy services to the local community surrounding their estate boundaries. However, the current status of clean energy development in agro-industries is not well covered in literature nor are the major barriers to the scaled-up involvement of agro-industries in sustainable energy development in sub-Saharan Africa well understood. Relying on a wide range of case examples, this paper will review the extent to which agro-industries have contributed to expanding the deployment of clean energy in the region as well as proposed options for promoting bio-energy and other sustainable energy options in sub-Saharan Africa's agro-industrial sector.

Keywords: Sustainable, agro-energy, Africa

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A - Biomass, Agronomy, Breeding, Other Energy Crops, Biotechnological Aspects of Plants Feedstocks

A.01 - Gene Silencing using Artificial miRNAs in sugarcane

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Loss-of-function mutants are useful tools to investigate the role of genes. In polyploid species, such as sugarcane, gene redundancy offers an extra obstacle for genetic analysis due to the difficulty of obtaining loss-of-function alleles. RNA interference (RNAi) is largely used for producing loss-of-function mutants, it presents some disadvantages, and the potential off-targets may be a major problem. The artificial microRNA technique (amiRNA) is an improved method for RNA silencing, presenting high specificity, reducing the issue with off-targets. In this work we present the potential use of amiRNA for sugarcane gene silencing. We developed a database of sugarcane assembled sequences that can be used to search the best amiRNA sequences and to evaluate the putative off-targets. We used the gene encoding the phytoene desaturase enzyme to design an amiRNA. An expression cassette using the maize ubiquitin promoter was used to overexpress the amiRNA, using an intron from rice to form the hairpin. Transgenic sugarcane plants overexpressing the amiRNA presented the expected phenotype of loss of chlorophyll. A varying degree of silencing was observed among independent events, as expected, and some events with severe loss of chlorophyll were also obtained. We believe this tool will be useful for the community working with functional genomics in sugarcane. Keywords: silencing, amiRNA, sugarcane Funding: FAPESP, CNPq and CAPES

Keywords: sugarcane, miRNA, silencing

Supported by: CAPES

A.02 - Agave multiomics: assessing the genetics of high productivity biomass plants that thrive in semiarid regions

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Plants from the genus *Agave* are studied because of their drought resistance mechanisms and for their bioenergy production potential. They are also used to produce alcoholic beverages and fibers. The cultivars most used to produce fibers are *Agave sisalana*, hybrid 11648 and *A. fourcroydes*. Brazil is the main producer and exporter of sisal fiber in the world and the production is done at the Brazilian Northeast semiarid region, employing around 500 thousand people on the production chain. This crop is essential for this region development, however, there are not many published works about Agave genetics and no genome available for any Agave species. We are working with transcriptomics, genomics and genotyping data in order to understand more about the genetics of these impressive plants. We are going to assemble draft genomes of the three fiber-producing cultivars, and we will use it to guide a genotyping experiment in the Bahia semiarid, aiming on building molecular markers for increased yield and disease resistance selection. Also, we have assembled the transcriptomes from leaf, stem and root from the same three cultivars collected from a germplasm bank located in Paraíba, a region with very low rainfall. We have found within these three transcriptomes around 10% of transcripts belonging to fungi, and the majority of root-specific transcripts are fungal. We believe they are endophytic fungi, as the exterior of the root samples has been adequately washed several times. We have used an in-house pipeline to separate these fungi and classify them between phyla. Among the most expressed transcripts, many were annotated as heat shock proteins, which are highly related to heat stress resistance. Other works have reported endophytic fungi in Agave's roots, but the presence of so many heat shock proteins is novel.

Keywords: drought resistance, genomics, transcriptomics; **Supported by:** CAPES, CNPq, FAPESP

A.03 - Foliar fertilizer with micronutrients in sugarcane

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Recent research with micronutrients in Brazil indicates positive responses of sugarcane to the application of higher doses than those recommended in the planting furrow. However, due to application difficulties, many farmers have opted for foliar application of these nutrients, without scientific criteria and efficiency. The aim of this study was to evaluate the effect of foliar application of micronutrients on the nutrition, yield and industrial quality of sugarcane in different production environments. For this, a net of sugarcane experiments was conducted in 8 locations in south-central Brazil, in the 2018/2019 crop. The experimental design was randomized blocks with 9 treatments and 5 replications. The treatments employed were: Control (without micronutrients), Zn (0.70 kg ha⁻¹), Cu (0.35 kg ha⁻¹), Mn (1.00 kg ha⁻¹), B (0.30 kg ha⁻¹), Mo (0.30 kg ha⁻¹), N (10.00 kg ha⁻¹), Complete-N (all nitrogen-free micronutrients) and Complete+N. The experiments were installed between December/2018 and January/2019, when the cane plant was about 1.5 m high. Were evaluated the effects on plant nutrition, stalk yield (SY) and industrial quality (total recoverable sugar, TRS). Joint statistical analysis of the data was performed by analysis of variance and subsequent comparison of means by the t-test (LSD) at 5% probability. There was an increase in the contents of all micronutrients studied in relation to the control treatment. There was an increase in the contents of all micronutrients studied in relation to the control treatment. Isolated applications of B and Mn increased SY by 5.7 t ha⁻¹ compared to control. The TRS increased with the application of Complete-N treatment, but did not differ from the control treatment. It can be concluded that the application of B and Mn at an advanced stage of development can increase the average yield of sugarcane. However, studies with application times, sources and doses of micronutrients should be conducted to establish a recommendation for sustainable cane fertilization for sugarcane.

Keywords: Plant nutrition, Micronutrients, Foliar application. **Supported by:** PPG-IAC, CAPES and PCEM.

A.04 - Soil erosion control on sugarcane farm roads and tracks by modifying ground surface coverage

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Uncovered farm tracks can reach 5% of the farm area. Although considered an important source of sediment that reaches the watercourses, areas of unpaved roads and lanes have been neglected in studies on soil erosion and environmental impacts of agricultural production activities. One of the limiting factors is the lack of data and methodology to evaluate soil losses in field scale to quantify the effects of proposed conservation practices, as track design, slope, drainage and surface cover. The objective of this study was to evaluate soil losses in dirt roads on sugarcane farm under some options of surface coverage. The experiment is being conducted in partnership with the Cofco Intl. Group, using the randomized block design, with 5 repetitions and 4 treatments (T1 - uncovered dirt road; T2 - newly implanted dirt road; T3 – dirt road covered with straw from sugarcane harvest; T4 – dirt road covered with rock powder). The soil is an Ultisols, with sandy/medium texture. The plots have 112.5 m² delimited by zinc plates. Near the plots, rain gauges were installed. Soil loss was evaluated by measuring the shape of the profile along the dirt road, taking the distance between a stem and the soil. Soil losses over one month in cm³ ha⁻¹ in each treatment were: T1 - 7.0; T2 - 7.9; T3 - 4.1 and T4 - 6.0. The total rainfall was 118.6 mm. T2 was the one with the highest soil loss, because it is a newly implanted dirt road, with no consolidate surface making it more susceptible to erosion. In straw-covered treatment, soil losses were reduced by 48% in relation to the newly implanted farm track and 41% in relation to the uncovered farm track. Cover the surface of farm track with straw has proved to be adequate to minimize soil erosion.

Keywords: sediment yield, dirty road, road maintenance. **Supported by:** FUNDAG

A.05 - Characterization of the promoter region of two drought-responsive SAS

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Synthetic promoters are a potential tool for the creation of technology that can be applied in the production of transgenic plants with the desired biomass characteristics, or in gene editing methodologies such as CRISPR-CAS. Thus, the characterization of the architecture of the promoters of target genes is essential to provide tools for transgene technology and gene editing. Characterize the promoter region of selected genes. In the present work, we selected two SAS, SASGMS07313 and SASGMS04367, based on their differential expression profile in sugarcane transcriptome experiments in response to drought. These SAS sequences were then aligned against the genome of SP80-3280 to retrieve their corresponding putative gene copies. An additional search based on the genome annotation was performed to find other possible putative genes for those SAS. The promoter was delimited as the region 1500 nucleotides upstream of the TSS, which was predicted by the software TSSFinder. The detection of candidate motifs followed by the evaluation of their significance was made using the tools available in the MotifSuite platform. Five different motifs (AT3G57600, CRF2, RAP211, ERF4 e ERF094) were found for SASGMS07313, all of which belong to the domain AP2/ERF. Except for one sequence, which only contained one predicted TFBS in its promoter architecture, all other sequences displayed an architecture with all five identified motifs. For SASGMS04367, we could detect nine different motifs, which belonged to the domains Basic and Winged helix-loop-helix; SWIM- and SBP-type zinc finger; AP2/ERF; and B3. As expected, both SAS datasets showed similar motifs within their respective sequences; however, the number of repeats of each motif differed for different sequences, which could potentially have an effect on their expression.

Keywords: gene regulation, motif discovery, TFBS. **Supported by:** FAPESP (2018/17561-9)

A.07 - Hydrothermal pre-treatment of eucalyptus forestry residues previously to obtaining xylooligosaccharides

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Brazil is the largest producer of cellulose from eucalyptus wood. However, industries such as pulp and paper and cellulosic biofuel production annually generate large amounts of waste throughout their production processes. In wood harvesting, the agroforestry residues generated are formed mainly by bark, branches and chips in considerable quantities. The final arrangement of these is mostly used to provide organic matter to the soil. In this sense, it is necessary to develop processes that value these materials, as they have low cost and high availability. Hydrothermal pre-treatments of in-natured bark and branches were developed following an experimental design. The tests were in stainless steel reactors with a capacity of 500 mL, and the reactions were made in a thermostatic bath. Approximately 20 g of dry biomass base was used for each experiment, maintaining a solids loading in the medium of 10% w/w. The chemical characterization of in-nature bark and branches showed a considerable difference for ash, extractives and lignin content for the biomasses. The hydrothermal pre-treatment results showed that the xylooligosaccharides (XOS) concentration can be affected by the severity factor and the chemical composition of the raw material. The hemicellulosic hydrolysate was characterized by HPLC, and the highest XOS content attached for branches and bark was 8.7 g/L and 5.4 g/L, respectively. The use of eucalyptus residues to produce high value-added chemicals represents a great opportunity for chemical, food, and pharmaceutical applications. **Keywords:** eucalyptus forestry residues, hydrothermal pretreatment, xylooligosaccharides. **Supported by:** FAPESP and CNPq

A.08 - Unearthing Agave secrets: transcriptome analysis of three fiber producing cultivars suitable for biochemicals and biofuels production in semiarid regions

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Agaves have been typically grown commercially for fiber or alcoholic beverages; currently, they are emerging as candidate crop for biochemicals and biofuels production in semiarid regions because of its high productivity in low rainfall areas, drought tolerance, and low lignin content. Brazil is the world's largest Agave's fiber producer, and its geo-economic context gives the country great opportunities to transition to a biorefinery system. Despite its importance, there is no genome available, and only a few transcriptomes published. In this context, we present the transcriptomic atlas of *Agave sisalana*, *Agave fourcroydes*, and agave hybrid 11648 (*A. amaniensis* x *A. angustifolia*) under prolonged drought in field conditions. We sequenced leaf, stem, and root, and correlated gene expression profiles with biomass composition, enzymatic hydrolysis, and non-structural carbohydrates. Although the cultivars presented differences in expression levels, all of them relayed on the same molecular mechanisms. The whole phenylpropanoid pathway is less expressed than other cell wall biosynthetic pathways. However, differences in biomass accessibility could, still, be attributed to either lignin content or lignin composition, possibly through modification of s/g ratio, mediated by COMT expression. Unlike most plants, the highly-expressed transcripts are not photosynthetic proteins, but proteins that are well known to be stress-responsive, e.g., for *A. fourcroydes* leaves, the most expressed transcript, a Chaperone, presented expression twenty-five times higher than the topmost photosynthesis-related gene. Furthermore, Agaves' main molecular strategies to cope with high-temperature and drought consist of overexpression of HSP, LEA, and proteolysis processes, as well as raffinose accumulation as an osmolyte. In conclusion, the data obtained is novel and provides new insights into cell wall architecture, recalcitrance, and resistance to high-light-intensity and water deficit. The comprehension of functional genomics in field conditions empowers molecular breeders to correspond to the emerging expectations for Agave as biorefineries feedstocks for dryland areas.

Keywords: Agave, Biomass, RNAseq

Supported by: FAPESP (2016/05396-8; 2017/04900-7) and CAPES

A.09 - Underlying efficiencies leading to biomass production by sugarcane and energy cane: a comparative study

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Using the same productive system as sugarcane, energy cane is able to produce more biomass per hectare. Such high biomass production of energy cane can be associated with its high tillering and with a vigorous root system. As consequence, energy cane better explores natural resources and then convert more energy into biomass as compare to sugarcane. Herein, we estimated the light interception and conversion efficiencies by energy cane and sugarcane. A field experiment was carried out using one sugarcane variety (IACSP95-5000) and two varieties of energy cane (Vertix3, type I; Vertix2, type II). Photosynthetically active radiation (PAR) above and below plant canopy was continuously measured during 270 days of the first ratoon and biomass production was quantified every 50 days. Biomass production and intercepted PAR were used to estimate the light interception efficiency (ϵ_i), light conversion efficiency into biomass (ϵ_c) and partitioning efficiency (ϵ_p). The average ϵ_i for sugarcane (0.81) was lower than one estimated for energy cane types I (0.89) and II (0.85) and led to lower dry biomass production (26 Mg ha⁻¹) as compared to energy cane type II (38 Mg ha⁻¹). Despite its higher ϵ_i , energy cane type I showed an intermediate biomass production (33 Mg ha⁻¹) and its ϵ_c (2.03 g MJ⁻¹) was similar to the others (1.89 and 2.37 g MJ⁻¹ for sugarcane and energy cane type II, respectively). Regarding the ϵ_p at the end of crop cycle, there was no significant differences between sugarcane (0.84) and energy cane types I (0.83) and II (0.78). Our findings revealed a higher photosynthetic capacity of energy cane when compared to sugarcane and also significant variation in canopy structure/morphology among genotypes, with important consequences for light interception. **Keywords:** biomass, light, Saccharum. **Supported by:** Capes

A.12 - Mixed linkage glucan contents in sugarcane tissues sampled at varied development stages

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Grasses present a complex cell anatomy and their cell walls recalcitrance is tissue specific. In a recent work, we demonstrated that parenchyma cell walls of sugarcane are considerably less recalcitrant than sclerenchyma fiber and vessels. Low recalcitrance in parenchyma has been associated with low lignification and high mixed linkage glucan (MLG) contents. Other studies indicate that MLG is progressively replaced by glucuronoarabinoxylans (GAX) throughout the cell development, suggesting that recalcitrance can increase along plant cell wall development. Understanding MLG occurrence in sugarcane tissues during cell wall development can help to understand, and perhaps manipulate, sugarcane recalcitrance. To address MLG occurrence in sugarcane at varied development stages, one low-lignin/high-MLG hybrid (H89) was sampled at three different development stages (internodes 3, 5 and 11 from the plant apices) and analyzed for MLG content. MLG was quantified across these internodes to represent the rind, pith and the pith-rind interface regions. Megazyme enzymatic kit designed for MLG quantification was used for a total of 27 samples (3 biological replicates x 3 internodes x 3 transversal samples across internodes). The highest MLG contents (11-14% w/w) were detected in the pith and pith-rind interface of all internodes, almost independently of the developing stage. In contrast, the rind region presented significantly lower MLG contents (1.5-4% w/w) that decreased from internodes 3 (4% w/w) to internodes 11 (1.5% w/w). Results obtained so far indicate that MLG occurs at low contents in the rind region, which is rich in vascular bundles and poor in parenchyma cells. In the rind region, the MLG contents decrease significantly from young to mature internodes, suggesting its replacement by GAX, or simply because cell walls density increases with development and lignification.

Keywords: Mixed linkage glucans, Sugarcane hemicelluloses, Recalcitrance

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A.13 - Unravelling the sucrose metabolism pathway of *Burkholderia sacchari*, a microbial cell factory
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Sucrose, the most abundant disaccharide in the world, is considered relatively inexpensive when compared to glucose. A crucial step on sucrose metabolism is the hydrolysis to fructose and glucose, by specific enzymes such as invertases or phosphorylases. Biological platforms that are able to consume sucrose and convert it into value-added bioproducts are economical advantageous over other platforms. *Burkholderia sacchari* consumes sucrose very well, and accumulates it in the form of various polymers. The mechanism of the whole process is not yet elucidated, but it is known that extracellular hydrolysis occurs, releasing fructose and glucose and preferential consumption of fructose. The aim of this work was to carry out an in silico analysis of the gene sequences related to sucrose consumption in *B. sacchari* and thus open exciting perspectives regarding the capacitation of other biological platforms to grow on sucrose as the single carbon source. Known Invertases (*Bacillus subtilis*, *Escherichia coli*) and phosphorylases (*Bifidobacterium adolescentis*, *Leuconostoc mesenteroides*) protein sequences were used as query entries to search for similarities in the *B. sacchari* genome. No degree of homology was obtained using phosphorylases. The invertase sequence showed 43% homology with a *B. sacchari* loci annotated as levansucrase. This putative levansucrase encoding sequence was found in other *Burkholderiaceae*, very conserved within the genus with identity that can reach up to 80% in *Burkholderia* spp. with amino acids fragments range from 483 to 453. In this work we describe, for the first time to our knowledge in bacteria, a new operon organization that encodes: a levansucrase, two regulatory proteins and an invertase. This in silico model described in *B. sacchari* appears to be unique and is currently being cloned and heterologously expressed in other bacteria, as *Pseudomonas* spp. and *E. coli*, in order to enable these bacteria to consume sucrose.

Keywords: sucrose, catabolism, catabolism. **Supported by:** CAPES

A.15 - Bud sprouting and shoot/root development: comparing sugar and energy canes

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Brazil today has about 9.5 million hectares of sugarcane planted, corresponding to just over 1% of the country's area. From the biomass harvested from this area are produced 35.48 million tons of sugar, 28.16 billion liters of ethanol and 25,482 GWh generated of electricity. These numbers represent the great potential of the sugar-energy sector. However, we still have about 190 million hectares of pasture in the country, most of which low productivity, with huge potential for implantation of sugarcane crop. Currently sugarcane is planted by vegetative propagation, a serious problem in the field is the low sprouting rate of some varieties, which results in a large loss of raw material and increases the cost of a sugarcane crop. Energy cane is a bioenergy crop which is characterized by high biomass production, higher rooting and lower sucrose content. It was verified that the sprouting in this variety was superior than the conventional sugarcane varieties, accompanied by a fast initial shoot development. Energy cane presented a differential tissue formation pattern than sugarcane, with the first appearance of the shoot and after ten days the appearance of the root, in conventional varieties, the root is emitted after 24 hours planting and the shoot between 7 - 14 days. The development of the internodes was also evaluated, the older internodes of energy cane sprouted and developed better than the younger ones, the opposite was verified in sugarcane. After 50 days of planting, the energy cane presented volume, area and root length, plant height, number of tillers and green leaves superior to sugarcane. Taking these results together, they indicate that energy cane is initially characterized by rapid development in the first days after planting, which characterizes in high productivity over time.

Keywords: Energy cane, Biomass, Sprouting. **Supported by:** FAPESP and CAPES

A.16 - Molecular diversity and genetic structure of *Saccharum* complex accessions as revealed by TRAP markers

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Sugarcane is a tropical grass and a significant component of the economy of many countries playing a central role as a primary sugar-producing crop and has major potential as a bioenergy crop. The use of molecular markers to evaluate the genetic variability in germplasm banks can contribute to the genetic improvement in the selection of parents for crossing and consequent obtaining of superior progenies. In order to evaluate the diversity and genetic structure of the Brazilian Panel of Sugarcane Genotypes (BPSG), 254 accessions of the *Saccharum* complex (75 accessions from *Saccharum* spp., six accessions from *Erianthus* spp. and 173 *Saccharum* spp. hybrids) were analyzed using eight TRAP markers combinations anchored in sucrose and lignin metabolism genes. A total of 595 fragments (584 polymorphic) were identified and an average value of polymorphic information content (PIC) and discriminatory power (DP) of 0.97 and 0.98, respectively. The first and second main components of the principal component analysis (PCA) explained 17.8% of the total variability expressed between the accessions. For AMOVA, the accessions were separated into three groups: i) ancestors (A), composed of accessions of *Saccharum* and *Erianthus* spp.; ii) Brazilian breedings (BB), containing the accessions of the Brazilian breeding programs; and iii) foreign hybrids (FH), composed of accessions originated from other countries. The fixation indexes were 0.14, 0.03 and 0.05 between A and BB, A and FH, and FH and BB, respectively. STRUCTURE was able to separate the ancestor and hybrids accessions into two subpopulations. The dendrogram divided the accessions in three clades, one of them mainly composed of ancestors and the other two composed by the hybrids. Thus, this work can help in organizing the genetic variability available and increasing the chance of obtaining superior progenies.

Keywords: genetic dissimilarity, molecular markers, sugarcane

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A.17 - Data Mining Based on Multi-Omics Factor Analysis Aimed at Validating To Differentially Expressed Genes Related To Sugarcane Development

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Sugarcane is considered one of the most sustainable crops among bioenergy crops. "Omic" level studies, such as transcriptomics and metabolomics, are important and complementary strategies that may provide information about metabolic pathways in order to increase sugarcane yield and to understand how sugarcane responds to differences in climatic conditions. For this particular purpose, tissues samples were collected from sugarcane variety SP80-3280, planted in two fields under different climatic conditions, were used to conduct molecular studies, namely transcriptomics and metabolomics. Thus far, DNA microarray and HPLC-MS data generated by our group were integrated via the use of Multi-Omics Factor Analysis (MOFA) leading to the identification of differentially expressed transcripts and altered metabolites throughout growth and development and in response to changes in different climatic conditions. The main metabolic pathways found to be altered were flavonoid biosynthesis (map00941), phenylalanine, tyrosine and tryptophan biosynthesis (map00400), phenylpropanoid biosynthesis (map00940) and pentose phosphate pathway (map00030). Based on this, 10 genes (identified from SUCEST-FUN: SASGMS00616, SASGMS11566, SASGMS06739, SASGMS22034, SASGMS18580, SASGMS27154, SASGMS00026, SASGMS00027 and 2 versions of SASGMS00025) were selected for future validation via qPCR in order to draw clear conclusions and identify possible metabolic markers with regards to sugarcane growth and development in the field, and to raise hypotheses on how to improve sugarcane yield.

Keywords: Transcriptomics, Metabolomics, MOFA. **Supported by:** Temático FAPESP - 2014 / 50921 - 8

A.19 - Phylogeny of putative BABY BOOM proteins controlling the somatic embryogenesis in sugarcane
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The roles of somatic embryogenesis (SE) require the expression of genes that stimulate cell proliferation and morphogenesis. One of the most common genes related to this process is BABY BOOM (BBM), which codifies the transcription factor AP2/ERF, responsible for the regulation of other genes. Despite BBM are already been reported in diverse species, in sugarcane this gene still unexplored. Thus, the present study aimed to find out putative amino acid (aa) sequences related to BBM family in sugarcane commercial variety SP80-3280. For this, aa BBM sequences from *Oryza sativa* (1), *Zea mays* (4), and *Arabidopsis thaliana* (2) were selected and submitted to NCBI, Gramene, Phytozome and UniProt platforms to obtain homologous sequences from *Sorghum bicolor* and other Poaceae species. Thus, sorghum sequences were used to find homologous in the sugarcane database (SUCEST-FUN) by BLAST searches. The predicted proteins (4) were aligned using multiple alignments with CLUSTALW software and, an unrooted neighbor-joining phylogenetic tree was constructed selecting p-distance, pairwise deletion and 1000 bootstrap replications as parameters. Besides, sequences were analyzed using BLAST tools implemented on SUCEST-FUN for detecting putative BBM sequences on the sugarcane (SP80-3280) gene space assembly. Results suggests that the four putative proteins found presents 3 - 6 homo(eo)logs. Phylogenetic analysis resulted in the detection of 6 groups based on high and moderate identity values. The sugarcane sequences were clustered with sorghum, with one exception (SP803280BBM7). This study provides evidence that SP80-3280 has four putative BBM loci (and its putative homo(eo)logs), which will be further tested as candidates related to SE pathways in sugarcane. **Keywords:** embryogenic competence, transcription factor, Saccharum sp. **Supported by:** CAPES (finance code 001)

A.21 - Evaluation of a biosensor based on reduced graphene oxide and glucose oxidase enzyme on the monitoring of second-generation ethanol production

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Bioethanol is a fuel derived from renewable energy sources. However, the main production processes use biomass that are also utilized as food. For this reason, new technologies are needed that employes alternative biomass sources as a raw material, such as the production of second-generation (2G) ethanol, which uses lignocellulosic biomass in its process. These biomass can be either in the form of bagasse, straw, bark, in other words, products that are residues from the agricultural chain production. An example of agricultural waste that may contribute toward this strategy is the coir fiber. In Brazil, coconut has a high consumption, accumulating about 240 thousand tons of husks per year, which can be used as biomass for the production of 2G ethanol. The coir fibers contain in its structure cellulose that can be degraded into glucose and later used as substrate for the production of ethanol. However, during the enzymatic hydrolysis of cellulose, glucose becomes a limiting factor, since its accumulation in the bioreactor causes inhibition in the cellulase enzyme, and this implies in a lower production of this hexose consequently a lower yield in ethanol production. In this context, an easily prepared biosensor based on reduced graphene oxide (rGO) and glucose oxidase (GOx) enzyme was developed to monitor the enzymatic hydrolysis process of the 2G ethanol process from green coconut biomass. The rGO-GOx biocomposite modified a glassy carbon (GC) electrode was characterized by morphological, electrochemical and spectrophotometric techniques showing that the GOx enzyme was immobilized on the rGO. The parameters for glucose determination were optimized by square wave voltammetry (SWV). The developed biosensor was applied for the determination of glucose during the enzymatic hydrolysis step, showing that the process can be stopped with 12 h of reaction. Thus, an important achievement of this analysis is the reduced time to get a valuable result for the test, saving time and reducing the cost of the 2G ethanol process.

Keywords: Graphene, Biosensor, 2G ethanol

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A.22 - Potential of soybean straw for production of fermentable sugars and carbonaceous structures.

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Waste from the agro-industrial sector is still barely explored and used. Brazil is one the pioneer countries in bio-resources field. In the case of soybeans, it can become the main producer, causing a considerable increase in waste generated and unused straw. The objective of this work is to study the potential of soybean straw (waste from soybean production chain residues) in the production of fermentable sugars and carbonaceous structures from primary biorefining. Studies on the performance of the delignification process by varying the temperature, solids charge and the concentration of the delignifying agent (organosolv and NaOH) are being performed, as well as the influence of the precipitating agent to recover lignin. Among the results we obtained that the straw showed a cellulose, hemicelluloses and lignin content of $38.6 \pm 0.98\%$, $13.6 \pm 0.08\%$ and $20.6 \pm 1.63\%$, respectively. Solubilization of hemicelluloses (by acid catalysis) reported a solubilization of about 68.49%. The recovery of solids, called cellulignin from $84.06 \pm 2.61\%$ where 69.01% of cellulose was recovered in this fraction. This study will provide an alternative source for use in biomaterials and biofuels.

Keywords: Soybean straw, biomaterials, biorefinery

Supported by: CAPES

A.23 - The formation of carbohydrates, organic acids and toxins during acid hydrolysis and removal of 5-hidroxymethylfurfural via overliming treatment, envisioning microbial fermentation

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The study of the potential use of algae-based biomass is increasing because of its fast-growing renewable industrial feedstock characteristic. In addition to its environmental advantages, such as low land occupation, and high carbon dioxide fixation, the rapid growth rate of algae makes them promising raw material candidates for bioprocesses. In an endeavor to better understand the hydrolysis process of the algae *Kappaphycus alvarezii*, and the removal of toxins that hinder bacterial growth, the algae biomass was hydrolyzed in various severity levels and the formation of sugars, organic acids, and toxins were plotted against reaction time for temperatures ranging from 70 up to 120°C. A 2⁴ factorial experiment design was performed, using temperature(70-80°C) acid concentration (1-5 %V/V), time and solid/solution(10-20%W/V) ratio as variables. For overliming treatments a three factor Box-Behnken design was used, with time(1-2h), temperature(25-50°C) and pH(9-11) as variables. Results show that higher temperatures and strong acid concentrations lead to the formation of more than 100% sugar monomers. The formation of 5-hydroxymethylfurfural (HMF) appeared to be closely related to the liberation of carbohydrates from the polymeric carrageenan structures. At low severity levels, the formation of HMF was significantly reduced. Data sets from experimental design indicate temperature and acid concentration as main factors for increasing HMF formation during hydrolysis. Overliming treatments were showed to reduce HMF/galactose ratio from 0.29 to 0,15, pH and temperature being the main factors for reducing of HMF without significant reduction of galactose. High sugar yields were registered, making *K. alvarezii* a potential biomass for fermentation processes.

Keywords: 3G biomass, Hydrolysis, Carrageenan

A.24 - Photosynthetic capacity varies significantly among sugarcane genotypes

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Sugarcane (*Saccharum* spp.) is one of the main alternatives for the bioenergy sector due to its great potential for biomass. This potential in biomass production is determined by sunlight energy that reaches in plants and the efficiency with which this energy intercepted is converted into biomass through photosynthesis. Thus, the crop yield could increase by developing cultivars with high photosynthesis, a heritable and relevant trait to breeding programs. The aim of this study was to investigate the photosynthetic variations in sugarcane cultivars developed by Brazilian breeding between 1948 and 2006, beyond the crosses *Saccharum officinarum*, *S. spontaneum* and *S. robustum*. CO₂ response curves of photosynthesis were performed and key photosynthetic traits were estimated. While *S. spontaneum*, IAC87-3396 and RB83-5486 presented higher maximum photosynthetic rates, the cultivars *S. officinarum* and IACSP94-2094 showed values until 36% lower. The increase of CO₂ atmospheric pressure, from natural condition ($p_a=38\text{Pa}$) to $p_a=150\text{Pa}$ resulted in high photosynthetic rate (L_{f150}) in all genotypes, with increments of 11% and presented stomatal limitation (L_s) of 10%. The carboxylation capacity of PEPC (V_{pmax}) is negatively related to L_{f150} and L_s. Our results suggest that Rubisco saturation is not achieved under normal environmental conditions (38Pa). Finally, our findings suggest that there is possibility for improving sugarcane yield through improvement of photosynthetic activity, a topic to be further explored by breeding programs.

Keywords: Saccharum spp., photosynthesis, yield

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A.25 - Evaluation of cell wall components of contrasting sugarcane progenies for biomass production

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The biotechnology process involving bioethanol production is in constant development and surrounded by great interest. Cellulosic bioethanol potentially will allow the use of more biomass from the plant and in the case of sugarcane the use of bagasse. This is especially important for this crop since productivity gains observed over the years are stagnant. Crosses between ancestral sugarcane genotypes are being analyzed to increase the genetic basis used in the development of modern commercial hybrids. This work is focused on obtaining biochemical data related to cell wall components of contrasting sugarcane genotypes for the number of tillers. The material used in this study comes from crosses aimed at developing an energy cane, evaluated in Araras, SP. First, biparental crosses between *S. officinarum*, *S. spontaneum* and *S. robustum*, besides two commercial varieties were done. From this, 31 contrasting genotypes for tillering, plus seven parental genotypes and other 2 varieties of energy canes were analyzed. All 40 genotypes have been evaluated regarding cell wall features at 8 and 12 months after planting. The analysis includes: lignin quantification, enzymatic saccharification, soluble sugars quantification - those related to first generation bioethanol -, starch dosage and TFA hydrolysis, and subsequently cell wall fractionation. The results at 8 months after planting do not show significant differences, just as the lignin content and the saccharification results for 12 month-old plants. The last analyzes are now being applied to the 12 month-old plant samples. All the steps are essential to clarify the differences between the progenies and support the study and subsequent production of new varieties of energy canes. **Keywords:** bioethanol, cell wall, biomass;

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A.26 - Effect of lignocellulosic inhibitors on the growth rate of bioethanol fermentation contaminating lactic acid bacteria

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The burning of fossil fuels is responsible for 73% of CO₂ emissions in the atmosphere, intensifying the greenhouse effect and global warming. The production of biofuels from lignocellulosic (LC) waste could supply the energy matrix in the near future. However, the fermentation of LC hydrolysates entails many scientific and technological challenges. Pretreatment processes generate a variety of molecules (furanic derivatives, phenolic compounds and organic acids) that act as inhibitors of microbial metabolism, and thus reduce the efficiency of fermentation. In this context, the present research proposal aims to investigate the effect of lignocellulosic inhibitors on contaminating lactic acid bacteria, normally present in industrial fermentation processes. In a 96 well microplate reader, growth kinetics were performed with homofermentative and heterofermentative lactic acid bacteria in semi-defined medium with inhibitors at certain concentrations where the OD₆₀₀ was read for 24h at 15 minute intervals. From the obtained results, it was possible to observe the toxicity of the organic acids, especially the formic acid, which caused the growth rate decrease in all tested strains. On the other hand, it was observed that the furanic compounds (HMF and furfural) had a positive effect on the growth rate in almost all heterofermentative lactic bacteria. This was not observed in the culture with homofermentative bacteria. The initial hypothesis is that these furanic compounds, even at high concentrations (2 to 4 g / L), function as electron acceptors, thus helping to redox balance bacteria with heterofermentative metabolism. With this work we aim to understand how different strains of contaminating bacteria respond to lignocellulosic inhibitors and with this information, we can propose alternative ways to control contamination or even apply genetic engineering techniques to the most inhibitor-resistant bacteria.

Keywords: bioethanol, lignocellulose, bacteria. **Supported by:** CNPQ

A.27 - Determining drivers of bioenergy technology adoption among livestock producers

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Global demand for Brazilian pork has grown exponentially in recent years. Unfortunately, manure disposal associated with demand has created negative externalities. Environmental pressures have presented a window of opportunity for biogas technology to be diffused as a strategy to recycle byproducts while capturing bioenergy from methane. To spread the technology, it is imperative to understand attributes that drive adoption. Preferences for technological attributes and their influence on adoption, however, have remained elusive. The objective of this study was to identify principal attributes that may drive adoption of covered lagoon digesters among medium-sized producers (users and potential users) in southeast Brazil. We facilitated a focus group with experts and employed semi-structured interviews with producers to identify these attributes. Thereafter, we conducted a systematic review of biogas studies to disconfirm, corroborate or explain qualitative findings. Results indicate that payback period, slurry quality and disposal, market access and maintenance were the most frequently cited attributes that may influence adoption. While payback period was the most cited attribute, the second most prominent attribute was how well a biodigester decomposed substrates, such that, it decreased the time and cost of disposal or provide future opportunities for profit (e.g., energy, biofertilizer). In addition, the capability of the technology to co-digest different types of byproducts (e.g., food waste, carcasses) was another attribute, affording opportunities to provide waste disposal services. The fourth attribute entailed if the technology could fulfill user-energy demands, and perhaps, provide surplus energy to local grids. Access to maintenance providers and spare part-suppliers was a final attribute of concern. These findings will inform a discrete choice experiment that will assess how pork producers make tradeoffs to adopt biogas technologies with specific attributes. The experiment will inform bioenergy disseminators how to make the most of this window of opportunity, supporting biogas diffusion.

Keywords: Biogas, Technology Adoption, Agricultural Producers

Supported by: The Fulbright Commission in Brazil

A.28 - Novel targets that can control the metabolic shift to triacylglycerol (TAGs) synthesis in microalgae under nitrogen starvation

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Microalgae have high potential as a feedstock for production of sustainable biofuels and chemicals. It stands out for the high capacity of lipid biosynthesis, mainly triacylglycerols (TAGs). However, it is not a commercially feasible option yet, due to the inversely correlation of high lipid concentrations and high biomass productivity in most microalgae species. Previous studies performed genetic manipulations either overexpressing or downregulating specific genes from lipids and starch biosynthetic pathways in order to increase TAGs production in mixotrophic growth. However, these approaches did not achieve the expected success, and did not contribute to the development of fully environmentally sustainable biomass production due to its dependency on organic carbon source. Therefore, we performed the present study with the aim of identifying molecular triggers and regulators that modulate TAGs biosynthesis. The microalgae model *Chlamydomonas reinhardtii* was cultivated in photoautotrophic condition and TAGs synthesis was enhanced by nitrogen (N) starvation. Time-course proteomics and metabolomics experiments were conducted in order to detect the alterations in abundance of proteins, primary metabolites and lipids. After 24 hours under N starvation the cells achieved the highest accumulation of TAGs. Our time-resolved metabolomics results showed that the metabolites arginine, 2-oxoglutarate, putrescine and ornithine were early upregulated under N starvation. The results of proteomics analysis corroborated with the metabolomics data since we also observed the enrichment of proteins annotated into pathways related to the synthesis of the metabolites identified. Furthermore, another result clearly observed was the redistribution of the percentage of plastid lipids and cell membrane lipids categories, with identification of a negative correlation between TAGs accumulation and all classes of membrane lipids in cells under nitrogen starvation, including the diacylglycerol (DAG), a known precursor of TAGs biosynthesis. Therefore, our results revealed the identity of metabolites and proteins that may participate as signaling molecules in the TAGs biosynthesis, representing novel targets of the regulation of this mechanism.

Keywords: metabolomics, proteomics, microalgae

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A.29 - Nitrate supply stimulates root NO synthesis and improves sugarcane tolerance to drought

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Nitric oxide (NO) is an important signaling molecule associated with many biochemical and physiological processes in plants under stressful conditions. Nitrate reductase (NR) not only mediates the reduction of NO_3^- to NO_2^- but also reduces NO_2^- to NO, a relevant pathway for NO production in higher plants. Herein, we hypothesized that sugarcane plants supplied with more NO_3^- as a source of N would produce more NO under water deficit. Such NO would reduce oxidative damage and favor photosynthetic metabolism and growth under water limiting conditions. Sugarcane plants were grown in nutrient solution and received the same amount of nitrogen, with varying nitrate:ammonium ratios (100:0 and 70:30). Plants were then grown under well-watered or water deficit conditions, in which the osmotic potential of nutrient solution was -0.15 and -0.75 MPa, respectively. Under water deficit, plants exhibited higher root $[\text{NO}_3^-]$ and $[\text{NO}_2^-]$ when supplied with 100% NO_3^- . Accordingly, the same plants also showed higher root NR activity and root NO production. We also found higher photosynthetic rates and stomatal conductance in plants supplied with more NO_3^- , which improved root growth. ROS accumulation was reduced due to increases in the activity of catalase in leaves and superoxide dismutase and ascorbate peroxidase in roots of plants supplied with 100% NO_3^- and facing water deficit. Such positive responses to water deficit were offset when a NO scavenger was supplied to the plants, thus confirming that increases in leaf gas exchange and plant growth were induced by NO. Concluding, NO_3^- supply is an interesting strategy for alleviating the negative effects of water deficit on sugarcane plants, increasing drought tolerance through enhanced NO production. Our data also provide insights on how plant nutrition could improve crop tolerance against abiotic stresses, such as drought.

Keywords: water deficit, nitrogen, nitrite

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A.30 - Effect of beneficial microorganisms on sugarcane plant growth, metabolism, biological control and soil bacterial community

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Beneficial microorganisms such as plant growth promoting bacteria (PGPB) and arbuscular mycorrhizal fungus (AMF), has been used in different plants of agricultural interest. Nevertheless, the effect these beneficial microorganisms in sugarcane plants are not completely clear, especially in sugarcane pre-sprouted seedlings (PSS). The main goal was to evaluate the effect of PGPB and AMF regards the nutritional state, nitrogen metabolism, biological control of *Colletotrichum falcatum*, plant interaction and influence on endophytic and rhizosphere bacterial community of sugarcane. Our data revealed that endophytic bacteria and rhizobacteria strains are were able to improve sugarcane plant growth, change the biochemical level of the foliar free amino acid and polyamine profiles. Sugarcane PSS inoculated with AMF have greater mineral nutrition, and co-inoculation of PGPB such as, *Kosakonia oryzae* or *Bacillus* sp. with AMF improved sugarcane seedling growth and mineral nutrition. Between the PGPB strains evaluated some bacteria such as *Herbaspirillum frisingense* (IAC-BEca-152) and *Pseudomonas fluorescens* (IAC-RBcr4) are able to control the pathogen *C. falcatum* on sugarcane PSS. PGPB strains impact on endo- and rhizosphere bacterial community, increasing the abundance of some family such as Xanthomonadaceae, Burkholderiaceae and Rhizobiaceae. Our results indicate that the use of beneficial microorganisms may be a viable practice in the production of sugarcane pre-sprouted seedlings.

Keywords: bacteria inoculant, biological control, sugarcane pre-sprouted seedlings

A.31 - SUCEST-FUN: The Sugarcane OMICs Data Mining Framework

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Molecular breeding is a promising field to assist in the process of developing improved varieties of sugarcane (*Saccharum* sp.). We have developed tools that enable the generation and integrative analysis of large datasets of genetic, molecular and physiological experimentation. Our framework and database for sugarcane -omics data mining (SUCEST-FUN; <http://sucest-fun.org>) makes it possible to deepen the genome analysis through the application of bioinformatics approaches. The gene space of SP80-3280 includes 373,869 putative genes and their potential promoter and regulatory regions. Our main goal is to organize the genomic resources and develop tools to interrogate them in an integrative way for breeding. The SUCEST-FUN framework was developed as a solution for the integration of -omics (genomics, transcriptomics, proteomics and metabolomics) data based on gene annotation, gene expression and functional genomics. The framework has been developed using Java Web and a Database System (MySQL). The database gathers core information about putative transcripts, assembled from Expressed Sequence Tags (ESTs) and contigs, assembled through full-length cDNA libraries from RNA-Seq technology. The SUCEST-FUN contains catalogs for the genome, and the transcriptomes from SUCEST and ORFEome for signal transduction-related transcripts composed of 21 categories, cell wall metabolism grouped in 10 categories and the transcription factors classified into 57 families. The framework also features a GBrowse, enabling dynamic navigation in the genome and integrated with other -omics information stored in the database. The whole Sugarcane -omics data has been stored and are available in the SUCEST-FUN, which has been improved by integrative analysis, by merging these heterogeneous data and integrated with public resources. The use of Systems Biology approaches for mining those data is now bringing new challenges for the development of new tools and integration into the SUCEST-FUN framework, besides granting for discoveries and insights for the development of new improved Sugarcane varieties.

Keywords: data integration, -omics data, systems biology

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A.32 - Assessment of pastureland changes on the net primary production-based exergy in São Paulo State, Brazil

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Land use and land cover change (LUCC) is a relevant topic in the context of environmental change and sustainability at several spatio-temporal scales. Pastureland covers around 3 billion hectares and is a complex land cover type with a variety of land use systems. Pasture areas have been recognized for intensification potential and its related alternatives for land sparing and land use diversification, as well as greenhouse gas emission mitigation and bioenergy expansion. In this context, quantitative analysis of these lands is important to understand trends and support land use policies to drive actual and future scenarios. The NPP-based exergy index is a new approach for the accounting impacts of land cover changes in spatial-temporal scales. This index is based on the integration of thermodynamics (exergy analysis) and ecology (Net Primary Production - NPP); relating variations in biomass surface with the use efficiency of the resources. The objective of this study was to analyze the effect of pastureland cover changes on NPP-based exergy (NPP_{EX}) in São Paulo State, Brazil from the year 2000 to 2014. The NPP_{EX} was estimated based on NPP extracted from MODIS sensor (MOD17A3 product) and the exergy conversion factor. The land cover transitions between pasturelands and other categories were analyzed associated with the dynamic of the NPP_{EX} changes. Pasture cover during this period decreased by about 62% between 2000 and 2014, mainly for gains in semi-perennial crops. Pastureland efficiency (NPP_{EX}) showed similar behavior of distribution between these years, with an increase from 41.6 to 43.3 MJex m⁻² yr⁻¹ in average values. Slight spatial negative effect on NPP_{EX} changes was observed during this period, which could be associated with decreasing biomass efficiency in the pasturelands that persisted during the period. It highlighted the necessity of analyzing pastureland cover dynamics and their influence on addressing land use policy.

Keywords: NPP, geospatial analysis, pasture;

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A.33 - The potential of C4 grass *Panicum maximum* cv. Mombaça for cellulosic biofuel production under future climate conditions

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Biomass produced by fast-growing grasses as *Panicum* genus, can be important renewable sources for energy production. However, is necessary to estimate their biomass production in simulated future climate situations, once plant physiological processes are influenced by environmental factors. Therefore, in this work, *Panicum maximum* was subject to four future climate conditions simulated using Trop-T-Face system: current temperature and CO₂ conditions (C), elevated temperature (eT)(+2°C), elevated CO₂ (eCO₂)(600ppm) or the treatments combination(eT+eC). In order to analyze the potential for bioenergy production of all treatments, it was measure the crystallinity index (CI) using X-ray diffractometer (XRD) and performed enzymatic hydrolysis using enzyme cocktails Cellic CTec2 and HTec (Novozymes[®]) with a total protein amount of 20 mg/g cellulose at 2% solids loading (50°C/48 hours). Carbohydrate-Binding Modules approach (CBM) using probes for crystalline(GC3a) and amorphous cellulose(CC17) was performed to support hydrolysis data. The XRD showed that CI was significantly lower for eT(53.15%) and eT+eC(57.82%) followed by C(64.69%) and eC(81,67%). Regarding to cellulose hydrolysis eT(34,5%) and eT+eC(32.58%) showed the highest hydrolysis yields when compared to C(30.72%) and eC(30.03%). In addition, the CBM showed that the warming leads to more surface exposure, once higher amount of Total Bounding Protein(μmoles/g substrate) was observed for eT(12.06) and eT+eC(9.95), compared to C(4.7) and eC(4.08). All the parameters analyzed in this work were reported as related with biomass recalcitrance. The lowest CI for eT and eT+eC is an advantage, since crystalline cellulose is a limiting step on cellulose hydrolysis due to its recalcitrance, which means that lower CI results in higher bioconversion rates as observed in hydrolysis performance. In addition, CBM data shows that the fiber surface for eT treatments is more accessible for enzymes once more CMBs was bound to the fiber in warming treatments. Finally, all the results reported here suggested that eT and eT+eC conditions should have a positive effect in the use of *P. maximum* for bioenergy applications.

Keywords: climate conditions, bioenergy, cellulose hydrolysis

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A.34 - Optimization of culture medium for recombinant Swollenin production by *Aspergillus niger* SWOUTR1 and its application in the deconstruction of lignocellulosic biomass

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The high recalcitrance of sugarcane bagasse, as well as the difficulty concerned to cellulases access to deconstruct this biomass represent challenges to be overcome. Some proteins (auxiliary proteins) can help in maximizing hydrolytic actions. Swollenin is reported to enter cellulose fibrils, disrupting hydrogen bonds and thus facilitating biomass amorphogenesis. In other words, swollenin works in synergism with cellulases once its action can increase the liberation of sugars from cellulose. The objective is the optimization of the culture medium to obtain a protein concentrate containing swollenin by *Aspergillus niger* SWOUTR1. Initially, seven media components (maltose; peptone; sodium-phosphate; magnesium-sulfate; Tween80; arginine and 2-N-morpholinoethanesulfonic-acid [MES]) were analyzed by experimental design Plackett-Burman. Four factors (peptone; arginine; sodium phosphate and Tween80) were statistically significant and they were optimized by Rotational Central Composite Design (DCCR) method. The concentrations of components were determined by analysis of the two experimental design techniques, namely (g.L⁻¹): maltose (10); magnesium-sulfate (5); MES (7.5 - use only in shake flasks); peptone (29); arginine (1.05); sodium-phosphate (0) and Tween80 (0.075). The optimal composition was validated and the total protein concentration obtained was 23.3 mg.L⁻¹, this value is as expected for recombinant proteins. The crude extract was concentrated using a hollow fiber membrane system (microfiltration and ultrafiltration) to a concentrate containing 887 mg.L⁻¹ of total protein. This concentrate was used to analyze the synergism degree with cellulases from *Talaromyces pinophilus* in the hydrolysis of pretreated sugarcane bagasse. The use of swollenin resulted in a synergism of 1.30, achieving a concentration of 49 g.L⁻¹ in a system containing a solid load of 10% and a protein load of 13 mg.g⁻¹ (10[cellulose] + 3[swollenin]). Therefore, the use of swollenin is promising, because it can increase the efficiency of lignocellulosic biomass hydrolysis, generating sugar-rich syrups that can be used to obtain molecules of industrial interest. **Keywords:** biomass, enzymes, swollenin. **Supported by:** CAPES

A.35 - Brachypodium as a proxy for investigating the nitrate uptake regulation of sugarcane

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Sugarcane (*Saccharum* spp.) presents a low response to nitrogen fertilization. Low N recovery is associated with low nitrate uptake by sugarcane roots, differing from other tropical grasses, such as sorghum and maize. Understanding the regulation of nitrate uptake is a key factor in improving the nitrogen use efficiency (NUE) in sugarcane. Given the lack of mutants and genomic complexity of sugarcane, we chose to use the model grass *Brachypodium distachyon*. We started by investigating the regulation of the NRTs genes (NITRATE TRANSPORTERS) responsible for the high affinity transport system (HATS) of nitrate in *B. distachyon* roots in response to nitrate resupply. *Brachypodium distachyon* (Bd21) plants were grown in N sufficient nutrient solution (+N, 2mM NH₄NO₃) for 21 d and subsequently in N (-N) deficient solution for 7 d. Soon after, plants were resupplied with nitrate (10mM KNO₃), and roots were collected at 0 (-N), 2, 4, 24 and 72 h after treatment. Influx analyses using ¹⁵N-nitrate and quantitative reverse transcript analyses (RT-qPCR) of the main genes responsible for nitrate uptake in *B. distachyon* roots were performed. Rapid induction of both BdNRT2.1 and BdNRT2.2 expression was observed after nitrate resupply. The same pattern of expression induction was observed for BdNRT3.1. ¹⁵N analyses showed induction of ¹⁵N-nitrate influx after nitrate resupply in the initial periods. Co-regulation of the genes coding for nitrate transporters and ¹⁵N influx demonstrates that a two-component complex is active after nitrate resupply, unlike that observed in sugarcane in previous experiments, indicating that the nitrate uptake process in grass roots is unique to each species. We are characterizing 11 T-DNA insertional lines of the NRT2 gene family to be used in further complementation studies of *Saccharum* genes. N analyses **Keywords:** HATS, co-regulation, T-DNA insertional lines. **Supported by:** CAPES

A.37 - Investigation of autohydrolysis of sugarcane bagasse and straw mixture to obtain a hemicellulosic hydrolysate.

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The sugar and alcohol sector can be inserted in the biorefinery model for converting biomass into energy and high-value chemicals. Therefore, biomass deconstruction is necessary to obtain the fractions of interest. Hemicellulose sugars have many applications in different processes, such as biotechnological production of xylitol, and, a common method used for recovering a pentose-rich solution is diluted-acid hydrolysis. However, concern for sustainability directed to the pursuit of green technologies, for example, the process of autohydrolysis, which dispenses the use of inorganics acids. Therefore, the objective of the present work is to analyze the efficiency of autohydrolysis, as an alternative methodology to diluted-acid hydrolysis to obtain a sugarcane bagasse and straw hemicellulosic hydrolysate. A fractional factorial design was elaborated using the Taguchi matrix (L9), with an evaluation of 3 factors: temperature, time and granulometry. A mixture of sugarcane bagasse and straw (1:1) was transferred to a rotating reactor with 650mL of working volume, a solid-liquid ratio of 1:10 (w/v). The liquid fraction was recovered (hemicellulosic hydrolysate) and the solid fraction was characterized to quantify the chemical composition of the remaining biomass. The determination of sugars was performed by high-performance liquid chromatography and the soluble lignin by UV-VIS spectrophotometry at 205nm. The results indicate that the acetyl, arabinosyl and xylan groups of hemicellulose were the most solubilized components in the tests, while lignin was not solubilized by the autohydrolysis process. The maximum sugar removal was 57.2% xylan and 84.2% arabinosyl, corresponding to the higher temperature condition, which had the greatest effect among the variations tested. Also, the observed decrease in hydrolysate pH values explains the solubilization effect of acetyl groups responsible for biomass autocatalysis. Thus, how the predominantly solubilized obtained was a hemicellulosic fraction, the results characterizing autohydrolysis as an adequate process for the utilization of its sugars constituted in several bioprocesses.

Keywords: sugarcane bagasse and straw, autohydrolysis, hemicellulose

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A.38 - Morphological and agrotechnological analysis of *Saccharum* spp progenies

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The study consisted of field evaluation of morphological and agroindustrial parameters of two energy cane genotypes and 31 progenies obtained from biparental crosses between sugarcane varieties (RB867515, and SP80-3280) and ancestral species including *Saccharum officinarum*, *S. robustum* and *S. spontaneum*. We directly counted the total number of tillers per plot, after which 10 plants of each genotype were randomly selected for the evaluation of height, diameter and weight biomass index estimation. In addition, we also evaluated agrotechnological parameters: Brix, cane pol, juice pol, fiber and purity. Finally, at eight months after planting, plots were also visually evaluated for disease incidence. Regarding morphological parameters, the genotypes C8-22, RB11999, C4-19, C8-27, C8-17, C8-13 presented the highest total biomass indices, while the lowest total biomass indices were observed for C6-20, C6-19, C4-29, C4-24, C6-23. Regarding agrotechnological analysis, genotypes C4-28, SP80-3280 presented the highest sucrose contents; whereas genotypes C6-20, C6-23 and IN84-58 presented the highest fiber content. Finally, we have observed that 36% of the evaluated plots exhibited symptoms of brown rust; genotypes C8-16 and C8-22 presenting the highest score average. In addition, 24% of the plots presented incidence of Orange Rust and the genotypes that showed the highest scores averages were C3-07 and C3-17. It was also possible to detect the presence of sugarcane smut, brown spot and SCMV sporadically across the field. This is a preliminary survey of collected data, which will be further explored for detecting genotypes with characteristics desirable for energy cane breeding.

Keywords: Sugarcane, Biomass, Fiber. **Supported by:** FAPESP

A.39 - Genome data mining for sugarcane improvement based on systems biology approach

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Introduction: Sugarcane is the main bioenergy crop in tropical and subtropical areas due to its potential high yield and smaller carbon footprint. One major restriction for sugarcane production is the water deficit. Breeding and biotechnology have important roles in obtaining cultivars with water stress tolerance. The recent availability of the SP80-3280 genome sequenced by our group and collaborators creates an extraordinary opportunity for studying sugarcane genes and their multiple copies. **Objectives:** In this scenery, we propose exploring genome information and manipulating sugarcane genetic architecture by selecting target genes useful for breeding and biotechnology. **Materials and Methods:** We analyzed data on sugarcane Transcriptomics, Genomics, and Physiology using a Systems Biology approach, increasing the chances of an assertive choice of candidate genes to be employed in biotechnology. Data mining was performed in field and greenhouse experiments with five sugarcane varieties subjected to drought conditions. A transcriptome dataset of 2889 drought-responsive SAS (Sugarcane Assembled Sequences) identified in three different experiments was used in the sequential steps of filtering data. Fifty-six SAS were selected based on their expression profile in sugarcane with distinct adaptability levels evaluated genotypes. Subsequently, each SAS was aligned to the SP80-3280 genome, ranging from 2 to 134 predicted genes. Ultimately, SAS matching up to 6 genes had their CDS and 5' UTR regions investigated to identify the distinction between copies. **Results:** Based on this pipeline analysis, two up-regulated SAS were selected as candidate genes, and their differential expression was confirmed by qPCR. Gene coding sequences were synthesized and inserted in the pGVG vector. SP80-3280 sugarcane *calli* were transformed via *Agrobacterium tumefaciens* and plantlets were regenerated. Over 180 putative events were obtained. **Conclusion:** The data mining of candidate genes was efficient. The effectivity of transformation will be assessed, and other candidate genes will be selected for future transformations.

Keywords: cisgenesis, drought, transcriptome

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A.40 - The SUSFACE Project Scientific Support for Agricultural Conversion to Energy Crops

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The Italian Ministry of Agricultural, Food, Forestry and Tourism Policies, acknowledging the increasing importance of the agro-energy sector, has committed significant resources by financing several research projects of national interest, with the aim of making available to the agricultural and agro-industrial entrepreneur knowledge and technologies to support the change in the organizational structure of the farm in order to foster the production of bio-energies in the agricultural sector. The SUSFACE Project, developed to meet the demand, advanced by the industrial properties promoting the reconversion of the sugar beet-sugar sector, aims to produce and make available technological innovations related to the production of both herbaceous and arboreal energy crops and the realization of prototypes and ready to use harvesting machineries, without which it would not be possible to reach the production of biomass to feed the processing plants at low costs. The project took into consideration agronomic, technological and mechanical aspects of fast-growing tree species (poplar, robinia, eucalyptus) and herbaceous oil species (rapeseed, sunflower, Brassica carinata, soya) and ligno-cellulosic herbaceous plants (common reed, sorghum from fiber, hemp). Once the research phase has been completed and the activities envisaged by the Project have been reached, the experimental results have been made available to research users (category associations, agricultural and agro-industrial entrepreneurs, agricultural machine manufacturers) and to the scientific community through an intense dissemination activity diversified according to the audience for which they are intended. Specifically, in order to comply with the need to transfer the results, an information dissemination system has been developed to use all the means available for each individual aspect studied. The model developed during these years of the project was aimed at producing the technological innovations requested by stakeholders and communicating the results with different forms of dissemination. This model overcomes the difficulty to translate the research results into innovative processes and products able of respond promptly to the needs of the agricultural and agro-industrial enterprise.

Keywords: Research projects, Energy crops, Technology transfer

Supported by: CREA Research Centre for Engineering and Agro-Food processing

A.41 - Effect of OFMSW on biochemical methane production of vinasse sludge from mesophilic anaerobic digestion process

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The amount of municipal solid waste (MSW) produced in Brazil has been increasing over time and their inappropriate destination can cause serious environmental problems. An alternative treatment is the biogas production by anaerobic digestion (AD), which allows recovery from methane content in biogas apart from the waste suitable disposal. The aim of this work was to evaluate the Biochemical Methane Potential (BMP) and the anaerobic digestibility of the Organic Fraction of MSW (OFMSW) in Campinas city (state of São Paulo, Brazil). MSW was classified into four fractions: paper (PFW), garden (GFW), food (FFW) and mixture of these three (OFMSW). The organic fraction accounted for 67.7% of the MSW. Food waste presented the highest ratio of volatile solids (VS) to total solids (TS) (96.1%) and the highest theoretical methane potential (507 NmL CH₄ gVS⁻¹). However, the greatest experimental value and biodegradability reached was from the mixture (OFMSW), 410.7 NmL CH₄ gVS⁻¹ and 86.6%, respectively. This study showed that Campinas has the potential for the production of methane from its waste. In addition, OFMSW studied was more advantageous than the other fractions analyzed since it was the one that had the best performance in methane production using AD. Besides that, this study showed an opportunity to treat and reduce MSW in landfills.

Keywords: Municipal Solid Waste, Anaerobic Digestion, Biochemical Methane Potential

Supported by: CAPES

A.42 - Gene expression analysis of sugarcane genotypes contrasting for biomass production

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The interest in bioenergy crops, such as those belonging to the Saccharinae subtribe, has increased due to improvements in technologies related to the production of cellulosic bioethanol. In this scenario, sugarcane stands out as one of the world's leading biomass crop with vast potential to mitigate climate change effects without affecting food security. The myriad of products that can derive from sugarcane biomass has been driving breeding programs towards varieties with a higher yield of fiber and a more vigorous and rustic performance: the energy cane. In order to obtain genotypes contrasting for biomass production, we crossed the commercial sugarcane variety SP80-3280 and IN84-58, a *Saccharum spontaneum* genotype. Plants of SP80-3280, IN84-58 and eight interspecific hybrids were planted under field condition. All genotypes were evaluated for culm height, culm diameter, stalk number, total biomass production and fiber and sugar content. In addition, tissue samples from the +1 leaf and upper and maturing internodes were collected from three biological replicates when the plants were 4, 8 and 12 months of age. A total of 240 stranded libraries were prepared and sequenced on the Illumina platform to obtain 150 bp paired-end reads. Clean reads from all samples were aligned to the SP80-3280 and *S. spontaneum* transcriptomes using Salmon. It was possible to quantify the expression levels of over 200,000 transcripts and Multidimensional scaling plots highlights tissue-specific expression patterns. Metabolic pathway enrichment analysis of differentially expressed genes points to changes in amino acid, lipid and carbohydrate metabolism among genotypes contrasting for biomass production. In addition, these changes are potentially regulated by phytohormones, such as ethylene, jasmonic acid, gibberellin and abscisic acid. Finally, co-expression analysis revealed AP2/ERF, NAC, MYB, GRAS and ARF transcription factors family members as putative regulatory elements underlying plant growth and biomass production in sugar/energy canes.

Keywords: Biomass, Transcriptome, Co-expression. **Supported by:** FAPESP

B - Biofuel Technologies, including biomass process engineering and biofuels production

B.01 - Use of electronic nose to evaluate the biodiesel oxidative stability

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Biodiesel is a renewable fuel that has been considered an important alternative to petroleum derivatives. Despite its advantages, biodiesel has a higher instability in relation to degradation process when compared to mineral diesel, due to the difference in chemical composition. Degradation reactions are commonly related to moisture, heat and exposure to air (oxidation). In this way, it is essential to monitor the biofuel quality. This work aimed to evaluate the use of electronic nose to determine the oxidative stability of biodiesel. The data obtained from the olfactive analysis were correlated with the results of the Rancimat method, described as official by international standards. The recognition of the signal behavior of the electronic nose sensors was performed through the differential stochastic modeling. The biodiesel was synthesized from waste cooking oil (WCO), previously characterized, in a batch reactor at 60 °C with 500 rpm mechanical agitation, using ethanol as acylant agent at molar ratio of 9:1 and 1% w/w potassium hydroxide (KOH) as catalyst. The biofuel samples were analyzed following standard methodologies (acidity, density, viscosity, ester content, olfactive profile and oxidative stability) and stored in AISI 1020 carbon steel and high-density polyethylene (HDPE) tanks. Analyses were also performed after 30 and 60 days of storage in order to monitor and verify change in the biodiesel properties. The results showed that after 60 days, the biodiesel has high degree of degradation and this process was more intensified in samples storage in carbon steel tanks. Moreover, the interpretation of the results obtained through the stochastic model revealed the existence of relationship between the olfactory profile and the oxidative stability measured, confirming the possibility of using the electronic nose as an alternative to the conventional techniques.

Keywords: Biodiesel, Electronic nose, Oxidative stability

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B.02 - Can yeast ferment cellobiose at high rates?

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Engineered yeast chassis are frequently used for the industrial production of biofuels and chemicals. However, after some initial metabolic engineering steps, further optimization of the microbial host is usually required to achieve high product yields and productivities. In this work, we aim to improve the conversion of cellobiose into ethanol by *Saccharomyces cerevisiae*. For this purpose, we first metabolically engineered five yeast strains by expressing a cellobiose transporter from *Neurospora crassa* and five different intracellular β -glucosidases from *Aspergillus niger*, *N. crassa* and *Podospora anserina*. However, since these strains displayed poor growth on cellobiose and low ethanol production, we subsequently submitted the engineered strains to laboratory evolution, via serial transfers in Falcon tubes containing synthetic media with 20 g/L initial cellobiose at 30 °C and 200 rpm. To evaluate the behaviour of the maximum specific growth rates (μ_{max}) throughout the course of evolution, growth profiles of cells isolated from single-colonies at different stages of the experiments (the “fossil record”) were obtained from microplate cultivations. After several cycles in cellobiose-containing media (between ~45 to 162 generations), single-colonies originated from these five strains reached $\mu_{max} > 0,1 \text{ h}^{-1}$, representing an important growth improvement over the parental strains. In spite of these increases, when two of the evolved phenotypes were characterized in shake-flask cultivations, cellobiose was completely consumed in ~22 h of cultivation, but no ethanol formation was observed, indicating respiratory metabolism. This behaviour was also observed for other evolved isolates selected from the microplate cultivations and is also supported by high biomass yields on cellobiose (between 0.55-0.61 gDW/g cellobiose). The physiological analysis of the remaining three evolved strains is still on progress and we also aim at sequencing the evolved phenotypes, once they start producing ethanol.

Keywords: Yeast, Cellobiose, Laboratory Evolution. **Supported by:** CAPES and FAPESP

B.03 - Cane bagasse combustion in reactor of Fluidized bed: modeling and simulation of the Nox emissions

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Introduction: A model is derived to predict the NO_x emission from a fluidized bed combustor burning biomass. The model is based in the general two-phase theory of fluidization and a two consecutive gas phase. The main characteristics of the model were the prediction of Nox formation, essentially generated from fuel-bound Nitrogen, from material balances. The numerical solution of these balances allows the No_x concentration profile to be predicted for various operating situations of the fluidized bed combustor: fluidization velocity, fluidized bed static height, particle diameter and combustor temperature. Two factors have been identified as crucial to the success of the model: (a) The relative importance of model parameters (eg means bed bubble size) and (b) mass transfer rate between bubble and emulsion phases. . The model was also tested for its sensitivity to changes in hydrodynamic parameters. Objectives: In this work, the importance of NO and N₂O formation was investigated with the formulation of a one-dimensional model for simulation of biomass combustion (sugarcane bagasse) in fluidized bed. The main characteristics of the model were the prediction of the formation of NO_x essentially generated from fuel-bound nitrogen from material balances. materials and methods: In the formation NO from fuel N in the as phase it is now generally that primary fuel N is first converted into a series of secondary intermediate N compounds (NO, N₂), which are then subject to a step parallel consecutive reaction mechanism. Conclusions The model was well developed to predict the fluidized bed sugarcane bagasse NO emissions. Conversion increases with excess air and concentration increases with increasing temperature. It was also evaluated the effect of bubble size parameter on NO concentration, it was observed that increasing bubble size decreases NO emission. And that emissions increase with increasing magnitude of the mass transfer coefficient. Keywords: sugarcane bagasse, fluidized bed combustion, emissions Nox Acknowledgements CNPq and CAPES.

Keywords: sugarcane bagasse, fluidized bed combustion, emissions Nox

B.04 - Evaluation of application of high pressure homogenizer in sugarcane bagasse as a pretreatment strategy to fermentable sugars production by enzymatic hydrolysis

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High Pressure Homogenization (HAP) is a non-thermal processing consisting of the high pressure pumping of a fluid through a narrow opening. The high pressure applied can provide desired modifications in a chemical structure of the lignocellulose biomass favoring the access of enzymes. The objective of this work was to evaluate changes on chemical composition of sugarcane bagasse (SB) after the application of HAP to be used as a pretreatment step to produce fermentable sugars by enzymatic hydrolysis. A central composite rotatable design (CCRD) was used to determine the optimal process conditions for the process of HAP application on SB. Two factors were evaluated, pressure (15-115 MPa) and temperature (19–62°C), with 2 axial points and 3 repetitions of the center point, totaling 11 runs. The dependent variables (responses) were % of Hemicellulose removing, % of cellulose and hemicellulose content in the solid after the process. Under optimized conditions enzymatic hydrolysis experiments were performed on pretreated biomass using two commercial enzyme cocktails separately, Cellic[®] CTec2 and HTec2 at 50°C, pH= 5.0 for 48h with an enzyme loading of 10 FPU/g. After this period, the supernatant was collected for analytical quantification of glucose and xylose by high-performance liquid chromatography (HPLC). Tests under high pressure and temperature (115 MPa and 62°C) favored the removal of hemicellulose reaching values of up to 21.29% removal, and consequently increased the cellulose content of 33.76 % to 36.67%. However, the mild conditions (30 MPa, 35°C) preserved both sources. Collectively, these findings demonstrated that the HAP can be considered an excellent pretreatment strategy to obtain satisfactory cellulose conversion yields in glucose if applied under high pressure and temperature conditions on the process as well to obtain higher values of hemicellulose in xylose conversion if applied under mild conditions of temperature and pressure. **Keywords:** sugarcane bagasse, pretreatment, enzymatic hydrolysis

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B.05 - Influence of glycine oxidase enzymatic activity on n-butanol production by *Saccharomyces cerevisiae*

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Butanol is considered the best substitute for gasoline because it has more physicochemical characteristics similar to gasoline when compared to ethanol. Furthermore, similar to ethanol, butanol can be used as a starting material or solvent in industrial reactions. However, although *Saccharomyces cerevisiae* yeast is capable of producing butanol, the low titers are still much lower than ethanol production, and to increase this production the yeast needs to be genetically modified. The aim of this work was to verify the influence of glycine oxidase gene (*goxB*) insertion in different *S. cerevisiae* strains on the increase of *n*-butanol production and enzymatic activity. The strains were transformed with the pYX212 *goxB opt* vector according to the lithium acetate (LiAc) method. Glycine oxidase activity was assayed spectrophotometrically via determination of hydrogen peroxide with an enzyme-coupled assay using horseradish peroxidase and *o*-dianisidine. The *n*-butanol production was evaluated after cultivation in synthetic medium (glucose 20 g/L, glycine 15 g/L and urea 2.3 g/L) in a microplate reader (Infinite[®] M200, Tecan, Männedorf, Switzerland) at 30 °C and 198 rpm for 72 h. The three transformed strains showed significant increase in *n*-butanol production, with 37%, 17% and 17% increase for CEN.PK113-7D, UFMG-CM-Y260 and UFMG-CM-Y267 strains, respectively. In addition, the strains also showed an increase in enzymatic activity, especially the modified CEN.PK113-7D strain which was able to produce 13.63 mg/L *n*-butanol with relative enzymatic activity of 152%. The results showed that the increase in *n*-butanol titers was directly proportional to the increase in glycine oxidase activity, which acts in the first step in the *n*-butanol conversion pathway using amino acid glycine as co-substrate. Although still presenting low *n*-butanol titer, in this work it was demonstrated that the insertion of the encoding gene for glycine oxidase enzyme has an influence on the increase of this alcohol production. **Keywords:** *Saccharomyces cerevisiae*, Butanol, Glycine oxidase. **Supported by:** FAPESP 2015/20630-4 and 2019/08542-3

B.06 - Ternary blends of renewable fast pyrolysis bio-oil, advanced bioethanol and marine gasoil offer potential to reduce GHG emissions in maritime transport sector

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Maritime transport sector is responsible for 90% of global trade and almost 15% and 13% of SOX and NOX worldwide emissions, respectively. The most feasible ways to reduce its environmental impact are by increasing energy efficiency of the vessel engines, reducing fuel consumption, and replacing fossil marine fuels (marine gasoil - MGO) by biofuels. A potential alternative to reduce pollutant emissions of MGO is blending it with bio-oil from fast pyrolysis of biomass. However, bio-oil is not miscible with MGO due to its high oxygen and water content. Despite the immiscibility of bio-oil and MGO regard to their difference of polarity, adding solvents as alcohols could improve blend miscibility. The objective of this study was to investigate the miscibility of two different ternary systems of bio-oil, MGO and bioethanol in different blend compositions. After blends reached the miscibility regions, the second objective was to investigate its fuel properties and to compare them to the standard fuel properties of MGO. The first system was build based on bio-oil from energy cane (BOECane), and the second system was build based on bio-oil from Eucalyptus (BOEuc). The Brazilian sugarcane bioethanol was chosen as solvent due to its potential to increase the miscibility and its benefits on reducing GHG emissions, since bioethanol is considered an advanced biofuel. Phase diagrams and binodal curves of ternary systems were built to represent the transition between heterogeneous and homogeneous regions. Finally, four homogeneous blends of BOEuc -bioethanol-MGO were characterized for the most important fuel parameters: water content, flash point, low heating value, viscosity and acidity. The best potential blend for application in marine engines was 10:60:30, which reached LHV of 28,85 MJ/kg, viscosity of 6 cSt, flash point of < 25°C, water content of 3 wt% and acidity of 8 mgKOH/g. **Keywords:** fast pyrolysis bio-oil, marine fuel, GHG emissions. **Supported by:** CAPES and U.S. Navy

B.07 - Butyric acid production from sugarcane straw hydrolysates by *Clostridium beijerinckii* Br21

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Butyric acid is widely employed in the chemical, pharmaceutical, and food industries. This compound is currently obtained from petroleum-derived raw materials, but it may become commercially more attractive if it is produced from renewable substrates such as lignocellulosic biomasses. In this context, we have used sugarcane straw (SCS), which is one of the main residues of the sugarcane industry, as a potential substrate. More specifically, we have pretreated and hydrolyzed SCS to obtain butyric acid by a sustainable process based on *Clostridium beijerinckii* Br21, a non-solventogenic strain. First, we conducted an experimental design to optimize the SCS pretreatment conditions (temperature and H₂SO₄ concentration) by microwave, assisted by acid. SCS was pretreated in a Multiwave PRO SOLV reactor 50 Hz (Anton Paar GmbH, Austria). SCS and H₂SO₄ solutions were mixed at a solid to liquid ratio of 7.5% w/v so as to maximize fermentable sugar recovery. After optimization, the liquid fraction was directly submitted to fermentation (hydrolysate 1). The pretreated solid fraction was submitted to enzymatic hydrolysis (10% w/v - Cellic CTec2 15 FPU/g) followed by fermentation (hydrolysate 2). Enzymatic hydrolysis of a mixture of the solid and liquid fractions was also performed before fermentation (hydrolysate 3). All the assays were carried out under anaerobic conditions in a 1.5-L Biostat B Plus reactor at 35 °C, 135 rpm, and controlled pH (6.0 ± 0.1); the assays were run in duplicate. The best pretreatment conditions were 162 °C and 0.61% H₂SO₄ (w/v), which provided 72.24% of total sugar recovery. The highest concentration of butyric acid, 14.35 g/L, was attained during fermentation of hydrolysate 3 (pretreated SCS mixed fractions) and represented a yield of 95.9% as compared to the expected theoretical yield. In all the assays, butyric acid was the only product resulting from the fermentation of pretreated hydrolyzed SCS, an abundant residual biomass, by *C. beijerinckii* Br21. Moreover, it was not necessary to separate the solid and liquid fractions, which makes the process even more attractive.

Keywords: lignocellulose, microwave, pH controlled

B.08 - Cellulase production by submerged fermentation using a wild strain of filamentous fungi isolated from Brazilian tropical forest soil

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Filamentous fungi are aerobic and chemoorganotrophic microorganisms widely distributed in nature, present in soils under different vegetations. Due to its metabolic versatility, fungi are capable of producing different enzymes, as cellulases, with potential for biotechnological purposes, and can be applied in the textile, food and bioenergy industries. Cellulases are carbohydrases produced by several microbial groups, especially filamentous fungi, capable of solubilize cellulose fiber to cellobiose and glucose. Therefore, the present study aimed to evaluate the production of cellulases by wild strain *Trichoderma* sp. I14-12, isolated from soil of Itatiaia National Park. The strain was initially grown in a fermentative medium containing 2.0% of sugarcane straw (SCS) as a main carbon source and 1.0% of corn steep liquor (CSL) as a main nitrogen source. The fermentation system was incubated for 5 days, 200 rpm at 28°C and aliquot was taken every day to determine kinetics parameter of enzyme production. The best time for endoglucanase (CMCase) and total cellulase (FPase) activities was the third day, when the enzyme activities observed were 1,283.2 U/L, for CMCase and 416.38 U/L for FPase. After determining the best enzyme production time (3 days), an experimental design was performed using SCS, CSL and temperature (°C) as independent variables, according to Central Composite Rotational Design (CCRD). The highest activity for CMCase (3,701.17 U/L) was obtained when the condition 2.0% (w/v) SCS and 1.0% (w/v) CSL at 28°C was used, while for FPase (553.43 U/L) was observed when the condition 3.0% (w/v) SCS and 1.5% (w/v) at 31°C was used. This study suggests the biotechnological potential of new strain *Trichoderma* sp. I14-12 in producing cellulase from agroindustrial by-products.

Keywords: sugarcane biomass, cellulases, filamentous fungi. **Supported by:** CNPq

B.09 - Characterization of invasive aquatic plants for bioenergy production

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In 2018, the consumption of energy and greenhouse gases emissions increased by 2.3% in the world. To reduce environmental impact, renewable energy sources with competitive prices have been preferred to non-renewable sources. A promissory resource available in Brazil is lignocellulosic biomass, such as aquatic plants, forestry and agro-industrial residues. The main advantage of biomass is its low-cost for energy production. In the region of Três Lagoas, aquatic plants, as macrophytes, there exist in large amount and have a high growth rate. The accumulation of plants into the hydrological systems have harmful effects on the aquatic ecosystem and to human activities, such as navigation and hydraulic energy generation. In 2017, the company CTG Brazil suffered a financial impact of BRL 3.8 million due to the presence of macrophytes at the reservoir, resulting in 10 turbines affected. Thus, the adequate destination of macrophytes for the generation of economic and environmental benefits is a promising alternative. A possible solution is biomass conversion through thermochemical route, obtaining energetic products, such as bio-oil, biochar and gas. This work aims to compare two different types of aquatic plants (*Typha domingensis* and *Eichhornia crassipes*) by analysis physical-chemical and pyrolysis yields at different temperatures. The results for moisture, volatiles, fixed carbon and ash content for dried samples of *T. domingensis* were 7.67%; 85.14%; 2.64 and 4.54%, respectively, for *E. crassipes* were 4.14%; 71.19%, 18.07% and 6.31%, respectively. The elemental analysis results were 47.38% C; 5.95% H; 1.15% N; 39.95% O for *T. domingensis* and 37.03% C; 4.98% H; 1.62% N; 35.83% O for *E. crassipes*, both plants did not present significant amounts of S (sulfur). The higher heating value were 19.82 MJ/kg for *T. domingensis* and 15.77 MJ/kg for *E. crassipes*. The bio-oil (35.65%) maximum yield was obtained for *T. domingensis* and the biochar (43.05%) was for *E. crassipes*, both at 400°C. **Keywords:** biomass, pyrolysis, bioenergy. **Supported by:** ANEEL, CTG Brasil, SENAI, SISTEMA FIEMS, EMBRAPPII

B.11 - Glycerol esterification for fuel additive production using ionic liquid as a green catalyst

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The majority of the energy used by humankind comes from fossil fuels – a limited resource that promotes harmful effects on the environment due to greenhouse gas emissions. As an alternative, scientists defend the use of biofuels as an energy source, stating it is possible to promote the reduction of carbon monoxide, hydrocarbons, sulfur, and aromatic compounds emissions to the atmosphere. In this scenario, a significant compound takes place: glycerol. Recognized as a substantial solid residue generated during biofuel production, it is mostly applied in pharmaceutical, food, and cosmetic industries. Furthermore, glycerol production increases as biofuel production intensify. Although having many applications, authors note a rising concern regarding glycerol production due to its large volume and low market absorption, making it necessary to promote new consumption routes for this material. One of these is triacetin production, which can be performed by the esterification of glycerol. It has been tested as a fuel additive for gasoline, diesel, and biodiesel, allegedly enhancing its cold flow, reducing the emission of hydrocarbons and carbon monoxide particles, and increasing the octane rating. Esterification reactions of glycerol along with acetic acid and ionic liquid as the catalyst were performed in a batched reactor at autogenous pressure. To investigate the adequate reaction parameters, a Design of Experiments` (DoE) was approached, ranging a series of parameters such as temperature, glycerol/acetic acid ratio, and catalyst percentage. The final analysis were achieved with gas chromatography and exhibited significant changes in triacetin production along with the parameters ranges, being the most significant one the increasing of triacetin production over the temperature rise. Responses obtained upon varying the parameter values made it possible to state the reaction's ideal parameters, leading to a more significant product formation.

Keywords: fuel additive, glycerol ester, ionic liquid. **Supported by:** CAPES

B.12 - Sugar cane bagasse pretreated by hydrodynamic cavitation and ozonation aiming to produce 2 G ethanol for biorefineries

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Nowadays, the production of biofuels and biomolecules from lignocellulosic materials are among the most important research topics. In Brazil, the development of ethanol production process from sugarcane bagasse (SCB) has great relevance. The economic viability of 2G ethanol production, however, requires the utilization of all fractions of raw material in a biorefinery context, and current research has been directed to the production of this alcohol from glucose present in enzymatically produced cellulosic hydrolysates. However, the utilization of cellulose present in biomass in fermentation processes requires an initial pretreatment step, which results in higher enzymatic digestibility of this polysaccharide in the subsequent hydrolysis. Even after many studies reported in literature, pretreatment remains as a critical bottleneck in biorefineries and, thus, evaluation of new alternatives is important for viability of the use of biomass. In our work, advanced HC (hydrodynamic cavitation) assisted oxidative processes were evaluated as a new approach to favor enzymatic hydrolysis of SCB carbohydrate fractions. HC-assisted technology was assessed considering the use of peroxide and NaOH along with the variation in ozone inlet flow to the reaction medium. The composition of the pretreated material showed the use of ozone in the presence of HC-assisted peroxide was effective in reducing SCB lignin content, removing until 41% of this fraction. This alternative was attractive considering the yield of glucan enzymatic hydrolysis reached up to 77% in 24 h in a subsequent enzymatic hydrolysis of biomass pretreated using 1% of peroxide and 76 mg/min of ozone inlet. Regarding to xylan, it was also hydrolyzed in the enzymatic step (hydrolysis yield of until 80% in 24 h), considering only 1% to 4% of this fraction was removed from SCB in pretreatment and the used commercial enzymatic preparation had xylanases. Obtained results showed the high potential of the technology for the pretreatment of biomass, helping to overcome one of the most important challenge in biorefineries development.

Keywords: Biofuels, Hydrodynamic Cavitation, Oxidative Process

B.14 - Deacidification of Amazonian oils by ethanol solvent extraction

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Vegetable oils typical from the Brazilian Amazonian region have been found to be potential alternative matrices for the oil industry. However, its use for biodiesel and edible oils production requires a series of refining steps. Among those steps, the removal of free fatty acids (FFA) is quite important because it determines the oil's quality, having a crucial economic impact on oil production feasibility. In this study, the solvent extraction was used as an alternative technique for the deacidification of pracaxi and patawa oils. Anhydrous ethanol and mixed hydroalcoholic solvents (ethanol + 6% and isopropanol + 13% of water mass) were used for extracting FFA from the oil phase. Experimental liquid-liquid equilibrium data for the systems vegetable oils + oleic acid + solvents were determined and correlated using thermodynamic equations (NRTL and UNIQUAC). The results obtained here showed that the ethanol is effective in removing FFA from the oil phase, since its use offers high FFA distribution coefficients. On the hand, it was found that the addition of water to the ethanol reduces the loss of neutral to the alcoholic phase, improving the solvent selectivity. Selectivity values obtained by the use of ethanol + water solvent were found to be three times higher than those values obtained using isopropanol + water solvent. Although the addition of water reduced the solvent capacity of extracting FFA, the loss of neutral oil was highly suppressed by the water content in the solvent and the heterogeneous region has been largely increased. Accordingly, NRTL and UNIQUAC models showed good correlation capacities (mean deviations of 0.68 and 0.66%, respectively), generating representative parameters for the evaluated systems. In conclusion, the results shown in this study endorse the use of liquid-liquid extraction for deacidifying vegetable oils and corroborate to extend the use of this technique for refining Amazonian oils. **Keywords:** liquid-liquid extraction, liquid-liquid equilibrium, vegetable oils. **Supported by:** FAPESP (207/16979-7, 2018/15737-2, 2014/21252-0, 2016/08566-1, 2017/23670-2), CNPq (305870/2014-9, 406963/2016-9) and CAPES (001)

B.15 - Use of response surface methodology to optimize the parameters of transesterification reaction from palm olein using Purolite A503 anionic resin as heterogeneous catalyst

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In the literature, studies involving the production of biodiesel using heterogeneous catalysts have been highlighted due to the advantages of being easily separated for reuse, reducing the steps of product purification. Taking this into consideration, this paper studied the performance of the Purolite A503S basic strongly anionic resin as a heterogeneous catalyst in the transesterification reaction for ethyl biodiesel production and to optimized the independent variables by the maximum conversion in ethyl esters. For the optimization, was used the response surface methodology (RSM) coupled to a factorial design (2³) with rotational central composite design (RCCD). The interactions of the effects of temperature, catalyst percentage and oil:ethanol molar ratio were evaluated using the 10 hours reaction time established by long reaction kinetics and stirring speed of 400 rpm determined by evaluating the conversion potential varying 250-1000 rpm. The reactions were performed in a jacketed reactor coupled to a thermostatic bath and at the end of the reactions, an aliquot was collected to calculate the conversion to biodiesel through quantifying the triacylglycerols, diacylglycerols, monoacylglycerols, ethyl esters and ethanol by the HPSEC method and glycerol by stoichiometry. After the reaction, the resin was separated and washed with ethanol and analyzed by nuclear magnetic resonance (NMR) and scanning electron microscopy (SEM) before and after the transesterification reaction. Through these analyses, it was possible to characterize the resin and identify the adsorption of fatty components in the resin. From the 17 factorial design trials, the results were adjusted to a second order model predictive and significant to explain the experimental data. From the optimized variables, a conversion of approximately 98% to ethyl esters was obtained. In conclusion, despite the long reaction time, Purolite A503S resin has proven to be a heterogeneous catalyst very useful for use in transesterification reactions. **Keywords:** transesterification, ethyl ester, ethanol. **Supported by:** FAPESP, CNPq and CAPES

B.16 - Energy efficiency evaluation of the supercritical water gasification of microalgae cultivated in vinasse

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Introduction: Microalgae are microorganisms that can be cultivated in agro-industrial residues like sugarcane vinasse, that can be used as a culture medium and nutrient source for their growth. With high lipid or carbohydrate content and an elevated growth rate, microalgae become an interesting good biomass feedstock for biofuel production. The supercritical water gasification (SCWG) is a promising technology to syngas production from wet or diluted biomass as feedstock like microalgae, avoiding the large amount of energy consumed to drying process. Although existing works assesses the process conversion efficiency, it remains to be verified its energy efficiency of the whole production systems, an important parameter when producing a biofuel. **Objectives:** This work aimed to evaluate the operational parameters influence in the syngas composition and the energy efficiency of the process. **Materials and Methods:** Through the process simulation in Aspen Plus software and considering a sludge with 20% microalgae content as the feedstock, the process operating conditions were determined for the production of high methane (case 1) or hydrogen-rich (case 2) syngas, as well as verify their influence on energy efficiency. Part of the obtained syngas was used to provide the energy required to the process. Energy integration is designed to maximize existing energy use. **Results and Discussion:** The results indicate a higher energy efficiency for methane production (55.1%) than the hydrogen-rich syngas production (47.5%). Due to the higher operating temperature of case 2 (750°C) compared to case 1 (450°C), more energy is needed by the process, consuming 41% of the product gas obtained as heat source, against 21% of the case 1. **Conclusions:** Thus, we can conclude that methane production has been shown to be more energy-efficient than hydrogen production for microalgae to syngas conversion.

Keywords: bioenergy, ethanol, pinch technology. **Supported by:** CNPq and CAPES

B.17 - Expression of enzymes involved in the butyric acid production pathway by *Clostridium beijerinckii* Br21

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Butyric acid is a compound of great industrial interest and is mainly employed in the pharmaceutical and food industries. Bacteria belonging to the genus *Clostridium* can produce butyric acid from carbohydrate fermentation. However, butyric acid biosynthesis decreases the medium pH, thereby inhibiting enzymes and increasing organic acid dissociation. As a result, the microorganism growth rate slows down, and substrate consumption is inhibited, to culminate in low butyric acid yields. Thus, pH control is important for butyric acid production. Here, we evaluate how pH control affects the expression of genes related to the butyric acid formation pathway by *Clostridium beijerinckii* Br21. Fermentative assays were conducted in a pH-controlled (pH = 6.4) bioreactor; Reinforced Clostridial Medium – RCM and 60 g L⁻¹ xylose were used. A control assay without pH control was also performed. After the fermentation samples were collected, at 4 and 28 h of fermentation, total RNA was extracted. After cDNA synthesis, qRT-PCR was accomplished for relative quantitation of the expression of the genes *buk* (butyrate kinase), *ack* (acetate kinase), and *hyd* (hydrogenase); helicase was employed as housekeeping. The expression of the genes *buk* and *hyd* increased with fermentation time in the pH-controlled assays, but it diminished in the non-controlled pH assay, which afforded 21.3 and 5.45 g L⁻¹ butyric acid, respectively. On the other hand, the expression of the gene *ack* decreased in the assay with pH control as compared to the non-controlled one. Therefore, controlled pH at 6.4 favors expression of the key enzymes involved in the butyric acid formation pathway by *C. beijerinckii* Br21 because the pH control prevents the expression of enzymes that could reduce the process yield.

Keywords: acetate kinase, butyrate kinase, hydrogenase. **Supported by:** FAPESP 2018/0789-7

B.18 - RNAi sugarcane lines with reduction in Glucuronic acid (GlcA) has higher yield in a saccharification process
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The global interest in the cultivation of sugarcane has increased significantly in recent years due to high demand for renewable energy production. Sugarcane provides high levels of fermentable sugars and its cell wall material, known as bagasse, is of a particular focus for biotechnology approaches. This can include fermentation of monosaccharides released from bagasse into 2G bioethanol. The efficiency of this process is hindered by the recalcitrance of sugarcane biomass to degradation. Recent evidence indicates that the reduction in xylan glucuronidation in eudicots, by mutagenesis of **GlucU**ronic acid substitution of **Xylan** (GUX) enzymes, can be a good strategy to reduce plant biomass recalcitrance. In this work we produced transgenic sugarcane in which we use the RNAi technology to reduce the expression of the sugarcane *GUX2* gene (*ScGUX2*). Using RT-qPCR we established that *ScGUX2* expression was successfully reduced in most transgenic lines. Polysaccharide Analysis using Electrophoresis Gel (PACE) showed a clear reduction in the amount of glucuronic acid on the xylan backbone and saccharification analysis indicates that in selected plants we can observe up to 30% increase in monosaccharide yield with no pre-treatment used. Importantly, reduction in *ScGUX2* expression did not reduce agronomic parameters such as plant height, number and diameter of culms, plant weight and Brix yield. Taken together, our data indicates that downregulation of sugarcane *GUX* expression may bring advantage to the bioenergy industry. **Keywords:** 2g ethanol, biomass, sugarcane. **Supported by:** FAPESP (2017/15895-4), CNPq (140868/2016-0) and CAPES (88887.364139/2019-00)

B.20 - Impacts of iron and nitrogen interplay on Chlamydomonas reinhardtii lipids and biomass production

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The global economy is heavily dependent on fossil fuels, therefore for replacing them, sustainable alternatives are required. There are several reasons why microalgae are one of the best options. One of the most conspicuous characteristics of some microalgae species is their ability to accumulate neutral-lipids, mainly Triacylglycerols (TAGs), which is important for future biofuel production. Although microalgae-based biofuels may provide a viable alternative to fossil fuels, there are a number of obstacles. The serious challenges we face are improving strains in terms of lipid and biomass productivity as these two are currently inversely correlated. Few detailed studies have systematically examined the combined effects of iron and nitrogen on microalgae molecular physiology and none of them deeply investigated the association of these responses to the accumulation of TAGs. To better understand this phenomenon in microalgae, we aimed to study the interplay of the cellular responses under varying iron and nitrogen availability. We selected different combinations of concentrations of Iron and Nitrogen using a Central Composite Rotatable Design (CCRD) and performed a time-course experiment of 7 days in constant light in mixotrophic conditions. We monitored cell growth, lipids and protein content and nitrogen and phosphate consumption. For selected conditions shotgun proteomics analysis was performed. The results showed that high nitrogen concentration is associated with high cell density and the inverse is associated with high lipid accumulation values in all time points ($p < 0.05$). Lipids accumulation in treatments with the same amount of nitrogen (3.675 mM) differs depending on the concentration of iron available. Protein content in all samples except the ones which have high amount of nitrogen were almost more than 3.5 to 2.5 folds lower within 48 hours and remains lower until the end of 144 hours. Our results indicate that high iron concentrations delay lipids production in cells under nitrogen deprivation. The results obtained from biomass characterization, lipids accumulation and proteomics data suggest that there is an interplay of Iron and Nitrogen in the regulation of growth and lipids biosynthesis in microalgae cells, possibly through the modulation of biological pathways related to oxidative stress. **Keywords:** CCRD, Chlamydomonas, TAG. **Supported by:** FAPESP, CNPq and CAPES

B.21 - Direct conversion of organic waste into electricity using a microbial fuel cell: influence of temperature and external resistance

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Introduction: Microbial fuel cell (MFC) is a novel bioelectrochemical system that, by using bacteria as biocatalyzer, directly converts the organic wastes into electricity. However, it still presents limited current generation and high costs that hamper large scale applications. Objective: Aiming at the performance optimization with low-cost electrodes, a novel tubular dual-chamber MFC prototype (1L) was design and assessed for the treatment of agricultural wastewater. Materials and Methods: Three MFCs with external resistance (R_{ext}) of 300 Ω were operated at 24°C, 35°C and 55°C. A fourth MFC at 24°C was operated with R_{ext} of 13 Ω . All MFCs were fed (0.307 L d⁻¹) with a synthetic substrate with initial Chemical Oxygen Demand (COD) of 2.5 g L⁻¹ further increased to 5 g L⁻¹, for 60 and 82 days, respectively, and monitored for physicochemical and electrochemical parameters. Results and Discussion: For treatment performance, with a hydraulic retention time of 33h, all reactors achieved average COD removal efficiency >93%. For electrochemical performance, the lowest anode resistance, between 0.61 and 0.76 Ω , was achieved at 55°C, but its total internal resistance (R_{int}) was the highest (23.4 Ω) due to cathode resistance. The lowest R_{int} (between 7.3 and 8.5 Ω) was observed at 24°C and R_{ext} of 300 Ω . In this condition, the maximum power density was 39.6 W m⁻³. However, the conversion, in terms of coulombic efficiency, was relatively low for the MFC with 300 Ω R_{ext} (R_{ext} with 2.5 gCOD L⁻¹ and 5 gCOD⁻¹, respectively). Conclusions: In conclusion, promising results were obtained for the prototype MFC with low-cost electrodes. Thermophilic conditions increased anode activity but decreased the overall performance due to the high cathode resistance. Despite the greater exoelectrogenic activity with lower R_{ext} , increased energy generation was achieved with higher R_{ext} .

Keywords: bioelectrochemical system, temperature, exoelectrogen

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B.22 - Parastillation reduces energy consumption in bioethanol distillation

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The increasing interest on renewable energy makes Brazil an important country in the global energy scenario, mostly because of its high bioethanol production. Although this is a sustainable process, the bioethanol production has a high energy consumption, associated with the distillation step. Parastillation is a promising technique to reduce energy consumption in distillation. This technique considers the vapor flow division into two or more ascending and parallel streams, which contact with a single downward stream of liquid. The parastillation structure enables the arrangement of a larger number of stages per column height, keeping the same tray spacing used in conventional distillation columns. In fact, a parastillation column with two vapor divisions may present almost twice the number of stages in comparison to a conventional distillation column of the same height; it also has a reduced tray area. In this context, the aim of this work is to present the parastillation as an alternative to bioethanol distillation. Simulations were carried out in MatLab®, adopting the rigorous methodology suggested by Naphtali-Sandholm, originally proposed for conventional distillation simulations. The mixture to be distilled were composed of ethanol, water, isoamyl alcohol, ethyl acetate and glycerol. Results show that the conventional distillation processes can be replaced by parastillation, resulting into energy savings up to 13%, which leads to total annual cost savings of 15%. Maintaining the same energy consumption, the parastillation column may reduce the capital and total annual costs in 16% and 9%, respectively, when compared with the conventional distillation. These results indicate that parastillation columns may be a good alternative to reduce energy consumption and/or investment cost in bioethanol distillation.

Keywords: parastillation, bioethanol, process intensification

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B.23 - BIOGAS PRODUCTION FROM SUGARCANE ENERGY OF DIFFERENT AGES AND PICKS

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The search for more sustainable technologies and clean energies has become more frequent and discussed. Replacing fossil fuels with less impacting options for the environment has been widely researched and, therefore, biogas became an attractive option. Biogas is produced through anaerobic digestion (AD) and this process is capable to convert into biogas a wide variety of biomasses, which are used as substrate. The product of interest in biogas is the methane and, considering this, the objective of this work is to evaluate the biochemical potential of methane (BMP) of sugarcane energy (EC) with different ages and millings. In this study, was selected EC of 5 ages (in months): 4, 6, 8, 10 and 12, and in each of these ages 3 different millings, that were made from this same sample. The base methodology used for biogas production was VDI4630/2006 and, for the characterization tests, was used ALPHA (1999). Until this moment, two experiments have been carried out, obtaining BMP values of 273 and 473 for EC 8 months (1 milling) and microcrystalline cellulose (CM), respectively; and, 395, 368, 384 and 355 for EC of 8 months (1, 2, 3 millings) and CM, respectively. These results are according to the literature, however, the second experiment showed better results. This difference of BMP between these assays with EC of 8 months occurred because, from the second experiment, the anaerobic inoculum was replaced by other with a higher amount of organic matter. The more organic matter present in the inoculum, the more efficient the hydrolysis of biomass will be and therefore higher biogas production will occur. Currently, the assays are following according to the collection of new EC samples, always trying to achieve higher BMP values.

Keywords: biogas, sugarcane energy, methane

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B.24 - Strategic planning of multi-feedstock biorefineries for market equalization in Brazil

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Ethanol as one of the viable solutions to replacing fossil fuels and reducing the climate change impact of the transportation sector is increasingly becoming part of the fuel mix of many nations. Brazil: the second largest producer of ethanol has sugarcane as its principal feedstock for ethanol production, however, increasing demand prompts a need for capital investments in new sugarcane biorefineries which can be expensive coupled with underutilization of biorefinery capacity during the sugarcane offseason. To tackle this, researchers propose the adoption of flex mill concept which is a multi-feedstock biorefinery capable of using more than one feedstock type to produce ethanol. Corn is one alternative feedstock that can be considered in Brazil to meet the increasing ethanol demand while optimizing total cost of retrofitting existing refineries and establishing new flex mills. Although this subject has become subject of discussion among researchers there has not been effort to carry out a strategic plan for the establishment of these flex mills in Brazil. Hence, this work presents a multi-period strategic design and planning model for multi-feedstock biorefineries which composes a supply chain superstructure that combines all the components of the biofuel supply chain. The model presented is a Mixed Integer Linear Programming (MILP) model used to identify regions where investments are required and the optimal configuration of the network while also taking into consideration inventory management and transportation logistics. The application of a dynamic capacity strategy also provides information on strategic capacity additions, expansions, contractions and closures. A case study of Brazil where investments in ethanol bio-refineries is required to equalize the market demand is considered. Results of the case study produce an optimal network configuration with minimal investments in biorefineries and optimal capacity utilization of plants while also ensuring demand satisfaction in all time periods.

Keywords: Strategic Planning, Biofuels, Multi-feedstock biorefineries

Supported by: Petroleum Technology Development Fund

B.25 - Porous CLEAs of lipases co-aggregated with bifunctionalized magnetic nanoparticle in soybean oil ethanolysis

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Biodiesel is an alternative to petroleum-derived fuels that can be produced enzymatically. The use of the enzyme in its immobilized form as highly porous magnetic crosslinked aggregates (CLEA) is a strategy for improving catalyst recovery, low mechanical strength and mass transfer limitations, inherent in this technique. In this work, the preparation of highly porous magnetic (pm-CLEA) of porcine pancreas lipase (LPP) and Eversa Transform (ETL) was carried out to evaluate some strategies to improve the immobilized biocatalyst's volumetric activity, such as the treatment of enzymes with modifying agents. (polyethyleneimine or dodecyl aldehyde), co-aggregation with co-feeders (soy protein (SP)), use of silica magnetic nanoparticles functionalized with amino groups (SMNPs) as a separation aid and starch as pore-making agent. The combined use of dodecyl aldehyde or polyethyleneimine, co-aggregation with SMNPs and/or SP, in the presence of 0.8% starch (followed by starch hydrolysis with α -amylase) produced LPP and ETL pm-CLEAs with yields of immobilization about 100%. In addition, pm-SP-LPP-CLEA and pm-ETL-CLEA exhibited high stability, maintaining approximately 80% of activity at 40°C, pH 8.0 and 10h incubation and 60°C, pH 7.0 and 96h incubation, respectively. Both biocatalysts were applied in soybean oil ethanolysis reaction and demonstrated maximum fatty acid ethyl ester conversion (FAEE) of approximately 65% (oil/ethanol molar ratio 1:7 and 750 TBU pm-SP-LPP-CLEA/g oil) and 80% (oil/ethanol molar ratio 1:6 and 3 Uest pm-ETL-CLEA/g oil) under 35°C conditions, shaking at 1500 rpm for 48h and 24h respectively. Thus, the strategies used in this work provided pm-CLEAs with improvements in intraparticle mass transport as well as being attractive for biotransformation applications due to their good performance in organic media and easy recovery by applying an external magnetic field.

Keywords: magnetic clea, biodiesel, lipases

Supported by: Fapesp, CNPq

B.27 - Soybean oil extraction using ethanol as solvent: effect of moisture of oleaginous solid and temperature of extraction

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Studies have been developed in order to make the soybean oil (SO) extraction process viable with the use of alcoholic solvents, since hexane isomers which are industrially used show considerable human and environmental toxicity. Soybean is the main oilseed produced in Brazil, which is the world's second largest producer of ethanol, a bio-renewable and certified as Generally Recognized as Safe (GRAS) solvent. Thus, Brazil presents several advantages in using this alternative solvent for the hexane replacement to obtain SO, however, for the same yield of SO extraction using ethanol, a flow of 280% higher is needed, compared to that using hexane, and that is a considerable factor that can make the process economically unfeasible. Therefore, this research aimed to study how temperature and solid moisture affect the soybean collets (SC) oil extraction using absolute ethanol. The extraction assays were performed in a fixed-bed column, at 60 and 70 °C, solid:solvent mass ratio of 1:1.5, considering two levels of moisture to SC (3 and 6 mass% of water - SC3 and SC6). It was observed that the increase of the temperature increased the SO in the extract phase as well as the reduction of moisture in the SC. For SC6/60 °C, 7.2% of SO in the extract was achieved; SC6/70 °C, 11.4%; and for SC3/70 °C, 14.7%. The levels of oil in the raffinate phase decreased as long as the temperature was increased, and also, this reduction was more pronounced for SC3. The extraction using SC3/70 °C showed less water content in the extract phase, less residual oil in the raffinate phase and presented the highest oil extraction yield value, 72%, compared to 36 and 48% for the SC6 at 60 and 70 °C, respectively. Thereby, SC3/70 °C can be proposed as a suitable condition for SO obtaining by ethanolic extraction. **Keywords:** ethanol, extraction, soybean

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B.28 - Increasing sugarcane straw pre-treatment efficiency using ionic liquid mixtures at low temperature

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One particularly application of Ionic Liquids (IL) is that for lignocellulose (LC) feedstock pre-treatment. The capacity to break hydrogen bond within LC and selectively solubilize its components are desirable for biorefinery applications. Lignin is a source of potential molecules, which can work as value added by-products to sugar platform convenient from cellulose and hemicellulose. Electricity is also a profitable source of revenue, so pre-treatment process' design must target the lowest energy consumption as possible. This work explored the potential of the ILs 2-hydroxyethylammonium acetate ([Mea][Ac]), 2-hydroxyethylammonium hexanoate ([Mea][Hex]) and their mixture for LC pre-treatment. Dried and milled sugarcane straw (SW) was mixed with IL at 6% (w w⁻¹) solid loading and pre-treated at 90 °C for 12 h without agitation, in convection oven. The chemical composition of residual solids were determined according to NREL methodology. Enzymatic hydrolysis were carried at 5% of solid loading, citrate buffer pH 4.8, 50 °C for 48 h using 10 FPU of Cellic CTec 2 (Novozymes®) per gram of biomass. [Mea][Ac] showed better aptitude to pre-treat SW in comparison to [Mea][Hex], leading to a enzymatic digestion of 69% for cellulose and 54% for hemicellulose, 1.5 and 1.87 folds higher than those obtained from [Mea][Hex] process, lignin remove was around 10% (w w⁻¹) for both. The best result for lignin remove was obtained by the IL mixture (50%, w w⁻¹) pre-treatment, in which 25% (w w⁻¹) of total lignin was solubilized, 2.5 folds higher than pure IL. The enzymatic digestibility of residual solids from IL mixture had the same efficiency of those from pure [Mea][Ac]. The tested ILs exhibited great potential for SW pre-treatment. Their mixture at a ratio of 50% (w w⁻¹) maintained the enzymatic digestibility efficiency and had a better performance on delignification than pure ILs without changing the processual conditions.

Keywords: biomass, ionic liquid, mixture. **Supported by:** FAPESP, CNPq and Be-basic

B.29 - Biodiesel synthesis using liquid lipase kinetic study: The importance of available interfacial area

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The enzymatic transesterification of triglycerides is a reversible reaction whose mechanism comprises a series of successive reactions for the production of alkyl esters of fatty acids and glycerol. Several kinetic models have been developed following the Ping Pong Bi Bi mechanism. However, most of them were developed focusing in the synthesis of biodiesel with methanol using immobilized lipases. When the enzyme is free, it is activated in the oil-water interface, so its kinetics cannot be described with a simple pseudo-homogeneous approach. Furthermore, available models using liquid lipase cannot determine concentrations of involved compounds. In these models, concentrations are calculated as a function of the reactor total volume. However, the relationship between the polar and non-polar phases can alter the reactor volume. The alteration in volume is associated to interfacial area extension, that in itself influences enzyme activity. Also, available models do not consider enzyme solubility. Therefore, for a given enzyme amount and interfacial area, as polar phase volume increases, enzyme activity decreases. A mechanistic model of the kinetics of transesterification of refined soybean oil with ethanol using the enzyme NS-40116 (liquid formulation of lipase *Thermomyces lanuginosus*) was developed considering the relationship between the amount of enzyme given and the interfacial area available. The model describes the enzymatic production of biodiesel for a heterogeneous reaction system predicting the concentration of the main species involved, as well as the influence of changes in the process conditions: oil composition, alcohol/oil molar ratio, amount of initial enzyme and amount of water. The kinetic study of biodiesel synthesis using liquid lipase provided a better understanding of the process and the resulting model can be used to optimize the biodiesel production. **Keywords:** enzymatic transesterification, soluble lipase, interfacial area. **Supported by:** FAPESP (grant #2016/10636-8 - #2018/04933-5) and CAPES (Finance Code #001)

B.30 - Influence of wood particle size of Eucalyptus spp in the torrefaction process

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Given the interest of using biomass as a renewable source for generation of energy, wood is a strong candidate because it is considered a neutral emission biomass. Eucalyptus spp. has a high productivity when coming from a traditional forest (20tDB/ha.year) and a short rotation energy forest (40tDB/ha.year). The objective of this work was to evaluate the influence particle size of Eucalyptus spp. Vale do Paraíba region in the torrefaction process - a thermochemical process that has been used as a heat pretreatment method that improves many of the limitations of in natura wood. The experiment was conducted with three particle sizes: the first being the chip as it comes out of the industrial chipper with size between 18 and 6mm and the other two sizes, the chip mill ground with 9 and 4mm sieve knives with particles between 4 and 0.4mm and 1.4 and 0.25mm, respectively. The samples were submitted to the torrefaction process at 260°C for 30minutes to evaluate the changes in the torrefied biomass. The results showed that there was loss of mass, and the torrefied biomass was 81.6%, 79.1% and 73.9% according to particle size reduction, while the fixed carbon and higher heating value had no significant changes as a function of particle size under study. After the torrefaction process, the fixed carbon increased by 1.38 times and the higher heating value increased by 1.14times compared to the raw biomass. Considering that wood chips rarely come in the same size, and studies have shown that there were no significant changes on the biomass after torrefaction, it is possible to use as raw material the wood chips that comes from the industrial chipping process, and to grind the torrefied biomass form during the milling process leading to energy savings for the purpose of particle reduction to smaller sizes.

Keywords: Biomass, Torrefaction, Renewable Energy

Supported by: Fapesp

B.32 - Evaluation of process alternatives for palm biodiesel production

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Biodiesel is a liquid mixture of alkyl esters obtained mainly through the alkali-catalyzed transesterification reaction of vegetable oils/fats with an alcohol, and has been widely studied as an alternative to substitute petroleum diesel. The utilization palm oil and ethanol as raw material guarantees the possibility of biodiesel production from renewable sources and can be easily coupled to a biorefinery. For large production, continuous process is needed, however only one continuous reactor may not achieve the required conversion. Intermediate separation of glycerol and series reactor association can increase the final conversion. In this context, the objective of this work was to investigate process alternatives for palm biodiesel production using the commercial Aspen Plus® simulator. To represent the palm oil triolein was used. For transesterification simulation, were used the kinetic parameters proposed by Narvaez, Noriega and Cadavid (2015). For liquid-liquid equilibrium the UNIF-LL thermodynamic model was used with a set of parameters proposed by Bessa et al. (2016). The continuous reactors used in this work were the Continuous Stirred-Tank Reactors (CSTR) and a Plug-Flow Reactor (PFR) and for liquid-liquid separation was used the model block DECANTER. An economic analysis was performed for the alternatives that achieved conversion higher than 98.5% using Aspen Process Economic Analyzer (APEA). The results showed that continuous reactors (CSTR and PFR) had conversions below 98.5% (93.9 and 96.0% respectively). Intermediate separation of glycerol presented better conversions when compared to the association of series of 4 reactors with residence time of 5 and 20 minutes. Among the alternatives, we chose to use the series of PFRs with intermediate glycerol separation, besides being cheaper and presenting good conversions (>99.1%) with short residence times (24 min).

Keywords: ethanolysis, biodiesel, process design

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B.34 - Economic and environmental assessment and optimization of advanced liquid biofuels production via combined gasification and Fischer-Tropsch synthesis integrated to Brazilian sugarcane mills

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The mitigation of greenhouse gas emissions (GHG) has become a subject of high importance at a global level. Large-scale deployment of both biochemical and thermochemical routes for advanced biofuels production can contribute to Brazil's compliance with the commitments assumed at the 21st Conference of the Parties (COP 21) to keep global warming below 2°C in relation to pre-industrial levels. Brazilian targets include increasing sustainable bioenergy participation in the country's energy matrix and reducing total GHG emissions. The expansion of advanced biofuels represents an important step to reduce GHG emissions and other relevant environmental impacts, such as land use, water depletion and biodiversity. In this study, we address technoeconomic and environmental assessment and optimization of advanced liquid biofuels production by combining biomass gasification and Fischer-Tropsch synthesis. Biorefinery configurations include the conversion of the residual lignocellulosic fraction of sugarcane (bagasse and straw) and other additional lignocellulosic biomasses, such as eucalyptus wood chips and energy-cane. We also evaluate plant scale, sugarcane season and off-season operation, and the possibility of integrating the thermochemical processes to a first-generation ethanol distillery. In this analysis, we develop simplified mathematical models (metamodels) enabling the assessment and optimization of the economic and environmental sustainability impacts of process alternatives. Both avoided greenhouse gases emissions and economic viability are taken into account for a multi-objective optimization. A pareto frontier shows the best performance possibilities and allows to identify the most suitable biorefinery configuration. Obtained results show that there is an economic benefit due to the economy of scale for the plant. However, our study shows that there is a limit imposed by the increasing transportation cost to collect scattered biomass options. Thermochemical processes may take advantage of the integration with the well-established Brazilian sugarcane mills, which help to mitigate the risks linked to the implementation of new technologies. **Keywords:** biorefinery, optimization, thermochemical. **Supported by:** CAPES

B.35 - Methane production by co-digestion of poultry manure and lignocellulosic biomass: kinetic and energy assessment

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Six typical Brazilian lignocellulosic biomasses (rice straw, corn cob, peanut shell, sawdust, coffee husk and sugarcane bagasse) were evaluated for methane production by solid-state anaerobic co-digestion (SS-AD) with poultry manure. SS-AD experiments were performed with three food to inoculum (F/I) ratios of 4, 2 and 0.5 $\text{gVS}_{\text{food}} \cdot \text{gVS}_{\text{inoculum}}^{-1}$, during 60 days for each condition. An energy analysis was carried out to evaluate the sustainability of the co-digestion process between poultry manure and lignocellulosic biomass; and a metagenomic characterization of poultry manure aimed to evaluate the suitability of the inoculum used in the process. The results showed the highest methane production was obtained with corn cob and poultry manure ($126.02 \text{ Nm}^3 \text{ CH}_4 \cdot \text{ton residue}^{-1}$), following by the co-digestions with rice straw ($113.4 \text{ Nm}^3 \text{ CH}_4 \cdot \text{ton residue}^{-1}$) and sawdust ($109.4 \text{ Nm}^3 \text{ CH}_4 \cdot \text{ton residue}^{-1}$), using a food to inoculum ratio of 0.5, which lowered volatile fatty acids accumulation. In the best condition, the thermal energy production ($1.73 \text{ MJ} \cdot \text{kg live chicken}^{-1}$) would be able to replace 53.2% of the energy with firewood in poultry farming. The high hemicellulose and low lignin content in corn cob seem to explain the biomethanation of such biomass, and this agrees with the microbial analysis which revealed the predominance of bacteria related to plant polysaccharides hydrolysis and carbohydrate conversion in the inoculum. The methane production was best modelled by Groot's multi-stage model, and the microbial adaptation to lignin might explain this.

Keywords: anaerobic co-digestion, lignocellulosic biomass, solid-state fermentation

Supported by: FUNASA, FAPEMIG, CNPq and CAPES

B.36 - LPMO AfAA9_B and cellobiohydrolase AfCel6A from *A. fumigatus* significantly boost the enzymatic saccharification of cellulolytic cocktail

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Several CAZymes were identified in *Aspergillus fumigatus* secretome after growth in sugarcane exploded bagasse, including LPMOs (AfAA9_B) and cellobiohydrolase (AfCel6A). To characterize, these enzymes were expressed in *P. pastoris* X-33 with native signal peptide and purified. The enzymatic properties of AfCel6A were determined with CMC-Na, and the AfAA9_B activities were determined by using 2,6-dimethoxyphenol as the substrate. The AfCel6A exhibited broad substrate specificity, including CMC-Na, Avicel, Xyloglucan and Birchwood xylan. The optimal conditions of AfCel6A is 55-60 °C and pH 5.5-6.0. AfAA9_B optimum temperature is 60°C or higher and pH 9.0. Both enzymes were highly stable at 50 °C. After 72 hours, AfCel6A and AfAA9_B retained 47% and 72% of residual activity, respectively. Additives have different effects on the enzymes activity. Both ones were resistant to SDS, Tween 20, DMSO, CaCl₂ and KCl. Other chemicals analyzed (MnCl₂, CoCl₂, FeSO₄, AgNO₃, MgSO₄, SLS, EDTA, ascorbic acid, DTT β-mercaptoethanol, ZnSO₄ and CuSO₄) had different effects on their activities. The inhibition by products on AfCel6A activity was analyzed after glucose and cellobiose additions. The enzyme retained 80% and 50% of residual activity in the presence of 200 mM glucose and 100 mM cellobiose, respectively. To analyze synergistic effects between enzymes, we evaluated the hydrolytic capabilities of AfCel6A in combination with AfAA9_B and cellulase cocktail (Celluclast® 1.5L) towards CM-Cellulose at different concentrations. Surprisingly, the AfCel6A and AfAA9_B enzyme combinations haven't increased significantly the releasing of reducing sugars. On the other hand, when Celluclast® 1.5L was supplemented with AfAA9_B or AfCel6A, the enzymes boosted by approximately 3.5 and 4.0 times the releasing of reducing sugars after 24 hours reaction, respectively (both when compared to the Celluclast® 1.5L alone). This result suggests a synergy of both enzymes with cocktail. The degree of synergy exhibited is currently being investigated on different biomasses.

Keywords: CAZymes, Cellobiohydrolases, LPMOs. **Supported by:** CAPES

B.37 - Minerals in sugarcane bagasse and straw: where and who are they?

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Introduction The conversion of agricultural residues into chemicals and biofuels has been restrained by several technological problems arising from the high loads of mineral impurities commonly present in the biomass. Wearing out of mechanical equipment is a well-known example of the harms caused by such impurities. **Objectives** In this work, we looked at the minerals present in bagasse and straw, the vast lignocellulosic feedstocks associated with the sugarcane industry. **Materials and Methods** We employed synchrotron X-ray microtomography (μCT) and image analysis to learn about the typical size, shape, and location of mineral particles adhered to the biomass. Moreover, we employed synchrotron micro X-ray fluorescence (μXRF) to investigate the elemental composition of the mineral particles. **Results and Discussion** Our analysis discriminated mineral particles within a characteristic range of size (circa 5-27 μm), found at the surface and inner cracked plant tissues. Soil aggregates were found adhered to the surface of straw with no equivalent observed in bagasse. Soil is the dominant source of mineral impurities in the biomass and we were able to trace a critical relationship between the characteristic type of the biomasses to the sizes of soil particles as well as soil mineralogy. **Conclusions** With the new insights obtained in this work, we expect to contribute for the development of more efficient cleaning ways of agricultural residues from their impurities, paving the way for more efficient industrial deployment of novel biomass valorization technologies.

Keywords: mineral impurities, sugarcane biomasses, soil. **Supported by:** FAPESP

B.38 - Residual gas for butanol/ethanol production by *C. carboxidivorans* in a dual impeller stirred tank bioreactor

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The increasing consumption of non-renewable natural resources and the need for sustainable management of solid waste produced by rising population in urban areas brings an enormous challenge. An important alternative for the disposal of solid urban waste, seeking the exploitation of its energy, would be the thermal process and subsequent valorization of the residual gas generated through gasification into an energy product with higher value. In this context a hybrid technology that combines the use of a thermochemical technology for low-cost production of syngas (mixture of CO, CO₂ and H₂), and biochemical route which consists in the transformation of this gas in biofuels would be a promising strategy. *Clostridium carboxidivorans* is a bacterial specie that derives energy for growth from the conversion of the reduced species, CO or H₂, to organic acids and solvents (ethanol and butanol) by the acetogenic pathway proposed by Ljungdahl and Wood. One of the major challenges in syngas fermentation is concerned to low mass transfer in these gas-liquid systems due to low solubility of its components in water. The increase of gas solubility in culture media may enhance the availability of gaseous substrate to cells, improving both cell's autotrophic growth and product conversion. It has been reported that the addition of Tween® 80 increased the volumetric mass transfer coefficient (kLa) for carbon monoxide in a dual impeller stirred tank reactor. In the present study, a dual impeller stirred tank bioreactor (STB) was used to produce butanol/ethanol from syngas using *C. carboxidivorans*. The addition of 0.15% of Tween® 80 to culture medium to increase the kLa of this system resulted in a significant increase in solvent production. This result indicates a new alternative to increase productivity in STB.

Keywords: hybrid process, syngas, biofuel

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B.40 - Increase of carbohydrate content of *Desmodesmus brasiliensis* microalgae for 3G bioethanol production

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Microalgae are eukaryotic microorganisms considered as the third generation (3G) of feedstocks for the production of biofuels. For its metabolism plasticity, microalgae can have its composition changed with the manipulation of the growth medium and conditions. This study aimed to increase the carbohydrate content of the microalgae *Desmodesmus brasiliensis* using the design of experiments (DOE) strategy in order to modify the standard medium used (BG-11). A Plackett-Burmann design was used to investigate the influence of the pH (7 to 9), nitrogen (0 to 1000 mg L⁻¹), phosphorus (0 to 35 mg L⁻¹) and sulphur (0 to 80 mg L⁻¹) concentration on the biomass growth and carbohydrate concentration. The cultivation experiments were carried out for 21 days with 135 rpm of agitation speed, 24 h of photoperiod and temperature of 26 °C in a shaker. The biomass growth and composition were followed by optical density (684 nm), cell counting (Neubauer chamber) and carbohydrate concentration (phenol- sulphuric method). The highest carbohydrate concentration (0.514 g L⁻¹) was achieved in the central point condition of the DOE with 500 mg L⁻¹ of nitrogen, 17.5 mg L⁻¹ of phosphorus, 40 mg L⁻¹ of Sulphur and pH 8 at the 21th day of cultivation. This condition also presented a high biomass concentration at the end of cultivation (0.989 g L⁻¹), indicating that a good productivity could be reached. The sugar profile of this condition will be investigated for further understanding of the microalgae composition and metabolism. An increase on the carbohydrate concentration was obtained by decreasing the nitrogen, phosphorous and Sulphur concentration in the growth medium, resulting in technical and economic advantages for the 3G bioethanol production.

Keywords: Microalgae, Carbohydrates, Bioethanol

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B.42 - Changes in bacterial community during ethanol production on different stages of productivity

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The presence of bacterial contaminants is common in all stages of Brazilian ethanol production. It is well known that the presence of those contaminants can lead to a decrease in ethanol productivity and cause financial losses for the ethanol industry. Great efforts have been made to identify those contaminants, but the majority of the studies used culture-dependent methods, which only represents a small fraction of the community. A more powerful approach is DNA metabarcoding, but we are unaware of any published work that has attempted to use this tool to explore the relation between the bacterial community and ethanol productivity during industrial production. In this work, we therefore aimed to evaluate if there is any relation between the bacterial community and ethanol productivity, in the industrial environment. Samples from must and fermentation tanks (1 and 3) were collected from an industrial ethanol production plant in Piracicaba region (SP-Brazil), that operated in continuous mode, between August and November 2017. Bacterial communities were identified through metabarcoding of the 16S rDNA gene. Based on the data generated, we were able to observe differences in the bacterial community from samples representing low and normal ethanol productivity. In the must we observe different proportions of bacterial genera. In the fermentation tanks, as expected, we observed a predominance of *Lactobacillus*. However, despite microbes being from this genus, we observe some differences in OTU patterns in Low and Normal productivity samples. Our preliminary results suggest that the must bacterial communities may be related to ethanol productivity rates.

Keywords: metabarcoding, contaminants, fermentation

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B.43 - Proposed use of next generation biofuels for the Brazilian transport sector

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Recently, Brazil has sought a prominent international role in climate change discussions and negotiations. A legal framework was built in the country whose objective is to encourage the use of renewable sources, especially biofuels. However, next-generation biofuels, those produced from lignocellulosic wastes from biochemical and thermochemical processes, are not yet fully used on an industrial scale. Thus, this research proposes, based on a review of the available literature and international experiences, five next-generation biofuels to decarbonize the Brazilian transportation sector: advanced bioethanol, algal biofuels, advanced biodiesel, biomethanol and bio-hydrogen. For this, the main technical challenges for their production were analyzed, such as: efficiency of biomass conversion, the cost and overall viability of the production process. The discussion presents first generation biofuels and the peculiar characteristics of the Brazilian transportation system, in addition to benefits of next generation biofuel, considering their impacts on economic, agricultural, environmental and energy policy. In order to make next generation biofuels production feasible in Brazil, it is necessary to invest in biomass management infrastructure, studies aiming at reducing high production costs (due to lack of mature technology), government initiatives for social acceptance of investments in biofuels and strong political support through combined goals and obligations aiming to replace diesel and gasoline with low carbon fuels for a sustainable solution.

Keywords: Brazil, transport sector, next generation biofuels

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B.44 - Enhancement of single-cell-oil and carotenoids production by *Rhodotorula glutinis* in bubble column bioreactors

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The present study aimed to evaluate the influence of Volumetric Oxygen Transfer Coefficient (k_{La}) and initial substrate concentration (S_0) on the production of single-cell-oil and carotenoids by the yeast *Rhodotorula glutinis* NRRL-Y-12905. The experiments were carried out using face-centered 2^2 full factorial design to evaluate the effects of substrate concentration (70 to 250 g.L⁻¹) and k_{La} (75 to 175 h⁻¹), on the production of lipids and carotenoids. The assays were performed in Bubble Column Bioreactor, with 1.8 L capacity, at 30 °C for 120 h. The culture medium was composed by MgSO₄.7H₂O (1,0 g.L⁻¹), KH₂PO₄ (20 g.L⁻¹), yeast extract (3,0 g.L⁻¹) and (NH₄)₂HPO₄ (3,0 g.L⁻¹) and glycerol (according to the experimental design). Within the evaluated region, the main effects of variables S_0 and k_{La} , as well as the quadratic effect of k_{La} , were statistically significant at 95% confidence level on lipid and carotenoids production. The quadratic effect of k_{La} on lipid accumulation and the interaction effect between k_{La} and S_0 on carotenoids production were also significant. In general, the model showed the increase in k_{La} and S_0 favored lipid accumulation, and that a lipid accumulation greater than 10g.L⁻¹ can be achieved for cultivation performed at S_0 and k_{La} greater than 210 g.L⁻¹ and 150 h⁻¹, respectively. For carotenoids production, the predicted maximum concentration was about 5.8 mg.L⁻¹, which can be achieved under k_{La} of 160 h⁻¹ and S_0 of 150 g.L⁻¹. Besides, overlapping models also made it possible to estimate a region of simultaneous optimization for both responses. Thus, cultivations performed with k_{La} of 150 h⁻¹ and 220 g.L⁻¹ of glycerol, can provide lipid and carotenoids concentrations greater than 10 g.L⁻¹ and 5 mg.L⁻¹, respectively. These results contribute to establishing operational conditions that maximize single-cell oil and carotenoids production from glycerol by *Rhodotorula glutinis* in pneumatically agitated bioreactors.

Keywords: Single-cell-oil, Oleaginous yeasts, Bubble Column Bioreactor

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B.45 - Technical and economic feasibility study of a recovery system for waste heat from a biogas-fuelled power plant in a sanitary landfill

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At a time when climate change is a worldwide struggle, sustainable energy production and energy efficiency are essential approaches to reduce greenhouse gas emissions. In 2019, the installed capacity of biomass-fuelled thermal power plants in Brazil reached 14.8 GW, i.e. 8.6% of the country's power generation capacity. Sanitary landfills provide 95% of the biogas-based generation capacity. Such thermoelectric plants, however, generally waste as heat 2/3 of the fuel energy content. A study case is presented here concerning a technical and economic evaluation of a recovery scheme of waste heat from a biogas-fuelled power plant in a sanitary landfill to dry sludge. The following assumptions were adopted for this study case: 15% of the landfill capacity of 1.8 thousand tons per day can be used for wet sludge deposition (up to 85% humidity), or 30% for dry sludge (up to 10% humidity); the revenue from the deposit of one ton of sludge in the landfill is R\$ 200.00. Assuming that the thermal energy required for the dryer is 1,000 kcal/liter of evaporated water and the engines exhaust gas power is 3421.1kW, it has been estimated that the drying system would need a minimum efficiency of 10% to be feasible. For higher efficiencies of the drying process, higher will be the revenue for the landfill from sludge deposition. By installing a commercial dryer operating with a 50% efficiency, up to 1635 kg/h of wet sludge could be dried, yielding a daily revenue of R\$ 14,536.00. The return on the investment would be in 15 years with an annual rate of return of 12%.

Keywords: biomass, cogeneration, energy

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B.47 - Techno-economic feasibility analysis to bio-oil production using eucalyptus residues from fast pyrolysis in São Paulo

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Forest residues, as a valuable feedstock, can be converted into product such as bio-oil and byproducts like biochar and acid extract through fast pyrolysis. We assessed the techno-economic analysis of bio-oil produced from fast pyrolysis of eucalyptus residues availability in the state of São Paulo, Brazil. The discounted cash flow analysis was conducted to calculate the minimum selling price (MSP) of the bio-oil. Geographical information system (GIS) research localized best potential eucalyptus forests with 106 km maximum radio in São Paulo. This work simulated one biorefinery, mass and energy balance, and techno-economic analysis to evaluate the feasibility through Microsoft Excel. Economy analysis showed the lowest MSP (194 USD/ton; in terms energetic 12 USD/GJ) for the simulated plant which was capable of processing 60000 ton/year of eucalyptus residues and producing 42000 L/day of bio-oil. This biorefinery costs 6 MM USD and reaches a peak operating expenditure (OPEX) of around 3 MM USD/year. Sensitivity analysis identified that capacity had the most significant impact on the MSP, while the byproducts (acid extract and bio-char) lowered the MSP up to 22%. Monte Carlo simulations showed that biorefinery presented an 80% change of resulting in an NPV > 0, and 80% probability of reaching IRR > 10%. This work demonstrates that the centralized configuration biorefinery is economically viable using the eucalyptus residues available in São Paulo.

Keywords: eucalyptus residues, techno-economic analysis, pyrolysis oil

B.49 - *S. cerevisiae* can express an AfAA9_B LPMO and improve the saccharification of lignocellulosic biomasses

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Lignocellulosic biomass is an important sustainable feedstock for the production of chemicals and fuels. *Saccharomyces cerevisiae* is the most used yeast in fermentation, but it is not able to degrade sugars from lignocellulosic biomass. The development of a yeast capable of simultaneous saccharification and fermentation (SSF) are gaining importance. In this work, we constructed a *S. cerevisiae* BY4741 strain expressing lytic polysaccharide monoxygenase (LPMO) from *A. fumigatus* (AfAA9_B) by the control of the TEF1 constitutive promoter by the CRISPR/Cas9 technique using the EasyClone-MarkerFree Kit (Addgene). The TEF1p::AfAA9_B was cloned at the XI-3 locus using the pCfB2904 integrative vector, and the constructed yeast was named TEF1p::AfAA9_B. AfAA9_B protein secretion was analyzed after yeast had grown in YPD medium at different times. After that, supernatants were concentrated and analyzed into the SDS-PAGE gel. Higher AfAA9_B secretion was observed at 48 hours, and no protein was detected in the control yeast. Fifty micrograms of total protein were analyzed by LC-MS/MS mass spectrometry, and the results confirmed the secreted protein as the LPMO (AfAA9_B). The AfAA9_B activity analyzed in the presence of 2,6-DMP as a substrate, showed an enzyme activity 2.0 times higher (8.5 U/g) in TEF1p::AfAA9_B yeast when compared to the control (4.5 U/g). Gene expression was analyzed by qRT-PCR in different carbon sources after 48 hours. The expression levels were 3.0, 4.0, 7.0 and 8.0 times higher in barley bagasse, rice straw, coffee pod and bean straw, respectively, when compared to those grown on glucose. To determine and optimize the best parameter values for the SSF process, we have been performing the design of experiments (DoE) using the DCCR and RSM. The yeast expressing a functional AfAA9_B obtained here can increase the saccharification of biomass and reduce the costs of the 2G ethanol production and other bio products.

Keywords: CRISPR/cas9, LPMOs, Yeast

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B.50 - A tool for obtaining Microbiological parameters from microplate screening: Kinetic study on sugarcane molasses

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Microplate assays are an effective method for screening several different initial conditions at once, allowing for dozens of simultaneous culture growths. However, the great amount of data obtained requires a considerable amount of work and is highly susceptible to bias. The main parameters that can be found through the analysis of the data obtained are the maximum specific growth rate (μ_{max}) and the duration of the lag phase (λ). Microbial and yeast growth curves have been largely described by empirical sigmoidal curves, which can have their parameters adjusted as to have a biological interpretation. The use of empirical sigmoidal curves allows for the growth parameters to be defined and obtained through analytical methods, reducing the amount of bias and work required. To that purpose, this study aims to compare different mathematical models describing growth curves and the development of a simple analysis tool to obtain the main growth parameters from the large amount of experimental data in microplate-readers. A case study was investigated in a kinetic study for different yeast strains using molasses. The kinetic study was assessed with two different industrial yeast strains (PE-2 and SA-1) and two laboratory yeast strains (CEN.PK113-7D and CEN.PK112) in six molasses in 96-wells microplate assays at 30°C. The methodology applied was successfully able to determine the important physiological parameters associated with microbial growth, μ_{max} and λ . Analyses of experimental data using this tool also showed that laboratory strains do not have well defined exponential growth phase in molasses as opposed to the industrial strains, which may suggest the adaptation of the industrial strains to the low nutrient concentration and stressful conditions in these industrial mediums.

Keywords: Mathematical modeling, Microplate screenings, Sugarcane molasses

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B.51 - Integrated Ethanol and Soybean Oil-Biodiesel Chain Influence on Carbon Footprint of Biodiesel production

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Due to Paris agreement, Brazil issued the Renovabio Program which is committed to reduce 37% of the greenhouse gases (GHG) emissions and to reach 45% of renewable energy sources on the national energy matrix. In addition, the replacement, in industrial process, of fossil-derived molecules by biomass based raw materials are also fundamental to reduce the CO₂ equivalent emissions in a low carbon economy. This work aims to evaluate the integration of the ethanol-biodiesel industry in relation to the carbon footprint effect. Researchers showed that ethanol can replace the conventional solvent hexane on soybean oil extraction. The produced oil would be employed on biodiesel production process. The biodiesel produced could be used on ethanol and soybean agricultural machinery and transportation, that represents the second most significant impact on bioethanol and soybean production. Besides, the sugarcane bagasse can be used as clean raw material to produce energy (vapour and bioelectricity). These technicalities indicate several synergies between the ethanol and the biodiesel production process. The soybean oil extraction, biodiesel and ethanol production process were simulated in the equation-oriented Environment for Modelling Simulation and Optimization (EMSO). The Life Cycle Assessment was performed considering the climate change impact category using the 100-year time horizon Global Warming Potential (GWP 100) under a cradle to grave scope, with energy-based allocation. The characterization factor database considered was IPCC (2006) for emissions in fuels burning and agricultural emissions and the Ecoinvent 3.1 database for raw materials inputs. As results, in the sugarcane production it was found a reduction of 19% of GHG emissions with 100% biodiesel (B100) in agricultural machinery and transportation; in soybean plantation it was obtained a reduction of 18% of GHG emissions with the use of biodiesel in the agricultural machinery and transportation and an overall reduction of 35% of CO₂ equivalent emissions between the traditional biodiesel production and the ethanol-biodiesel integrated production. **Keywords:** life cycle assessment, biodiesel production, ethanol

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B.53 - Acetate and butyrate assimilation by solventogenic and non-solventogenic *Clostridium beijerinckii* strains

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Bioprocesses make industrial development more sustainable. Employing lignocellulosic biomass and specific biocatalysts enables bioproducts to be manufactured. However, lignocellulose saccharification produces not only carbohydrates, but also other byproducts, such as acetic acid. Solventogenic *Clostridium* are remarkable biocatalysts that can consume glucose and xylose, and organic acids to generate solvents via Acetone-Butanol-Ethanol (ABE) fermentation. The biphasic metabolism of clostridia comprises acidogenesis, to give acetate and butyrate as intermediates, which are reabsorbed during solventogenesis, to yield butanol as the main product. Nevertheless, some *Clostridium* strains lose the capacity to migrate from the acidogenic to the solventogenic phase, which changes the fermentation product profile. Here, we compare xylose fermentation in the presence of acetate and butyrate by two *Clostridium beijerinckii* strains: Br21 (non-solventogenic, lacking the enzyme acetoacetate decarboxylase) and *C. beijerinckii* ATCC 35702 (solventogenic). Fermentative assays were performed with xylose alone (33 mmol L⁻¹) and with xylose supplemented with acetate (25 mmol L⁻¹), butyrate (25 mmol L⁻¹), or both, at 25 mmol L⁻¹ each. During fermentation, the fermentation samples were analysed based on the optical density at 600 nm, pH, and xylose, organic acids, and butanol concentration. Both strains consumed xylose completely, but they differed in terms of acetate consumption: 65.49 and 10.84 mmol L⁻¹ for Br21 and ATCC 35702, respectively. When acetate was added, the Br21 strain afforded butyric acid as the only product, at the highest concentration of 43.73 mmol L⁻¹. As expected, upon extra addition of butyrate, the ATCC 35702 strain provided butanol as the main product, at the highest concentration of 12.98 mmol L⁻¹. Although *C. beijerinckii* Br21 cannot form butanol, it is a promising biocatalyst for the fermentation of lignocellulose hydrolysates. Besides consuming xylose, it can use up acetate, a biomass hydrolysis derivative, to form butyrate as the only bioproduct. **Keywords:** xylose, organic acids, acetoacetate decarboxylase. **Supported by:** FAPESP 2018/12471-1, FAPESP 2020/ 03168-3. CNPq

B.54 - Using byproducts from the hydrolysis of lignocellulosic substrates to generate energy in a microbial fuel cell.

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Microbial Fuel Cells (MFCs) are devices that convert chemical energy directly into electricity through catalytic activity of exoelectrogenic microorganisms that promote the oxidation of compounds to generate electric current. The energy generation in MFC is even more interesting and sustainable if the oxidized compounds are wastes or wastewater. After pretreatment and hydrolysis of lignocellulose are generated sugars, which are employed in bioprocesses, but pretreatments can also generate byproducts, such as acetate, furfural, and phenolics that can inhibit fermentative microorganisms. Here we aimed to generate energy in a MFC using a mixed culture of microorganisms as a biocatalyst of an anode and acetate as the main substrate. In addition, coumaric acid, a phenolic derivative of lignocelulose hydrolisis, was added to the medium in order to investigate its effect on energy generation. A single-anodic-chamber MFC model with a carbon electrode was employed, in which nitrogen was sparged to maintain anaerobiosis and the cathodic compartment was in direct contact with air. The MFC was fed with a culture medium contained 12mM acetate, refed whenever the system voltage dropped. When the voltage was stable, 0.05 mM coumaric acid was added to the system. The tension was then measured as a function of time with an Arduino[®] system. Polarization curves and cyclic voltammetry of the biofilm on the anode were performed. The MFC fed only with acetate-promoted an averaged voltage at 450 mV. Addition of coumaric acid in the medium does not change MFC voltage, but voltage decreases faster compared with acetate alone. Qualitative spectrophotometric analysis showed that coumaric acid is consumed during energy generation. Based on these results, it was assumed that coumaric acid at 0.05 mM may be used as substrate or mediator by the biocatalists, or it is even inhibiting exoelectrogens and therefore interfering with the generation of energy.

Keywords: coumaric acid, acetate, bioelectricity. **Supported by:** FAPESP

B.55 - Anhydrous bioethanol purification using extractive distillation with a deep eutectic solvent by means of rate-based model simulation

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Bioethanol is one of the main renewable automotive biofuel, therefore, the search for more energy efficient production rates is constant, in order to ensure the best performance of its production chain. The purification of hydrated bioethanol to anhydrous bioethanol is still a challenge regarding the energy consumption of the process and the purity. Moreover, the study of new green technologies to improve and to make the process more environmental friendly is necessary. The use of extractive distillation is a well-known process to provide the separation of the azeotrope and generate anhydrous bioethanol, but the conventional solvents currently used are expensive, toxic and corrosive to equipment. In this way, deep eutectic solvents (DES) are new effective, easy to produce, non-toxic and of low cost. The potential use of such DES-based solvents has already been proved by literature, but the study of the best process conditions is required. In order to evaluate different operating strategies and separation process configurations, simulations emerge as a fundamental tool. Most of the works involving the simulation of these systems consider the equilibrium stage model, which significantly simplifies the process representation and also may not provide realistic results. Thus, the aim of this study was to simulate an extractive distillation system considering the non-equilibrium stage model to produce anhydrous bioethanol using a deep eutectic solvent. Simulations were developed in the software Aspen Plus® with rate-based model and rad-frac columns, using ChCl/Urea (1:2) deep eutectic solvent. The system was composed by an extractive column and a solvent recovery column. The simulations of the rate-based were compared with the equilibrium stage model approach and showed differences in their energy requirements and stream results. The system showed a potential for application in large scale processes indicating that ChCl/Urea (1:2) can be a suitable solvent for bioethanol dehydration.

Keywords: anhydrous bioethanol, deep eutectic solvents, rate based model

Supported by: FAPESP

C - Biorefineries, Bio-Based Chemicals and Biomaterials

C.01 - Evaluation of prebiotic potential of cello-oligosaccharides produced by enzymatic hydrolysis using different agro-industrial wastes

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In recent years, oligosaccharides and their derivatives have become useful for health applications in various fields because of their specific biological activities. In the food industries, several oligosaccharides have received increasing attention as key components for functional foods and nutraceutical products. The objective of this work was verify prebiotic potential evidences of cello-oligosaccharides (CELOs) produced by enzymatic hydrolysis of wastes rich in cellulose provide from sugarcane straw (SS) and coffee peel (CP) using an optimized enzymatic cocktail formulation. CELOs production was performed by enzymatic hydrolysis of wastes rich in cellulose, which were obtained by alkali extraction from these agricultural wastes. Central Composite Design was used to optimize the enzymatic concentration of three different enzymes: endoglucanase, exoglucanase and Feruloyl Esterase, all required from Megazyme[®]. The other hydrolysis conditions were fixed in temperature (50°C), solid-to-liquid ratio (5% w/v), pH (5.0) for 48 h, in a reactional volume of 1 mL. Optimized the concentration of enzymes “in vitro” digestion tests were performed using digestive tract enzymes (Salivary Amylase, Porcine Pepsin e Pancreatin) Sigma Aldrich[®] in order to verify the prebiotic potential of the CELOs produced. The CELOs profile concentration was quantified by HPAEC-PAD using standard (C2-C6) from Megazyme[®]. Our strategy was satisfactory for developing efficient enzymatic mixture achieving total CELOs concentration (265.76 ± 0.76 g/L) using SS cellulose (SSc) and (302.45 ± 0.85 mg/L) using CP cellulose (CPC). The digestibility tests revealed a considerable resistance to hydrolysis of digestive enzymes of the CELOs produced achieving the total digestibility percentage using SSc (12.56 ± 0.97 %) and CPC (14.23 ± 1.23%), thus meeting one of requirements to be possible prebiotic agents.

Keywords: agro-industrial waste, cello-oligosaccharides, enzymatic hydrolysis

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C.02 - Assessment of the addition of ethylic biodiesel as a co-solvent of ethanol in the soybean oil extraction

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In the last few years, studies have been developed in order to increase the solubility between vegetable oils and alcoholic solvents during the oil extraction process aiming to replace the solvent commonly used in industrial practice, a fossil solvent that is a mixture of hexane isomers, by a renewable and safer solvent, like as ethanol. The addition of co-solvents to ethanol can be considered as a strategy to enhance the process yield once, in the soybean oil extraction process, the use of ethanol requires a flow 280% higher than the hexane. Considering the concept of biorefinery, this research had as the main aim to study the technical viability of using soybean oil based ethylic biodiesel as co-solvent of ethanol. The extraction experiments were accomplished in a fixed-bed column under 60 and 70 °C, solid:solvent mass ratio of 1:1.5, absolute ethanol or added from biodiesel (5% in mass, B5), for soybean collets (SC) with 3 or 6 mass% of moisture (SC3 or SC6). For SC6/60 and SC6/70, the addition of the co-solvent B5 promoted greater reduction of residual oil in the raffinate phase, 15.35 to 11.64% and 12.80 to 9.59%, respectively. Hence, the extract phase oil content increased by the addition of B5, from 6.04 to 8.95% for 60 °C and 8.00 to 10.51%, at 70 °C. For SC3, significant differences were not observed by the addition of B5, being the extract phase oil contents ranging from 11.01 to 11.38% and 13.11 to 12.30%, for 60 and 70 °C. In general, the SC oil extraction yield values increased with the addition of B5, being the more prominent effects observed for SC without reduction of moisture, from 34.56 to 52.46% for SC6/60 and 45.20 to 62.18% for SC6/70, against 61.31 to 64.30% for SC3/60 and 71.90 to 68.57% for SC3/70.

Keywords: soybean oil, extraction, fatty acid ethyl esters

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C.03 - Approaches and challenges for specific rate estimations in a fed-batch culture with production of polyhydroxyalkanoate by *Ralstonia eutropha*

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In a bioprocess, measured extracellular rates are critical inputs for formulation of kinetic relationships, obtaining preliminary estimates of the parameters, establishing the basis for intracellular flux calculations and online monitoring and control of biotechnological processes. In a fed-batch culture, the specific rates are derivative quantities, which means that is necessary to differentiate the batch biomass and product concentration data to estimate the specific growth and product formation rates. However, the differentiation of measured concentrations amplifies the experimental error. Most existing approaches for specific rate estimations assume the population to be in a state of balanced exponential growth, in which the cell population accumulates at a constant growth rate and in which the rates of exchange reactions are constant, simplifying the problem as it reduces rate estimation to a standard linear regression problem. Therefore, having reliable specific rates is essential to accurately represent the biological process. In this context, this work aims to evaluate different approaches for specific rate estimation while handling the measurement uncertainties. For that, production of poly-3-hydroxybutyrate (P3HB) by *Ralstonia eutropha* was selected due its well-established knowledge of the process and the fact that this microorganism is a model in P3HB production. Fed-batch experiments were conducted in which was noted the impact of different methods for cell mass determination and some discrepancies in some analytical procedures, especially PHB quantification. The later was pointed out in a further carbon mass balance analysis. Improvements in sampling collection and glucose concentration determination were made in order to reduce data inconsistency and those were found to be satisfactory.

Keywords: specific rates, fed-batch process, PHB production. **Supported by:** CNPq

C.04 - Turbo yeasts: Catabolism of sugars in wild type, lab and industrial strains

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Yeast glycolysis is the central pathway to relevant bio-based building blocks, therefore high glycolytic fluxes are of great industrial interest. Surprisingly, the glycolytic rate of specific yeast strains is higher on sucrose than on glucose, although the pathway is basically the same. Despite these observations, sucrose metabolism in *Saccharomyces cerevisiae* has not been studied in depth. *S. cerevisiae* metabolizes sucrose differently from glucose in that the disaccharide is first hydrolyzed by invertase, to release the hexose monomers that will then enter the glycolysis pathway. Substrate consumption in *S. cerevisiae* is characterized by a glucose repression mechanism that leads to preferred consumption of glucose. Thus, in principle growth on glucose should be faster than on sucrose or any other carbon source. In the present work we studied the quantitative physiology of *Saccharomyces cerevisiae* strains on sucrose and compared to that on related carbon and energy sources. Wild type, industrial and laboratory strains were screened in microplate and shake-flask cultivation systems and the strains that presented the most diverse profiles with respect to maximum specific growth rates on sucrose were subjected to deeper investigation. For this purpose, a set of well-controlled aerobic batch bioreactor cultivations were performed and relevant physiological parameters determined. The efficiency of sucrose utilization revealed to be strain- and condition- dependent. Remarkably, the sucrose hydrolytic capacity of the strains diverged from one to another, leading to distinct levels of hexoses accumulation in the cultivation broth. Our work provides quantitative data on the physiology of different *S. cerevisiae* strains on sucrose, and emphasizes the capability of particular strains to display high glycolytic flux on this substrate. We are working further to investigate the underlying mechanism(s) behind the enhanced growth on sucrose displayed by some strains.

Keywords: sucrose, glycolysis, glucose repression. **Supported by:** FAPESP

C.05 - An integrated approach to obtain xylo-oligosaccharides from sugarcane straw for bioethanol production

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The replacement of energy from fossil fuels over renewable sources cannot be delayed any longer. Given this scenario, sugarcane straw is a widely available brazilian feedstocks with potential for biofuels and biochemicals production, besides energy use. In particular, bioethanol production from both cellulosic and hemicellulosic sugars still faces some bottlenecks which hinder its economic feasibility. In this work, we propose to study operational parameters to maximize the production of low degree of polymerization xylo-oligosaccharides (XOS) for later use in fermentative processes using an industrial yeast strains engineered to offer a novel perspective to produce bioethanol by minimizing issues of microbial contamination and focused in low severity pretreatments of sugarcane straw with low energy consumption and toxic fermentation compounds production. A two-stage pretreatment (mild alkaline deacetylation prior hydrothermal) followed by enzymatic hydrolysis was optimized to generate a cellulose rich-fraction and a XOS rich-hydrolysate. Afterwards, the scaling-up of the process and comparative material balances and yields was performed. According to our results, from laboratory to pilot scale, the two-stage pretreatment promoted 81.5% and 70.5% of hemicellulose solubilization and yielded up to 9.7% and 9.1% w/w of initial straw into XOS, respectively. A GH10 xylanase from *Aspergillus nidulans* efficiently converted high molecular weight XOS into mainly xylobiose and xylotriose with minimal xylose formation. Moreover, it was obtained a cellulose-rich fraction and a high-quality lignin from the deacetylation black liquor that can be converted into cello-oligomers sugars and aromatic high-value compounds, respectively.

Keywords: pretreatment, xylo-oligosaccharides , enzymatic stoning

Supported by: FAPESP

C.06 - Production of the copolyester P3HB-co-3HV by Burkholderia sacchari prp mutants using xylose and propionate and evaluation of the modulation of its monomeric composition

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Burkholderia sacchari, a non-pathogenic Gram-negative bacterium isolated from sugarcane soil in Brazil, is able to use carbohydrates accumulating up to 70% of cell dry biomass as polyhydroxyalkanoates (PHA) granules. PHA are biodegradable and biocompatible polyesters extensively studied for their great potential for the pharmaceutical and biotechnological industries. When cultivated under excess of a carbohydrate, under limitation of nitrogen, phosphorous or other nutrient, *B. sacchari* accumulates the homopolymer poly-3-hydroxybutyrate (P3HB) and, if also propionate is provided, the copolymer poly-3-hydroxybutyrate-co-3-hydroxyvalerate (P3HB-co-3HV) is accumulated. P3HB-co-3HV is more malleable, increasing its possibilities of application. Nevertheless, the elevated price of propionate as substrate and low conversion to 3HV represent obstacles to market competitiveness. The low natural conversion to 3HV is attributed to the presence of at least two propionate catabolic routes: α -oxidation and 2-methylcitrate (2MCC), which preferentially convert it to biomass, CO₂ and H₂O. While the presence of 2MCC has been well studied, the α -oxidation pathway remains indicated only by phenotypical evidences. In this work we tested previously obtained *prp* mutants deficient on propionate consumption and affected in α -oxidation and its intermediates, meaning inability to use propionate for growth, directing it to 3HV units. In two-phase shaker cultivations, with xylose and different propionate proportions supply during polymer accumulation phase, the efficiency of the mutants in converting propionate to 3HV units (YHV/Prop) increased up to 0.34 g/g compared to values up to 0.13 g/g obtained with the control wild type, with much lower substrate waste, representing more than 2.5-fold increasing efficiency. Bioreactor fed-batch cultivation currently under development are expected to further increase the efficiency, evaluating detailed carbons source consumption, conversion efficiency to 3HB and 3HV with xylose and propionate establishing kinetic parameters of the bioprocess. Molecular studies of regions affected on those mutants will endorse the presence and role of α -oxidation on propionate catabolism and copolymer production.

Keywords: P3HB-co-3HV, xylose, α -oxidation. **Supported by:** CAPES

C.07 - High-value aromatic alcohols directly from lignocellulosic biomass: A consolidated Biodegradation and transformation strategy

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Modern society is hugely dependent on finite oil reserves for the supply of fuels and chemicals. Moving our dependence away from these unsustainable oil-based feedstocks to renewable ones is, therefore, a critical factor towards the development of a low carbon bioeconomy. However, in order to compete with current petrochemical refinery processes, alternative biorefinery processes must overcome significant costs and productivity barriers. Objectives: Herein, we demonstrate the biocatalytic production of the versatile chemical building block, coniferol (i.e. fragrance precursor), directly from lignocellulosic biomass. In this study we explored the use of both an in vitro enzymatic and whole-cell biocatalytic route to release ferulic acid from biomass sources and convert it directly into coniferol. Following the biocatalytic treatment of lignocellulose to release and convert ferulic acid with feruloyl esterase (XynZ), carboxylic acid reductase (CAR) and aldo-keto reductase (AKR). This whole cell catalytic cascade not only achieved equivalent release of ferulic acid from lignocellulose compared to alkaline hydrolysis, but also displayed efficient conversion of ferulic acid to coniferol. The generation of these chemicals from abundant biomass sources has many advantages including use of mild reaction conditions, compatibility with aqueous media, sourcing of catalysts from renewable feedstocks and chemo, regio and enantioselectivity. **Conclusions:** This system represents a consolidated biodegradation-biotransformation strategy for the production of high value fine chemicals from waste plant biomass, offering the potential to minimize environmental waste and add value to agro-industrial residues.

Keywords: Biocatalysis, Coniferol, Lignocellulose. **Supported by:** FAPESP

C.08 - Soybean protein addition during the sugarcane bagasse saccharification in the context of a biorefinery: effect and techno-economic analysis

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The biochemical conversion of lignocellulosic biomass into biofuels and other bioproducts still have some technological challenges such as the low yield of the enzymatic hydrolysis step and the high cost of cellulolytic enzymes. The use of additives during the enzymatic hydrolysis has shown significant positive effects, since they decrease unproductive adsorption of cellulases in lignin and reduce the loss of enzyme activity. However, the use of low-cost additives is necessary to make the process economically feasible. Thus, the present study evaluated the process conditions and the techno-economic analysis of the addition of soybean protein as a low-cost additive during the enzymatic saccharification of hydrothermally pretreated sugarcane bagasse in the context of an integrated biorefinery. Firstly, a statistical experimental design methodology was used to select the solids loading and soybean protein concentration, allowing to set these variables for the subsequent steps of this study at 15% (w/v) and 12% (w/w), respectively. The time profile evaluation for glucose release using different enzyme dosages showed that it was under the conditions of 10 FPU/g and 24 h of hydrolysis that soybean protein addition was more effective in relation to the control (hydrolysis without additive). The saccharification at these set of conditions allowed to increase the glucose released by up to 26%. The Retro-Techno-Economic Analysis was used to evaluate the feasibility of soybean protein addition to the process, enabling the definition of performance targets within the context of an integrated ethanol biorefinery, in order to make it economically feasible. The performance targets obtained include: the increase in biomass conversion, the reduction of the additive cost, the decrease of the enzyme dosage and reduction of its cost. If these targets were reached, the use of soybean protein as a hydrolysis additive will help to increase the 2G ethanol process efficiency, contributing to its economic feasibility. **Keywords:** Biorefinery, Lignocellulosic biomass, Soybean protein
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C.09 - Influence of medium composition and initial pH in pullulan production by *Aureobasidium pullulans* LB83

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The use and improper disposal of low biodegradability materials like petroleum derived polymers has raising concerns regarding to environmental pollution. In this context, biopolymers production in lignocellulosic biorefineries could be a sustainable alternative to favor an increasing availability of environmentally friendly products. Among the biopolymers, pullulan is a non-toxic, non-carcinogenic and non-mutagenic exopolysaccharide produced by the fungus *Aureobasidium pullulans*, with applications in food and pharmaceutical sector. Usually it is produced from starch hydrolysate. However, cellulosic hydrolysate from lignocellulosic biomass is a potential low-cost carbon source that can be used in bioprocess for pullulan production. *A. pullulans* LB83, a strain isolated in Brazil, has been recently tested for production of this biopolymer in a lignocellulosic biorefinery context; however, some of the main parameters as medium composition and pH requires study. Thus, experiments were performed according to a Box-Behnken design to evaluate the influence of the concentration of glucose, yeast extract and initial pH in the pullulan production. Cells were grown in Erlenmeyer flasks in commercial glucose-based medium at 200 rpm and 28 °C for 96 hours. Statistical analysis of data indicated that pH had linear negative effect in pullulans production. Also, higher yeast extract concentration was beneficial for the both biopolymer and biomass production. Higher pullulan production of 42 g/L was obtained using 105 g/L of glucose, 5 g/L of yeast extract and initial pH of 6. This value of pullulan concentration was at least 1,68 times higher than the reported in previous studies for this strain and highlight its potential for production of this biopolymer. **Keywords:** Biopolymer, Biorefinery, Pullulan. **Supported by:** PRONABEC, FAPESP, CNPq and CAPES

C.10 - Lignosulfonates as an alternative to add value to 1G2G biorefineries

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Alkaline sulfite pretreatment demonstrates high efficiency for reducing recalcitrance in lignocellulosic materials. However, the techno-economic evaluation of this process, integrating a 1G2G sugarcane bagasse biorefinery to produce ethanol and electricity, indicates that the cost of chemicals is critical for the economic viability of the entire process. One interesting alternative to improve the economics of the process is to produce new products in the 1G2G portfolio. In this context, we evaluated the production of lignosulfonates as a side product of the alkaline sulfite pretreatment. The original pretreatment liquor, containing lignosulfonates at low concentration and residual sulfite ions, was used to convert additional lignin to lignosulfonates by reacting the liquor with residual solids resulting from the enzymatic hydrolysis of the pretreated sugarcane bagasse. A factorial experiment was performed and indicated the maximum lignosulfonate production (26.3 g/L) when 13% (w/w) NaOH was added to reaction mixture and temperature was adjusted to 160 °C. Under these conditions, 64.2% of the lignin contained in the enzymatic hydrolysis residual solids was converted to lignosulfonate. The molar mass distribution of the produced lignosulfonate was dependent on the reaction conditions, with a mixture of macromolecules with Mw values ranging from 3600 to 8200 g/mol. The mass balance of the whole process indicated that 67.9% of the polysaccharides were converted to monomers and 51.5% of the original lignin was converted to lignosulfonate. The current process optimization indicated that lignosulfonates can be produced within the 1G2G sugarcane bagasse biorefinery at an approximate level of 17 kg lignosulfonate/ton of processed sugarcane, adding value to the entire process.

Keywords: biomass, lignosulfonates, biorefinery

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C.11 - Plasmid stabilization by *icd*^{NAD} gene complementation and bioproduction of 1,3-propanediol in recombinant *Escherichia coli*

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1,3-propanediol (1,3-PDO) is a primary component for the production of high added value biopolymers. This compound can be produced by some bacteria using glycerol as a carbon source. 1,3-PDO-producing bacteria such as *Klebsiella pneumoniae* are not of interest to the industry as they are considered pathogenic and produce secondary compounds that hampers the purification of the product of interest. To this end, an *Escherichia coli* strain was transformed with a plasmid containing the *K. pneumoniae* genes (operon *dha*) necessary for the conversion of glycerol to 1,3-PDO (pBBR1MCS2::*dha*). However, the plasmid maintains in the population by adding an antibiotic to the culture medium, resulting in an additional cost for large-scale production. To overcome this problem, it was proposed a strategy of *icd*^{NAD} gene complementation. The gene codes for NAD-dependent Isocitrate dehydrogenase, a key enzyme to the Tricarboxylic Acid cycle (TCA). Although the gene is necessary for growth in minimal medium, the mutant bacteria is still able to grow in rich medium. This project aimed to construct an *E. coli* strain lacking *icd*^{NAD} in its chromosome, complemented with the same gene in the production plasmid pBBR1MCS2::*dha*. As this plasmid is required for the conversion of glycerol to 1,3-PDO, the new strain was used in bioreactor assays, in order to compare the production from complementation strategy to the use of antibiotic. The results were promising, as the *icd*^{NAD} complemented strain produced only slightly less 1,3-PDO in a culture without antibiotic, compared to the added one.

Keywords: 1,3-propanediol, *icd*, plasmid stabilization. **Supported by:** CAPES

C.12 - Evaluation of hydrothermal pretreatment for valorization of olive mill solid waste through bio-based volatile fatty acids production

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A lignocellulose residue produced in a large amount in the world is the olive mill solid waste, which is generated during olive oil extraction. In this work, the olive mill solid waste (OMSW) was pretreated by hydrothermal pretreatment (HP). The pretreated olive mill solid fraction (OMSF) and pretreated olive mill liquid fraction (OMLF) were evaluated as potential substrates for acidification during the anaerobic digestion. Three HP conditions (D1: 125°C, 53min; D2: 161°C, 62min; D3: 191°C, 83min) were selected using desirability tool of the Statistica® software to produce substrates for acidogenic anaerobic digestion (AD) in liquid (L-AD), semi-solid (Ss-AD) and solid (S-AD) phases. Batch acidogenic AD tests were carried out in mesophilic conditions using 120 mL glass bottles as reactors. The inoculum used in AD tests consisted of a mixture of 50% (grams of total volatile solids, VS) of anaerobic sludge and 50% (grams of VS) of fresh bovine manure (FBM). The bottles were loaded with 15 wt.% of total solids (TS) for S-AD and 12 wt.% of TS for Ss-AD. A food-to-microorganism (F/M) ratio of 2.0 gVS gVS⁻¹ was used in the S-AD and Ss-AD tests. For L-AD tests (1.5 wt.% of TS), the F/M ratio was 0.5 gCOD gVS⁻¹. HP improved the acidification efficiency (AE) of all AD systems evaluated. The presence of polyphenols changed the metabolic pathway of microorganisms, increasing the production of longer chain volatile fatty acids (VFA) in the L-AD and S-AD systems. For Ss-AD, condition D2 (161°C, 62min) was the most profitable scenario for bio-based VFA production. The typology of AD in terms of solid concentration and HP severity had great influence on VFA production profiles. Therefore, HP of OMSW followed by AD for VFAs production can be an attractive method for the olive oil production chain in a biorefineries concept.

Keywords: hydrothermal pretreatment, olive mill solid waste, volatile fatty acids

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C.13 - Sugarcane straw as feedstock for biorefineries: process design and simulation for different pretreatments

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With Brazil's new public policy RenovaBio, biofuel producers will receive emission certificates according to their products' carbon footprint, in comparison to their fossil counterparts. This way, it is of interest to explore alternatives to reduce emissions. Harvest residues are a potential biomass source to increase production whilst maintaining the culture area. An example is sugarcane straw (SS). With the implementation of green harvesting on sugarcane fields and the restriction of burning procedures, layers of SS stay on field, providing coverage and nutrient recycle. While important, studies point that a fraction of this biomass can be retrieved and use as a new feedstock, with remarkable energy and lignocellulosic contents. This work aims to evaluate the potential of SS as the raw material in different stand-alone biorefinery process routes, considering ethanol (hydrous and anhydrous) and electricity as main products. Three scenarios were built on Aspen Plus, based on different pretreatments (PT): Hydrothermal, Steam Explosion, and Alkaline. These simulations are based on a PSSF arrangement (pre-saccharification and simultaneous saccharification and fermentation), that allows increased solids loading on reactors and, thus, higher ethanol titers. The scenarios also feature an anaerobic digestion unit, to generate methane from aqueous streams such as the pentose liquor and vinasse. This was crucial to improve the processes' overall energy balance and increase the electricity surplus, avoiding the need to diverge part of the SS feedstock to be burnt in the furnace. The Alkaline scenario returned the highest ethanol yield, of 153.3 L/dtSS (dry ton of SS); and Hydrothermal, the lowest, of 120.5 L/dtSS. As for electricity, Steam Explosion showed the highest surplus (655.3 kWh/dtSS); and Alkaline, the lowest (401.8 kWh/dtSS). Therefore, there is a tradeoff between ethanol and electricity outputs, with the PT choice being a key factor, since it alters the process overall arrangement and performance.

Keywords: biorefinery design and simulation, ethanol, sugarcane straw

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C.14 - Multi-omic characterization of a novel red yeast strain with biotechnological potential for lignin valorization strategies

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Rhodospiridium fluviale, known as “red yeast”, because of their pigment colony related to carotenoids production, is an oleaginous yeast, capable of accumulating more than 20% of their dry cell weight as lipids. The species of this gender show native adaptability to a wide variety of carbon source and high tolerance to hydrolysate inhibitory compounds, representing a significant biotechnological potential. In this work, *R. fluviale* was isolated from a lignin-degrading microorganism consortium (Moraes et al., 2018). The isolated yeast strain grows in lignin as the sole carbon source in the media, and it was characterized based on genomic, transcriptomic and secretomic strategies. The genome size was estimated to be 50 Mb encoding 17,936 protein sequences, including 28 peroxidases, a key enzyme for lignin degradation, and 345 CAZymes. The flow cytometry and pulsed-field gel electrophoresis analysis showed that the strain is polyploidy with approximately 16 chromosomes. For the transcriptomic and secretomic analysis, *R. fluviale* was cultivated in two different culture media (Kraft lignin + glucose and glucose). Lignolytic enzymes, as catalases, heme peroxidases and lignin-modifying peroxidases (AA2) were identified up-regulated with a Log2FoldChange value above two. Moreover, auxiliary enzymes as AA5 were identified in the secretome, using a 5% False Discovery Rate (FDR) index. This strain, apart from growing in lignin containing media without glucose, it can use phenolic compounds as the carbon source, such as ferulic acid. In conclusion, this is the first report of *R. fluviale* genome, and the omics analyses revealed important pathways of interest for rational use of this microorganism in lignin valorization strategies.

Keywords: Lignin valorization, yeast, omics. **Supported by:** FAPESP; CAPES

C.15 - Selection and isolation of CO2-fixing bacteria for flue gas treatment and biogas/natural gas purification systems

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The increase in atmospheric CO2 levels due to emissions resulting from the industrial use of fossil fuels is one of the major causes of the global warming. The burning of hydrocarbons, specially oil and coal, produces flue gases containing high CO2 concentrations (10-20%). Alternatively, replacing oil and coal for fuels with lower carbon contents, such as biogas and natural gas, has become a widespread option for mitigating CO2 emissions. However, raw biogas and natural gas may have high CO2 contents, thus requiring expensive purification processes prior to their use. Aiming the efficient CO2 removal from flue gases and fuel gases, many studies focused on techniques for carbon capture. Among these techniques, biological carbon uptake have demonstrated great potential. In this context, this study concerns the isolation of chemoautotrophic bacteria capable of fixing CO2 in highly concentrated gas streams. The bacteria were isolated in liquid batch cultures inoculated with Antarctica’s soil samples and maintained under a mixed gas atmosphere (CO2 and air). CO2 was the only carbon source and thiosulfate or ammonium ions were used as potential energy sources for bacterial metabolism. The growth of the isolates under different CO2 concentrations was determined by measuring OD600. Bacterial consumption of thiosulfate and ammonium was accessed through ion chromatography. Seven different bacterial strains were selected and compared by MALDI-TOF MS. Two of these strains were capable of growing under 20% and 30% of CO2, and showed no increase in OD600 when cultured at 0% CO2. Ion chromatography demonstrated the reduction of thiosulfate and ammonium in the culture media, indicating bacterial consumption of these ions. Additionally, one of the isolates was identified as a biofilm-forming bacterium. Therefore, this study resulted in the isolation of bacteria that can be applied in industrial purification systems for CO2 removal from gases with CO2 contents up to 30%.

Keywords: CO2-fixing bacteria, Flue gas, Biogas. **Supported by:** CNPq

C.16 - Holistic approach for valorization of sugarcane bagasse through the thermochemical conversion route: from bagasse characterization to upgraded bio-oil.

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Sugarcane crops play a significant role in ethanol and sugar production. Approximately 1.6 billion tons of sugarcane is annually produced worldwide, from which 635.51 million tons are produced in Brazil. Consequently, 448 tons of sugarcane bagasse generated worldwide, 178 million just in Brazil. Currently used for electricity generation, new alternatives have been proposed for conversion of sugarcane bagasse into ethanol. However, higher ethanol yields and reduced costs are still required. Hence, fast pyrolysis is an interesting alternative, due to its simplicity and the possibility to produce another range of chemicals. Considering the complexity of the pyrolysis product or bio-oil, an additional step is required in order to “upgrade” its properties. Then a catalytic treatment at high temperatures, high pressure of hydrogen over heterogeneous catalysts can be employed, resulting in a less viscous product, with lower oxygen content, higher pH and a broad molecules range. So far, a very limited number of studies have considered the upgrading of sugarcane bagasse pyrolysis-oil. The present work brings a complete investigation from sugarcane bagasse characterization to the final upgraded products. The biomass is characterized applying a variety of analytical methodologies, such as proximate and ultimate analysis and Py GC/FID. The bio-oil composition is correlated to the bagasse building blocks, and the hydrotreatment reaction pathways are identified. Despite the ash content of 6.75 wt.%, 60.1 wt.% of bio-oil was obtained by fast pyrolysis, attributed to low concentration of potassium (0.08 wt.%) of the bagasse. Upgraded bio-oil with 60.3% less water and 43.3% less oxygen were obtained with Ni/SiO₂, resulting in an HHV 63% higher compared to bagasse. Ni-Cr/SiO₂ showed the highest hydrogenation activity and the highest conversion of acids, while Ni/SiO₂ was more active for conversion of compounds containing aromatic groups. Hence, sugarcane bagasse is an attractive feedstock with an overall yield of 30.5 wt% of the upgraded product.

Keywords: biomass, thermochemical conversion, hydrotreatment. **Supported by:** CNPq

C.18 - Economic and environmental assessment of the production of a low-cost lipase

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Enzymatic catalysis has been shown to be an interesting alternative for chemical catalysis due to higher selectivity and mild temperature and pressure conditions. However, enzyme cost is regarded as a bottleneck for its broader application. Vegetable enzymes can be an interesting alternative to microbial enzymes, due to their higher availability and ease of purification. In this context, castor bean lipase is an interesting alternative for the industrial processing of oils, since it presents high catalytic activity at acidic pHs. Thus, low cost feedstocks, such as crude vegetable oils, can be directly processed, without the need of purification steps, upstream the enzymatic reactor. This study aims to perform a techno-economic and environmental assessment of the production of solid seed extract (SSE) of castor bean for the hydrolysis of low-cost oils and fats. The SSE can be obtained after milling castor bean seeds, followed by crushing the seeds in contact with refrigerated acetone to remove castor oil, and solid/liquid separation. Process simulation and techno-economic-environmental assessment was performed in EMSO (Environment for Modeling, Simulation, and Optimization), which is an equation-oriented simulator. The economic and environmental metrics evaluated were the minimum selling price (MSP) and the global warm potential (GWP), respectively. The results showed that the acid castor bean lipase could be an economic and environmentally feasible solution for oil hydrolysis reactions.

Keywords: enzyme, economic analysis, Life Cycle Assessment

Supported by: FAPESP, CNPq and CAPES

C.19 - Performance of enzymatic hydrolysis at high loading of rice straw in a non-conventional reactor

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The use of high solids load during the enzymatic hydrolysis of lignocellulosic biomass makes it possible to obtain a solution with high sugars concentration, which is required to attain high levels of ethanol in the fermentation stage. However, operating the enzymatic hydrolysis at high solids (> 15% w/v) is a great challenge due to technical problems associated with the initial viscosity of the reaction medium that in turn, affect the process yield. Recently, we have demonstrated the potential of a non-conventional reactor, specifically a vertical ball mill (VBM) reactor, for use in the enzymatic hydrolysis of pretreated rice straw at 8% w/v solids. In the present study, we assessed the performance of this reactor for enzymatic hydrolysis of pretreated rice straw at higher solids loads (16, 20 or 24% w/v) in batch process. A fed batch mode at 24% (w/v) solids was also evaluated. The reactor was equipped with three flat round plate's impellers and 30 units of glass spheres (23 mm) as grinding elements. All the experiments were conducted at 46 ° C and 100 rpm, using an enzyme load (21.5 FPU cellulase and 26.5 IU β-glucosidase/g cellulose) corresponding to 29.5 FPU/g cellulose. The results shown that, after 36 h, glucose concentration increased from 76 to 100 g/L and hydrolysis yield decreased from 80 to 72% when the solid loading increased from 16 to 24% (w/v). By using the higher solid load (24%), fed-batch operation did not improve enzymatic digestibility when compared to the batch process, resulting in a hydrolysate with approximately 100 g/L of glucose. These results are promising and demonstrate that the VBM reactor can be used with success for high consistency hydrolysis of lignocellulosic substrates. Further studies are still necessary to investigate others enzymes commercial blends as well as its proper loadings.

Keywords: rice straw, enzymatic hydrolysis, high solids load

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C.20 - Application of residual brewer's yeast in the detoxification of sugarcane bagasse hemicellulosic hydrolysate for xylitol bioproduction

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Sugarcane bagasse is rich in polysaccharides that can be converted in fermentable sugars and used in a biorefinery context. The hemicellulosic fraction of bagasse is rich in xylose, a precursor of xylitol, a high value-added product in the industry. Hemicellulose solubilization is achieved by diluted acid hydrolysis which simultaneously releases toxic compounds to microorganisms (mostly phenolic). This study aims to employ a residual brewer's yeast to detoxify the sugarcane bagasse hemicellulosic hydrolysate to be used in xylitol bioproduction. A factorial design 2² was performed with the variables time (30 or 60 minutes) and residual biomass condition (dry or wet cells). Both biomasses were inactivated by autoclaving and dried. The dried biomasses were macerated in a mortar with pestle and sieved at 35 Mesh. Detoxification was based on overliming and adsorption with dried brewer's yeast. In the overliming step, CaO was added to the hydrolysate until pH 7. Thus, 1% (w/v) of dry or wet biomass was transferred to the hydrolysate for 30 or 60 minutes under 100 rpm at 30°C. As a control experiment, activated charcoal adsorption (1% w/v) was performed at 60°C for 30 minutes. Determination of total phenolic compounds was performed by UV-VIS spectrophotometry at 280 nm. Among the detoxification procedures using dried brewer's yeast, the highest phenolics removal (40.02%) was achieved with the dry biomass for 60 minutes. On the other hand, charcoal adsorption allowed removing 66.61% of phenolics. Results showed that dry yeast biomass, a residue from a large process (beer production), can be potentially used as detoxification agent, however further research is necessary to effectively use it instead of activated charcoal.

Keywords: Biorefinery, Sugarcane bagasse, Residual Yeast

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C.21 - Evaluation of *Methylocystis hirsuta* growth in stirred tank and bubble column bioreactors with variation of culture medium and gas phase recycle

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Methanotrophic bacteria are microorganisms capable of using methane as source of carbon and energy. The ability to consume a greenhouse gas, coupled with the world recent efforts to find new sources of protein, make them promising fermentative agents for the generation of food supplements for both animals and humans. Several advantages can be observed in the use of this source: reduced protein synthesis time in unicellulars compared to superior organisms, high contents of protein, facility to genetically modify the microorganism and independence of seasonal factors. The present work aimed to compare *Methylocystis hirsuta* growth utilizing methane as carbon source, exploring aspects related to the conduction of the bioprocess and the type of bioreactor. Assays were performed on stirred tank (STR) and bubble column (BCR) bioreactors, with a new system of three vessels developed at the laboratory, using Pall rings to fill the BCR system. Variations in the conduction of the experiments were done, using recycle in the STR assays in order to improve the mass transfer of methane and oxygen from the gas to the liquid phase. Results obtained in STR showed the highest efficiency of the recycled system in cell growth. Recycle assays showed cell growth from 0.2 to 1.0 g/L, a 5-fold increase in final concentration, while non-recycle assays showed only 1-fold increase, from 0.2 to 0.4 g/L. The concentrations obtained in BCR assays were 1.1 and 2.1 g/L with mineral and supplemented medium, respectively, and an assay with mineral oil and supplemented medium exhibited a cell concentration of 4.5 g/L, the highest value obtained so far. Further experiments should be performed to optimize cell production in the two systems, but both have shown promise in the utilization of methane as a substrate by the microorganism and in their efficient mass transfer to the liquid phase.

Keywords: Methanotrophic, Methane, Bubble column bioreactor. **Supported by:** CAPES; CNPQ

C.22 - Comparison of 1,3-propanediol production by *Clostridium beijerinckii* Br21 and *Clostridium pasteurianum* DSMZ 525 from glycerol fermentation

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Many alternatives have been sought for the application of residual glycerol (GLY) from the biodiesel industry. 1,3-Propanediol (1,3-PDO) is one of the highest value-added products that can be obtained from glycerol fermentation by *Clostridium* species. Glycerol fermentation occurs through two pathways: an oxidative and a reductive one. In the reductive pathway, glycerol dehydratase converts glycerol to 3-hydroxypropionaldehyde, which is reduced to 1,3-PDO by a NADH + H⁺ - dependent step. In the oxidative pathway, glycolytic pathway metabolites are formed and further converted mainly into organic acids and H₂. The latter pathway generates reducing equivalents, which can contribute to 1,3-PDO production in the reductive pathway. In this work, fermentation kinetic assays were performed with *Clostridium beijerinckii* Br21, a non-solventogenic strain isolated in our lab, and *Clostridium pasteurianum* DSMZ 525. Glycerol at different concentrations was used as substrate (75, 170, 390, and 1100 mM). *C. beijerinckii* at 170 mM and 390 mM gave the highest 1,3-PDO concentrations and yields: 48 ± 0.3 and 44 ± 0.4 mM and 0.66 ± 0.00 and 0.47 ± 0.02 mol of 1,3-PDO/mol of GLY, respectively. For *C. pasteurianum*, 390 mM glycerol provided the highest 1,3-PDO concentration and yield: 77 ± 1.7 mM and 0.44 ± 0.02 mol of 1,3-PDO/mol of GLY. Although *C. pasteurianum* DSMZ 525 afforded higher 1,3-PDO concentrations from glycerol fermentation, *C. beijerinckii* Br21 generally furnished higher 1,3-PDO yields, especially for 170 mM glycerol. This was probably because the strain Br21 cannot produce solvents and the carbons are directed to 1,3 PDO formation. Thus, *C. beijerinckii* Br21 is a promising candidate to produce 1,3 PDO with high yields from low glycerol concentrations. **Keywords:** biodiesel, butyric acid, reducing equivalents.

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C.23 - Integrated biorefinery of bioethanol and bioplastic – conceptual design and economical evaluation

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Biorefineries were envisioned as an alternative to diminish the global dependency on fossil resources and to replace fossil-based chemical products with more environmentally friendly ones. Different added-value chemicals were aimed to be produced from the biomass such as xylitol, itaconic acid and bioethanol. One class of these added values chemicals are the polyhydroxyalkonates (PHA) more specifically the polyhydroxybutyrate (PHB) which has properties similar to polypropylene and can be used for medical applications and nanotechnology. A significant part of the cost of this bioplastic is associated with the use of a noble substrate (glucose), hence cheaper alternatives could have significant economic impact. Consequently, this work aims to assess the economic viability of introducing an alternative pathway to produce PHB from xylose, a less noble sugar, in an integrated biorefinery that has already ethanol and electricity as end products. This could serve as a conceptual design for new integrated plants. This goal is achieved by formulating a superstructure to account for different alternatives that is translated into an optimization problem, solved in GAMS environment with data collected from literature. Not only was an optimal configuration identified and the mass flows determined but it was also observed that inclusion of a new process alternative enhanced the economic performance of the integrated process in this first approach although a more detailed considerations need to be made to fully assess its competitiveness in an industrial scenario.

Keywords: Biorefinery, Polyhydroxybutyrate, Process Synthesis

C.24 - Assessment of Nb₂O₅ pellet as catalyst for 5 hydroxymethylfurfural production

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Great efforts have been made to establish competitive technologies for use of lignocellulosic biomass in the production of alternative fuels, high value products and chemical intermediates like 5-hydroxymethylfurfural (HMF). Among the products of interest that can be produced, HMF is one of the most interesting as it is within the top ten key platform molecules for the production of high value chemicals by the US Department of Energy. In this context, the present study aimed to evaluate the production of HMF from glucose using the heterogeneous catalyst Nb₂O₅ in the pellets form. A study on the reaction conditions that could affect the HMF yield was performed. For this study, the variables: percentage of catalyst in the reaction (1% or 5% w/w), pellets diameters (1 or 2 mm) and reaction time (30 or 60 minutes) were evaluated, which were combined through a Taguchi L8 experimental design. The reactions were carried out in a 300-mL pressurized stainless steel reactor (Parr reactor) at 160 °C, using 50 g/L of glucose, acetone:water ratio of 1:1 (v/v), and agitation of 300 rpm. Statistical analysis of the results indicated that the main effects of catalyst dosage (A), diameter (B) and reaction time (C), as well as the second order effect AC and ABC were significant at 95% confidence level. The best performance in terms of HMF production was obtained using catalyst Nb₂O₅ in pellets of 1 mm, 5% (w/w) catalyst dosage, during 30 minutes, being achieved a HMF concentration of 5.0 g/L, approximately 100% glucose conversion and 14.3% HMF yield. Finally, assays to evaluate the catalyst recycling under the optimized reaction conditions revealed that the HMF yield remained in 13.5% in the first and second recycle reactions. It was concluded that the developed catalyst has potential for application in the conversion of glucose to HMF.

Keywords: 5-Hydroxymethylfurfural, Pellet, Heterogeneous catalyst

C.26 - The potential of co-processing lignocellulosic biocrude oil from hydrothermal liquefaction in petroleum refineries

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Brazil has committed to an ambitious Nationally Determined Contribution (NDC) target of reducing its greenhouse gas (GHG) emissions by 37% and 43% below 2005 levels, in 2025 and 2030, respectively. In the transport sector, although some alternatives have been identified for light duty vehicles, liquid fuels are still needed for long hauls, e.g., to fuel airplanes, ships and trucks. For instance, efforts from the international aviation sector such as the Carbon Offsetting and Reduction Scheme for International Aviation (CORSIA) are encouraging research on technologies able to deliver renewable jet fuel. Hydrothermal liquefaction (HTL) has been pointed out as one of the most promising technologies for the production of biofuels with low carbon footprint. HTL is a feedstock-flexible thermochemical process that converts wet biomass and residues into biocrude oil and other co-products. When compared with bio-oil from pyrolysis, HTL biocrude presents higher heating values in the range of 30-40 MJ/kg, as well as relatively low oxygen (5-10 wt.%) and water (0-5 wt.%) contents. These properties, along with other characteristics, reveal the HTL biocrude as an alternative to pyrolysis bio-oil for co-processing as a biobased feed along with petroleum in a conventional refinery. In this sense, a study of the co-processing of lignocellulosic HTL bio-crude is proposed to assess the advantages of using an existing refinery scheme and downstream infrastructure. This study is part of a doctoral project inserted in BioValue Fapesp Project, which will evaluate different conversion technologies to produce advanced biofuels.

Keywords: co-processing, hydrothermal liquefaction, lignocellulosic biocrude

Supported by: FAPESP and CAPES

C.27 - Utilization of alkaline-sulfite pretreated sugarcane bagasse for xylo-oligosaccharides production assisted by enzymes

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The production of xylo-oligosaccharides (XOS) had increased importance in recent years due to their functional, antioxidant and prebiotic properties. The objective of this study was to obtain and purify XOS from alkaline-sulfite pretreated sugarcane bagasse by enzymatic extraction. Sugarcane bagasse was pretreated by 5% sulfite/10% NaOH w/w (120°C/2 h) to facilitate enzymatic extraction of XOS. XOS extraction was performed by xylanase (5U Luminase PB-200/g bagasse), at pH 6, 70°C and 5% solid loading, resulting in enzymatic hydrolysis yields of 30% of the original xylan. Crude prepared XOS were fractionated by adsorption in activated charcoal followed by elution with an ethanol gradient (10 to 70% v/v). Activated charcoal adsorbed more than 80% of the sugars present in the enzymatic extract. Adsorbed XOS were eluted with ethanol gradient, providing fractions composed of dimmers up to hexamers, where high degree of polymerization XOS eluted at the highest ethanol concentrations. All the XOS fractions exhibited antioxidant capacity, which was evaluated by three tests: phospho molybdenum to measure total antioxidant capacity (TAC), and ABTS^{••} or DPPH[•] antioxidant parameters based on total radical-trapping. The XOS fraction eluted by 70% ethanol presented the highest antioxidant capacity in all assays, followed by XOS extracted by 50% ethanol. XOS fractions also contained phenolic constituents and the antioxidant activity matched with higher phenolic contents. Such data will allow for investigation the relation between XOS structure and antioxidant capacity. The potential antioxidant of xylo-oligosaccharides from sugarcane bagasse demonstrated in this study shows the enzymatic extraction of XOS as an attractive alternative for the Biorefineries.

Keywords: antioxidant, xylanase, xylan. **Supported by:** FAPESP, CNPq and CAPES

C.28 - Production of xylitol from switchgrass and Eucalyptus globulus hydrolysates obtained by steam explosion pretreatment

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Xylitol is a compound of great interest for incorporation in lignocellulosic biorefineries due to its emerging market and numerous applications in food, healthy, and energy areas. This study investigated the production of xylitol from hemicellulosic hydrolysates obtained by steam explosion of two feedstocks, switchgrass (*Panicum virgatum*) and Eucalyptus globulus, with the aim of producing a valuable compound with potential to be integrated in an ethanol biorefinery. Initially, the raw materials were submitted to steam explosion pretreatment to recover hemicellulose sugars, especially xylose. Continuous steam explosion pretreatment was performed in a pre-pilot plant under previously optimized conditions (200 °C, 10 min of residence time). Characterization of the produced hydrolysates revealed the presence of significant amount of oligomeric sugars and phenolic compounds. Then, a post hydrolysis with sulfuric acid was performed to increase the amount of monomeric sugars. However, the concentration of inhibitors like acetic acid and furfural, was also increased during this stage. Therefore, an evolved strain of the yeast *Kluyveromyces marxianus*, with improved ability to tolerate inhibitor compounds, was selected for use in the fermentation experiments. For comparison, fermentation assays in chemically defined media simulating the composition of the hydrolysates (with and without inhibitors) were also carried out. Although an evolved yeast strain was used for fermentation, the results revealed that a detoxification of the hydrolysates was still needed to improve their fermentability. After detoxification, the hydrolysates contained minimum amounts of formic and acetic acids and phenolic compounds, and the production of xylitol was significantly improved -reaching up to 13,7 g/L in the case of switchgrass and 28,1 g/L for Eucalyptus- when compared to the non-detoxified media -which was 0 g/L.

Keywords: Fermentation, Steam explosion pretreatment, Xylitol

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C.29 - Advanced Oxidative Process: a lean method for hydrolysate detoxification

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The use of lignocellulosic biomass plays a key role in the consolidation of biorefineries. The main fractions of these materials, such as cellulose and hemicellulose, can be converted into hydrolysates rich in fermentable sugars. In the process of hemicelluloses hydrolysis with dilute acid, together with sugars, compounds potentially inhibiting microbial metabolism such as furans and phenolic compounds are generated. Thus, detoxification processes of these hydrolysates are of great importance for their application as substrate in bioconversion processes. This study aimed to compare the detoxification of rice straw hemicellulosic hydrolysate by Advanced oxidative processes (POAs) with two traditional detoxification methods, Overliming and Activated Carbon adsorption. The original rice straw hemicellulosic hydrolysate has a 120 g/L of total sugar and potentially microbial metabolism inhibiting compounds such as furans (146 mg/L) and low molecular weight phenolic compounds (1600 mg/L). The results showed that, the POAs detoxification process was the most efficient in reducing phenolic compounds and furans concentrations. Treatment of hydrolysate by POAs and adsorption by active charcoal led to reductions in phenolic compound concentrations of 85% and 76%, respectively. Regarding Overliming treatment, no reduction in the concentration of phenolic compounds was observed. With respect to furans, concentration reductions up to 70%, 20% and 100% were achieved for hydrolysate treated by POAs, Overliming and Activated Carbon, respectively. In both detoxification treatments, by POAs or Activated Carbon, there were no decrease in the sugars concentration in the treated hydrolysate. Treatment by POAs was the only one that provided full recovery of the treated hydrolysate volume. Both treatments, Overliming and Activated Carbon, led to a loss of about 35% of the initial volume of hydrolysate. Thus, the POA hydrolysate detoxification method can be considered a lean method, capable of reducing the concentration of initiating compounds preserving both sugar concentration and the volume of treated hydrolysates. **Keywords:** Advanced Oxidative Processes, Destoxification, hemicellulosic hydrolysate. **Supported by:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES

C.30 - Effect of nitrogen sources on butyric acid production by *Clostridium beijerinckii* Br21

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Butyric acid is a compound of high relevance in the chemical, pharmaceutical, and food industry. Approximately 8×10^4 tons of this acid were commercialized worldwide in 2018. Currently, butyric acid is almost exclusively produced by organic synthesis: a petroleum derivative is used as precursor, which generates pollutants such as chlorides and cyanides. Strategies for the clean production of this acid have been studied, and the fermentation route is an attractive option to solve this issue. In this context, *Clostridium beijerinckii* Br21 is promising, it can produce exclusively butyric acid from various substrates. This work aims to compare how different nitrogen sources influence butyric acid production since these sources are fundamental in several metabolic pathways, using sucrose as a carbon source as this substrate has low cost and high availability. The assays were conducted in 100 mL batch flasks. Different culture mediums containing simple nitrogen sources as ammonium acetate, ammonium sulfate, ammonium chloride, and urea and complex sources as casaminoacids, peptone, beef extract, and yeast extract were tested and 15 mmol.L⁻¹ sucrose, as carbon source, was used. The tests were conducted at carbon/nitrogen ratios (g/g) of 1, 10, and 20, at an initial pH of 6.5 and 35 °C, for 48 h. During the fermentative assays, samples were taken for organic acids and sucrose quantification by HPLC. In general, complex sources showed better results of cell growth and butyric acid production when compared to simple sources. The highest final concentration of butyric acid for complex sources was 36.8 mmol.L⁻¹ in the assay using yeast extract C/N 1. For simple sources, the tests containing C/N 10 and C/N 20 urea showed the most promising results, reaching concentrations of 25.3 and 25.1 mmol.L⁻¹, respectively. In all the assays, butyric acid was the main fermentation product and was achieved in very promising yields.

Keywords: Nitrogen, Yeast extract, C/N ratio. **Supported by:** FAPESP 2018/00789-7 and CAPES 001

C.31 - Bioinformatic analysis of transporter proteins and enzymes related to *Candida tropicalis*' slow L-arabinose assimilation

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One crucial aspect of economically viable bioprocesses is the entire use of the sugars available in the raw material, which is in line with the strategies to maximize the conversion of lignocellulosic materials into bioproducts with high added value, as xylitol. The efficient utilization of pentoses by yeasts depends on the existence of a cellular machinery that includes the association between transporter proteins and specific enzymes responsible for their integration with cellular metabolism. Several studies have discussed the slow L-arabinose assimilation by *Candida tropicalis*, yeast with high capacity to assimilate others pentoses like xylose. Therefore, considering that *C. tropicalis* genome was available on the NCBI platform (*National Center for Biotechnology Information*), the aim of this work was to use computational tools to assess the presence and frequency of transporters and enzymes related to L-arabinose metabolism in this yeast. For this, a set of proteins related to L-arabinose assimilation by yeasts was selected based on manual search. The selected protein sequences were used as a template on BLASTp for identification of proteins with similar sequence on *Candida tropicalis* MYA-3404 proteome, applying an e-value of 10e-30. These sequences were subsequently added in the PFAM data bank and TMHMM and SignalP softwares. After alignment by MUSCLE, phylogenetic analysis were carried out using the Maximum-Likelihood method through the PhyML program and visualized through iTOL software. The results evidenced the presence of two L-arabinose transporter proteins (ART1 and ART2) from 17 initial candidates and four putative aldose reductases (AR1, AR2, AR3, and AR4) related to arabitol generation from arabinose, which were highlighted according to the homology with Aldose Reductases from ascomycetes yeasts. Thus, the computational tools evidenced the presence of a cellular machinery related to arabinose assimilation in *Candida tropicalis* but further experiments are required to confirm the participation of these proteins on this biological process.

Keywords: L-arabinose, Transporters, *Candida tropicalis*. **Supported by:** FAPESP, CNPq and CAPES

C.33 - Recover of extractives, hemicellulose and lignin as byproducts in ethanol production

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The extraction of byproducts co-generated in the production of 2G ethanol and their conversion into high value-added products are key factors to enable more green and sustainable processes in a future biorefinery. In this work, we combined and modulated chemical and physical treatments to propose different scenarios for a biorefinery using elephant grass as starting material. Alkaline treatments applied directly to non-treated biomass (Scenario 1) were more efficient considering 2G ethanol production alone, due to the higher solid content remaining after the treatment. Though both lignin and hemicellulose can be recovered from the alkaline liquid fraction in Scenario 1, the use of diluted acid and diluted alkaline treatments sequentially (Scenario 2) allowed the isolation of these components (hemicellulose from acid liquid fraction and lignin from alkaline liquid fraction) in amounts ca. two times higher. In contrast, ethanol production decreased in Scenario 2. In both scenarios, physical treatments using ball milling applied after the alkaline step reduced the biomass crystallinity and, consequently, improved the enzymatic digestibility. Finally, extractives were recovered in Scenario 3 using green methodologies, such as supercritical carbon dioxide (SFE) and pressurized liquid extractions (PLE) using water and ethanol as solvents, and resulting in extracts richer in fatty acids and sterols (for SFE) or alcohols and phenolics (for PLE). The highest extract yield was obtained using PLE (ca. 7%), and the post-extraction solid of this process was sequentially treated using a diluted alkaline solution aiming at the improvement of the sugar release and the lignin recovery. Therefore, besides the conversion of cellulose into glucose and the further fermentation to ethanol, the byproducts can be forwarded to applications as chemicals, materials or biofuels, which can contribute to the transition towards a more sustainable industry and enable the production of 2G ethanol. **Keywords:** biorefinery, ethanol, lignin. **Supported by:** Fapesp

C.34 - adaptive mutations to ethanol tolerance in *Saccharomyces cerevisiae* PE-2

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Four populations of *Saccharomyces cerevisiae* Pe-2, from same progenitor, were submitted to an Experimental Evolution protocol where increasing ethanol concentration shocks gave selective pressure leading to mutations related to ethanol tolerance. Through Genomic sequence we could compare progenitors versus evolved populations genomes and found possible adaptive mutations during the course of the experiment. Those mutations were reverse engineering on the progenitor background tested phenotypically, providing candidates genes or pathways that could improve the tolerance of ethanol in yeast. AIM: The major goal of the present work is to conduct a molecular and phenotypic characterization of mutations conferring yeast *Saccharomyces cerevisiae* PE- 2 ethanol tolerance. Progenitor and evolved yeasts were sequenced by Illumina/Pac Bio at the initial and final point of the experiment, respectively, and also in intermediated points for the search of alleles candidates as adaptive genes by Sanger sequencing. We performed phenotypical evaluation experiments, such as competitions, viability tests and threalose quantification to compare progenitors and evolved yeasts. The alleles found were genetically engineered using knockouts or Crispr Cas9 on the progenitor background and tested. Genome sequencing of evolved populations revealed 72 mutations. Using Sanger sequencing, we identified the time in which 22 of these mutations appeared along the experiment time-line. We found that some mutations got fixed on their respective populations early at shock number 20, whereas other appeared later on experiment (treatments 40, 60). Thirteen of these mutations were reverse engineered into the parental background. The resulting strains and the evolved populations were tested comparatively to the parental types by cell viability assays, competition experiments and trehalose quantification allowing to estimate the contribution of each 13 mutations to the phenotype of ethanol adaptation. The data shows that genes related to AMPc/PKA/MAPK and threalose pathways may be related to ethanol tolerance. **Keywords:** Experimental Evolution, ethanol tolerance, *Saccharomyces cerevisiae*. **Supported by:** FAPESP 2017/13972-1 and 2017/24453-5

C.35 - Extracellular antioxidant production from microalgae under increased CO₂ concentrations

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Microalgae have been used to transform CO₂ into a whole range of interesting metabolites, such as fatty acids, polyhydroxyalkanoates, polysaccharides, and carotenoids that present antioxidant activity. Among the later, astaxanthin is the highest-value bioproduct produced from microalgae that has achieved commercial success. In a previous work, fast-growing and high CO₂-tolerant microalgae were pre-selected under a 30% CO₂ atmosphere. From these cultivations, cells were isolated, identified and evaluated under CO₂ concentrations ranging from 0.04% to 30%. Among the isolates, *Parachlorella kessleri* presented the highest biomass production and growth rate under all conditions tested, besides producing an extracellular reddish pigment that displayed an antioxidant activity. The production of antioxidants in some cases occurs when microalgae are subjected to stress, apparently as a protective device. In the present work, we aimed to evaluate the extracellular antioxidant production from *P. kessleri* in well-controlled stirred tank photobioreactors under 5%, 15% and 30% CO₂. In the 10th day of cultivation, CO₂ supply was interrupted until the end of the cultivation (14th day), as a way to impose a stressful condition required for pigment production. Total carotenoid content was approximately 16% higher under 5% CO₂ compared to 15% CO₂, which was almost 140% higher compared to 30% CO₂. Total lipids in the final biomass was also higher, almost 20% (~250 mg/g dw), under 5% CO₂ when compared to 15% CO₂ (~200 mg/g dw), which was also higher when compared to 30% CO₂ (~170 mg/g dw). Lipids composition analysis showed different relative amounts of the 5 major fatty acids (16:0, 18:0, 18:1, 18:2 and 18:3) encountered under different CO₂ conditions. Higher amounts of palmitic acid (16:0) were detected under 30% CO₂ (~60%), whereas higher amounts of oleic acid (18:1) were detected under 15% CO₂ (~30%). To our knowledge, this is the first report of an extracellular carotenoid produced by this species.

Keywords: carotenoid, microalgae, biomolecules

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C.36 - Comparative genomics of industrial yeasts strains used in bioethanol production

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The production of bioethanol in industrial scale relies primarily on yeasts (*S. cerevisiae*) to conduct the fermentation processes that convert biomass into fuel. There are multiple strains of this species currently in use for industrial processes, with each of them possessing unique characteristics and genetic backgrounds that can heavily impact the overall efficiency of the process. For example, some strains are used to process biomass from corn (such as EthanolRed) while others are used for sugarcane (CAT1). In order to further understand the biological and genetic aspects associated with particularities of different industrial yeast strains, we conducted a comparative genomics study of 10 different strains commonly used in bioethanol production: BG1, CAT1, EthanolRed, FaliES1, JAY291, NCIM3186, SA1, ThermosaccDry, VR1 and ZTW1. Additionally, we also included S288C as a reference strain, CEN.PK113-7D as a control and *S. paradoxus* as an outgroup. Our pipeline was able to annotate an average of ~6000 genes per sample and, using a combined approach of orthology, positional homology and sequence similarity, identify known equivalents in ~95% of predicted genes with high accuracy. Preliminary phylogenetic analysis on the strains indicated that biomass substrate is the most important factor for grouping the samples. Additionally, we also identified a total of 5336 high-impact short variants across the 11 *S. cerevisiae* samples. Lastly, 13-way multiple genome alignment allowed us to identify genomic rearrangements that are shared amongst the strains or unique to a certain group (e.g. sugarcane-fermenting yeasts). At the individual level, 5 industrial strains (BG1, SA1, EthanolRed, VR1 and ZTW1) exhibited large-scale genomic rearrangements which include both chromosomal translocations and inversions. Taken together, our results allowed a deeper understanding the differences between industrial strains at a molecular level, identifying chromosomal regions, genes and variants that can be associated with observed differences amongst industrial yeast strains. **Keywords:** yeast, genomics, bioethanol. **Supported by:** BBRSC, FAPESP and CNPq

C.37 - Xylooligosaccharydes (XOs) enzymatic production from sugarcane bagasse hydrothermal and organosolv liquors: process optimization and prebiotic activity

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Xylooligosaccharides (XOs) are small oligomers of xylose (2–10 units of monomers), with high added value and important prebiotic properties, which makes it interesting for pharmaceutical and food industries. These compounds can be produced through a mild hydrolysis of xylan catalyzed by xylanases. The enzyme mechanism action and XOs production profile will depend of enzyme and substrate characteristics. Sugarcane bagasse, on the other hand, is the major agro-industrial residue in Brazil and represents a rich source of xylan that could be used for XOs production. Due sugarcane bagasse recalcitrant characteristics, a pretreatment step is necessary to make enzyme access possible. Between pretreatment methods, hydrothermal and organosolv pretreatments stands out in hemicellulose solubilization in the form of oligomers. In this context, the present work aimed to evaluate XOs production from hydrothermal and organosolv liquors and its prebiotic activity. For this, hydrothermal and organosolv pretreatments conditions were optimized, being defined as 185°C/10min and 170°C/60min/50% ethanol, respectively. The obtained liquors were concentrated at 70°C under vacuum, till a xylooligomers concentration of 25 g/L. Enzymatic XOs production were carried out using hydrothermal and organosolv liquors at 50°C and pH 5.5, using 3.8 IU/mL of a pre-selected xylanase (NS22036). The produced XOs were quantified by liquid chromatography mass spectrometry (LC-MS) and a conversion of 65% and 37% was observed for hydrothermal and organosolv liquors, respectively. In this way, hydrothermal XOs were selected and its prebiotic activity was evaluated in the growth of probiotic lactic bacteria (*L. reuteri*, *L. acidophilus* and *L. rhamnosus*). The presence of XOs improved bacteria growth, reaching the same growth of MRS control experiments, besides the presence of microorganism inhibitors (furfural and hydroxymethylfurfural). This shows XOs obtained from sugarcane bagasse prebiotic potential, needing further studies of purification to optimize its action. **Keywords:** Xylooligosaccharydes, bagasse pretreatment, prebiotics. **Supported by:** FAPESP #2018/14552-9; #2016/10636-8 and #2018/06696-0, CNPq and CAPES

C.38 - Direct conversion of cellulose to 5-hydroxymethylfurfural using a heterogeneous acid catalyst based on heteropolyacid

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One aspect of great importance for the development of biorefineries based on lignocellulosic biomass is the production of platform molecules, such as 5-hydroxymethylfurfural (HMF). This study aimed at evaluating the production of HMF from cellulose using a heterogeneous acid catalyst based on H₃PW₁₂O₄₀ supported on Nb₂O₅ (30% w/w). Initially, a screening of the main variables that could influence the HMF yield from cellulose was performed using a Taguchi L16 experimental design. Namely, the effect of temperature (160 or 200 °C), acetone (50% or 75% v/v), cellulose (5% or 10% m/v) and catalyst dosage (1 or 5% m/v) on the production of HMF was evaluated. The reactions were carried out in a 300-mL pressurized stainless steel reactor (Parr reactor) during 30 minutes. Statistical analysis of the results indicated that the main effects of temperature (A), acetone (B) and catalyst concentration, as well as the second order effect AB were significant at 95% confidence level. The highest HMF yield (17.0%) was obtained when using 200 °C, 75% (v/v) acetone, 100 g/L of cellulose and 5% (m/v) of catalyst. In a second step, efforts were done to optimize the conditions of temperature (160 to 240 °C) and acetone (50% to 90% v/v) to be used on the production of HMF by using a 22 full factorial rotational design, maintaining the cellulose and catalyst dosage fixed at 100 g/L and 5% m/v, respectively. Analysis of these results indicated high statistical significance ($p < 0.05$) for the quadratic effects of temperature and acetone. The condition able to maximize the HMF yield (20.6%) within the studied region was defined as 200 °C and 70% (v/v) acetone. These results show the potential of application of catalysts based on heteropolyacid for direct conversion of cellulose into HMF.

Keywords: Hydroxymethylfurfural, Cellulose, Heteropolyacid catalyst

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C.39 - Pullulan production from xylose: a promising alternative for hemicellulose valorization

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Hemicellulose is the second most abundant polysaccharide in nature. Some studies have been suggested that the viability of biorefineries depends on the hemicellulosic fraction valorization, mainly by obtaining high added-value products. An attractive alternative to add value to hemicelluloses is the pullulan production. Pullulan, an exopolysaccharide (EPS) that has diversified applications in the food, pharmaceutical, agricultural, and cosmetic sectors, is industrially produced from starch by the microorganism *Aureobasidium pullulans*. The present work aimed the use of xylose, a monosaccharide from hemicellulose, for pullulan production. The use of xylose as carbon source has a great potential in the production of this EPS, but this option has been only scarcely studied until now. Influence of xylose concentration (45-115 g/L), sodium nitrate concentration (0.2-3.8 g/L) and initial pH (4.0-8.0) in the pullulan production by *A. pullulans* ATCC 9348 was evaluated, using a design of experiments (Box-Behnken) followed by response surface methodology analysis, towards the optimization of the composition and initial pH of the medium. Obtained results shown xylose and sodium nitrate concentrations and initial pH of the medium are significant factors in the pullulan production. The maximal pullulan concentration obtained was in the range of 32-35 (g/L), with a yield and productivity of 0.41-0.46 g/g and 0.35-0.39 g/(L.h), respectively. Such results were obtained employing 80 g/L xylose and 2 g/L sodium nitrate at pH 6.0. This work showed the potential of xylose as carbon source for pullulan production as an interesting alternative for hemicellulose valorization.

Keywords: Hemicellulose, Pullulan Production, Xylose. **Supported by:** CNPq and FAPESP

C.40 - Laccase catalyzed cross-linking of ferulates attached to arabinoxylan extracted from sugarcane bagasse

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Within the concept of biorefineries, hemicellulose derived from lignocelulosic materials has a great potential for biotechnological applications. Considering sugarcane bagasse hemicelluloses, arabinoxylan is the main component of secondary cell walls. In alkaline-sulfite chemothermomechanical pretreatment, a significant fraction of this hemicellulose is retained in pretreated materials. Enzymatic xylan extraction from these materials can generate a C6-rich residual fraction useful for saccharification steps, besides fractions of low molar mass xylans with low lignin content. Enzyme catalyzed cross-linking of low molar mass xylans through hydroxycinnamates oxidative coupling represents an alternative to promote an increase in molar mass of enzymatically extracted xylan with potential applications for the cross-coupled polymeric materials. In the present study, the main goal was to evaluate laccase-catalyzed cross-linking of enzymatically extracted xylans. For this, sugarcane bagasse was initially pretreated with alkaline-sulfite chemothermomechanical process at 7.5% (w/w) sulfite load. Xylans contained in pretreated bagasse were partially extracted with aid of commercial xylanases (8 IU.g⁻¹) at 50°C for 24h. Recovered xylan corresponded to 38.4% (w/w) of the available xylan in pretreated material. The extracted material contained 52.8% xylan, 7.1% arabinosyl and 0.5% acetyl substituents. Ferulates were also detected at 1.3%. Lignin, p-coumarate and glucan contaminations were also detected in the xylan fraction. FTIR and UV/Vis characterization of the xylan fraction indicated typical arabinoxylan structures containing hydroxycinnamate substitution. Molar mass distribution pointed for predominance of a low molar mass fraction with 1.4 kDa. After oxidative treatment of extracted xylan with commercial laccase (45 IU.mg⁻¹, for 2h and 27°C), xylan cross coupling was confirmed, once the molar mass distribution identified a minor fraction (approximately 15% of the eluted xylan) with a high molar mass (50 kDa). Current data suggest that a new cross-coupled xylan with promising application can be produced, adding value to the use of sugarcane bagasse in biorefineries. **Keywords:** biorefinery, xylan, cross-linking. **Supported by:** CAPES and FAPESP (2014/06923-6; 2018/17417-5)

C.41 - Beyond the levulinic acid: An accurate and efficient estimation of kinetic parameters by means of an optimization procedure based on genetic algorithm.

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On this work, a mechanistic model was developed in order to simulate the kinetics of the levulinic acid (LA) production from sugarcane bagasse (SB), rice husk (RH) and soybean straw (SS). The production of LA from this agro-industrial waste (AW) followed the methodology of bio-refining in three stages. Experimental data of the third stage (catalytic depolymerization of cellulose) obtained under a wide range of operating conditions were used to estimate the parameters of the model. An optimizing procedure based on genetic algorithm was used to determine the optimum values of the parameters. The prediction of glucose concentrations, 5-HMF and LA using the mechanistic model was particularly precise, as demonstrated by R² and RMSE. Therefore, a satisfactory concordance between the high yields of levulinic acid calculated by the model of 61.1 mol% 67.7 mol% and 61.4 mol% and the experimental yields of 60.5 ± 2.1 mol%, 65.2 ± 2.9 mol% and 61.5 ± 4.0 mol% ± 4.0 mol% (under the optimum conditions of 190°C, 7.0% m/v of H₂SO₄, 75 min) to SB, RH and SS, respectively. The biorefining of AWs under optimum operating conditions allowed a satisfactory catalytic depolymerization of the cellulose, independent of the degree of crystallinity. The estimation of yields led to propose a strategy, from the viewpoint of the synthesis and the project of the process, which integrates SB, RH and SS to supply the off season of them and, so, to ensure the supply of raw material in the production of LA. **Keywords:** agro-industrial waste, levulinic acid, mathematical modeling. **Supported by:** FAPESP; CNPq

C.42 - Construction of a recombinant library of *Burkholderia sacchari* for improved production of polyhydroxyalkanoates of mixed composition

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Polyhydroxyalkanoates (PHA) are biodegradable, biocompatible bacterial polyesters with thermoplastic properties. Thus, they constitute an environmentally friendly alternative to the use of plastic of petrochemical origin. P(3HB-co-3HHx) is a member of the PHA family that contains 3-hydroxyhexanoate copolymerized with 3-hydroxybutyrate, which has recently been studied as a scaffold or molds in tissue engineering and other medical applications. Two relevant points to develop application of this copolyester are: (i) control of the copolymer's 3HHx content, which determines its properties, and (ii) to increase the conversion of the carbon source supplied to 3HHx units. The bacterium *B. sacchari* accumulates high levels of PHA but low 3HHx is naturally incorporated, while *Aeromonas sp.* has enzymes for high levels of 3HHx but accumulates low PHA content and moreover uses few range of carbon sources. This work aimed to overexpress the production of this biopolymer in *Burkholderia sacchari* with the insertion of the *phaPCJ* operon of *Aeromonas sp.* TSM81 in its chromosome. A library of stable, plasmid-free, antibiotic-resistant bacterial mutants for PHA production was generated using the Tn5 mini-transposon. A total of 3000 recombinants from the library were screened regarding PHA production. DNA sequencing analysis of the improved recombinants selected, concerning the chromosomal site of transposon insertion, is expected to identify the most favourable region to express heterologous genes in *B. sacchari*, a non-model bacterium considered a promising microbial cell factory.

Keywords: *Burkholderia sacchari*, polyhydroxyalkanoates, recombinant library

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C.44 - Co-expression of xylose isomerase and xylulose kinase as strategy to increase xylose conversion rate to poly(3-hydroxybutyrate) in *Burkholderia sacchari*

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Polyhydroxyalkanoates (PHA) are alternative materials to petrochemical plastics, with the advantage of being biodegradable and biocompatible. There are several types of PHA, the most studied being poly(3-hydroxybutyrate) [P(3HB)], differing from each other by monomer composition. One of the limitations to achieve mass production of PHA is the price of raw material. The utilization of xylose (the major component of hemicelluloses) from the lignocellulosic biomass left from ethanol production is considered an alternative path to lower PHA production cost. In this scenario, *Burkholderia sacchari* emerges as a potential tool for the production of PHA due to the fact that it is capable of converting xylose into P(3HB) with high efficiency. Our objectives were to further increase *B. sacchari* performance [growth rate and P(3HB) production] through increased expression of genes related to xylose catabolism, a possibility suggested by previous studies. To reach that goal, we obtained recombinant strains expressing the genes of interest (*xylA* and *xylB*) in pBBR1MCS-2, a broad-range vector, and evaluated performance in shake flask experiments. Xylose isomerase (*xylA*) from five bacterial strains were evaluated regarding enzymatic activity. *B. sacchari* recombinant strain expressing *xylA* from *Burkholderia xenovorans* LB400 presented 113% increase in enzymatic activity. However, that increase in activity did not translate to increased strain growth performance, since the recombinant presented a decreased maximum specific growth rate in xylose (0,05 1/h, compared to the wild-type 0,10 1/h. The decrease was even higher (0,03 1/h) for recombinants harboring both *xylA* and *xylB* (xylulose kinase encoding gene). In conclusion, we hypothesize that the bottleneck for xylose consumption lies on another point of the catabolic network, possibly in the pentose phosphate pathway. **keywords:** *Burkholderia sacchari*, poly-3-hydroxybutyrate, xylose. **Supported by:** FAPESP, CNPq and CAPES

C.45 - Fuzzy Logic integrated with the Geographic Information System to study the potentiality of sites for biorefinery location

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Currently, due to environmental damage generating scarcity of natural resources, the diversification in the energy matrix has been studied and is required, reducing the use of fossil fuels, because these could soon come to an end. The renewable energy appears as alternative, enabling the commercialization of bioproducts, such as biofuels and bioelectricity, and has the main source of raw material the agricultural residues. However, for the processing of these biomass are not carried in their collection sites, but in biorefineries. These industries face some problems for their creation, especially in its initial stage, like the ideal location for implementation of the industry. This paper is based on the application of *Fuzzy* logic integrated to the Geographic Information Systems, proposing the identification of potential areas for the implementation of a biorefinery in the Administrative Region of Campinas. The criteria adopted for choosing the optimal location were proposed based on economic and environmental criteria, which were relevant for the mount of the geospatial database. After the mount of the geospatial database, maps were made to reclassify the criteria to be used in fuzzification, immediately afterwards they were reclassified and thus obtain defuzzification as the final results. In multicriteria analysis, the use of *Fuzzy* logic integrated with Geographic Information Systems proved to be an important tool to help decision making of potential areas for location of biorefineries in Administrative Region of Campinas.

Keywords: biomass, Fuzzy logical, GIS

C.47 - Retro-techno-economic-environmental analysis (RTEEA): a new approach for defining research & development process targets applied to a sugarcane biorefinery case

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Economic and environmental analysis is an important field in (bio)process engineering, supporting R&D decisions, enhancing the chance of successful industrial implementation of innovative or unconventional processes. Process systems engineering tools (PSE) allied with techno-economic analysis (TEA) and life cycle assessment (LCA) can be useful to verify the techno-economic feasibility and environmental impact of new processes, identifying possible bottlenecks and providing targets for R&D teams. Recently, retro-techno-economic analysis (RTEA) was expanded into retro-techno-economic-environmental analysis (RTEEA), combining LCA metrics with economic ones. RTEEA is constituted by four steps: construction of a base case, incorporation of TEA and LCA analysis into the simulation of the process, selection of key variables through global sensitivity analysis (GSA) and delimitation of feasible regions. RTEEA was applied to a first and second-generation ethanol-from-sugarcane process. The process net present value (NPV) and the CML-IA midpoint indicators were chosen as performance and environmental metrics. For the global sensitivity analysis, process variables from the pretreatment, cellulose hydrolysis and xylose fermentation stages were chosen. GSA indicated two groups of metrics influenced by two sets of variables. After the retro analysis in group 1, it was identified that PO limits the feasible region. For group 2, ozone layer depletion is the limiting metric. Threshold values were obtained for two of the main process variables that influence the process economic and environmental performance, enzyme load and hydrolysis conversion: 10.8 FPU/gcellulose and 20.6%, respectively. Additionally, RTEEA methodology was able to make explicit the relations between these variables through windows of feasible operation.

Keywords: Global sensitivity analysis, life cycle assessment, techno-economic analysis

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C.48 - Lignin-enhanced laccase production from *Pycnoporus sanguineus* 2512 in instrumented bioreactors

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The white-rot fungus *Pycnoporus sanguineus* is capable of excreting ligninolytic enzymes in solid-state and submerged cultivation. Fungal laccase is considered a powerful green biocatalyst, envisioned for industrial production and application; their high oxidative capacity allows them to be applied in various industrial fields, in particular in the biodegradation of industrial waste rich in phenolic and non-phenolic compounds. The current work evaluated laccase production excreted by *P. sanguineus* 2512 in a bubble column (BCR) and a stirred tank bioreactor (STR) under the inductive action of promptly available lignin (lignin soda and Kraft) and acid cellulignin (29% of lignin). Addition of 1.2 g/L lignin to the culture medium of *P. sanguineus* 2512 improved laccase production, with acid cellulignin being the most efficient inducer. In shake flasks, laccase activity achieved 10632 U/L on the sixth day of cultivation, an increase of 348% compared to the control (3053 U/L). The best aeration in BCR was 0.67 vvm, resulting in a laccase activity of 3249 U/L; associated with agitation (200 rpm) in STR, an increase of 158% was recorded in the enzymatic activity. The enzymatic cocktail was concentrated in a hollow fiber system and partially characterized by the size of the protein bands, pH and temperature effects, and thermal stability. The molecular masses of two bands obtained by SDS-PAGE, named Lcc1 and Lcc2, were 54.6 kDa and 76.53 kDa, respectively. The optimum temperature and pH (CCD - composite central design) were 50 °C and 5.0. The enzyme displayed thermal stability with a half-life of 3-4 days at 30 °C, 1-2 days at 40 and 50 °C, and 60-120 minutes at 60 °C. The described characteristics of the laccase production by *P. sanguineus* 2512 are similar to those reported in the literature, and make it a good candidate for further investigation and possible application in biotechnology.

Keywords: white-rot fungus, enzymes, thermal stability

Supported by: CAPES and CAPES

C.49 - Effects of PILs with hexylamine cations on biomass pretreatment

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Introduction: Pretreatment is a crucial and much-discussed step within biorefinery once market preferences of these process do not separate lignin from cellulose and hemicellulose properly, which makes the use of all macromolecular fractions of biomass difficult. Therefore, studies which are based on increasing sugars conversion and lignin solubilization, which directly results in higher enzymatic hydrolysis conversion, are important. **Objective:** In this sense, this study had the goal to evaluate the use of acetic and formic acids combined with hexylamine to form two protic ionic liquids to be applied in the sugarcane bagasse pretreatment. **Materials and Methods:** Pretreatment conditions was 2 h, 135°C (temperature chosen according to thermogravimetric analysis results) and 10% solids loading. Pretreated material was subjected to enzymatic hydrolysis assays at 150 rpm, 50°C, for 48 h, 10% solids loading, loadings of 15 FPU.g⁻¹ dry biomass (Cellic Ctec2) and the pH 4.8, adjusted with 0.05 mol.L⁻¹ sodium citrate buffer. **Results and Discussion:** sugarcane bagasse pretreated with hexylamine acetate resulted in higher cellulose and hemicellulose conversion, 53.5% and 26.5%, respectively. On the other hand, lignin solubilization was higher for hexylamine formate, 42.5%. **Conclusions:** Both PILs were efficient for the design of the material obtained, increasing the options for the desired product.

Keywords: Biorefinary, Enzymatic hydrolysis, Lignin solubilization

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C.50 - Fungal lignin peroxidases from Atlantic forest soils for biomass valorization

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Biomass is a known renewable source for bioenergy, products and materials. This also contributes as alternative compounds to fossil carbon sources, decreasing environmental pollution. In this perspective, routes for biomass valorization, especially looking for lignocellulosic materials are specially important. Chemoenzymatic approaches for pretreatment of lignocellulosic biomass applying oxidative enzymes, like lignin peroxidases (LiP) can be considered as real industrial solutions. In this context, our study aimed at the bioprospection of fungal isolates from Atlantic forest soil samples, enriched by lignocellulosic residues, looking to select microbial LiP for applications at biorefineries. A total of 190 filamentous fungi were evaluated. Applying high throughput enzymatic screenings, thirteen (13) fungi were selected. Among these strains, three (3) fungi were evaluated about their ligninolytic activities. They were characterized as *Mucor* sp., *Byssoschlamys nivea* and *Paecilomyces saturatus*. These fungi were evaluated by enzyme kinetic analysis, defining optima conditions for their activities, including pH, salinity and temperature. Apparent kinetic constants were determined from enzymatic crude extracts. In this sense, the influence of pH (range 2.0 to 9.0), temperature (4 °C, 25 °C, 30 °C and 65 °C) and salinity (0.5 - 4.0 M NaCl) at enzymatic activity and stability were investigated. Our results revealed alkaline-halotolerant lignin peroxidases, stable at 65 °C. Although, LiP from *Mucor* sp. (KM 55.65 mM, V_{max} 414.75 mmol min⁻¹, k_{cat} 7.45 min⁻¹) was more active at pH 3.0 and 25 °C. It is important to know that LiP from *Mucor* sp. was not well described by the literature.

Keywords: lignin peroxidase, biorefinery, biomass

Supported by: CAPES, FAPESP

D - Biofuel Engines and Applications to Aviation

D.02 - Amperometric detection of antioxidants in aviation biokerosene by HPLC using nanostructured detectors

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Current global challenges require a major effort to use new sources of energy. The aviation industry has developed biokerosene with characteristics similar to fossil kerosene. However, biofuels have low oxidation stability, that influence in the physicochemical properties these fuels. The antioxidants addition to biofuels is considered indispensable and has been effective in ensuring greater oxidation resistance and longer shelf life. In this work, the effectiveness of two nanostructured detectors was compared: GCE/rGO-AuNPs and GCE/rGO-AgNPs. Chromatographic separation was optimized, the best conditions were obtained with a C18 column, isocratic elution occurred with acetonitrile:water, and a post-column pump containing 0.3 mol L⁻¹ NaOH. The optimum potential for amperometric determination of antioxidants was determined under hydrodynamic conditions, 0.40 V showed better sensitivity. Analytical curves were constructed, a linear relationship was obtained between peak areas versus added concentration of antioxidants BPA, TBP, BHT and BMP. The limits of detection obtained were: (I) GCE/rGO-AuNPs detector 2.4x10⁻⁷ (BPA), 2.6x10⁻⁷ (TBP) and 2.2x10⁻⁷ mol L⁻¹ (BHT); (II) GCE/rGO-AgNPs detector 2.9x10⁻⁷ (BPA), 6.1x10⁻⁷ (TBP), 3.8x10⁻⁷ (BHT) and 4.2x10⁻⁷ (BMP). These methods were applied for antioxidants simultaneous determination in biokerosene samples. Peaks identifications were based on the antioxidants retentions times, and confirmed by addition of analytes standards in the samples. The determined concentrations of TBP were: 3.1x10⁻⁵ mol L⁻¹ (AuNPs) and 3.8x10⁻⁵ mol L⁻¹ (AgNPs); BHT: 3.3x10⁻⁵ mol L⁻¹ (AuNPs) and 2.7x10⁻⁵ mol L⁻¹ (AgNPs), and BMP 2.0x10⁻⁵ mol L⁻¹ (AgNPs), BPA was not detected. The average recoveries obtained varied from 97.3 to 101.5% (AuNPs) and 96.7 to 102.8 (AgNPs), confirming the accuracy of the methods. Precision was estimated from the relative standard deviation (RSD), values between 1.7 to 2.1% (AuNPs) and 1.2 to 4.5% (AgNPs) were obtained for all the compounds. **Keywords:** biokerosene, antioxidants, HPLC. **Supported by:** FAPESP

D.03 - ELECTROCHEMICAL DETERMINATION OF ORGANIC CONTAMINANTS IN AVIATION BIOKEROSENE Max Fabrício Falone Varelo^{1,2}, Edervaldo Buffon^{1,2}, Nelson Ramos Stradiotto^{1,2}

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The air sector is highly dependent on the use of fossil fuels; this practice effectively contributes to the emission of air pollutants, such as CO₂. In contrast, aviation biokerosene has emerged as an effective sustainable alternative to minimize this environmental problem. One of its main production routes is via sugarcane fermentation using genetically modified yeasts to produce farnesene, which after a hydrogenation process converts to farnesane (biokerosene). During the biokerosene production process, in addition to farnesane, other potential biokerosene contaminants are generated, such as hexahydrofarnesol (HHF) and mercaptan (MCP). To be commercialized, this biofuel must meet regulatory agency specifications, such as ASTM and ANP, which recommend a 1.5% (m:m) percentage for HHF and a maximum of 0.003% (m:m) MCP. In this context, it is necessary to develop simple, sensitive, selective and stable methods to determine these organic contaminants in biokerosene. In this context, molecularly imprinted polymers (MIPs) are an interesting analytical approach to the development of electrochemical sensors, since they are materials that selectively recognize a molecule of interest. Thus, the objective of this work is the development of MIP-based electrochemical sensors for the determination of organic contaminants in biokerosene. The sensors for HHF and MCP were prepared from pyrrol and o-phenylenediamine electropolymerization on the GCE surface, respectively. The experimental parameters that influence the analytical performance of these sensors were optimized. The developed electrodes were characterized by cyclic voltammetry, electrochemical impedance spectroscopy, electron scanning microscopy, atomic force microscopy and RAMAN spectroscopy. Analytical curves for HHF and MCP determination were constructed in a range of 4.0x10⁻⁸ to 1.5x10⁻⁶ and 1.0x10⁻¹² to 1.0x10⁻¹¹ mol L⁻¹, with limits of detection of 1.2x10⁻⁸ and 6.1x10⁻¹³, respectively. The proposed methods were successfully applied for HHF and MCP determination in aviation biokerosene sample.

Keywords: Aviation Biokerosene, Electrochemical Sensor, Organic Contaminants

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D.04 - Developing a Database Management System for Sustainable Aviation Fuels deployment in Brazil: a case study for Fischer-Tropsch pathway using eucalyptus in São Paulo state.

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The Carbon Offsetting and Reduction Scheme for International Aviation (CORSA) is an international agreement in the ambit of the International Civil Aviation Organization (ICAO) aiming to limit greenhouse gas emissions of civil aviation from 2020 onwards, also fostering the use of sustainable aviation fuels (SAFs). Brazil is one of the global leaders in biofuel production and presents significant potential for biokerosene production. In this context, a Database Management System (DBMS) is being developed in order to support decision-makers to obtain information related to potential feedstocks for SAFs production in Brazil. The framework has been developed to combine aspects such as edaphoclimatic suitability for six crops with topography, current land use and land cover, land price and existing infrastructure (e.g. roads, railways, refineries, airports, etc.) and socio-economic parameters (e.g. HDI, education level, wealth, health infrastructure). All information is spatially defined, and results can be obtained through reports or maps. The user can identify suitable areas for biomass production or rank areas based on indicators like productivity and production costs. This work presents results of a case study considering the production of SAF through Fischer-Tropsch pathway, based on eucalyptus, in São Paulo state. Preliminary results are available for co-locating industrial units located at Henrique Lage Refinery (REVAP), in São José dos Campos; and at a pulp industry, in Mogi-Guaçu. Due to the high costs of biomass, other cases have been developed, considering the production of eucalyptus in a region where land price is not expensive and yield could be high, transporting biomass through railway to an oil refinery (alternatives have been explored). Alternatively, a greenfield industrial plant has been also considered, and in this case, the aim would be defining the sites for minimizing the production costs. In these two cases, supply curves of eucalyptus at the industrial site have been built.

Keywords: biokerosene, database management system, eucalyptus

Supported by: Joint Research Center for Sustainable Aviation Biofuels – Boeing/Embraer

E - Sustainability, Environmental, Social, Economic and Public Policy Issues

E.01 - Native oilseeds as a potential of development by Brazilian biodiversity value chains

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This paper analyze and evaluate Brazilian biodiversity oils as potential triggers for the development of a new value chain of no-timber forest products that will contribute for a transition to bioeconomy. Firstly the paper analyzes the production, processing, and market models of two different vegetable oils. The model based on the olive oil in EU indicates paths for the improvement for Brazilian biodiversity oils value chain. Then, state of art of Brazilian biodiversity oils market and public policies that supports the socio-biodiversity production chain is analyzed. In addition to that, market and technological characteristics of the main raw materials for the production of oils from no-timber forest products are described with emphasis in Brazil nuts and babassu oils. The results indicate that those oils can play a relevant role for the improvement of no-timber forest products value chain, however they have identified a gap of scientific and technological for vegetable oil extraction and refining process. These technological progresses contribute for the Brazilian biomes conservation by adding value in processed products. Beyond the technological and market challenges of Brazilian biodiversity oils production, the value chain still demands institutional, legal, cross-sectorial and trusting framework that encourage actors to overcome the present difficulties of extractive activities. As an example of a cross-sectorial framework, it is possible to mention the personal hygiene and pharmaceutical Brazilian industries. These industries develop a market differentiation by vegetable assets as Brazilian biodiversity oils, extracts and tinctures. For this development demand high investments in R,D&I. For the Brazilian characteristics, the partnerships and alliances between companies, universities or research institutes and small local enterprises become relevant. In conclusion, the paper recommends social productive arrangements that integrate cooperatives and associations of local communities, universities and research institutes, industries and government institutions in order to make Brazilian biodiversity a strategic axis for sustainable development. At the local level, this social arrangement could promote decentralized vegetable oil processing units and a specific Ecolabel for the products of the Brazilian socio-biodiversity.

Keywords: local development, non-timber forest resources, sociobiodiversity.

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E.02 - RenovaBio: the responsibility involved in the development of the policy since its beginning

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Brazil is launching the National Biofuels Policy (RenovaBio), which will start operating in 2020, an impressive timing to implement a new regulatory system in the Brazilian context. The bill was approved by the congress in 2017, with a lack of discussions among the parties. The initiative aims to recognize the strategic role of all types of biofuels in the fuel matrix, as well as their importance for energy security and environmental sustainability. The policy - aligned with the Paris Agreement -, creates the Biofuel Decarbonization Credit (CBIO), an asset that will be traded on the stock exchange. The assets should be issued by biofuel producers and will be bought by fuel distributors that will have to comply with a decarbonization target set by the government. Based on the importance of carrying on initiatives related to the bioeconomy responsibly, generating socioeconomic development and environmental sustainability, this work aims to verify the responsibility involved in the development of the RenovaBio Policy since its beginning. We based our discussion in the framework of Responsible Research and Innovation (RRI). This theoretical and normative approach highlights the importance to find new ways to respond to the main challenges faced by society. According to RRI, the different stakeholders (scientists, educators, governments, private sector, policymakers, etc.) should anticipate the consequences of initiatives, being reflexive, responsive, and inclusive. The hypothesis we propose is that some aspects of responsibility pointed by the RRI framework were not attended due to the fast approval process of the RenovaBio policy. Inclusion, reflexiveness, and anticipation, for example, could be more explored. However, the policy can not be considered as irresponsible. On the contrary, since its beginning the guiding values were equity, credibility, dialog, efficiency, predictability, and sustainability. In this sense, this work could contribute with some reflexiveness regarding RenovaBio.

Keywords: Public policies, RenovaBio, Responsible Research and Innovation

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E.04 - Sustainable model of rural communities through bioenergy

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In Brazil, approximately 1 million families still live without electricity, which represents 0.48% of the total population. Although this percentage indicates the attendance of almost the entire Brazilian population, 82% of families without access to electricity live in rural areas. And it is precisely in this environment that bioenergy can play an important role in the universalization of this service, with the use of biomass in the generation of electricity. Given this scenario, the study presents guidelines for rural communities to become sustainable models with the deployment of biogas plants by harnessing the existing biomass on site, as well as being attractive to the government and entrepreneurs to invest in the project profitably, both financially and environmentally by offering a reduction in the costs with the electricity grid, with the commercialization of carbon credits and CBios by the new Brazilian program RenovaBio focused on biofuels. The rural settlement Estrela da Ilha was used as a model; The potential to produce electricity using a biodigester coupled to the generator was simulated in the conversion of existing biomass, a scenario was created with the planting of an energy crop to increase the energy potential and a mathematical model was used to analyze economic viability. It is concluded, according to the analysis made from the application of the software, a recovery of the initial investments within a period of 0.55 years with the own resources. Given the scenario with the cultivation of the Pennisetum purpureum (elephant grass) energy crop in only 10% of the pasture area without affecting settlement productivity, it is estimated that the potential for biogas production will increase by more than 10 times compared to the value of biogas production with available biomass, which may be appropriate to the RenovaBio program with the purification and commercialization of biomethane as biofuel.

Keywords: bioenergy, rural communities, sustainable model. **Supported by:** CAPES

E.05 - RenovaBio impacts on fuels prices and demand in Brazil in 2020 estimated by an input-output analysis in three scenarios of capital appropriation by the biofuels industry

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RenovaBio is the Brazilian national biofuels policy in force since 2017. In 2020, decarbonization credits (CBIOs) are expected to start to be issued. Fuels distributors are enforced to buy CBIOs from biofuels producers to meet decarbonization goals established by CNPE. The objective of this work was to estimate potential impacts on fuels prices and demand levels in 2020, considering 2020 decarbonization goal, in three scenarios of capital appropriation of CBIOs value. The estimate was conducted by an input-output analysis, using the Brazilian matrix of 2015 and Leontief's price model. Some premises behind this are that technology had insignificantly changed since 2015 and that the economy level of production would not respond endogenously to price changes. CBIOs price was assumed as USD10 and their volume issued in 2020 equivalent to the goal of 29.1 million CBIOs. They were considered as a tax-like payment by the fossil fuels sector and the shock was performed at the price of this 'tax'. This 'tax' was also used to perform a shock as a subsidy to biofuels sector, as a negative entry in the added value account. The three scenarios simulated were 0%, 50% and 100% of capital appropriation of CBIOs value by the biofuels sector, accounted also in the added value entry. According to these premises, the resulting variation of biofuels sector prices would be -2.2%, -1.1% and 0.04% in each of these scenarios; in the fossil fuels sector, of 0.4%, 0.6% and 0.7%. Considering elasticity-prices of -0.63 and -1.03 for biofuels and fossil fuels respectively, their demand would increase in 1.4%, 0.7% and -0.02% and in -0.5%, -0.6% and -0.7% for the three scenarios. This shows the relevance of the percentage of capital appropriation by the industry to enhance biofuel consumption. This result can contribute to policy improvement and biofuels sector planning. **Keywords:** Biodiesel, Biofuels policy, Ethanol. **Supported by:** Embrapa and Unicamp

E.06 - Peanut Oil Production Integrated to Sugarcane Industry: A Techno-Economic and Environmental Assessment

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A low-carbon society implies the replacement of non-renewable raw materials by renewable ones. Peanut is cultivated in São Paulo state as a green fertilizer to renovate the sugarcane culture soil. Hexane is used nowadays by the industry for the extraction of oil from leguminous cultures. However, bioethanol is a renewable production input with potential to replace hexane in the peanut oil extraction. In this sense, a techno-economic and environmental assessment is required to analyze the feasibility of this process. This work evaluates experimentally the use of ethanol as solvent for peanut oil extraction and presents an environmental and economic assessment of peanut oil production integrated to the sugarcane industry. Ethanol was used as solvent to extract peanut oil in a fixed bed column at 70 °C, with the extract recirculation during 4 h. Both crude and toasted peanut were used as grounded raw materials, being composed of 46% and 48% oil, and 5.0% and 3.5% water, respectively. The industrial peanut oil extraction process was simulated in the equation-oriented Environment for Modelling Simulation and Optimization (EMSO). The Life Cycle Assessment was performed considering the climate change impact category using the 100-year time horizon Global Warming Potential (GWP 100) under a cradle to grave scope, with energy-based allocation. The characterization factor data base was IPCC (2006) for emissions from fuels burning and for agricultural emissions, and the Ecoinvent 3.1 database for raw materials inputs. As results, the oil content in the extract obtained was 18 – 26% for ethanol. Both processes are economically feasible. Ethanol showed to be environmentally cleaner than hexane, but the net present value of the plant using hexane is higher than for ethanol. **Keywords:** life cycle assessment, peanut oil production, ethanol **Supported by:** FAPESP (grants #18/13207-6, #2017/13993-9, #2014/21252-0, #2016/10636-8), CNPq, and CAPES.

E.07 - Process Simulation and Economic Analysis of a Mango Kernel Biorefinery

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Introduction: The development and implementation of modern, sustainable biorefinery processes is a key factor towards meeting the vision of a biobased economy. The development of such processes requires information on the entire value chain, from production, transport and logistics to final products. This information is usually not available at an early stage of development. To this end, process simulation tools can offer a valuable alternative. Such tools have been in use in the chemical industry for many decades to facilitate the development of new processes and optimize the performance of existing ones. Similar benefits can be derived from the use of such tools in the industrial biotechnology arena. **Objective:** this work aimed to model and economically evaluate the operation of a biorefinery that utilizes mango kernels to generate energy and specialty bioproducts. **Materials and Methods:** the biorefinery was modeled and economically assessed using software SuperPro Designer, assuming that the plant processes 5 MT/h of mango kernels. Firstly, the seed coat is removed, and then the kernels are subjected to a steeping procedure. Next, the oil, protein, fiber and starch fractions are separated in dedicated process sections. In addition, the seed coat is burned to produce heat and electricity. **Results:** The simulated biorefinery produces approximately 901 MT of oil, 4,331 MT of starch, 771 MT of fiber and 643 MT of protein per year. The annual operating cost is estimated in \$5 million, and the total capital investment, in \$19 million. The operating cost is largely due to facility-dependent expenses (56%). The gross margin is expected to be 36%, and the payback time, roughly 5 years. **Conclusions:** This analysis indicates that a mango kernel biorefinery would be technically feasible and an attractive investment. It also demonstrates how process simulation can aid in the design and techno-economic evaluation of biorefinery systems.

Keywords: biorefinery, process simulation, techno-economic analysis

E.08 - Family Farming and biodiesel in Brazil: an overview and trajectory assessment of the Social Fuel Seal

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Family farming in Brazil is heterogeneous, formed mainly by a group of small producers marginalized in the 1970s by the agriculture modernization process. Since the 1990s several politics are being developed to support this social category. Strengthening the Brazilian family farming involves a set of social, economic, political, and cultural factors. Among the initiatives, the National Biodiesel Production and Use Program (PNPB) stands out. This program has as the main instrument the Social Fuel Seal, created in 2004 aiming to promote social inclusion by inserting small farmers in the agro-industrial biodiesel productive chain. In this sense, this work assesses the inclusion of the Brazilian family farmers in the biodiesel productive chain, focusing on the Social Fuel Seal as the main instrument to foster this process. We verified the changes in the requirements for the Seal's concession, use, and maintenance over time. Besides, we identified the impacts of this instrument concerning four aspects: a) production volume, b) income generation, c) type of raw-material offered, and d) the number of families benefited. A systematic literature review of studies about the Seal, as well as official data from the Brazilian federal government, pointed that the characteristics of family farming in some Brazilian regions do not allow these farmers to meet specific norms of the program. It was also possible to verify that there was legislation loosening to grant the Social Fuel Seal to the mills, once the raw-material purchases did not necessarily represent a real increase in family farming participation. Therefore, one of the main conclusions of this paper is that the changes in the Social Fuel Seal criteria enabled the mills to be strengthened rather than focusing on inclusion. In other words, the Seal has moved from a social inclusion instrument to one of strengthening the agro-industrial sector of biodiesel.

Keywords: Family Farming, Public policies, Social Fuel Seal

Supported by: CAPES

E.10 - The role of sustainable biomass in a low-carbon economy in the Netherlands

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The Netherlands has an ambitious goal to reduce Green House Gas emissions (49% in 2030) and 85% in 2050. To achieve this a major transition in the material and energy supply is required. Such a transition requires a vision, roadmaps, innovation and a policy and commitment from the society to achieve this. This presentation will present the potential contribution of biomass to this low carbon development. IEA scenario calculations [1] have indicated an important role of biomass to reach greenhouse gas reduction targets by 2050-2060. Biomass availability is not unlimited, so efficient use will be key. There is a clear motivation for policy to speed the transition towards the bioeconomy – grand challenges and the associated policy goals of climate change mitigation, energy security, the circular economy concept, rural regeneration, smart specialization and, paradoxically, reindustrialization. The new Dutch government has formulated in 2017 a target of 49% GHG reduction in 2030. All sectors; industry, housing, transport and agriculture will have to contribute. Electrification from renewable energy (solar and wind) and renewable heating by geothermal and a limited amount of biomass will enable this transition.[3] The Policy of the Circular Economy was presented in 2017 and roadmaps for several transition paths have been formulated. For this paper the main roadmap for Biomass and Food presents the integrated development in the bioeconomy and the role of biomass.[4] Biorefineries are generally expected to play an important role in decarbonizing energy and transport sectors with bioenergy and biofuel products. On the other hand, national and regional economies would benefit the most from a production portfolio that favors high-added value products, like advanced materials and chemicals. Industrial symbioses and increased integration with a versatile production of added-value biobased products and bioenergy can have highest impact both for climate goals and economic growth. However, sustainability of the biomass is crucial to ensure the role of biomass in this development and to avoid negative impact of the increase of biomass on agriculture and forestry. Therefore the Netherlands developed a framework for sustainability of biomass for electricity and heat production. This paper will present an overview and present promising concepts in the biobased economy and business opportunities in different sectors. The concept of sustainable biomass, both for production as well utilization will be presented based on experiences with national and European regulation for energy.

Keywords: Policy, Bioeconomy, Sustainability

E.11 - Assessment of soil conservation practices in sugarcane using SWAT modelling

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Soil conservation practices in sugarcane areas are efficient alternatives to mitigate erosion processes. However, the understanding of the impact of these practices on sediment yield and runoff as well as their efficiency in watersheds is still scarce. In this context, hydrosedimentological modelling is an effective and low-cost alternative for these studies. The objective of this study was to evaluate the efficiency of terracing and no-tillage system in reducing sediment yield and runoff in sugarcane cultivation. Eight scenarios were proposed: conventional sugarcane with terraces (CST) and without terraces (CSW), no-tillage system with terraces (NTST) and without terraces (NTSW), and all scenarios with and without the recovery of riparian vegetation, as established by the Brazilian Forest Law. The Soil and Water Assessment Tool model (SWAT) was used for hydrosedimentological modelling. The scenarios were applied in a sugarcane representative watershed, located in Limeira, state of São Paulo. The results showed that the NTSW reduced the sediment yield by up to 78% when compared to the CSW. CST reduced sediment yield by 60%, while in NTST this reduction was around 76% in comparison to the cultivation without terraces (CSW and NTSW). A decrease of 20% in runoff was observed in the NTSW scenario. The riparian recovery in CSW resulted in remarkable reduction in runoff and sediment yield, being 34 and 28%, respectively. Terracing and no-tillage system, when well implemented, showed similar behaviours in terms of sediment and runoff decrease when compared with the riparian vegetation recovery. These results represent a step forward in soil erosion modelling in watershed scale, as well as support agro-environmental planning of sugarcane activity. **Keywords:** terracing, no-tillage, sediment yield **Acknowledgments:** The authors thank the Coordination for the Improvement of Higher Education Personnel (CAPES) and the São Paulo Research Foundation (FAPESP, grant number 2018/26366-5) for funding the research on which this study is based. **Keywords:** terracing, no-tillage, sediment yield. **Supported by:** CAPES e FAPESP

E.12 - Life Cycle Inventory of Frozen Açai Pulp Production in Brazil

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Açaí (*Euterpe oleracea*) is an important specie in Amazonian region with diversified applications in food and pharmaceutical industries. Its industrial processing generates large quantities of organic residue which, currently, are disposed in uncontrolled landfills. In addition, the region supply of electricity relies on small hydroelectric (SH) local facilities and eventually, depending on the distance from SHs, on fossil fuel (diesel) engines for electric and thermal energy production. There are no published studies related to life-cycle inventory (LCI) and life-cycle assessment (LCA) for açai processing and distribution. The objective of this work is to provide a comprehensive LCI of açai's production chain to support further research on its environmental impacts. The system boundary for the production chain was set from the extraction of açai in natura and its further pulp processing and freezing, including the transportation and waste destination/treatment. The functional unit is kilograms of frozen açai pulp. Data for all the input flows were gathered from interviews with local managers, previous researches and government regulations. The LCI allows the identification of the major flows for açai processing and distribution and its ranking. Despite the magnitude of the input flows, a further LCA considering the suitable impact categories in a cradle-to-gate approach will provide guiding for a more sustainable destination of açai residues. For the LCA, emissions factors for the all of flows related to field and industrial activities such as chemicals, equipment, electricity, fossil fuels will be obtained from EcoInvent 3.9 database. The LCI results will support future açai processing LCA, what could be a strategic management tool for stakeholders, decision-makers and public policies related to sustainable agro industrial development.

Keywords: Life Cycle Inventory, Lignocellulosic Organic Residues, Waste Management

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E.13 - Are greener policies able to make biofuels sustainable? An expert's opinion analysis using the SDG Framework

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In September 2015, all UN Member States adopted the 2030 Agenda, in the form of the 17 Sustainable Development Goals (SDGs) and 69 targets. With the potential to contribute to this Agenda by reducing emissions with low-cost technology, bioenergy strategies have spread in countries seeking for energy security. However, the same strategy might contribute to the achievement of some SDGs while undermine the achievement of others. The objective of this study was to identify bioenergy's risks and benefits for the SDGs in Brazil, using Brazil's new biofuel policy (Renovabio) as a case study. The policy designs a system of tradable carbon savings credits, with the quantity of credits assigned reflecting the emissions saved by the biofuel's production process. We identified the most affected targets using literature review and expert surveys. The survey was conducted with 41 Brazilian experts from scientific community, civil society, producers & farmers, and policy-makers. Our results suggest that while targets for clean energy and climate policies are strongly supported by Renovabio, environmental targets are subject to negative trade-offs. This is the case for the SDG15 (life on land), with 5 on the top 11 negatively affected targets, along with water and food-related targets. Land-use change is a concern due to the decision on opening the Amazon region to sugarcane. In general, the policy has rather a positive effect in the SDG targets than negative, with greater intensity (6 times higher), according to experts. The policy's benefits seem to be clear to the different expert groups, what remains controversial are the risks. Biodiversity protection, water pollution, and inequalities are aspects of high relevance for policy monitoring in the coming years. This paper outlines which SDGs need special attention when implementing a bioenergy policy and the results add to the debate on SDG trade-offs inside of a policy.

Keywords: Renovabio, bioenergy, expert survey. **Supported by:** DAAD, BMUB

E.14 - Global land sparing due pasture intensification for bioenergy production

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Global pasture area is around 3 billion ha, which correspond to double of the area used for croplands, but has low contribution to food consumption. Sustainable intensification of pastureland is desirable to increase food production, improve economic returns, mitigate greenhouse gases emissions, and to spare land, e.g. to bioenergy crops. The aims of this study were to estimate global and regional potentials to increase livestock productivity in grazing systems and consequent spare land for bioenergy crops. Global datasets were used from pasture area, net primary production (NPP) from grassland and livestock at 5 x 5 arc-minute spatial resolutions near year 2000. The potential to intensify grazing systems was estimated considering the biomass from grassland available to ruminant, grass consumption and improving feed conversion rate (FCR) to most efficient 10th percentile of each climate, livestock product and region. Grass available to ruminant had higher potential to increase livestock production than improving FCR. Global production can increase up to 8 times considering grass available and animal consumption, and 2 times if improve FCR. The high potential to increase yield can suggest large area to be spared from pasture if keep the same final production. In this way, the potential of land sparing was 410 to 917 Mha of pasture worldwide. Developing regions showed higher potential to spare land than developed countries. Sub-Saharan Africa can spare up to 300 Mha, Latin America around 56 Mha and Eastern Asia can spare 144 Mha. In other hand, North America and Europe showed smaller potentials, and can spare less than 2 Mha. The potential to spare land can contribute to the global need of land expansion for bioenergy production, estimated in 200 Mha for 2050. The land use change in pastureland globally offer opportunity to other crops as to produce bioenergy, without threatening food security.

Keywords: land use, sustainability, pastureland. **Supported by:** FAPESP

E.15 - Collaborative networks structure the analysis of the bioenergy paradigm evolution: determinants in the upgrading process of technological frontiers

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Although a previous work has raised the existence of co-occurrence among research clusters in bioethanol that use biomass originated from sugarcane cultivation and from a cluster of research areas on marine and freshwater algae biomass and oceanography (Figure 1, sugarcane, cluster B), the results raise methodological questions of what could have led to the phenomenon of interdependence of areas of knowledge among clusters. Thus, before attempting to explain the result, this article first tests whether the result is maintained even when other knowledge areas of bioenergy are investigated (in this article, algae and biogas), which addresses some concerns about: → a) what the role of the interdependence of knowledge areas is and how international collaborative networks can be explained. In other words, can networks explain the development and evolution of bioenergy research, suggesting that the paradigm is expanding?; → b) whether there is an emerging standard in scientific collaboration and, simultaneously, topic specialization for the generation of technological frontiers. In addition to this introduction, the second section which has two subsections about knowledge networks and bioenergy. This paper is comprised of three sections more. Section three presents the methodology in two stages: knowledge networks (cumulativeness) and technological frontiers. The idea of cumulativeness used in this work is represented by research areas and links of research collaboration networks across national borders (Bueno et. al., 2018a); and the issue of technological frontiers is represented by co-occurrence clusters among areas of knowledge identified through patents (Bueno et. al., 2018b). The methodology section also presents the concept of networks and provides analytical treatments, using the software for the co-occurrence matrix, and the software applications Pajek, Odysseýs (Masago, 2013) and R for the analysis of metrics. Section fourth presents the results, allowing an analysis of the evolution and patterns of change of scientific collaboration and a correspondence among the collaborative networks of countries and areas of knowledge for the formation of emerging frontiers. The final sections present the discussions and conclusions. Keywords: technological frontiers, bioenergy, low carbon transition. Funding: FAPESP.

Keywords: Bioenergy, Technological frontiers, low carbon transition

Supported by: FAPESP

E.16 - Prediction of soil stresses due to agricultural machines in different sugarcane cultivation systems

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One strategy to eliminate or minimize occurrence of soil compaction in sugarcane production is through modelling soil stresses, which can identify the risk associated with different operations and machine equipment. In this study, the magnitudes and distribution of stresses produced in the soil through the loads carried by the axles of the machines used in sugarcane cultivation systems were analysed and modelled. The Tyres/Track and Soil Compaction (TASC) tool took the soil and machine data, estimated contact areas and mean contact pressures at the soil-tyre/track interface, and associated with preconsolidation stress data obtained in the uniaxial test modelled the propagation of the applied stress into the soil. There was a risk of soil compaction for three operations before sugarcane harvesting: crop rotations planting, harvesting of the peanut rotational crop, and planting of the sugarcane billets. The peanut harvester can caused compaction to 0.33 m depth. This high potential to cause soil compaction is associated with the high load carried by the axle, combined with fact, of this harvester promotes the grubbing of the plants, and intensively disaggregates the soil surface, which causes the stress applied by the wheels to extend in the vertical profile of the soil. The sugarcane planting was predicted to be the highest risk of soil compaction operation, by applying stresses of 406 and 274 kPa by the front and rear axle, respectively. On harvesting, the trailer has the higher potential to cause soil compaction, with a compressive stress of 157 kPa, at 0.21 m depth. Crop rotation systems associated with soil tillage that promote intense soil disaggregation increase the risk of soil compaction, which can not compensate other advantages of such systems. Adjustments on machine loads and changes in tillage and management design are strategies indicated to avoid soil compaction.

Keywords: soil compaction, preconsolidation pressure, soil stress distribution

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E.17 - Biogas generation from hybrid anaerobic treatment of dairy industry using lipolytic fungal cells and anaerobic sludge

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Introduction: The dairy industry generates a significant amount of liquid effluents with high organic load and nutrients from milk processing, which are highly polluting if discharged without proper treatment in water bodies. Objectives: In this context, the present work proposes the simultaneous use of lipolytic fungal cells with anaerobic sludge to treat these effluents. The main objective is to integrate the action of lipolytic enzymes present in fungal cells and anaerobic sludge to reduce the organic load and increase the generation of biogas. Materials and Methods: The fungal cells were prepared using the *P. citrinum* URM 4216 (UFPE) filamentous fungus and the culture medium was composed of 30 g L⁻¹ of carbon source (residual oil, olive oil or cheese whey) and 70 g L⁻¹ of nitrogen source (soybean peptone, malt residue or okara residue) supplemented with salts NaNO₃ (1 g L⁻¹), KH₂PO₄ (1 g L⁻¹) and MgSO₄·7H₂O (0.5 g L⁻¹). A sample of 0.3 mL spore suspension inoculum (about 10⁶ spores) were added into flasks containing 100 mL of culture medium and the cells grew during 96h at 30 °C under shaking bath. The cells were separated from the medium by vacuum filtration and dried. Hybrid treatment occurred in shaken bottles (30 °C, 60h) containing 50 mL of dairy effluent (Queijos Bandeira, Bandeira do Sul/MG) at pH 7.0 (adjusted with NaHCO₃), 15 mL of anaerobic sludge (Avícola Dacar, Tietê/SP) and 2 g of dried cells. Results and Discussion: Each 2 hours the biogas were measured and the results showed that the hybrid system increasing the production of biogas in 53% compared to the control experimente (without cells). Conclusions: The addition of cells cultivated in residual oil and soyben peptone provided the highest production of biogas 4.73 L L⁻¹ (biogas/sludge), but only 18% of removal organic matter. Additional studies are being conducted to optimize hybrid anaerobic treatment employing these cells.

Keywords: dairy effluent, fungal cells, biogas

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E.18 - Use of biochar, vinasse, and nitrogen fertilization in energy cane: implications for N₂O emissions

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Agro-industrial residues recycling is a differential of the sugar-energy sector, as it promotes cost reduction, prevents incorrect disposal and contributes to the sustainability of agricultural soils. However, several studies indicate that residues contribute to increasing soil greenhouse gas (GHG) emissions. This study aimed to assess the effect of nitrogen fertilization and vinasse and biochar application on soil N₂O emissions. The study was performed under control conditions with pots of 20 dm³, filled with clayey soil, consisted by four replications and five treatments: i) control (without N-fertilizer); ii) N (ammonium sulfate); iii) N + vinasse; iv) N + biochar; and v) N + vinasse + biochar, arranged in a randomized design. The N fertilizer, vinasse, and biochar were applied at rates of 120 kg ha⁻¹, 300 m³ ha⁻¹ and 5 g kg⁻¹, respectively. A significant effect was found for N₂O emission as a function of treatments, with the highest daily mean values observed for treatments with vinasse application (157.50 ug N m⁻² day⁻¹) and the lowest ones in the treatment with biochar (38.09 ug N m⁻² day⁻¹) and the control (19.74 ug N m⁻² day⁻¹). Cumulative N₂O emission showed that the use of biochar reduced by 125 and 164% when the treatments with N and N + vinasse, respectively, was added. The PCA indicated that a high N₂O emission in the treatment with vinasse application is associated with a high concentration of nitrate and ammonium, organic matter, and enzymatic activity in this soil. The vinasse use associated with N fertilization should be avoided to obtain better sustainability indicators of energy cane production. Biochar addition to soil should be considered a strategy to mitigates N₂O emissions caused by N fertilizer and vinasse addition and long-term studies are encouraged to understand how biochar acts to reduce N₂O formation and emission in tropical soils. **Keywords:** Byproducts, GHG emissions, Sustainability

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E.19 - The impact of sugarcane expansion in Brazil: local stakeholders' perceptions

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Sugarcane expansion in Brazil during the 2000s was partly restricted by several discussions about the sustainability aspects of its cultivation. These discussions were mainly based on surveys that sometimes use highly aggregated data not including local perspectives and particularities, and sometimes used case studies with small samples which, while listening to local perspectives, cannot be considered representative of the whole sector. This work aims at filling this gap by considering both the perceptions of the local community, which add primary data on impact, and a large sample, to increase the research representativeness. To do so, we present the results of 353 interviews, covering 33 municipalities in five states of the Center-South region of Brazil (the largest cultivation area in the country). The results show that the expansion of biofuels has generated conflict mostly related to environmental and social issues, although there is good acceptance of the sugarcane mills in general. Our conclusions point to the importance of including local voices for a deeper understanding of the advantages and limitations of the expansion of biofuels. Then, we highlight the main findings as: a) Wide field research in the top 5 states in area of expansion to fill methodological gaps on studies about sugarcane expansion in Brazil; b) Differences between global and local stakeholder's perceptions; c) Themes with different meaning or relevance for local stakeholders such as deforestation, air quality or food security; d) Themes with strong positive impact as job creation, decent work, income generation and tax collection and e) Importance of inclusiveness to policy makers on the improvement of biofuels social and environmental impacts.

Keywords: Sugarcane expansion, stakeholders' perceptions, social-environmental impacts

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E.21 - The role of bioenergy and livestock integration for the Brazilian sustainable future

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Brazil is among the land-rich countries with predicted expansion of agricultural commodities production, including food and energy crops. Appropriate land management practices will be fundamental for optimizing land-based outputs under climate mitigation scenarios. In this context, bioenergy-livestock integrated model is particularly interesting for Brazil and an important option for future land use management strategies. To facilitate the understanding of future demands of land, food and energy, a new generation of scenarios, the Shared Socioeconomic Pathways (SSPs), were established by the climate research community. This study presents a bottom-up approach to assess the potential contribution of some alternatives of bioenergy-livestock integration in Brazil for the future demands (2030 to 2100) of energy derived from the SSPs. The bioenergy-livestock integration was designed taking into consideration pasture intensification options and use of biofuels byproducts as animal feed supplement, with a land management perspective. We consider land use restrictions for sugarcane expansion on pastureland based on Sugarcane Agricultural Zoning (ZAE). The bioenergy-livestock integration alternatives were modeled using the Virtual Sugarcane Biorefinery, developed at LNBR/CNPEM. Our results show that in 2030, the major contribution of biofuels production to the demands of liquid fuel for transportation happens in SSP1, reaching 64%, while in 2050 it reaches 60% in SSP 3. Proper bioenergy-livestock integration has great potential to meet a large extent of future demands of food, feed and energy, as it may reduce the additional pressure for future land use demands in the country while staying beyond some sustainable limits for expansion (e.g. ZAE). We believe these results might help to design more assertive public policies regarding biofuel production in Brazil and contribute to achieve the ambitious targets assumed by the country in the Paris agreement.

keywords: land use intensification, climate change, integrated assessment models

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