
**PROGRAMA INTEGRADO (UNESP, USP E UNICAMP) DE PÓS-GRADUAÇÃO
EM BIOENERGIA**

**ORGANOSOLV PRETREATMENT ASSESSMENT ON FRUIT WASTE TO OBTAIN
PLATFORM CHEMICALS AND BIOPLASTICS**

HERNÁN DARÍO ZAMORA ZAMORA



**Rio Claro, SP
Maio - 2023**

**PROGRAMA INTEGRADO (UNESP, USP E UNICAMP) DE PÓS-GRADUAÇÃO
EM BIOENERGIA**

**ORGANOSOLV PRETREATMENT ASSESSMENT ON FRUIT WASTE TO OBTAIN
PLATFORM CHEMICALS AND BIOPLASTICS**

HERNÁN DARÍO ZAMORA ZAMORA

Tese apresentada ao Instituto de Pesquisa em Bioenergia de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutor em Ciências.

Orientador: Prof. Dr. Michel Brienzo

Z25o Zamora, Hernán Darío Zamora
Organosolv pretreatment assessment on fruit
waste to obtain platform chemicals and bioplastics /
Hernán Darío Zamora Zamora. -- Rio Claro, 2023
210 f. : il., tabs., fotos

Tese (doutorado) - Universidade Estadual Paulista
(Unesp), Instituto de Pesquisa em Bioenergia, Rio
Claro
Orientador: Michel Brienzo

1. Pretreatment. 2. Organosolv. 3. Hemicelluloses.
4. Lignin. 5. Bioplastics. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp.
Biblioteca do Instituto de Pesquisa em Bioenergia, Rio Claro. Dados
fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: ORGANOSOLV PRETREATMENT ASSESSMENT ON FRUIT WASTE TO OBTAIN
PLATFORM CHEMICALS AND BIOPLASTICS

AUTOR: HERNÁN DARÍO ZAMORA ZAMORA

ORIENTADOR: MICHEL BRIENZO

Aprovado como parte das exigências para obtenção do Título de Doutor em Bioenergia, área:
Bioenergia pela Comissão Examinadora:

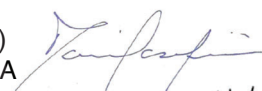
Prof. Dr. MICHEL BRIENZO (Participação Virtual)

Laboratório de Caracterização de Biomassa / Instituto de Pesquisa em Bioenergia UNESP Rio Claro



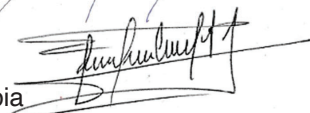
PROFESSOR ADJUNTO DANIEL PASQUINI (Participação Virtual)

Instituto de Química / UNIVERSIDADE FEDERAL DE UBERLÂNDIA



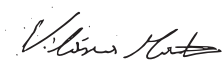
Prof. Dr. JESÚS DAVID CORAL MEDINA (Participação Virtual)

Departamento de Engenharia / Universidade Cooperativa de Colômbia



Profa. Dra. VILÁSIA GUIMARÃES MARTINS (Participação Virtual)

Escola de Química e Alimentos / Universidade Federal do Rio Grande (FURG)



Rio Claro, 29 de maio de 2023

To God for the opportunity to live.

To my parents Orlando and Enriqueta for their support, wise advice, and
unconditional and vast love.

To my brothers Daniel and Leo because they inspired me to become the person that
I am.

To my wife Adriana for her infinite love, for listening every time that I needed, and for
teaching me that God is in every place and that the life secret is in the small things.

ACKNOWLEDGEMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001.

To the Organization of American States (OAS) and the Coimbra Group of Brazilian Universities (GCUB) because contributed to the beginning of my research path.

To the São Paulo State University (UNESP) for having allowed me to be part of its academic family that contributes to the society development.

To the Institute for Research in Bioenergy (IPBEN) for having given me the opportunity to research topics of global significance.

To the Professors of the Graduate Program in Bioenergy, and to Professor Michel Brienzo for his advising and for having allowed me to be part of the Biomass Characterization and Conversion Laboratory.

To all the laboratory colleagues for their help

And, to my neighbor and friend country!

RESUMO

Esta pesquisa consistiu em duas fases principais, a primeira relacionada com avaliar os efeitos de um pré-tratamento organossolve etanólico catalisado por álcali (OHEOP) na composição e estrutura lignocelulósica (LC) do pseudocaule de bananeira e da torta de semente de goiaba, e na obtenção de uma fração rica em celulose (CL), hemiceluloses (HC) e lignina (LG). E, a segunda fase relacionada com a produção e caracterização de bioplásticos com base em quitosana (Ch) e adição de HC e LG extraídos de pseudocaule de bananeira e da torta de semente de goiaba. Na primeira fase, a avaliação do OHEOP foi realizada por meio de um planejamento composto central 2^3 . Os fatores do planejamento foram a concentração do álcali (OHC) em relação à massa do resíduo, temperatura (T), e concentração da solução aquosa de etanol (EC), e as variáveis de resposta foram o rendimento mássico de HC extraídas (HCy), o rendimento mássico de LG extraída (LGy) e a conversão de celulose (CLX). Além disso, o OHEOP envolveu caracterizações químicas dos resíduos pré-tratados e não tratados, e hidrólise enzimática (Cellic® CTec2). As HC e LG extraídas foram analisadas por espectroscopia de infravermelho com transformada de Fourier (FTIR), comparando-as com produtos comerciais. Com um nível de confiança de 95%, a T foi o fator com maior influência nas variáveis de resposta. Para o pseudocaule de bananeira, 78% correspondeu ao máximo HCy (a 170 °C, EC de 30%, e OHC de 60%), 35.2% para LGy (a 170 °C, EC de 30%, e OHC de 10%) e 96.6% para CLX (a 120 °C, EC de 70%, e OHC de 60%). E, no caso da torta de semente de goiaba, 97.3% foi o mais alto HCy (a 170 °C, EC de 30%, e OHC de 60%), 45.0% para LGy (a 170 °C, EC de 30%, e OHC de 60%), e 50.3% para CLX (a 170 °C, EC de 30%, e OHC de 60%). Na segunda fase, bioplásticos foram produzidos pelo método casting, apresentando uma estrutura homogênea, flexível, e sem rachaduras, e tendo a intensidade da cor aumentando proporcionalmente com a massa de HC e LG adicionadas. Os bioplásticos com adição de 10, 5, e 25% de HC apresentaram maior teor de umidade (22.3%), solubilidade em água (22.4%), e opacidade (1.95 mm^{-1}), respectivamente. A adição de 10% de LG no bioplástico resultou nas maiores percentagens de umidade (19.4%) e opacidade (4.75%). A adição de HC e LG melhorou a resistência à tração e o módulo de Young, enquanto o inchamento dos bioplásticos reduziu. A análise termogravimétrica revelou que a degradação dos bioplásticos ocorreu em quatro etapas diferentes; a primeira de evaporação de água e ácido acético, a segunda de degradação de glicerina, a terceira de despolimerização de HC, Ch e LG, e a última correspondente à etapa de carbonização. De acordo com os resultados o OHEOP permitiu extrair HC e LG semelhantes aos encontrados comercialmente, e estes componentes como aditivos na produção de bioplásticos contribuíram com a melhoria em algumas propriedades, com potencial para aplicação na indústria de alimentos ou na medicina.

Palavras-chaves: Pré-tratamento; Organossolve; Hemiceluloses; Lignina; Bioplásticos.

ABSTRACT

This research consisted of two main phases, the first one related to assess effects of an ethanolic organosolv pretreatment catalyzed by alkali (OHEOP) on the lignocellulosic (LC) composition and structure of banana pseudostem and guava seed cake, and on the obtaining of a rich fraction in cellulose (CL), hemicelluloses (HC) and lignin (LG). And the second one linked to produce and characterize bioplastics based on chitosan (Ch) and addition of HC and LG extracted from banana pseudostem and guava seed cake. In the first phase, the OHEOP evaluation was performed through a central composite design 2³. The factors were the alkali concentration (OHC) in relation to the waste mass, temperature (T), and concentration of the ethanolic aqueous solution (EC), and the response variables were the mass yield of extracted HC (HCy), the mass yield of extracted LG (LGy), and the cellulose conversion (CLX). Additionally, the OHEOP involved chemical characterizations of the pretreated and untreated wastes, and enzymatic hydrolysis (Cellic® CTec2). The extracted HC and LG were analyzed by Fourier-transform infrared (FTIR) spectroscopy, comparing them with commercial products. With a confidence level of 95%, T was the factor with the greatest influence on the response variables. For banana pseudostem, 78% corresponded to the maximum HCy (at 170 °C, EC of 30%, and OHC of 60%), 35.2% for LGy (at 170 °C, EC of 30%, and OHC of 10%) and 96.6% for CLX (at 120 °C, EC of 70%, and OHC of 60%). And, in the case of guava seed cake, 97.3% was the highest HCy (at 170 °C, EC of 30%, and OHC of 60%), 45.0% for LGy (at 170 °C, EC of 30%, and OHC of 60%), and 50.3% for CLX (at 170 °C, EC of 30%, and OHC of 60%). In the second phase, bioplastics were produced by the casting method, presenting a homogeneous, flexible, and without cracks structure, and having the color intensity increasing proportionally with the mass of added HC and LG. Bioplastics with the addition of 10, 5, and 25% of HC had the highest moisture content (22.3%), water solubility (22.4%), and opacity (1.95 mm⁻¹), respectively. The addition of 10% of LG in the bioplastic resulted in the highest percentages of moisture (19.4%) and opacity (4.75 mm⁻¹). The addition of HC and LG improved tensile strength and Young's modulus, while swelling was reduced. The thermogravimetric analysis (TGA) revealed that the bioplastics degradation occurred in four different stages; the first of water and acetic acid evaporation, the second of glycerin degradation, the third of HC, Ch, and LG depolymerization, and the last one corresponding to the carbonization stage. According to the results, the OHEOP allowed to extract HC and LG similar to the commercial ones, and these components as additives in the production of bioplastics contributed to improve some properties, with potential for application in the food or medical industry.

Keywords: Pretreatment; Organosolv; Hemicelluloses; Lignin; Bioplastics

ABBREVIATIONS

1G	First generation
2G	Second generation
ADH	Alcohol dehydrogenase
AI	L-arabinose isomerase
AR	Arabinose
AR(XR)	Aldose reductase
ATP	Adenosine 5'-triphosphate
BRF	Biorefinery
BS	Biomass
Ch	Chitosan
CL	Cellulose
CLX	Cellulose conversion/Glycose yield
D-XY	D-xylose
DNA	Deoxyribonucleic acid
EC	Ethanol concentration
EDTA	Ethylenediamine tetraacetic acid
FTIR	Fourier Transform Infrared
GSH	Glutathione
HC	Hemicelluloses
HCy	Mass yield of extracted hemicelluloses
HDPE	High density polyethylene
L-AR	L-arabinose
LAD	L-arabitol-4-dehydrogenase
LC	Lignocellulosic
LCH	Lignocellulosic hydrolysate
LG	Lignin
LGy	Mass yield of extracted lignin
LXR(ALX)	L-xylulose reductase
MFS	Major facilitator superfamily
OHC	Alkali concentration
OHEOP	Alkali ethanolic organosolv pretreatment
PB	Pretreated biomass

PDC	Pyruvate decarboxylase
PP	Polypropylene
PPP	Pentose-phosphate pathway
QTL	Quantitative trait loci
R5PE	L-Ribulose-5-P-4-epimerase
RK	L-Ribulokinase
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SEM	Scanning electronic microscopy
SNPs	Single nucleotide polymorphisms
T	Temperature
TCA	Tricarboxylic acid
TFs	Transcription factors
VB	Vegetal biomass
XDH	D-xylulose reductase
XI	Xylose isomerase
XK	Xylulokinase
XR	Xylose reductase
XDH	Xylitol dehydrogenase
XY	Xylose

CONTENT

GENERAL INTRODUCTION	15
Objectives	17
Scope of this thesis	17
Figure 1 - General structure of research	18
References	19
CHAPTER 1 - Biomass Fractionation Based on Enzymatic Hydrolysis for Biorefinery Systems.....	21
Abstract.....	21
1.1. Introduction to a Biorefinery	22
1.2. Biomass Composition and Recalcitrance	24
1.3. Biomass Pretreatment to Improve Cellulose Accessibility.....	27
1.4. Lignocellulosic Biomass Hydrolysis	29
1.5. Enzymatic Hydrolysis.....	31
1.5.1. Cellulases Performance	33
1.5.2. Xylanases Performance.....	34
1.5.3. Cellulolytic Enzymes Synthesized by Fungi and Bacteria	36
1.5.4. Hemicellulolytic Enzymes Synthesized by Fungi and Bacteria	37
1.5.5. Enzymes Purification Systems	40
1.5.6. Interference Among Enzymes.....	45
1.5.7. Assistance Elements for Improving Enzyme Activity	48
1.6. Challenges on industrial scale of cellulose hydrolysis via enzymatic	49
1.7. Concluding remarks	50
References	51
CHAPTER 2 - Hemicelluloses Role in Biorefinery Systems of Cellulosic Bioethanol, Particleboard, and Pulp and Paper Industries.....	64
Abstract.....	64
2.1. Introduction.....	65
2.2. Hemicelluloses valorization in the cellulosic bioethanol production	69
2.2.1. Hemicelluloses recovery in 2G bioethanol process	71
2.3. Hemicelluloses valorization in the Pulp and Paper Industry	72
2.4. Hemicelluloses valorization in particleboard fabrication.....	74
2.5. Challenges of Biological Aspects of Pentoses Fermentation of Lignocellulosic Hydrolysates using <i>Saccharomyces cerevisiae</i>.....	78
2.5.1. Yeast Engineering For 2nd Generation Bioethanol	79

2.5.1.1.	Xylose Fermentation	80
2.5.1.2.	Arabinose Fermentation	83
2.5.1.3.	Engineering Pentose transports in <i>S. cerevisiae</i> strains.....	84
2.6.	Yield loss through the formation of inhibitors during the hydrolysis of lignocellulose materials.....	87
2.6.1.	Genetic advances to improve yeast tolerance to lignocellulosic hydrolysates	90
2.7.	Concluding Remarks.....	94
	References	95
CHAPTER 3 - Assessment of organosolv pretreatment catalyzed by alkali as fractionation strategy of banana plant pseudostem		105
	Abstract.....	105
	Statement of Novelty.....	106
3.1.	Introduction.....	106
3.2.	Material and methods.....	108
3.2.1.	Lignocellulosic (LC) biomass	108
3.2.2.	Alkali ethanolic organosolv pretreatment (OHEOP) for hemicelluloses (HC) and lignin (LG) production/extraction.....	109
3.2.3.	Characterization of pretreated biomass (PB), and extracted hemicelluloses (HC) and lignin (LG).....	110
3.2.4.	Enzymatic hydrolysis	110
3.3.	Results and discussion.....	111
3.3.1.	Raw biomass characterization.....	111
3.3.2.	Extraction, and characterization by FTIR spectroscopy of hemicelluloses (HC) and lignin (LG).....	112
3.3.3.	Hemicelluloses (HC) extraction and characterization by FTIR.....	115
3.3.4.	Hemicelluloses (HC) molecular weight	118
3.3.5.	Lignin (LG) extraction and characterization by FTIR	120
3.3.6.	Enzymatic hydrolysis (Biomass digestibility)	122
3.4.	Conclusions	124
3.5.	Supplementary Information	124
	References	127
CHAPTER 4 - Hemicelluloses added to chitosan-based bioplastic improved the tensile strength and allowed to control the material swelling.....		132
	Abstract.....	132
4.1.	Introduction.....	132
4.2.	Experimental	134

4.2.1.	Materials	134
4.2.2.	Hemicelluloses (HC) extraction	135
4.2.3.	Preparation of bioplastics	135
4.2.4.	Characterization of bioplastics	136
4.2.4.1.	Moisture content and solubility in water	136
4.2.4.2.	Thickness and opacity	136
4.2.4.3.	Water absorption (swelling).....	137
4.2.4.4.	Tensile assays	137
4.2.4.5.	Thermogravimetric analysis (TGA)	137
4.2.5.	Statistical analysis	138
4.3.	Results and discussion.....	138
4.3.1.	Preparation of bioplastics	138
4.3.2.	Characterization of bioplastics	139
4.3.2.1.	Moisture content and solubility in water	140
4.3.2.2.	Thickness and opacity	142
4.3.2.3.	Water absorption (swelling).....	143
4.3.2.4.	Tensile strength.....	145
4.3.2.5.	Elongation at break	146
4.3.2.6.	Young's modulus	148
4.3.2.7.	Thermogravimetric analysis	149
4.4.	Conclusions	150
	Statements and Declarations	151
	Acknowledgments.....	151
4.5.	Supplementary Information	151
	References	152
	CHAPTER 5 - Fractionation of guava seed cake through organosolv pretreatment catalyzed by alkali	156
	Abstract.....	156
	Statement of Novelty.....	156
5.1.	Introduction.....	157
5.2.	Material and methods.....	159
5.2.1.	Biomass preparation	159
5.2.2.	NaOH-catalyzed organosolv pretreatment using ethanol (OHEOP), for hemicelluloses (HC) and lignin (LG) extraction.....	160
5.2.3.	Characterization of pretreated biomass (PB), and extracted hemicelluloses (HC) and lignin (LG).....	161

5.2.4.	Digestibility of the pretreated biomass (PB).....	161
5.3.	Results and discussion.....	162
5.3.1.	Chemical characterization of guava seed cake	162
5.3.2.	Extraction, and characterization of hemicelluloses (HC) and lignin (LG) 163	
5.3.3.	Hemicelluloses (HC) extraction and characterization by FTIR.....	166
5.3.4.	Molecular weight of extracted hemicelluloses (HC).....	170
5.3.5.	Lignin (LG) extraction and characterization by FTIR	171
5.3.6.	Biomass digestibility through enzymatic hydrolysis.....	174
5.4.	Conclusions	175
5.5.	Supplementary Information	176
	References	179
	CHAPTER 6 - Incorporating lignin to chitosan-based bioplastics enhances the tensile properties and provides control of swelling and opacity.....	184
	Abstract.....	184
6.1.	Introduction.....	184
6.2.	Experimental	186
6.2.1.	Materials	186
6.2.2.	Extraction of lignin (LG)	187
6.2.3.	Preparation and formulation of bioplastics	187
6.2.4.	Characterization of bioplastics	188
6.2.4.1.	Moisture and water solubility	188
6.2.4.2.	Thickness and opacity	189
6.2.4.3.	Swelling (water absorption).....	189
6.2.4.4.	Tensile test.....	189
6.2.4.5.	Thermogravimetric analysis (TGA)	190
6.2.5.	Statistical analysis	190
6.3.	Results and discussion.....	190
6.3.1.	Appearance of the bioplastics	190
6.3.2.	Characterization of bioplastics	191
6.3.2.1.	Moisture and water solubility	192
6.3.2.2.	Thickness and opacity	194
6.3.2.3.	Swelling (water absorption).....	195
6.3.2.4.	Tensile strength.....	197
6.3.2.5.	Elongation at break	198
6.3.2.6.	Young's modulus	200

6.3.2.7. Thermogravimetric analysis (TGA).....	200
6.4. Conclusions	202
Statements and Declarations	203
Acknowledgments.....	203
6.5. Supplementary Information	203
References	204
GENERAL CONCLUSIONS	208

GENERAL INTRODUCTION

Currently, it is no longer a secret that lignocellulosic (LC) biomass is being considered as a strong and potential option to substitute fossil resources, since the last ones, despite being used to obtain different types of products (fuels, plastics, clothing, chemicals, and so on) have been commonly pointed out for causing negative alterations to natural ecosystems and adverse effects on human health. When LC biomass comes from vegetal waste, this is regarded as a better alternative, due to it does not interfere with crops destined for human food, shows wide availability, is relatively low cost, represents the key for the bioeconomy development, and can be harnessed in a similar route than oil through refinery scheme (SHIMIZU *et al.*, 2020). The generation of biomass-based products (also called bioproducts) using a refinery scheme is known as biorefinery, which effectively uses biomass for the combined development of several categories of products such as biofuels, biopolymers, biomaterials, carboxylic acids, pharmaceuticals, sweeteners, and platform chemicals (FLÓREZ; LÓPEZ; LOZANO, 2018).

The biggest challenges of a biorefinery are related to achieve high conversion yields through technologies with suitable cost-effective, which means, low cost, low production time, low energy consumption, cheap raw materials, high value-added bioproducts, and environmentally friendly processes (CORAL; MAGALHAES, 2020). Much research experiences involving biorefinery have been completed using diverse LC wastes come from sugarcane (straw and bagasse), rice (spent grounds, husk, and straw), corn (cob and leaves), coffee (pulp and husk), fruit (peel, seeds, and vegetal plant byproducts), etc. In every case, LC material was conditioned through pretreatment processes in order to generate a loose biomass allowing better accessibility to its main constituents (CL, HC, and LG) (MANKAR *et al.*, 2021). Thus, the generation of bioproducts via biorefinery depends on pretreatment performance and use related to what constituent(s) is(are) desired for preservation, solubilization, or even breaking down (ZAMORA *et al.*, 2021). Organosolv pretreatment is a promissory process with great versatility among the pretreatments used in a biorefinery since it can take several configurations and thus obtain CL-rich, HC, and LG individual solid fractions as a result of the LC biomass deconstruction (PURKAIT; HALDAR, 2021; VAIDYA *et al.*, 2022).

In the whole world exist many types of LC waste and its vast generation should be harnessed. Among them, fruit wastes are LC materials that have provoked great interest in research and industries (MANHONGO *et al.*, 2022; TSEGAYE; JAISWAL; JAISWAL, 2021). In 2020, banana was the most produced fruit in the world with 119.83 million tons, followed by watermelon, apple, grape, orange, and guava with 101.62, 86.44, 78.03, 75.46, and 54.83 million tons, respectively (SHAHBANDEH, 2022a). It is estimated that 1 ton of harvested banana generates 3 tons of pseudostem (CHANG *et al.*, 2014; SOUZA *et al.*, 2014). Brazil stands out in the fruit world production being the third global producer, which leads to great amounts of waste generated from fruits (SHAHBANDEH, 2022b). In 2020, were produced around 6.64 million tons of banana (IBGE, 2022), which supposes an estimated generation of 19.9 million tons/year of pseudostem, becoming a potential LC source to obtain diverse bioproducts using a biorefinery scheme. On the other hand, fruit wastes are also generated in industries, such as the guava industry, which is stood out for its economic importance in various regions worldwide, owing to the high agricultural yield of the fruit and the permanent demand for its products that are industrially obtained (ANGULO-LÓPEZ *et al.*, 2021). During the industrial process of pulp extraction from guava, seeds (mostly) and peel are generated as byproducts (commonly called as guava seed) (BIBWE *et al.*, 2022). Despite being commonly discarded, guava seed is a LC waste that contains approximately 13% of oil, which can be extracted in order to valorize the waste (ANGULO-LÓPEZ *et al.*, 2021). From the extraction process, the cake is another waste generated, which likely will have a similar composition of cellulose, HC, and LG to the guava seed (PEREIRA *et al.*, 2022).

The research interest to extract/recover CL-rich fractions, HC and LG from banana pseudostem and guava seed cake is because those molecules are considered as platform chemicals (also named intermediate products) or direct feedstock to obtain biofuels and bioplastics. For instance, from the CL-rich (pretreated material) fraction can be obtained fermentable sugars, which are the base to obtain 2nd generation bioethanol (THANGAVELU; AHMED; ANI, 2016). On the other hand, cellulosic fibers can be used as reinforcing elements in polymeric matrices and are considered highly promising fillers in composite preparation such as particleboards (MONTEIRO *et al.*, 2016; XIE *et al.*, 2016). Additionally, holocellulose, xylan, and cellulose extracted from sugarcane bagasse have been used as additives in starch-based bioplastics, which are considered alternatives to substitute common plastics and thus avoid

environmental issues (ABE *et al.*, 2022). LG upgrading and valorization processes are significantly less-developed than those related to HC and CL, however, LG has been used as additive in polymeric films too, but based on chitosan (Ch) (ROSOVA *et al.*, 2021).

Objectives

General:

To assess the ethanolic alkali organosolv pretreatment on fractionation of fruit wastes aiming to obtain a CL-rich fraction, HC and LG. To evaluate the extracted HC and LG as additive to produce bioplastics based on Ch.

Specific:

- To characterize (chemically) the banana plant pseudostem and guava seed cake (also after pretreatments);
- To evaluate different organosolv configurations (central composite design 2³) for biomass fractionation into HC, LG, and cellulose-rich material;
- To determine the pretreated biomass digestibility through enzymatic hydrolysis for fermentable sugars production;
- To produce bioplastics with extracted HC and LG as additive, respectively, using chitosan/glycerol combination.
- To characterize the obtained bioplastics through tests of moisture, thickness, opacity, water solubility, water absorption, mechanical properties (tensile strength, elongation at break and Young's module), and thermogravimetric analysis

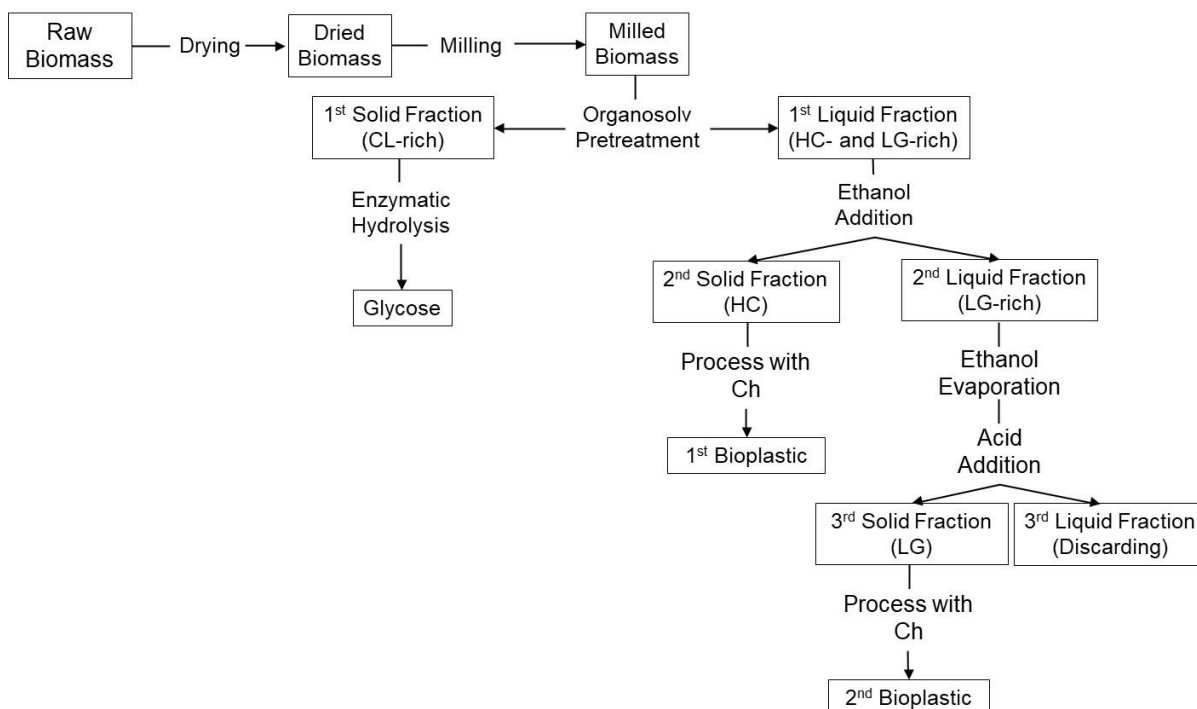
Scope of this thesis

This doctoral research involved a biorefinery approach using banana pseudostem and guava seed cake as feedstocks to obtain two different biopolymers (Figure 1). In the first phase, it was assessed the influence of different configurations of an ethanolic alkali organosolv pretreatment on LC composition and structure of the fruit wastes, and on obtaining CL-rich fractions, HC and LG. And in the second one,

the extracted HC and LG were used to obtain two different types of polymers based on Ch.

The untreated and pretreated wastes were characterized through chemical composition of CL, HC, and LG. The digestibility of the pretreated biomass was assessed using enzymatic hydrolysis with Cellic® CTec2 cocktail of Novozymes. The extracted HC and LG were analyzed through Fourier Transform Infrared (FTIR) spectroscopy. And, bioplastics based on commercial Ch with addition of the extracted HC and LG were obtained using the casting method.

Figure 1 - General structure of research



CL: Cellulose; HC: Hemicelluloses; LG: Lignin; Ch: Chitosan

Source: By author

This thesis document includes 6 chapters:

1. **Biomass Fractionation Based on Enzymatic Hydrolysis for Biorefinery Systems.** Book section published in the book: “Biorefineries: A Step Towards Renewable and Clean Energy”. Here, the biorefinery concept, chemical composition and recalcitrance of LC biomass, pretreatments to improve CL access, enzymatic hydrolysis, and challenges on industrial scaling of enzymatic hydrolysis were reviewed.

2. **Hemicelluloses Role in Biorefinery Systems of Cellulosic Bioethanol, Particleboard, and Pulp and Paper Industries.** Book section published in the book: "Hemicellulose Biorefinery: A Sustainable Solution for Value Addition to Bio-Based Products and Bioenergy. Here, the HC-based biorefinery concept as a key aspect to improve the feasibility of bioproduct generation, different types to extract HC for its valorization, pentoses fermentation, and inhibitory effects were reviewed.
3. **Assessment of organosolv pretreatment catalyzed by alkali as fractionation strategy of banana plant pseudostem.** This chapter showed the impact of alkali ethanolic organosolv pretreatment on the fractionation of banana plant pseudostem aiming to concomitantly extract HC and LG.
4. **Hemicellulose additive to chitosan-based bioplastic improved the tensile strength and allowed to control the material swelling.** This chapter summarized the production and characterization of bioplastics from commercial Ch and HC (extracted from banana plant pseudostem) using mixtures in variable proportions.
5. **Fractionation of guava seed cake through organosolv pretreatment catalyzed by alkali.** This chapter summarized the assessment of an alkali ethanolic organosolv pretreatment as fractionation strategy of the industrial waste generated after oil extraction process on guava seed (waste referred to as guava seed cake).
6. **Incorporating lignin to chitosan-based bioplastics enhances the tensile properties and provides control of swelling and opacity.** This chapter summarized the production and characterization of bioplastics using different mass proportions of Ch (commercial) and LG (extracted from guava seed cake).

References

ABE, M. M.; BRANCIFORTI, M. C.; NALLIN MONTAGNOLLI, R.; MARIN MORALES, M. A.; JACOBUS, A. P.; BRIENZO, M. Production and assessment of the biodegradation and ecotoxicity of xylan- and starch-based bioplastics. **Chemosphere**, Amsterdam, vol. 287, no. Part 3, p. 1–10, 2022. DOI 10.1016/j.chemosphere.2021.132290. Available at: <https://www.sciencedirect.com/science/article/pii/S0045653521027624>. Accessed on: 15 Sep. 2022.

ANGULO-LÓPEZ, J. E.; FLORES-GALLEGOS, A. C.; TORRES-LEÓN, C.; RAMÍREZ-GUZMÁN, K. N.; MARTÍNEZ, G. A.; AGUILAR, C. N. Guava (*Psidium guajava* L.) Fruit and Valorization of Industrialization By-Products. **Processes**, Basel, vol. 9, no. 6, p. 1–17, 2021. DOI 10.3390/pr9061075. Available at: <https://www.mdpi.com/2227-9717/9/6/1075>. Accessed on: 19 Jul. 2022.

BIBWE, B.; MAHAWAR, M. K.; JALGAONKAR, K.; MEENA, V. S.; KADAM, D. M. Mass modeling of guava (cv. Allahabad safeda) fruit with selected dimensional attributes: Regression analysis approach. **Journal of Food Process Engineering**, Hoboken, vol.

45, no. 3, p. 1–11, 1 Mar. 2022. DOI 10.1111/jfpe.13978. Available at: <https://onlinelibrary.wiley.com/doi/full/10.1111/jfpe.13978>. Accessed on: 21 Feb. 2023.

CHANG, Y. P.; TAN, M. P.; LOK, W. L.; PAKIANATHAN, S.; SUPRAMANIAM, Y. Making use of guava seed (*Psidium guajava* L.): The effects of pre-treatments on its chemical composition. **Plant Foods for Human Nutrition**, Berlin, vol. 69, no. 1, p. 43–49, 2014. DOI 10.1007/s11130-013-0396-3. Available at: <https://link.springer.com/article/10.1007/s11130-013-0396-3>. Accessed on: 8 Jul. 2022.

CORAL MEDINA, J. D.; MAGALHAES, A. I. J. Ethanol Production, Current Facts, Future Scenarios, and Techno-Economic Assessment of Different Biorefinery Configurations. In: INAMBAO, F. L. (ed.). **Bioethanol Technologies**. London: IntechOpen, 2020. p. 1–14. DOI 10.5772/intechopen.95081. Available at: <https://www.intechopen.com/chapters/74288>. Accessed on: 6 Jul. 2022.

FLÓREZ PARDO, L. M.; LÓPEZ GALÁN, J. E.; LOZANO RAMÍREZ, T. Saccharide Biomass for Biofuels, Biomaterials, and Chemicals. In: VAZ JR., S. (ed.). **Biomass and Green Chemistry: Building a Renewable Pathway**. Cham: Springer International Publishing, 2018. p. 11–30. DOI 10.1007/978-3-319-66736-2_2. Available at: https://link.springer.com/chapter/10.1007/978-3-319-66736-2_2. Accessed on: 21 Jun. 2022.

IBGE. Produção Agropecuária. 2022. Available at: <https://www.ibge.gov.br/explica/producao-agropecuaria/>. Accessed on: 8 Jul. 2022.

MANHONGO, T. T.; CHIMPHANGO, A. F. A.; THORNLEY, P.; RÖDER, M. Current status and opportunities for fruit processing waste biorefineries. **Renewable and Sustainable Energy Reviews**, Amsterdam, vol. 155, p. 111823, 2022. DOI 10.1016/j.rser.2021.111823. Available at: <https://www.sciencedirect.com/science/article/pii/S1364032121010911>. Accessed on: 7 Jul. 2022.

MANKAR, A. R.; PANDEY, A.; MODAK, A.; PANT, K. K. Pretreatment of lignocellulosic biomass: A review on recent advances. **Bioresource Technology**, Amsterdam, vol. 334, p. 1–12, 2021. DOI 10.1016/j.biortech.2021.125235. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852421005745>. Accessed on: 7 Jul. 2022.

MONTEIRO, S.; MARTINS, J.; MAGALHÃES, F. D.; CARVALHO, L. Low Density Wood-Based Particleboards Bonded with Foamable Sour Cassava Starch: Preliminary Studies. **Polymers**, Basel, vol. 8, no. 10, p. 1–11, 2016. DOI 10.3390/polym8100354. Available at: <https://www.mdpi.com/2073-4360/8/10/354>. Accessed on: 15 Sep. 2022.

PEREIRA, B. S.; DE FREITAS, C.; VIEIRA, R. M.; BRIENZO, M. Brazilian banana, guava, and orange fruit and waste production as a potential biorefinery feedstock. **Journal of Material Cycles and Waste Management**, Berlin, vol. 24, no. 6, p. 2126–2140, 2022. DOI 10.1007/s10163-022-01495-6. Available at: <https://link.springer.com/article/10.1007/s10163-022-01495-6>. Accessed on: 21 Dec. 2023.

PURKAIT, M. K.; HALDAR, D. Chapter 3 - Conventional pretreatment methods of lignocellulosic biomass. In: PURKAIT, M. K.; HALDAR, D. B. T.-L. B. to V.-A. P. (eds.). **Lignocellulosic Biomass to Value-Added Products**. Amsterdam: Elsevier, 2021. p. 31–46. DOI 10.1016/B978-0-12-823534-8.00009-0. Available at: <https://www.sciencedirect.com/science/article/pii/B9780128235348000090>. Accessed on: 7 Jul. 2022.

ROSOVA, E.; SMIRNOVA, N.; DRESVYANINA, E.; SMIRNOVA, V.; VLASOVA, E.; IVAN'KOVA, E.; SOKOLOVA, M.; MASLENNIKOVA, T.; MALAFEEV, K.; KOLBE, K.; KANERVA, M.; YUD, V. Biocomposite Materials Based on Chitosan and Lignin: Preparation and Characterization. **Cosmetics**, Basel, vol. 8, no. 1, p. 1–17, 2021. DOI 10.3390/cosmetics8010024. Available at: <https://www.mdpi.com/2079-9284/8/1/24>. Accessed on: 15 Sep. 2022.

SHAHBANDEH, M. Global fruit production in 2020, by selected variety. 2022a. Available at: <https://www.statista.com/statistics/264001/worldwide-production-of-fruit-by-variety/>. Accessed on: 8 Jul. 2022.

SHAHBANDEH, M. Major producers of fresh fruit worldwide 2020. 2022b. Available at: <https://www.statista.com/statistics/279164/global-top-producers-of-selected-fresh-fruit-worldwide/>. Accessed on: 8 Jul. 2022.

SHIMIZU, F. L.; DE AZEVEDO, G. O.; COELHO, L. F.; PAGNOCCA, F. C.; BRIENZO, M. Minimum Lignin and Xylan Removal to Improve Cellulose Accessibility. **BioEnergy Research**, Berlin, vol. 13, no. 3, p. 775–785, 2020. DOI 10.1007/s12155-020-10120-z. Available at: <https://link.springer.com/article/10.1007/s12155-020-10120-z>. Accessed on: 1 May 2020.

SOUZA, E. L.; LIEBL, G. F.; MARANGONI, C.; SELLIN, N.; MONTAGNOLI, M.; SOUZA, O. Bioethanol from fresh and dried banana plant pseudostem. **Chemical Engineering Transactions**, Milan, vol. 38, p. 271–276, 20 Sep. 2014. DOI 10.3303/CET1438046. Available at: <https://www.cetjournal.it/index.php/cet/article/view/CET1438046>. Accessed on: 8 Jul. 2022.

THANGAVELU, S. K.; AHMED, A. S.; ANI, F. N. Review on bioethanol as alternative fuel for spark ignition engines. **Renewable and Sustainable Energy Reviews**, Amsterdam, vol. 56, p. 820–835, Apr. 2016. DOI 10.1016/J.RSER.2015.11.089. Available at: <https://www.sciencedirect.com/science/article/pii/S1364032115013568>. Accessed on: 15 Sep. 2022.

TSEGAYE, B.; JAISWAL, S.; JAISWAL, A. K. Food waste biorefinery: Pathway towards circular bioeconomy. **Foods**, Basel, vol. 10, no. 6, p. 1–21, 2021. DOI 10.3390/foods10061174. Available at: <https://www.mdpi.com/2304-8158/10/6/1174/htm>.

VAIDYA, A. A.; MURTON, K. D.; SMITH, D. A.; DEDUAL, G. A review on organosolv pretreatment of softwood with a focus on enzymatic hydrolysis of cellulose. **Biomass Conversion and Biorefinery**, Berlin, , p. 1–16, 2022. DOI 10.1007/s13399-022-02373-9. Available at: <https://link.springer.com/article/10.1007/s13399-022-02373-9>. Accessed on: 7 Jul. 2022.

XIE, J.; HSE, C.-Y.; SHUPE, T. F.; PAN, H.; HU, T. Extraction and characterization of holocellulose fibers by microwave-assisted selective liquefaction of bamboo. **Journal of Applied Polymer Science**, Hoboken, vol. 133, no. 18, p. 1–8, Jan. 2016. DOI 10.1002/app.43394. Available at: <https://onlinelibrary.wiley.com/doi/full/10.1002/app.43394>. Accessed on: 15 Sep. 2022.

ZAMORA, H. D. Z.; DE FREITAS, C.; BUENO, D.; SHIMIZU, F. L.; CONTIERO, J.; BRIENZO, M. Biomass Fractionation Based on Enzymatic Hydrolysis for Biorefinery Systems. In: VERMA, P. (ed.). **Biorefineries: A Step Towards Renewable and Clean Energy**. Singapore: Springer Singapore, 2021. p. 217–254. DOI 10.1007/978-981-15-9593-6_9. Available at: https://link.springer.com/chapter/10.1007/978-981-15-9593-6_9. Accessed on: 23 Sep. 2021.

CHAPTER 1 - Biomass Fractionation Based on Enzymatic Hydrolysis for Biorefinery Systems

ZAMORA ZAMORA, H. D.; DE FREITAS, C.; BUENO, D.; SHIMIZU, F. L.; CONTIERO, J.; BRIENZO, M. Biomass Fractionation Based on Enzymatic Hydrolysis for Biorefinery Systems. In: VERMA, P. (ed.). **Biorefineries: A Step Towards Renewable and Clean Energy**. Singapore: Springer Singapore, 2021. p. 217–254. DOI 10.1007/978-981-15-9593-6_9. Available at: https://link.springer.com/chapter/10.1007/978-981-15-9593-6_9.

Abstract

A biorefinery system scheme has developed great interest in researchers and industries to obtaining products. A biorefinery is based on efficient use of different type of raw material, such as biomass, for integrated or simultaneous obtaining of several kinds of value-added products. Among used biomass, it is highlighted the vegetable sources, which can come from agroindustrial crops or from waste of biomass processing. There is an extensive variety of biomass that implicates diverse conversion process. Lignocellulosic biomass, in particular, can be transformed into bio-based products by different conversion stages. Biofuels, biopolymers, and others bioproducts come from several types of biomass and various process. One of the most important phases in the transformation process is the hydrolysis, which can be performed by conventional (chemical) and non-conventional (biological) routes. Enzymatic hydrolysis process allows to breakdown chemical structures into smaller molecules, which can be took in advantage for generation of value-added products at subsequent stages in biorefineries.

Keywords Biomass disruption · Renewable energy · Bioresources refinery

Abbreviations

BRF Biorefinery

CL Cellulose

DEAE Diethylaminoethyl

EDTA Ethylenediamine tetraacetic acid

HC Hemicelluloses

LC Lignocellulosic

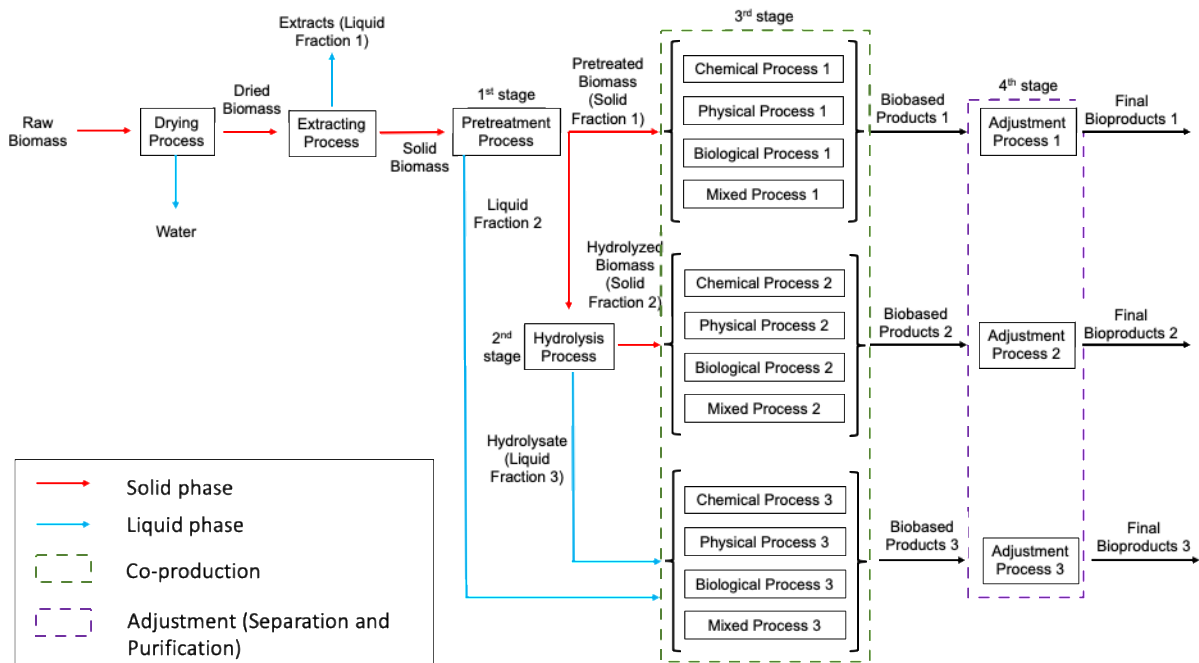
LG Lignin
VB Vegetal biomass
XOS Xylooligosaccharides

1.1. Introduction to a Biorefinery

In 2018, non-renewable fossil sources share 84.7% to sustain the world energy consumption matrix, among those, oil was the main contributor with 33.6% (BP, 2019). Thanks to its chemical composition, oil is considered as a versatile raw material that allows to obtain different chemical products through refinery process (U.S. ENERGY INFORMATION ADMINISTRATION, 2012). Despite this, crude oil systems for refining are great CO₂ producers and are generally placed in water-stress sites (GUEDES *et al.*, 2019). In addition, oil has serious problems related to its depletion, uniform distribution lack of reserves, price fluctuations and various negative impacts in human health and environment caused mostly by burning of oil derivatives (HOEGH-GULDBERG; JACOB; TAYLOR, 2018; STATISTA, 2020). According to this context, world and local researches have been developed to replace oil with renewable sources (biological material) that can be used in a similar process route than oil (refinery). Refinery operating with biological raw material (biomass) and basing on integrated or simultaneous generation of different types of products is named “biorefinery” (BRF).

BRF system can be described through the basic process scheme shown in Figure 1.1, where vegetal biomass (VB) is turned into value-added products by four main stages: pretreatment, hydrolysis, co-production process (conversion or transformation stage) and adjustment process (separation or purification stage). Raw VB generally is dried before get in BRF system in order to eliminate water content avoiding microorganism activity that causes modifications in physical-chemical properties and thus can be stored (RENTIZELAS, 2016). In some cases, when VB is waste from any type of productive chain like fruits, flowers or another type of vegetal species, an extraction step into a BRF can be considered important. Some fruit peels, barks, leaves and others vegetal waste (petals, seeds, bagasse, etc.) have special substances in their chemical structure that can be extracted (MCCABE; SMITH; HARRIOT, 2007; SEADER; HENLEY; ROPER, 2011). According to some researches, from fruit peels, vegetal barks and leaves is possible to obtain essential oils, antioxidants or repellent substances, which have an high interest for some industrial sectors (ASADOLLAHI *et al.*, 2019; ASHA *et al.*, 2015; ORTIZ *et al.*, 2017).

Figure 1.1 - Basic representation of a biorefinery general system



Source: By author

After extracting stage, BRF process obtains its first value-added products (extracts in liquid fraction 1) and solid biomass, which can be either directly sent to transformation (third stage) or be transported to pretreatment process (first stage) where biomass chemical structure is modified. Pretreatment outflows are solid and liquid fractions of chemically modified biomass.

The main characteristic of pretreated biomass is that access to compounds of its chemical structure is enhanced, which is really helpful for subsequent procedures in BRF (CORAL *et al.*, 2015; SHIMIZU; ZAMORA; *et al.*, 2020a). Liquid fraction generated after pretreatment (identified with number 2) incorporates soluble substances such as oligosaccharides and others that can be sent to third stage for transformation (SILVA *et al.*, 2017). Pretreated biomass can be directly sent to conversion and obtaining of new products (biobased products 1) or be transported to hydrolysis process (second stage), where chemical structure is broken into smaller molecules such as monosaccharides (LOOW *et al.*, 2016). After hydrolysis, a hydrolyzed biomass (solid fraction 2) and hydrolysate (liquid fraction 3) are generated.

Depending on applied pretreatment and hydrolysis procedures, hydrolyzed solid fraction and liquid hydrolysate will have specific chemical compositions, which

will establish the conversion route at co-production stage for product obtaining. From hydrolyzed solid fraction can be obtained products (biobased products 2) such as active carbon, lignin, biofilms, adsorption material, microfibrillated cellulose, biochar, bio-oil, electricity, etc. (CORAL MEDINA *et al.*, 2015; LI; KUMAR, 2016; NANDA *et al.*, 2014; ROSALES-CALDERON; ARANTES, 2019; VASSILEVA *et al.*, 2016; VIRTANEN *et al.*, 2017). From liquid hydrolysate that contains soluble substances such as monosaccharides can be obtained a huge variety of products (biobased products 3), among them are bioethanol, furfural, hydroxymethylfurfural, carboxylic acids (itaconic, glutamic, lactic and succinic), biopolymers, etc. (DULIE *et al.*, 2020; ROSALES-CALDERON; ARANTES, 2019; SOROKINA *et al.*, 2017; STEINBACH; KRUSE; SAUER, 2017). After any kind of transformation process (chemical, physical, biological or mixed) each crude outcome generally is sent to an adjustment stage, where separation operations occur for product quality improvement (GEANKOPLIS, 1993; SEADER; HENLEY; ROPER, 2011).

Aside to known processes involved in BRF, it is essential to find the best type of raw material (biomass) to employ in the process. Raw VB can come from agroindustrial crops or from waste of biomass processing, the last one usually is burnt in situ at rural fields as practice for solving accumulation problem (ALMSATAR, 2020). This burning practice caused by human activities has generated significant concerns as result of greenhouse gases and others chemical substances emitted into the atmosphere, both considered pollutants (ZHANG; BUEKENS; LI, 2016). Although burning raw VB waste solves accumulation problem in situ, this condition enlarges negative effects on environment (ONOJA *et al.*, 2018). To avoid this disadvantage, it is convenient to use mainly VB waste for transformation into value-added products by a BRF process.

1.2. Biomass Composition and Recalcitrance

Biomass is defined as any biological resource that can be turned into value-added products (including those energy producers). Biomass can be used in a direct way, when biological matter is consumed in its natural form; such as wood for firewood, and indirectly, when biological material undergoes some conversion process for product obtaining, such as biodiesel from oilseeds, carboxylic acids and biopolymers from lignocellulosic (LC), and bioethanol from starchy sources (PERVEZ *et al.*, 2014;

ROSALES-CALDERON; ARANTES, 2019; SOROKINA *et al.*, 2017). In addition, any kind of biomass is originated from spontaneous or induced biochemical processes occurring in biological sources that belong to vegetal, animal and algae kingdoms (ALLEN *et al.*, 2018; JHA; KUMAR, 2019; SHARMA; XU; QIN, 2019). Due to huge availability of biomass, chemical compositions are vast and different from one another, depending on source.

In the biosphere, VB is the largest biological biomass; around 450 gigatons of carbon are distributed over the Earth, meaning that 81.82% of the world biomass belongs to dominant kingdom of (mostly terrestrial) plants. Besides this, it is estimated that its renewal rate rises 3% per year. Bacteria biomass shares 12.73%, fungi 2.18%, archaea 1.27%, protists 0.73%, animal (predominantly oceanic) 0.36%, and others 0.90% (BAR-ON; PHILLIPS; MILO, 2018; GANDINI; BELGACEM, 2008). Thus, biomass from plants has caused great interest for scientific and industrial sectors. All plants have the common characteristic of being constituted by millions of cells, which are considered as the basic functional unit and the fundamental unit of life. These have enormous variety in shape, structure and functions allowing existence of huge diversity of plant species (REVILLA; ZARRA, 2013). Plants are multicellular organism constituted by eukaryotic cells, which have a nucleus, other organelles, a plasm membrane and a cell wall.

The plant cell wall encloses three main layers; the middle lamella, the primary wall and the secondary wall (together with plasm membrane), respectively from external to internal side. The first one is plentiful in pectic compounds and protein. The primary wall is subsequent to middle lamella and comprises of cellulose microfibrils inserted in a matrix made of pectic compounds, hemicelluloses, and glycoproteins. The secondary wall is the thickest layer made of cellulose (CL), hemicelluloses (HC) and lignin (LG) (MANAVALAN; MANAVALAN; HEESE, 2015). This three-layer structure is responsible for allowing the transit of benefit substances into cell and for protection against different kind of chemical, thermal, physical or biological attacks that can negatively affect the cell functions (ALBERTS *et al.*, 2014). As the thickest layer makes up most of cell wall, then, the CL, HC and LG content is a determinant factor for using it as raw material in a BRF. Considering the latest, the Table 1-1 shows different mass compositions (dry basis) of CL, HC and LG of several VB.

Table 1-1 - Cellulose (CL), hemicelluloses (HC) and lignin (LG) composition in some vegetal biomass (VB)

Biomass		CL (%)	HC (%)	LG (%)	Reference
Tropical	corn	25.6	34.6	20.5	Chen <i>et al.</i> , 2020
bagasse					
Sweet	sorghum	30.0	17.3	21.4	Chen <i>et al.</i> , 2020
bagasse					
Sugarcane	bagasse	14.8 - 42.8	15.5 - 27.7	16.3 - 24.28	Chen <i>et al.</i> , 2020; Hirunsupachote; Chavalparit, 2019; Silva <i>et al.</i> , 2017
Wheat	bran	32	26	20	Annamalai; Sivakumar, 2016
Sunflower	stalk	38.5	33.5	21.9	Kim <i>et al.</i> , 2016
Waste	triticale	31.9	25.4	18.4	Kucharska <i>et al.</i> , 2020
Meadow	grass	27.1	22.6	16.8	Kucharska <i>et al.</i> , 2020
Beech		38.6	19.9	28.3	Kucharska <i>et al.</i> , 2020
Mixed	municipal	49.5	23.0	18.4	Rosen; Mamane; Gerchman, 2019
trimming					
Corn	cobs	38.8	44.4	11.9	Pocan <i>et al.</i> , 2018
Pomegranate	peels	26.2	10.8	5.7	Pocan <i>et al.</i> , 2018
Walnut	shell	32.2	26.2	36.9	Özsin; Pütün; Pütün, 2019
Orange	bagasse	19.8	6.4	4.2	Cypriano <i>et al.</i> , 2017
Napier	grass	26.7	37.9	3.8	Hirunsupachote; Chavalparit, 2019
Acacia	leave	18.8	14.3	17.4	Hirunsupachote; Chavalparit, 2019
Empty	fruit bunches	26.9 - 28	22.7 - 24.1	14.5 - 20	Hirunsupachote; Chavalparit, 2019; Medina <i>et al.</i> , 2016

CL: Cellulose; HC: Hemicelluloses; LG: Lignin

Source: By author

Three-layer structure is rigid in order to act as protection system, which is very important when it comes to cell preserving, however, this cell wall characteristic is not useful when it is intended to use VB waste as raw material in a BRF (BICHOT *et al.*, 2018). This drawback is named recalcitrance, which is generated by complex interactions among LC fibers made of CL, HC and LG. Besides, it is well-known that recalcitrance is a crucial issue when using plant biomass (preferably waste) in a BRF system (HOLWERDA *et al.*, 2019). For solving this obstacle, BRF incorporates a pretreatment stage that allows disrupt rigid LC fibers into a looser material, improves component (CL, HC and LG) accessibility for its processing in subsequent steps and thus increases yields in the whole process (ANTUNES *et al.*, 2019; MELATI *et al.*, 2019).

1.3. Biomass Pretreatment to Improve Cellulose Accessibility

Many kinds of processes can be employed before pretreatments, such as milling, extraction and drying. Material can be milled in order to reduce particle size and increase the exposed surface area, and thus raising effectiveness of pretreatment, and subsequent enzymatic hydrolysis (DA SILVA *et al.*, 2013). The most commonly employed mills are ball mill (crushes material using spheres) (LIN *et al.*, 2010), and discs mill (employs serrated plates to pulverize biomass) (HIDENO *et al.*, 2009). However, this milling step is expensive and hard to achieve in large-scale. Some factors such as dry material and milling coupled with pretreatments affect hydrolysis performance. Milling process is pivotal to enable efficient pretreatments.

Pretreatments employing acid are known for solubilizing HC bound to CL. These processes commonly use sulfuric acid at high temperature and pressure conditions (BRIENZO *et al.*, 2017). This pretreatment is sectioned roughly in these events (HERRERA *et al.*, 2003):

- Proton scattering through damp LC material;
- Protonation of ether-oxygen binds chaining monomeric sugars;
- Rupture of glycosidic bind;
- Generation of intermediate carbocation;
- Solvation of carbocation in liquid;
- Reconstruction of protons and sugars;

- Distribution of products in liquid phase.

Looking at CL, this pretreatment can remove up to 20% of its content in biomass (TRAJANO; WYMAN, 2013). Acid process enhances acid hydrolysis accomplishment, but generates degradation products such as furfural and hydroxymethylfurfural (ALONSO *et al.*, 2013). Enzymatic hydrolysis is considerably influenced by HC and LG contents (MENG *et al.*, 2017); lowering these contents is an essential step of biomass transformation. HC does not possess a linear structure, making it more exposed to acid pretreatments than CL. Therefore, HC content can be totally removed with mild pretreatments that will not degrade CL within biomass (AGUILAR *et al.*, 2002). Steam explosion pretreatment uses SO₂ at high temperatures to reduce HC content in corn straw up to 65%, being an alternative to acid pretreatments (OHGREN; GALBE; ZACCHI, 2005). The kind of acid employed can also enhance HC removal. LC materials pretreated with sulfuric acid showed complete HC removal while steam explosion using SO₂ showed minor promising results. Even though it removed most of HC, acid pretreatment produces more degradation products due to its severity, damaging obtained sugars and producing furfural (MARTÍN *et al.*, 2002). Sugars present in HC are solubilized into oligomers or monomers (influenced by reagent concentration, temperatures and reaction times) (TRAJANO; WYMAN, 2013). Higher temperatures (EXCOFFIER; TOUSSAINT; VIGNON, 1991) and acid concentrations improve removal of HC content (Table 1-2) (KUMAR *et al.*, 2009).

Table 1-2 - Acid, alkaline and oxidative pretreatment effect on chemical composition after enzymatic hydrolysis of sugarcane biomass (node).

Pretreatment	Glucan (%)	Hemicelluloses (%)	Lignin (%)
Untreated	38.61 (± 0.91)	29.47 (± 1.53)	30.46 (± 2.61)
Acid 5%	38.47 (± 2.82)	9.65 (± 0.87)	33.59 (± 2.14)
Acid 10%	42.20 (± 2.69)	7.56 (± 0.32)	41.96 (± 13.39)
Acid 20%	41.96 (± 3.67)	5.20 (± 0.17)	42.67 (± 0.72)
Alkaline 5%	39.61 (± 5.29)	13.72 (± 1.50)	27.28 (± 1.53)
Alkaline 10%	40.75 (± 2.00)	11.18 (± 0.93)	17.33 (± 0.14)
Alkaline 20%	53.59 (± 6.65)	14.08 (± 0.73)	12.59 (± 0.21)
Alkaline 30%	42.55 (± 10.55)	13.11 (± 1.70)	7.63 (± 0.04)

Oxidative 0.5 h	38.20 (± 1.95)	29.02 (± 1.95)	34.21 (± 4.26)
Oxidative 1 h	38.50 (± 0.03)	28.47 (± 1.56)	28.90 (± 5.70)
Oxidative 2 h	39.38 (±0.26)	28.49 (± 2.80)	23.40 (± 1.82)
Oxidative 3 h	41.08 (± 3.55)	28.45 (± 1.64)	11.65 (± 5.21)

Lignin = soluble + insoluble lignin; Oxidative pretreatment was executed by adding new charges of acetic acid and sodium chlorite at longer reaction times.

Source: By author

Acid pretreatments are much less effective concerning LG removal regardless of acid employed (KUMAR 2009). Normally, severe conditions cause lower LG solubilization, resulting condensation in fiber surface (SCHMATZ; TYHODA; BRIENZO, 2020a). Removing LG content usually brings generation of aromatic molecules, different types of phenolic are determined by which biomass and pretreatment were used (DU *et al.*, 2010). Solid fraction microscopy shows considerable change in LG structure and content, presenting LG droplets within plant cells (SCHMATZ; TYHODA; BRIENZO, 2020a). These characteristics suggest a trend of rupture and aggregation that could cause less efficiency in LG content removal (SELIG *et al.*, 2007).

Many substances can be employed during alkaline pretreatments (NaOH, Ca(OH)₂, KOH, etc.). During these pretreatments, solvation and saponification processes happen first, resulting in swelling and higher accessibility for enzymes to act within LC material. Aggressive alkaline pretreatments can cause dissolution, peeling, decomposition and degradation of polysaccharides (HENDRIKS; ZEEMAN, 2009). The focus of alkaline pretreatments is LG content reduction, clearing a path for enzymatic hydrolysis and enhancing polysaccharide reactivity (Table 1-2). Studies show that this trend is related to intermolecular saponification of ester links among HC and LG, increasing pore density (DA SILVA *et al.*, 2013). Peroxide pretreatments are also quite capable of HC and LG removal, generating a pretreated biomass with improved enzymatic digestibility (MONTE; BRIENZO; MILAGRES, 2011). This pretreatment can successfully solubilize HC and leaves only residual LG (BRIENZO, M; SIQUEIRA; MILAGRES, 2009).

1.4. Lignocellulosic Biomass Hydrolysis

Many variables are involved in LC biomass conversion into sugars through enzymatic hydrolysis. For example, accessibility issues and costs of employed technologies are obstacles often encountered. CL accessibility is crucial since it is closely tied to enzymatic hydrolysis (CROWE; ZARGER; HODGE, 2017). Shortly, accessibility is a way to measure how much CL can be reached by enzymes during hydrolysis. Biomass on its own has an impact on accessibility since it is a heterogenic material, has variable crystallinity, polymerization degree, and HC and LG contents creating a physicochemical barrier. Actually, one of the hardest obstacles to jump is the difficulty to reach a large portion of CL covered by robust and organized microfibrils.

It is safe to say that there is a close connection between internal surface area and enzymatic hydrolysis (COSGROVE, 2005), being one of the obstacles in the way of biomass hydrolysis (HUANG *et al.*, 2010). Accessibility is also heavily tied to pore size distribution, significantly more than what is observed with external surface area (WANG *et al.*, 2012). With that in mind, enzymes are able to reach internal surface through big enough pores (HARMOKO *et al.*, 2016). Pores smaller than 5.1 nm are not wide enough for enzymes to hydrolyze LC material. It is important to note that most studies use dried biomass, which shows lower digestibility due to shrinkage of pores (LUO; ZHU, 2011).

LG and HC contents have a close relation to accessibility, influencing pore distribution, pretreatment efficiency and enzymatic hydrolysis (ZHAO *et al.*, 2017). LC biomasses are heterogeneous, possessing its area sectioned into external and internal areas, presenting different characteristics. Regarding internal surface, accessibility can be inferred by looking at its rifts, voids and openings generated by HC and LG removal by employing many kinds of pretreatments (steam explosion, peroxide, diluted acid, alkaline, and others) (KARIMI; TAHERZADEH, 2016). It has been reported that HC removal could be a more important asset to accessibility than delignification (LEU; ZHU, 2013). HC present barriers to enzymatic hydrolysis as it is placed between and around fibers within cell walls (ZHU; PAN; ZALESNY, 2010).

LG inherently hinders enzymes by forming a physicochemical wall that adsorbs enzymes (XIMENES *et al.*, 2011). By employing correct pretreatments, lower contents of LG and HC may diminish or stop enzymatic hydrolysis hindrance (CHEN *et al.*, 2015). While a complete removal of LG would be too expensive in large scale (YANG *et al.*, 2015), mild delignification increases accessibility and can increase enzymatic hydrolysis yield dramatically (SHIMIZU *et al.*, 2020).

Digestibility can also be influenced by particle size. Quantifying particle size is a problem since they have variable shapes and commonly agglomerate (EK *et al.*, 1994). Particle size can be visualized by employing microscopy, image analysis or mechanized particle analyzers. On the down side, these techniques do not discriminate between material's topology and ruptures that can increase exposed surface areas. However, it is expected that small particles provide higher digestibility by presenting a wider exposed surface area for enzyme action. In sum, HC and LG removal is related to accessibility just as much as physicochemical properties of biomass (SHIMIZU *et al.*, 2020).

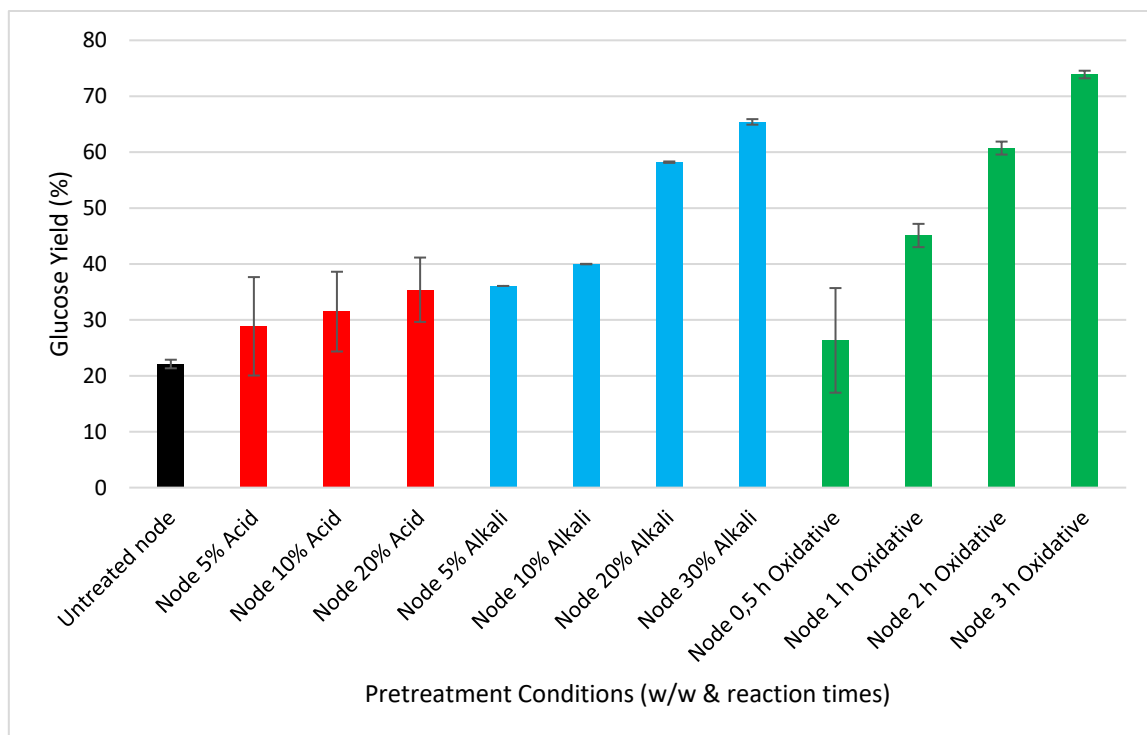
Lower degrees of polymerization present an increase the number of linkage sites for enzymes, enhancing biomass hydrolysis. Regardless, CL needs to be exposed to be reached by enzymes. Altering of polymerization degree usually involves meddling with porosity and crystallinity, as the milling process rips fibers and increases pore size distribution (ZHAO; ZHANG; LIU, 2012). Depolymerization happens when polysaccharides are broken into monomers (GOUFO; MUGISHA, 2018). Depolymerization of CL chains within LC biomasses is a step of enzymatic hydrolysis process. For example, cellulases are capable of depolymerize CL in order to obtain glucose (a specific monomer) (YÜCEL; GÖYCINCIK, 2015). Enzymes used and substrate employed are also important. The spread of enzymes through pores, the binding sites occupied during hydrolysis, inhibition products and other enzymes used to attack cell wall are properties related to accessibility (ZHAO *et al.*, 2017).

1.5. Enzymatic Hydrolysis

Cellulases are an enzymatic cocktail of various enzymes that depolymerize CL into sugars by breaking its glycosidic links. Cellulases are actually three enzymes: exo- β -1,4-glucanases, endo- β -1,4-glucanases and β -glucosidases. These enzymes work in synergic manner, where their efficiency combined is higher than if they were used separately (YÜCEL; GÖYCINCIK, 2015). Among these enzymes, endoglucanases are capable of breaking chains through interchain, exo- β -1,4-glucanases attack free ends of chains created by endo- β -1,4-glucanases deploying water-soluble cellobiose and β -glucosidases finish the process by hydrolyzing cellobiose into monomers. Difficulty is faced if LC materials possess HC and LG, which makes enzymes unable to reach and hydrolyze CL. Endo- β -1,4-glucanases role is most likely affected during hydrolysis

since it starts the action on CL. The exposed surface area by pretreatments accessible to enzymes is closely related to glucose yield (Figure 1.2) (BRIENZO *et al.*, 2017). Enzymatic hydrolysis of CL chain can be sectioned in these parts (WALKER; WILSON, 1991): Moving enzymes from liquid medium to LC material; Adsorption and generation of enzyme-substrate complex; Hydrolysis of CL chain; Moving of sugars obtained from biomass surface to the liquid medium; Hydrolysis of cellobiose and oligomers into monomers in liquid medium.

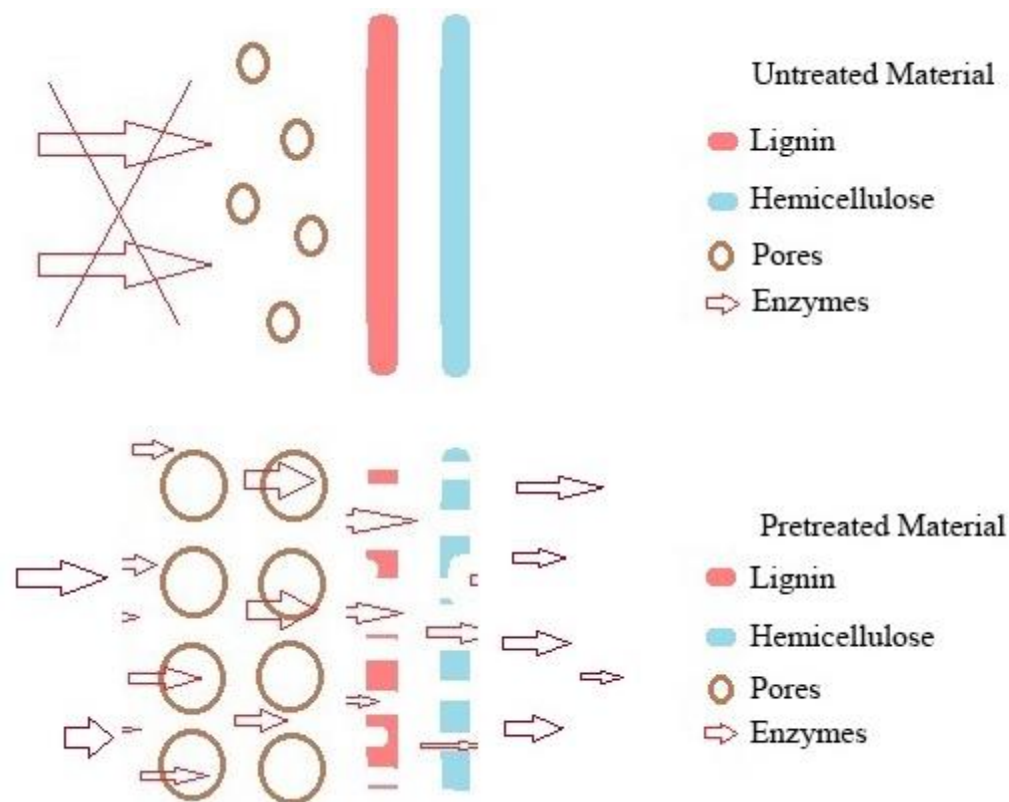
Figure 1.2 - Increased cellulose accessibility by pretreatment allows an efficient enzymatic hydrolysis (sugarcane bagasse node)



Source: By author

Many factors influence the enzymatic hydrolysis process, such as HC and LG contents present in LC material, generating a physicochemical barrier against enzymes (Figure 1.3). Furthermore, during hydrolysis LG content increases, slowing down the process. While enzymatic hydrolysis process occurs, the material loses CL content, increasing LG/CL ratio and hindering hydrolysis (WALLACE *et al.*, 2016).

Figure 1.3 - Representation of hemicelluloses - lignin barrier before and after of pretreatment



Source: By author

Mild delignification of VB enhances glucose yield, more so when LG content reaches 10% (MOONEY *et al.*, 1998). Other than that, it increases hydrolysis efficiency due to larger quantity of enzymes retrieved, caused by less unproductive binding to LG. High LG contents can cause redepositing, occupying pores that could be penetrated by enzymes. Enzymes are unproductively adsorbed by LG within biomass, severely hindering enzymatic hydrolysis (SCHMATZ; TYHODA; BRIENZO, 2020a). In addition, LG amounts in LC material can be used as a discrimination parameter for enzymatic hydrolysis (BRIENZO *et al.*, 2014).

1.5.1. Cellulases Performance

Enzymes that play a synergistic important role on CL depolymerization into cellobiose and glucose molecules at saccharification step are denominated cellulases or cellulolytic enzymes, which can be synthesized during growth of some microorganism (fungi or bacteria) in LC substrates through solid or submersed fermentations (ARUWAJOYE; FALOYE; KANA, 2020; BARBOSA; SILVELLO;

GOLDBECK, 2020). CL hydrolysis requires participation of three types of cellulolytic enzymes, all of them characterized by cleaving glycosidic linkages β -(1 $\rightarrow$ 4).

Cellulases performance is synergistic, which means that if one of them does not act, then hydrolysis stage will have low yield and therefore, BRF process will not be technically feasible. These drawbacks can occur by different factors such as temperature, pH, chemicals (that can come from pretreatment) or products generated by own cellulases action (HSIEH *et al.*, 2014; TEIXEIRA *et al.*, 2016). Recent studies have confirmed inhibitory effects on cellulases by cellobiose and some monosaccharides like glucose, mannose and galactose (HSIEH *et al.*, 2014). Although inhibitory compounds concentration can vary, there is evidence of a generality; CMCase activity can be inhibited by cellobiose, CBHs can be deactivated by glucose and β -glucosidases sensibility can be greater in glucose presence than mannose and galactose (HSIEH *et al.*, 2014; MURPHY *et al.*, 2013; VIANNA BERNARDI *et al.*, 2019). Nevertheless, one of the principal challenges is focus on researching minimum level of inhibitory concentration that can affect negatively the cellulases activities and that inhibitory effects can be reversible or irreversible.

In addition to mentioned inhibitors, others chemical compounds can affect cellulohydrolytic system performance, among them are ionic liquids, some salt and metal ions, phenolics and furans (SUMMERS *et al.*, 2017; TEIXEIRA *et al.*, 2016; ZHAI; HU; SADDLER, 2016).

1.5.2. Xylanases Performance

Because HC is a branched heteropolysaccharide constituted by several kind of molecules, a multienzyme system that works synergistically is required for its depolymerization. These enzymes are denominated xylanases or xylanolytic enzymes, which can be synthesized by some microorganism (fungi, bacteria, yeast or algae) using xylan-containing substrates through solid or submersed fermentations (BHARDWAJ; KUMAR; VERMA, 2019). Xylan hydrolysis needs contribution of various types of xylanolytic enzymes, which acted both on xylan-main chain cleaving chemical bonds β -(1 $\rightarrow$ 4) and on main-chain sides cleaving linkages α -(1 $\rightarrow$ 2) and α -(1 $\rightarrow$ 3) principally (CHAKDAR *et al.*, 2016). In main-chain, endoxylanases (EC 3.2.1.8) are responsible for depolymerizing the xylan backbone (β -(1 $\rightarrow$ 4) bonds) to obtain

xylobiose and shorter xylooligomers. β -xylosidases (EC 3.2.1.37) breakdown xylobiose and other soluble xylooligomers into xylose molecules (BOSETTO *et al.*, 2016; PUCHART *et al.*, 2018). Accessory enzymes act on xylan side chains, among them are α -arabinofuranosidases (EC 3.2.1.55), α -glucuronidases (EC 3.2.1.131), β -galactosidases (EC 3.2.1.23), acetyl xylan esterases (EC 3.1.1.6), ferulic acid esterases (EC 3.1.1.73) and p-coumaric acid esterases (EC 3.1.1.73) (MALGAS *et al.*, 2019).

The first one, also called α -arabinosidases, break α -(1 $\rightarrow$ 2), α -(1 $\rightarrow$ 3) and α -(1 $\rightarrow$ 5) bonds, and release arabinose from xylose-arabinose linkage (TERRONE *et al.*, 2020; YEOMAN *et al.*, 2010). α -glucuronidases disrupt α -(1 $\rightarrow$ 2) bonds and split off methyl glucuronic acid from acid-xylose linkages (DASHNYAM *et al.*, 2018). β -galactosidases are responsible to cleave β -(1 $\rightarrow$ 4) linkages, which allows galactose to unlink from galactose-xylose bonds (KHOSRAVI *et al.*, 2015). Acetyl xylan esterases attack acetyl groups located at xylan branches, these type of enzymes release acetyl groups allowing endoxylanases to work on the main chain (PAWAR *et al.*, 2016; RAZEQ *et al.*, 2018). Feruloyl and p-coumaroyl esterases are capable to cleave ester bonds in arabinose-ferulic acid linkages and in arabinose-p-coumaric acid linkages, respectively (LOPES; FERREIRA FILHO; MOREIRA, 2018).

The complex system of xylanolytic enzymes act to breakdown xylan (as is mentioned above), the process can develop some difficulties related to inhibitory effects in xylanases activities. Among factors that can negatively modify their activities are temperature, pH, chemicals (that can come from pretreatment) or products generated by own xylanases action (BAJPAI, 2014a). Although inhibitory substance concentration can differ, it exists an overview; endoxylanases activity can be susceptible to xylobiose and xylotriose presence, and β -xylosidases can be deactivated by xylose (FU *et al.*, 2019; YEOMAN *et al.*, 2010). It is necessary to expand and deepen studies of inhibitory effects on accessory enzymes; these could include analysis of dimmers and monomers produced in pretreatment stage.

Although xylanases and cellulases can be synthesized extracellularly by different fungi or bacteria species, few microorganisms are able to produce the whole set of hydrolytic enzymes. For this reason, it is important to continue with researches involving analysis of microorganisms, methods and variables for hydrolytic enzymes production.

1.5.3. Cellulolytic Enzymes Synthesized by Fungi and Bacteria

CL is the main constituent of plant cell wall, in addition to being the most abundant natural polymer available (GEORGE; SABAPATHI, 2015). CL is chemically composed of linear chains of 7000 to 15000 glucose residues joined by β -1,4 glucosidic bonds, without branches. β -type bond causes a 180° rotation alternating glucose unit, resulting in a linear chain. CL is hydrolyzed by the action of microbial enzymes, such as endoglucanases, exoglucanases and β -glucosidases (BAGEWADI; MULLA; NINNEKAR, 2017; PINO *et al.*, 2018; SØRENSEN *et al.*, 2014)

Cellulases are produced by several species of fungi (NARRA *et al.*, 2014). Endoglucanases can be produced by *Myceliophthora sp.* (ZANELATO *et al.*, 2012), *Streptomyces sp.* (CHELLAPANDI; JANI, 2008), *Trichoderma viride* (IRFAN; NADEEM; SYED, 2012), *Trichoderma harzianum* (BAGEWADI; MULLA; NINNEKAR, 2017) and *Aspergillus oryzae* (KOTAKA *et al.*, 2008). Exoglucanases, as well as endoglucanases, are also produced by a wide variety of fungal species, such as *Rhizopus oryzae* (MUKHERJEE; KARMAKAR; RAY, 2011), *Aspergillus niger* (CHANDRA; ESTEGHLALIAN; SADDLER, 2008), *Aspergillus fumigatus* (MAHMOOD *et al.*, 2013), *Trichoderma reesei* (ELSHAFEI *et al.*, 2014), *Trichoderma viride* and *Ganoderma lucidum* (SHAHZADI *et al.*, 2014).

In addition to endo and exoglucanases, β -glucosidases can be produced by *Trichoderma reesei* (JUHÁSZ; EGYHÁZI; RÉCZEY, 2005), *Penicillium funiculosum* (RAMANI *et al.*, 2012), *Aspergillus niger* (GARCÍA-KIRCHNER; SEGURA-GRANADOS; RODRÍGUEZ-PASCUAL, 2005), *Debaryomyces pseudopolymorphus* (BARBOSA *et al.*, 2010), *Pichia pastoris* (BATRA; BERI; MISHRA, 2014) and *Aspergillus saccharolyticus* (SØRENSEN *et al.*, 2014). Besides fungi, bacteria also produce cellulases of industrial importance. Endoglucanases can be produced by *Bacillus agaradhaerens* (HIRASAWA *et al.*, 2006), *Clostridium thermocellum* (ROMANIEC *et al.*, 1992), *Clostridium thermocellum* (FAUTH *et al.*, 1991). Exoglucanases have also been identified in bacteria of species *Clostridium stercorarium* (CREUZET; BERENGER; FRIXON, 1983), *Celullomonas fimi* (DUEDE; FRENCH, 2016), and *Xanthomonas oryzae* pv. *oryzae* (TAYI *et al.*, 2017). Additionally, β -glucosidases can be produced by bacteria of species *Dyella koreensis* sp. nov. (AN *et al.*, 2005), *Burkholderia ginsengisoli* sp. nov. (KIM *et al.*, 2006), *Microbacterium*

ginsengisoli sp. nov. (PARK *et al.*, 2008), *Lactobacillus kimchicus* sp. nov. (LIANG *et al.*, 2011), *Brevibacillus panacihumi* (KIM *et al.*, 2009), most of which are species that had never been described before.

Cellulases, in general, are enzymes that are of great use in several industrial sectors. Endoglucanases, exoglucanases and β -glucosidases are enzymes widely used in bioethanol production (KOTAKA *et al.*, 2008; SINGHANIA *et al.*, 2017). Endoglucanases are used in textile and food industries (NARRA *et al.*, 2014). Exoglucanases are used mainly in textile and in pulp and paper industries (CASTRO; PEREIRA JR, 2010).

Beside from cellulases, other enzymes play fundamental roles in the development of products with high added value, such as hemicellulases, which for industrial sector may be essential in obtaining medicines, packaging and products of relevance in the medical field. Hemicellulases, like cellulases, can also be produced by fungi (LIU *et al.*, 2010) and bacteria (WALIA *et al.*, 2014).

1.5.4. Hemicellulolytic Enzymes Synthesized by Fungi and Bacteria

Basically, xylan hydrolysis, the most abundant compound of HC in nature, occurs through endo-enzymes action, which act internally on main chain, and exo-enzymes or auxiliary enzymes that hydrolyze oligosaccharides and produce monosaccharides (KALOGERIS *et al.*, 2001). Some enzymes produced by microorganisms hydrolyze xylan in specific regions of its main chain and side chains, leading to monosaccharides production that are used by microorganisms themselves as carbon source for cellular metabolism.

Among microbial enzymes that hydrolyze xylan into monosaccharides, it can be highlighted endo- β -1,4-D-xylanases (EC 3.2.1.8), which acts on xylan main chain and generates low polymerization xylooligosaccharides that are substrates for exo- β 1,4-xylanases (EC 3.2.1.37), that act through non-reducing terminals generating D-xylose (BEG *et al.*, 2001; WONG *et al.*, 1988).

In addition to the enzymes that hydrolyze xylan main chain, there are still some enzymes that hydrolyze the pendant-side groups. Among these accessory enzymes, α -arabinofuranosidases (EC 3.2.1.55) stands out, which removes arabinose, α -glucuronidases (EC 3.2.1.131) which removes glucuronic acids, acetyl-xylan esterases (EC 3.1.1.72), which removes acetyl groups (JUHÁSZ; EGYHÁZI; RÉCZEY,

2005), and feruloyl esterases which remove ester bonds between hydroxycinnamic acids and sugars (MATHEW; ABRAHAM, 2004). The consequent action of accessory enzymes produces debranched xylan, which is of great interest for industrial applications.

α -glucuronidases are classified into two families: GH 67, which cleaves uronic acids linked to non-reducing terminals of xylosyl residues of small xylooligosaccharides, and GH 115, which hydrolyze 4-O-D-methylglucuronic acids linked to non-reducing terminals of xylopropanosyl residues and also those linked to internal xylosyl residues. They are produced by bacteria (about 70 kDa) and fungi (about 90kDa) (DIMAROGONA; TOPAKAS, 2016; YEOMAN *et al.*, 2010).

Acetyl-xylan esterases are enzymes produced by bacteria and fungi. They are responsible for cleaving acetyl groups from heteroxylans. Microorganisms that produce these enzymes become capable of degrading acetylated xylans. Feruloyl esterases cleave bonds between esterified hydroxycinnamic acids for arabinoxylans. They are also produced by fungi and bacteria, and its production can be induced according to presence of certain substrates in microbial growth (WONG *et al.*, 1988).

Complete hydrolysis of xylan requires joint action of enzymes that hydrolyze its main chain (xylanases and β -xylosidases) and accessory enzymes responsible for hydrolyzing its branches (α -arabinofuranosidases, acetyl-xylan esterases, α -glucuronosidases and feruloyl esterases) (YANG, Yi *et al.*, 2017). Several microorganisms have the capacity to produce xylanolytic enzymes, which in turn have great potential for industrial uses. Below, more details of some xylanase-producing microorganisms are presented.

Xylanolytic enzymes are usually best induced by xylan-containing substrate (FERNÁNDEZ-ESPINAR *et al.*, 1994). However, some authors show inducing effect with xylan fragments (ARO; PAKULA; PENTTILÄ, 2005) and its repression by readily assimilable sugars such as glucose, lactose and xylose (GASPAR *et al.*, 1997). LC substrates are used to obtain xylanolytic enzymes on a large scale and at low cost in submerged or solid culture (MISHRA, Chittra *et al.*, 1990).

Biochemical properties of endoxylanases from bacteria and fungi vary in terms of molecule size (8.5 to 85 KDa) and isoelectric point (4.0 to 10.3 pI) (CHAKDAR *et al.*, 2016). Table 1-3 shows some xylanase-producing microorganisms found in specialized literature (CHAKDAR *et al.*, 2016; GOSWAMI; PATHAK, 2013; WALIA *et*

al., 2014), showing optimal conditions for action of such hemicellulases and then, its respective molecular sizes.

Table 1-3 - Hemicellulases from bacteria and fungi.

Microorganism	Optimal conditions		Molecular Weight (kDa)	Reference
	pH	Temperature (°C)		
Bacteria				
<i>Bacillus pumilus</i> SSP 35	6	50	20	Subramaniyan, 2012
<i>Bacillus halodurans</i>	9	78	40	Kumar; Satyanarayana, 2011
<i>Cellulosimicrobium cellulans</i> CKMX1	8	60	58	Walia <i>et al.</i> , 2014
<i>Paenibacillus macerans</i> IIPSP3	4.5	60		Dheeran <i>et al.</i> , 2012
<i>Paenibacillus</i> sp. NF1	6	60	37	Zheng <i>et al.</i> , 2014
Fungi				
<i>Aspergillus niger</i> ANL-301 9	5.5	45	13.5-14	Okafor <i>et al.</i> , 2007
<i>Geotrichum candidum</i>	4	50	60-67	Rodionova <i>et al.</i> , 2000
<i>Trichoderma reesei</i>	5 – 5.4, 4 - 4.5	45	20. 19	Tenkanen; Puls; Poutanen, 1992
<i>Penicillium capsulatum</i>	5 – 5.5, 4 - 4.5	48	22	Ryan <i>et al.</i> , 2003
<i>Penicillium</i> sp. CGMCC 1669	3.8	40	21	Liu <i>et al.</i> , 2010

Source: By author

Among the products of high added value obtained by hemicellulases use, it can be highlighted: xylooligosaccharides (XOS), xylitol, hydrogel, dressings and substitutes for human skin, films for biodegradable packaging, medicine encapsulation, substitute for fat in cheeses, additives in paper production, substitute for gelatin and in food gums, additives in textile and cosmetic industries, production of new biomaterials such as glucagel, etc (EBRINGEROVÁ, 2006).

The study of HC applications is constant, such as XOS with high purity (REDDY; KRISHNAN, 2016), XOS for fermentation (CHEN; LIU, 2017), mucus adhesive film (HANIF; ZAMAN, 2017), xylan-based thermoplastic - lactate copolymer (ZHANG *et al.*, 2017) and also desirable for biomedical applications (CANTU-JUNGLES *et al.*, 2017).

1.5.5. Enzymes Purification Systems

Over the past five decades, it has been a great development of techniques and methods for separation and purification of biological macromolecules such as enzymes. As a result, advances in biosciences and biotechnology have become increasingly important (ERSSON; RYDÉN; JANSON, 2011; YIMER; TILAHUN, 2018).

In order to acquire knowledge about structural and functional properties of enzymes, the purification process is an essential step. Enzyme purification often requires the use of multiple purification steps applying different methods in suitable order to obtain the highest possible yield. However, it should be kept in mind that the more steps of purification, the lower the yield. Therefore, knowledge of enzyme's properties is of utmost importance to reduce purification steps (GENERAL ELECTRIC HEALTHCARE, 2010).

The most common method for enzyme purification is chromatography. These methods separate molecules according to interest enzyme properties and other substances in the sample (GENERAL ELECTRIC HEALTHCARE, 2010). Enzyme characteristics that should be taken into consideration when choosing a purification method are solubility, size, charge and specific binding affinity (BERG; TYMOCZKO; STRYER, 2002; YIMER; TILAHUN, 2018).

Cellulases purification, an enzyme group that play an important role in hydrolysis of renewable LC materials, can be carried out using different methods. Purification efficiency is generally analyzed in terms of yield and purification fold, as is presented in Table 1-4, along with purification methods used for cellulases reported in specialized literature.

Table 1-4 - Purification methods, fold and yield of cellulases from different microorganisms.

Microorganism	Purification methods	Yield (%)	Purification Fold	Reference
<i>Aspergillus oryzae</i>	DEAE-cellulose	65.7	15.0	Begum; Absar, 2009
	Sephadex G-75	27.4	41.4	
<i>Bacillus vallismortis</i>	Ammonium Sulfate	83.8	4.7	Gaur; Tiwari, 2015
	Q-Sepharose	75.4	11.6	

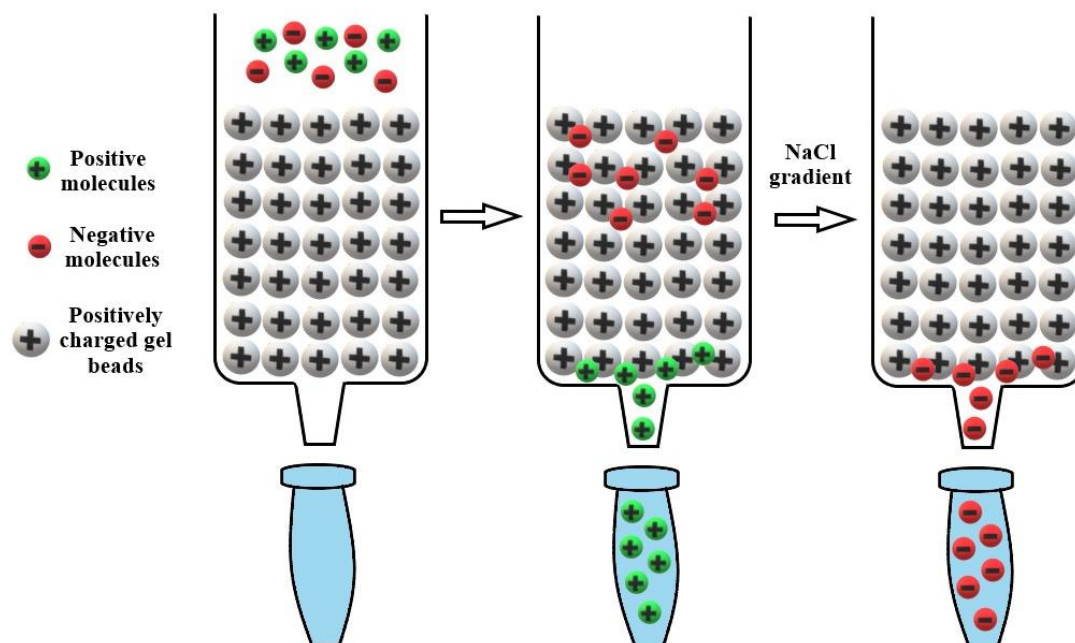
	Sephadex G-75	28.8	39.1	
<i>Aspergillus terreus</i>	Ammonium Sulfate	96.2	16.0	Megha <i>et al.</i> , 2015
	DEAE-cellulose	42.6	135.5	
<i>Anoxybacillus gonensis</i>	Ammonium Sulfate	30.7	55.1	Genç <i>et al.</i> , 2014
	DEAE-sepharose	20.7	74.4	
<i>Paenibacillus</i> sp.	Ammonium Sulfate	91.8	1.7	Islam; Roy, 2018
	DEAE-cellulose	78.9	5.9	
	CM-cellulose	35.7	9.7	
<i>Trichoderma longibrachiatum</i>	Ammonium Sulfate	95.8	1.1	Hamdan; Jasim, 2018
	DEAE-cellulose	78.1	7.8	
	Sephadex G-200	68.8	9.7	
<i>Pseudomonas</i> sp.	Ammonium Sulfate	37.7	4.1	Goel <i>et al.</i> , 2019
	DEAE-cellulose	33.4	14.5	

Source: By author

Salting out and dialysis are methods that are usually used as first steps. Protein solubility usually decreases at high salt concentrations, causing its precipitation. Different proteins precipitate in different concentrations of salt, therefore, salting out can be used to fractionate proteins (DUONG-LY; GABELLI, 2014; WINGFIELD, 2016). Ammonium sulfate is commonly used as a precipitate agent to separate other proteins from cellulase (FARINAS *et al.*, 2011). Dialysis is usually used after salting out to remove salt or other small molecules through a semipermeable membrane, which can come in a variety of pore sizes (BERG; TYMOCZKO; STRYER, 2002; YIMER; TILAHUN, 2018).

Different types of chromatography can be used for cellulases purification. Ion exchange chromatography (Figure 1.4) separates proteins based on their charge. Protein with positive charge will usually bind to a column negatively charged with sulfopropyl (SP) or carboxymethyl (CM). Likewise, negatively charged proteins will bind to a positively charged column containing quaternary amines (Q) or diethylaminoethyl (DEAE) (BERG; TYMOCZKO; STRYER, 2002). When above its isoelectric point (pI), an enzyme tends to bind to a positively charged anion exchanger, and when below its pI, a cation exchanger negatively charged matrix is where the enzyme will bind (BAUER; SCHNAPP, 2007; GENERAL ELECTRIC HEALTHCARE, 2010).

Figure 1.4 - Ion exchange chromatography

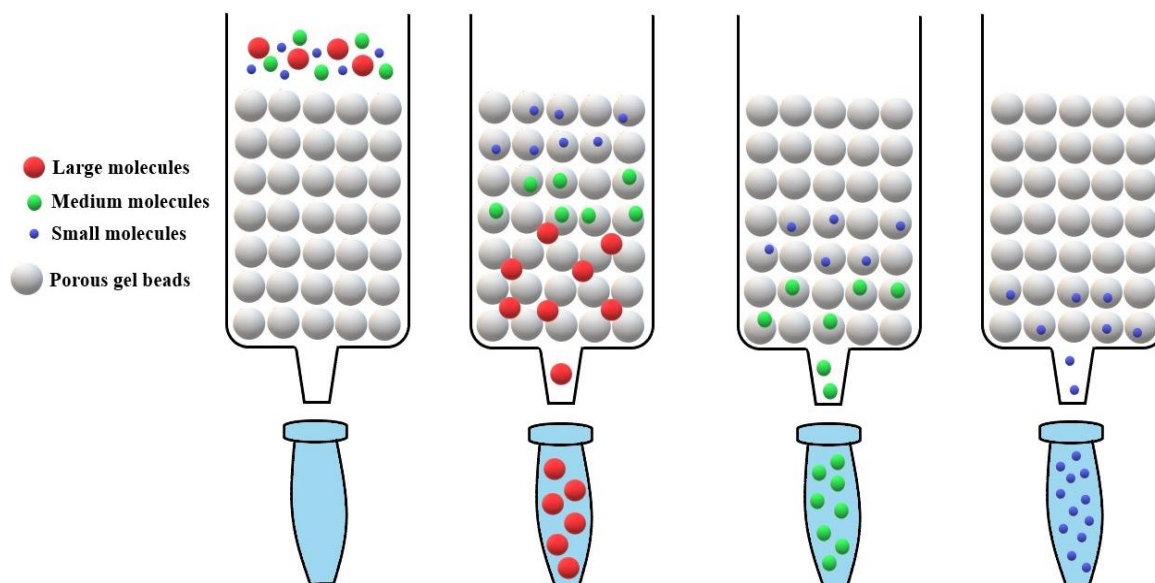


Source: By author

Depending on pH, cellulases can be negatively or positively charged, however several researches show that most of them present better purification when using anion exchange chromatography (BEGUM; ABSAR, 2009; GAUR; TIWARI, 2015; GENÇ *et al.*, 2014; GOEL *et al.*, 2019; MEGHA *et al.*, 2015). DEAE is a positively charged matrix commonly used as the stationary phase, where cellulases will bind if negatively charged. In order for cellulases to be eluted from the matrix, a linear sodium chloride gradient from zero to 1 mol/L is generally used. Chloride ions will compete with negatively charged groups on cellulases for binding to the column (BAUER; SCHNAPP, 2007).

Size exclusion chromatography (Figure 1.5) allows separations of substances with different molecular sizes. Large molecules that cannot enter into pores of the chromatography beads will elute first, while smaller molecules will penetrate the pores and therefore will take longer to elute (GENERAL ELECTRIC HEALTHCARE, 2010) Sephadex is the most popular matrix for gel exclusion chromatography. Molecular weight of cellulases produced by different microorganisms can vary from 12 to 126 kDa, therefore, column packing material with different beads sizes can be used for purification (CHANDRA; MADAKKA, 2019; TAO *et al.*, 2010).

Figure 1.5 - Size exclusion chromatography



Source: By author

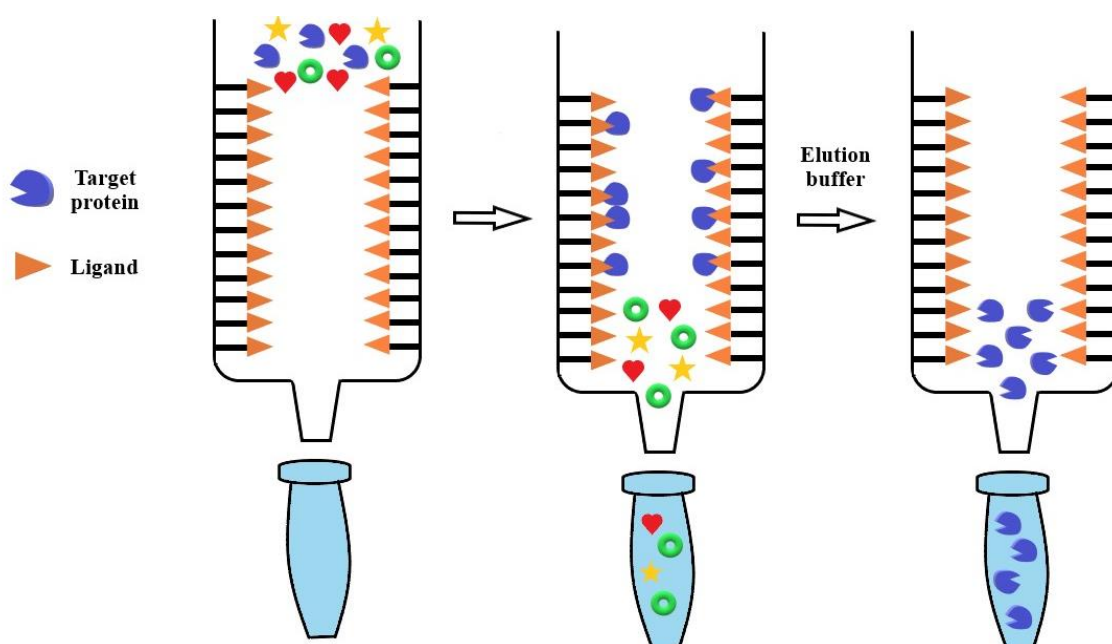
Specific activity of cellulases is usually two-fold lower than of other hydrolytic enzymes. For industrial purposes, large quantity of cellulases is needed and this production becomes a “metabolic burden” even for native cellulolytic organisms. Therefore, heterologous expression of cellulases in industrial microorganisms, such as *E. coli*, *Pichia pastoris* and *Saccharomyces cerevisiae*, enables robust and cost effective production (VINUSELVI, 2012). Several researches with this goal have been carried out in the past years, mainly focusing on heterologous expression of cellulases produced by different microorganisms and termites in *E. coli* or other model microorganisms (CHAHED *et al.*, 2018; CHUAN WEI *et al.*, 2015; RAZA *et al.*, 2020; TROLLOPE; NEL; VOLSCHEK, 2018; ZEESHAN *et al.*, 2018; ZENG *et al.*, 2016; ZHANG, 2018).

Purification of recombinant proteins can be influenced by molecular biology of gene isolation and expression. For an economical production, efficient implementation of purification methods to maintain a high yield of the heterologous protein is of great importance. Affinity chromatography is one of the most efficient methods for heterologous proteins purification due to its high recovery yield and purity achieved (EVANGELISTA; SUTTNAR, 1997; MAHMOODI *et al.*, 2019; PINA; LOWE; ROQUE, 2014).

Affinity chromatography (Figure 1.6) separates molecules based on a reversible interaction between the protein of interest and a specific ligand attached to a chromatography matrix. In this method, specific tag sequence is attached to the protein

of interest at DNA level, generally fused to N- or C- terminal, which leads to expression of a tagged protein. Besides facilitating the purification process, these tags also enhance protein stability and increase expression levels. Metal ions such as zinc, copper and nickel have been found to bind favorably with histidine residues in proteins (EVANGELISTA; SUTTNAR, 1997; GENERAL ELECTRIC HEALTHCARE, 2010; MAHMOODI *et al.*, 2019; PINA; LOWE; ROQUE, 2014).

Figure 1.6 - Affinity chromatography



Source: By author

For purification of heterologous cellulases, the use of a nickel-nitrilotriacetic acid matrix, in which metal ions interact with histidine residues in the affinity tag, is commonly found in literature. High concentration of imidazole buffer, use of strong chelating agents such as Ethylenediamine tetraacetic acid (EDTA) and lowering pH in order to protonate histidines, and they no longer have affinity for the nickel resin, are methods that can be used to elute cellulases from the matrix (AMAIKE CAMPEN *et al.*, 2017; NI; TAKEHARA; WATANABE, 2005; REVERBEL-LEROY *et al.*, 1997; SHI *et al.*, 2013; ZENG *et al.*, 2016).

There are several strategies for purifying cellulases. It must be taken into consideration the best system to obtain the highest yield with the highest catalytic activity of the enzymes. Purity degree will depend on its end use, however most of

purification systems used in laboratory researches can be scaled up to industrial processes (BAJPAI, 2014a, b).

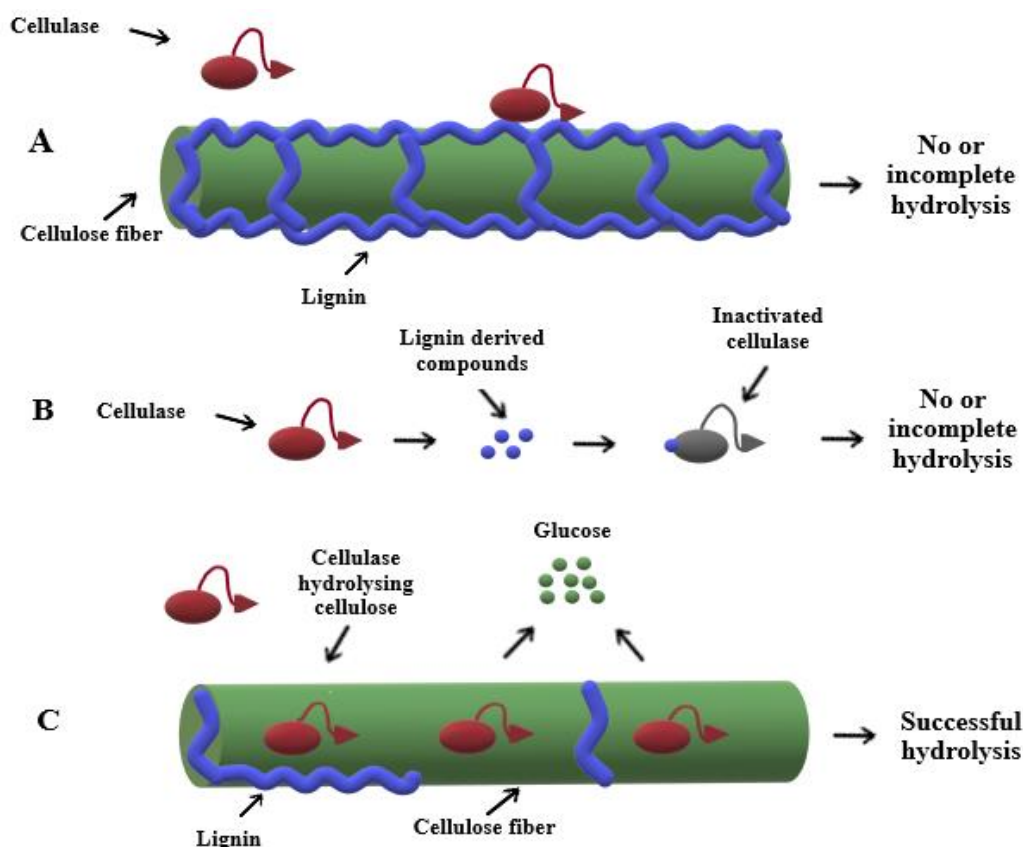
1.5.6. Interference Among Enzymes

Conversion process of polysaccharides on LC biomass into simple sugars through enzymatic hydrolysis is a crucial and limiting step for biofuel production (SHELDON, 2014). Enzymatic hydrolysis is a heterogeneous process that involves close contact between enzyme and substrate, enzyme activity and reaction conditions such as temperature and pH (SAINI *et al.*, 2016).

Enzymatic hydrolysis of lignocellulose can be affected by several factors, mainly substrate-related since the presence of LG and HC in biomass can decrease accessible area to cellulases (SHIMIZU *et al.*, 2020). Polymerization and crystallinity degrees of CL also play a role on enzymatic hydrolysis yield. Interaction between CL and cellulases can be inhibited by LG in different ways depending on its structural properties (YING *et al.*, 2018).

Inhibition of cellulases by LG can occur by three major factors: exposure to soluble lignin-derived compounds, non-productive binding of cellulase into LG and physical barrier of cellulases progress (Figure 1.7). Enzyme inhibition due to LG is a complex process and will depend on biomass botanical origin and pretreatments applied to it, since both affect localization and chemical properties of LG (SAINI *et al.*, 2016).

Figure 1.7 - Cellulase inhibition by LG. A) Physical barrier of cellulase progress on CL fibers and non-production binding of cellulase to LG. B) Inhibition due to exposure to LG derived compounds. C) Normal hydrolysis where cellulase binds to CL releasing glucose.



Source: By author

Phenolic compounds can cause loss of cellulases activity as the length of exposure to them increases. This occurs due to cellulases precipitation and can vary depending on microbial source of enzymes, type of cellulases and type of phenolic compound (SAINI *et al.*, 2016). In addition to act as a shield preventing enzymes to reach CL, LG can also adsorb cellulases irreversibly by hydrophobic, electrostatic or hydrogen-bonding interactions (KUMAR *et al.*, 2012).

All of these factors led to decrease of glucose release in enzymatic hydrolysis process. Therefore, a way to reduce cellulases-lignin interaction is widely studied. Some of the strategies used are chemical modification of LG or its complete removal, cellulases modification and addition of LG blocking additives. Pretreatment of LC biomass can remove or modify LG and HC contents, which can raise accessibility for cellulases (MELATI *et al.*, 2019; SAINI *et al.*, 2016; SHIMIZU *et al.*, 2020).

The rate of LC biomass enzymatic hydrolysis usually decreases during hydrolysis. Steam pretreated sugarcane bagasse was enzymatic hydrolyzed for 72 hours, showing an initial phase with fast conversion of 61.7% glucose and a final conversion of 86%. As hydrolysis progressed, rate of conversion decreases as well as

glucan content while LG, phenolic compounds and ash increased. In addition, enzyme activity was lost during the first 24 h of hydrolysis. Recalcitrance increasing during hydrolysis and deactivation of enzymes due to thermal stability are factors that contribute for slowdown and incomplete hydrolysis (WALLACE *et al.*, 2016).

After different pretreatments, sugarcane biomass (external fraction, node, internode and leaf) presented improve on CL conversion into glucose after enzymatic hydrolysis. A complete conversion of CL into glucose was possible after oxidative pretreatment of the internode fraction. LG removal of 15% and 10%, respectively, resulted in at least 60% of glucose yield (SHIMIZU *et al.*, 2020). Addition of a lignin-blocking agent to sugarcane bagasse can increase hydrolysis yield by 40% or even 60%, depending on agent concentration. They prevent non-productive adsorption of cellulases by blocking exposed LG surfaces (ÁZAR *et al.*, 2020).

Steam explosion and alkaline sulfate pretreatments on sugarcane bagasse resulted in more accessible substrate, with approximately 90% of CL hydrolyzed using high enzyme loadings. Besides, after pretreatment with sodium sulfate and sodium hydroxide hydrolysis using low loading of a modern enzyme preparation used in industrial processes, Cellic CTec3, resulted in 70% conversion into glucose (SIQUEIRA *et al.*, 2017). In contrast, the presence of vanillin, a lignin-derived phenolic, can cause cellulases deactivation and precipitation, resulting in a decrease of 20% in CL conversion with a concentration of 5 mg/mL vanillin and 50% with 10 mg/mL (QIN *et al.*, 2016).

The majority of β -glucosidases from commercial cellulases (Cellic CTec 2), derived from *T. reesei*, adsorb to LG, whereas the same enzyme derived from *A. niger* presents less inhibition. Therefore, engineering robust cellulases that are highly active and stable is a strategy to mitigate non-productive adsorption. Modification of cellulase from *T. reesei* surface charge by succinylation, converting positively charged primary amine groups to negatively charged acid and neutral acetyl groups, resulted in greater than twofold increase in Avicel conversion after 170 h (NORDWALD *et al.*, 2014).

Several techniques can be used to improve enzymatic hydrolysis of LC biomass, however for economic feasible process a multi-disciplinary research involving areas of plant biotechnology, enzyme engineering, lignin-celullases interactions and process development is important (SIPPONEN *et al.*, 2017).

1.5.7. Assistance Elements for Improving Enzyme Activity

For industrial applications, knowledge about enzymes activators and inhibitors is relevant. Many compounds can influence on enzymes activities and may be present in water and/or other reagents employed in industrial processes or can be a result of equipment corrosion. Metal ions can activate or inhibit enzyme activity by interacting with amine or carboxylic acid group of amino acids. They can act like electron donors or acceptors by forming complexes with other molecules linked to enzymes, besides help reduce non-productive adsorption of enzymes. Ionic charge and ion radius size are properties that can influence activity and stability of enzymes (PEREIRA *et al.*, 2017).

Effects of each metal ion can differ from cellulases, depending on its type and source. Addition of Cu^{2+} , Fe^{2+} , Co^{2+} and Mn^{2+} at 2 mmol/L in the reaction medium increased activity of endoglucanases from *A. niger*, especially manganese, which improved activity by 57%. However, β -glucosidases from the same microorganism showed increase of activity by 33% after Co^{2+} addition. Glucose releasing after enzymatic hydrolysis of acid pretreated bagasse increased 34% after addition of 10 mM Mn^{2+} (VASCONCELLOS *et al.*, 2016).

Some metal ions can be cellulases inhibitors. Chelating agents such as EDTA (ethylene diamine tetra acetic acid) can be used to enhance cellulases activity since they act by sequestering inhibitors metal ions and as protease inhibitor. EDTA forms a complex with metal ions in the reaction medium, allowing the enzyme site to react with the substrate. After an addition of 2 mmol/L EDTA, endoglucanases from *A. aculeatus* presented increasing of 1.5-fold in its enzymatic activity and enzyme stability also increased (NAIKA; TIKU, 2011).

The high crystallinity of CL structure makes cellulases penetration difficult. Surfactants tend to decrease surface tension of aqueous systems and their addition during enzymatic hydrolysis can modify cellulases surface and improve CL conversion. CL conversion increased 38.4% after addition of 0.5% (v/v) Triton X-100 and 60% after addition of azobenzene-based surfactant (NURUL ADELA; LOH; NASRIN, 2015; SEIDEL; LEE, 2020).

Although there are few studies about the effect of ultrasound on enzymes performance, the use of ultrasonic treatment at appropriate frequencies and intensity levels can increase enzymatic activity and favorably change enzymes without altering

its structural integrity. After a 17.33 W/cm² intensity and time of 30 min, ultrasonic treatment was able to increase cellulases activity by 25% (SUBHEDAR; GOGATE, 2014).

Some HC, mostly xylan, can still remain in LC biomass after mild pretreatment conditions and this can influence cellulases accessibility. Xylanases supplementation along with cellulases can potentially improve glucose yield. The partially replacement of cellulases with xylanases in enzyme mixture, increased CL conversion to glucose from 70% to 86%. This improvement shows that there is a synergistic interaction between xylanases and cellulases, since xylanases can hydrolyze the remaining xylan improving CL accessibility (HU; ARANTES; SADDLER, 2011).

1.6. Challenges on industrial scale of cellulose hydrolysis via enzymatic

Comparing biological hydrolysis with its chemical homologous process, it can be observed that the former presents more favorable characteristics, among them are: slight temperature conditions (up to 50 - 55 °C), possibility to raise sugar yields (especially when commercial enzymes are used), low operation costs, low energy suppling, no use of corrosive chemicals (like inorganic acids) and no production of inhibitory substances (such as furfural and hydroxymethylfurfural principally) that contribute with conversion low yields (AMEZCUA-ALLIERI; SÁNCHEZ DURÁN; ABURTO, 2017; CHEN; LIU, 2017; JAHNAVI *et al.*, 2017; STEINBACH; KRUSE; SAUER, 2017). Nevertheless, enzyme hydrolysis has difficulties related to elevated process time, high cost of enzyme (in synthesis and purification, or purchasing), enzymatic loads, pH and temperature sensibility, mixing modes, stirring intensity, low degree of synergy, recovery system of used enzymes and some good experiences obtained in laboratory has not been tested in large scale (BALAN, 2014; HU *et al.*, 2016; KADIĆ, 2017; KUMAR *et al.*, 2018; SINGHVI; GOKHALE, 2019).

In the particular case of CL hydrolysis by biological route, it can be enlisted several strains found mainly in pilot-scale experiences. Elevated viscosity, unsuitable mass and heat transference, and significant inhibitory concentrations are reasons for decreasing in enzyme hydrolysis yields specially when high solid biomass loads are used (AGRAWAL *et al.*, 2018). According to this, the research principal challenges are to focus on different solutions to design non-conventional units that let a continuous or fed-batch process; free-fall horizontal devices and helical impellers could be promising

(DA SILVA *et al.*, 2020). Also regarding reactor design, it could be researched a reaction-separation system that allows a simultaneous extraction of intermediate-products while these are obtained, avoiding inhibitory effects (KISS, 2014). However, if conventional BRF process (hydrolysis and adjustment stages separately) is carried on, a recovery system of enzymes and how many times they could be used again as a recycling strategy into the BRF would be a great approach (MESA *et al.*, 2016).

On the other hand, physical issues associated with surface tension and viscosity are also treated. Currently, to correct surface tension problems in the system of solubilized enzymes in aqueous medium - pretreated biomass, different surfactant agents that does not destabilize enzymatic activity and reduces superficial tension in the aqueous medium can be added to improve enzymes access into biomass (WANG *et al.*, 2018; YUSUF; KRESNOWATI, 2019; ZHOU *et al.*, 2019). Taking viscosity into account, an intermittent or split feed of biomass and aqueous medium enzymes can improve hydrolysis yield since system viscosity decreases and enzyme contact to inhibition chemicals is reduced (AGRAWAL *et al.*, 2018).

In addition to above, techno-economic studies are necessary to analyze the best configuration of BRF systems; considering variety, availability and quality of raw material (biomass), desired outflows (bio-based products), type and yield of pretreatments and co-production and adjustment stage category (separation and purification process). Finally, it should remind that enzymes are sensible to temperature and pH, therefore it is suggested to conserve constant thermal and pH ranges in pretreatment and hydrolysis stages or to continue investigating thermal and pH tolerance in both processes working in sequential way.

1.7. Concluding remarks

In each stage of proposed BRF, there is flexibility to use several types of processes (chemical, physical, biological or combined). However, biological pretreatment and hydrolysis presents a special interest for new researches due to its mild conditions and lesser energy inputs compared with chemical route. These factors could be taken into account as potential for large-scale production. Different stages configurations (extraction, pretreatment, hydrolysis, co-production and adjustment) and types of processes (chemical, physical, biological and combined) in the proposed

BRF approach, should be further researched and investigated in order to analyze limitations for BRF efficiency improvement.

Despite of its recalcitrance behavior, LC biomass remains as a significant alternative to generate biobased products such as biofuels, biopolymers and others biochemicals. Breaking CL and HC to obtain smaller molecules (monomers) is not a stress-free activity, the specific chemical bonds in both macromolecules and its curious configuration in the cell wall leads to continuous scientific developments in this area. The LC biomass is the most abundant resource in the world and is found in several categories; agroindustrial and industrial residues are attractive to take advantage and obtain different biobased products.

Cellulases purification can be carried out by different methods, however all of them should aim for high yield and catalytic activity. Although addition of pure cellulases in LC biomass enzymatic hydrolysis process is an important factor to obtain great conversion to glucose, LG can negatively interfere in this process.

LG creates a barrier that undermines cellulases action by non-productive binding and its derived compounds can inhibit their activity, therefore its removal increases enzymatic hydrolysis yield. Some other strategies as cellulases modification and addition of LG blocking additives are also known to minimize this problem. In the other hand, some compounds can enhance cellulases activities such as few metal ions, surfactants and auxiliary enzymes as xylanases.

The co-work multisystem of hydrolytic enzymes in pilot-scale units for hydrolysis should be more explored in order to analyze how to increase mass and heat transfer efficiency, how to reduce viscosity effects (rheology), how to decrease surface tension effects and thus improve hydrolysis performance.

Acknowledgement: The authors are thankful for the support of Brazilian Council for Research and Development – CNPq (grant 401900/2016-9), and São Paulo Research Foundation (FAPESP, grant 2017/11345-0 and 2017/22401-8).

References

- AGRAWAL, R.; BHADANA, B.; MATHUR, A. S.; KUMAR, R.; GUPTA, R. P.; SATLEWAL, A. Improved Enzymatic Hydrolysis of Pilot Scale Pretreated Rice Straw at High Total Solids Loading. **Frontiers in Energy Research**, Lausanne, vol. 6, p. 115, 2018. DOI 10.3389/fenrg.2018.00115. Available at: <https://www.frontiersin.org/articles/10.3389/fenrg.2018.00115/full>. Accessed on: 19 May 2020.
- AGUILAR, R.; RAMÍREZ, J. A.; GARROTE, G.; VÁZQUEZ, M. Kinetic study of the acid hydrolysis of sugar cane bagasse. **Journal of Food Engineering**, Amsterdam, vol. 55, no. 4, p. 309–318, 2002. DOI 10.1016/S0260-8774(02)00106-1. Available at: <https://www.sciencedirect.com/science/article/pii/S0260877402001061>. Accessed on: 1 May 2020.

ALBERTS, B.; BRAY, D.; HPKIN, K.; JOHNSON, A.; LEWIS, J.; RAFF, M.; ROBERTS, K.; WALTER, P. **Essential Cell Biology**. 4th ed. New York: Garland Science, 2014.

ALLEN, J.; UNLU, S.; DEMIREL, Y.; BLACK, P.; RIEKHOF, W. Integration of biology, ecology and engineering for sustainable algal-based biofuel and bioproduct biorefinery. **Bioresources and Bioprocessing**, Berlin, vol. 5, no. 47, p. 1–28, 2018. DOI 10.1186/s40643-018-0233-5. Available at: <https://link.springer.com/article/10.1186/s40643-018-0233-5>. Accessed on: 8 May 2020.

ALMSATAR, T. Environmental Issues of Biomass-Burning in Sub-Saharan African Countries. In: MAMMINO, L. (ed.). **Biomass Burning in Sub-Saharan Africa**. Dordrecht: Springer Link, 2020. p. 1–14. DOI 10.1007/978-94-007-0808-2_1. Available at: https://link.springer.com/chapter/10.1007/978-94-007-0808-2_1. Accessed on: 3 May 2020.

ALONSO, D. M.; GALLO, J. M. R.; MELLMER, M. A.; WETTSTEIN, S. G.; DUMESIC, J. A. Direct conversion of cellulose to levulinic acid and gamma-valerolactone using solid acid catalysts. **Catalysis Science & Technology**, Cambridge, vol. 3, no. 4, p. 927–931, 2013. DOI 10.1039/C2CY20689G. Available at: <https://pubs.rsc.org/en/content/articlelanding/2013/CY/C2CY20689G>. Accessed on: 1 May 2020.

AMAIKE CAMPEN, S.; LYNN, J.; SIBERT, S. J.; SRIKRISHNAN, S.; PHATALE, P.; FELDMAN, T.; GUENTHER, J. M.; HIRAS, J.; TRAN, Y. T. A.; SINGER, S. W.; ADAMS, P. D.; SALE, K. L.; SIMMONS, B. A.; BAKER, S. E.; MAGNUSON, J. K.; GLADDEN, J. M. Expression of naturally ionic liquid-tolerant thermophilic cellulases in *Aspergillus niger*. **PLOS ONE**, San Francisco, vol. 12, no. 12, p. 1–15, 27 Dec. 2017. DOI 10.1371/journal.pone.0189604. Available at: <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0189604>. Accessed on: 1 Jun. 2020.

AMEZCUA-ALLIERI, M. A.; SÁNCHEZ DURÁN, T.; ABURTO, J. Study of Chemical and Enzymatic Hydrolysis of Cellulosic Material to Obtain Fermentable Sugars. **Journal of Chemistry**, London, vol. 2017, p. 1–9, 2017. DOI 10.1155/2017/5680105. Available at: <https://www.hindawi.com/journals/jchem/2017/5680105/>. Accessed on: 17 May 2020.

AN, D.-S.; IM, W.-T.; YANG, H.-C.; YANG, D.-C.; LEE, S.-T. *Dyella koreensis* sp. nov., a β -glucosidase-producing bacterium. **International Journal of Systematic and Evolutionary Microbiology**, London, vol. 55, no. 4, p. 1625–1628, 2005. DOI 10.1099/ijs.0.63695-0. Available at: <https://www.microbiologyresearch.org/content/journal/ijsem/10.1099/ijs.0.63695-0>. Accessed on: 1 May 2020.

ANNAMALAI, N.; SIVAKUMAR, N. Production of polyhydroxybutyrate from wheat bran hydrolysate using *Ralstonia eutropha* through microbial fermentation. **Journal of Biotechnology**, Amsterdam, vol. 237, p. 13–17, 2016. DOI 10.1016/j.jbiotec.2016.09.001. Available at: <http://www.sciencedirect.com/science/article/pii/S0168165616314894>. Accessed on: 10 May 2020.

ANTUNES, F. A. F.; CHANDEL, A. K.; TERÁN-HILARES, R.; INGLE, A. P.; RAI, M.; DOS SANTOS MILESSI, T. S.; DA SILVA, S. S.; DOS SANTOS, J. C. Overcoming challenges in lignocellulosic biomass pretreatment for second-generation (2G) sugar production: emerging role of nano, biotechnological and promising approaches. **3 Biotech**, Berlin, vol. 9, no. 6, p. 1–17, 2019. DOI 10.1007/s13205-019-1761-1. Available at: <https://link.springer.com/article/10.1007/s13205-019-1761-1>. Accessed on: 12 May 2020.

ARO, N.; PAKULA, T.; PENTTILÄ, M. Transcriptional regulation of plant cell wall degradation by filamentous fungi. **FEMS Microbiology Reviews**, Oxford, vol. 29, no. 4, p. 719–739, 1 Sep. 2005. DOI 10.1016/j.femsre.2004.11.006. Available at: <https://academic.oup.com/femsre/article/29/4/719/492673>. Accessed on: 3 May 2020.

ARUWAJOYE, G. S.; FALOYE, F. D.; KANA, E. G. Process Optimisation of Enzymatic Saccharification of Soaking Assisted and Thermal Pretreated Cassava Peels Waste for Bioethanol Production. **Waste and Biomass Valorization**, Berlin, vol. 11, no. 6, p. 2409–2420, 2020. DOI 10.1007/s12649-018-00562-0. Available at: <https://link.springer.com/article/10.1007/s12649-018-00562-0>. Accessed on: 12 May 2020.

ASADOLLAHI, A.; KHOODEL, M.; ZAHRAEI-RAMAZANI, A.; AZARMI, S.; MOSAWI, S. H. Effectiveness of plant-based repellents against different *Anopheles* species: a systematic review. **Malaria Journal**, Berlin, vol. 18, no. 1, p. 436, 2019. DOI 10.1186/s12936-019-3064-8. Available at: <https://link.springer.com/article/10.1186/s12936-019-3064-8>. Accessed on: 4 May 2020.

ASHA, A.; MANJUNATHA, M.; REKHA, R. M.; SURENDRANATH, B.; HEARTWIN, P.; RAO, J.; MAGDALINE, E.; SINHA, C. Antioxidant activities of orange peel extract in ghee (butter oil) stored at different storage temperatures. **Journal of Food Science and Technology**, Berlin, vol. 52, no. 12, p. 8220–8227, 2015. DOI 10.1007/s13197-015-1911-3. Available at: <https://link.springer.com/article/10.1007/s13197-015-1911-3>. Accessed on: 4 May 2020.

ÁZAR, R. I. S.; BORDIGNON-JUNIOR, S. E.; LAUFER, C.; SPECHT, J.; FERRIER, D.; KIM, D. Effect of Lignin Content on Cellulolytic Saccharification of Liquid Hot Water Pretreated Sugarcane Bagasse. **Molecules**, Basel, vol. 25, no. 3, p. 1–12, 2020. DOI 10.3390/molecules25030623. Available at: <https://www.mdpi.com/1420-3049/25/3/623>. Accessed on: 1 Jun. 2020.

BAGEWADI, Z. K.; MULLA, S. I.; NINNEKAR, H. Z. Optimization of endoglucanase production from *Trichoderma harzianum* strain HZN11 by central composite design under response surface methodology. **Biomass Conversion and Biorefinery**, Berlin, vol. 8, no. 2, p. 305–316, 2017. DOI 10.1007/s13399-017-0285-3. Available at: <https://link.springer.com/article/10.1007/s13399-017-0285-3>. Accessed on: 1 May 2020.

BAJPAI, P. Chapter 3 - Microbial Xylanolytic Systems and Their Properties. In: BAJPAI, P. (ed.). **Xylanolytic Enzymes**. Amsterdam: Academic Press, 2014a. p. 19–36. DOI 10.1016/B978-0-12-801020-4.00003-2. Available at: <http://www.sciencedirect.com/science/article/pii/B9780128010204000032>. Accessed on: 15 May 2020.

BAJPAI, P. Chapter 6 - Purification of Xylanases. In: BAJPAI, P. B. T.-X. E. (ed.). **Xylanolytic Enzymes**. Amsterdam: Academic Press, 2014b. p. 53–61. DOI 10.1016/B978-0-12-801020-4.00006-8. Available at: <https://www.sciencedirect.com/science/article/pii/B9780128010204000068>. Accessed on: 1 Jun. 2020.

BALAN, V. Current Challenges in Commercially Producing Biofuels from Lignocellulosic Biomass. **ISRN Biotechnology**, London, vol. 2014, p. 1–31, 2014. DOI 10.1155/2014/463074. Available at: <https://www.hindawi.com/journals/isrn/2014/463074/>. Accessed on: 17 May 2020.

BAR-ON, Y. M.; PHILLIPS, R.; MILO, R. The biomass distribution on Earth. **Proceedings of the National Academy of Sciences**, vol. 115, no. 25, p. 6506–6511, 19 Jun. 2018. DOI 10.1073/pnas.1711842115. Available at: <https://www.pnas.org/content/115/25/6506>. Accessed on: 1 Feb. 2020.

BARBOSA, A. M.; GIESE, E. C.; DEKKER, R. F. H.; BORSATO, D.; BRIONES PÉREZ, A. I.; ÚBEDA IRANZO, J. F. Extracellular β -glucosidase production by the yeast *Debaryomyces pseudopolymorphus* UCLM-NS7A: optimization using response surface methodology. **New Biotechnology**, Amsterdam, vol. 27, no. 4, p. 374–381, 2010. DOI 10.1016/j.nbt.2010.05.013. Available at: <https://www.sciencedirect.com/science/article/pii/S1871678410004486>. Accessed on: 2 May 2020.

BARBOSA, F. C.; SILVELLO, M. A.; GOLDBECK, R. Cellulase and oxidative enzymes: new approaches, challenges and perspectives on cellulose degradation for bioethanol production. **Biotechnology Letters**, Berlin, vol. 42, no. 6, p. 875–884, 2020. DOI 10.1007/s10529-020-02875-4. Available at: <https://link.springer.com/article/10.1007/s10529-020-02875-4>. Accessed on: 12 May 2020.

BATRA, J.; BERI, D.; MISHRA, S. Response Surface Methodology Based Optimization of β -Glucosidase Production from *Pichia pastoris*. **Applied Biochemistry and Biotechnology**, Berlin, vol. 172, no. 1, p. 380–393, 2014. DOI 10.1007/s12010-013-0519-1. Available at: <https://link.springer.com/article/10.1007/s12010-013-0519-1>. Accessed on: 2 May 2020.

BAUER, M. M. T.; SCHNAPP, G. 3.19 - Protein Production for Three-Dimensional Structural Analysis. In: TAYLOR, J. B.; TRIGGLE, D. J. B. T.-C. M. C. I. I. (eds.). **Comprehensive Medicinal Chemistry II**. Oxford: Elsevier, 2007. vol. 3, p. 411–432. DOI 10.1016/B0-08-045044-X/00092-4. Available at: <https://www.sciencedirect.com/science/article/pii/B008045044X000924>. Accessed on: 1 Jun. 2020.

BEG, Q.; KAPOOR, M.; MAHAJAN, L.; HOONDAL, G. Microbial xylanases and their industrial applications: a review. **Applied Microbiology and Biotechnology**, Berlin, vol. 56, no. 3, p. 326–338, 2001. DOI 10.1007/s002530100704. Available at: <https://link.springer.com/article/10.1007/s002530100704>. Accessed on: 2 May 2020.

BEGUM, M. F.; ABSAR, N. Purification and Characterization of Intracellular Cellulase from *Aspergillus oryzae* ITCC-4857.01. **Mycobiology**, London, vol. 37, no. 2, p. 121–127, 1 Jun. 2009. DOI 10.4489/MYCO.2009.37.2.121. Available at: <https://www.tandfonline.com/doi/abs/10.4489/MYCO.2009.37.2.121>. Accessed on: 2 May 2020.

BERG, J.; TYMOCZKO, J.; STRYER, L. **Biochemistry**. New York: W.H. Freeman, 2002.

BHARDWAJ, N.; KUMAR, B.; VERMA, P. A detailed overview of xylanases: an emerging biomolecule for current and future prospective. **Bioresources and Bioprocessing**, Amsterdam, vol. 6, no. 1, p. 1–36, 2019. DOI 10.1186/s40643-019-0276-2. Available at: <https://link.springer.com/article/10.1186/s40643-019-0276-2>. Accessed on: 12 Feb. 2021.

BICHOT, A.; DELGENÈS, J.-P.; MÉCHIN, V.; CARRÈRE, H.; BERNET, N.; GARCÍA-BERNET, D. Understanding biomass recalcitrance in grasses for their efficient utilization as biorefinery feedstock. **Reviews in Environmental Science and Bio/Technology**, Berlin, vol. 17, no. 4, p. 707–748, 2018. DOI 10.1007/s11157-018-9485-y. Available at: <https://link.springer.com/article/10.1007/s11157-018-9485-y>. Accessed on: 10 May 2020.

BOSETTO, A.; JUSTO, P. I.; ZANARDI, B.; VENZON, S. S.; GRACIANO, L.; DOS SANTOS, E. L.; DE CÁSSIA GARCIA SIMÃO, R. Research Progress Concerning Fungal and Bacterial β -Xylosidases. **Applied Biochemistry and Biotechnology**, Amsterdam, vol. 178, no. 4, p. 766–795, 2016. DOI 10.1007/s12010-015-1908-4. Available at: <https://link.springer.com/article/10.1007/s12010-015-1908-4>. Accessed on: 15 May 2020.

BP. **BP Statistical Review of World Energy**. London: Pureprint Group Limited, 2019. Available at: <https://www.bp.com/content/dam/bp/business-sites/en/global/corporate/pdfs/energy-economics/statistical-review/bp-stats-review-2019-full-report.pdf>. Accessed on: 1 May 2020.

BRIENZO, M.; SIQUEIRA, A. F.; MILAGRES, A. M. F. Search for optimum conditions of sugarcane bagasse hemicellulose extraction. **Biochemical Engineering Journal**, Amsterdam, vol. 46, no. 2, p. 199–204, 2009. DOI 10.1016/j.bej.2009.05.012. Available at: <https://www.sciencedirect.com/science/article/pii/S1369703X09001569>. Accessed on: 29 Sep. 2021.

BRIENZO, Michel; FERREIRA, S.; VICENTIM, M. P.; DE SOUZA, W.; SANT'ANNA, C. Comparison Study on the Biomass Recalcitrance of Different Tissue Fractions of Sugarcane Culm. **BioEnergy Research**, Berlin, vol. 7, no. 4, p. 1454–1465, 2014. DOI 10.1007/s12155-014-9487-8. Available at: <https://link.springer.com/article/10.1007/s12155-014-9487-8>. Accessed on: 1 May 2020.

BRIENZO, Michel; FIKIZOLO, S.; BENJAMIN, Y.; TYHODA, L.; GÖRGENS, J. Influence of pretreatment severity on structural changes, lignin content and enzymatic hydrolysis of sugarcane bagasse samples. **Renewable Energy**, Amsterdam, vol. 104, p. 271–280, 2017. DOI 10.1016/j.renene.2016.12.037. Available at: <https://www.sciencedirect.com/science/article/pii/S096014811631093X>. Accessed on: 1 May 2020.

CANTU-JUNGLES, T. M.; IACOMINI, M.; CIPRIANI, T. R.; CORDEIRO, L. M. C. Isolation and characterization of a xylan with industrial and biomedical applications from edible açai berries (*Euterpe oleracea*). **Food Chemistry**, Amsterdam, vol. 221, p. 1595–1597, 2017. DOI 10.1016/j.foodchem.2016.10.133. Available at: <https://www.sciencedirect.com/science/article/pii/S0308814616318118>. Accessed on: 2 May 2020.

CASTRO, A. M. de; PEREIRA JR, N. Produção, propriedades e aplicação de celulases na hidrólise de resíduos agroindustriais. **Química Nova**, São Paulo, vol. 33, no. 1, p. 181–188, 2010. DOI 10.1590/S0100-40422010000100031. Available at: <https://www.scielo.br/qn/a/HbPYKnpgwSf4jhH4dtVSMhr?lang=pt#>. Accessed on: 2 May 2020.

CHAHED, H.; BOUMAIZA, M.; EZZINE, A.; MARZOUKI, M. N. Heterologous expression and biochemical characterization of a novel thermostable Sclerotinia sclerotiorum GH45 endoglucanase in *Pichia pastoris*. **International Journal of Biological Macromolecules**, Amsterdam, vol. 106, p. 629–635, 2018. DOI 10.1016/j.ijbiomac.2017.08.062. Available at: <https://www.sciencedirect.com/science/article/pii/S0141813017314502>. Accessed on: 1 Jun. 2020.

CHAKDAR, H.; KUMAR, M.; PANDIYAN, K.; SINGH, A.; NANJAPPAN, K.; KASHYAP, P. L.; SRIVASTAVA, A. K. Bacterial xylanases: biology to biotechnology. **3 Biotech**, Amsterdam, vol. 6, no. 2, p. 1–15, 2016. DOI 10.1007/s13205-016-0457-z. Available at: <https://link.springer.com/article/10.1007/s13205-016-0457-z>. Accessed on: 15 May 2020.

CHANDRA, M. R. G. S.; MADAKKA, M. Chapter 11 - Comparative Biochemistry and Kinetics of Microbial Lignocellulolytic Enzymes. In: BUDDOLLA, V. B. T.-R. D. in A. M. and B. (ed.). **Recent Developments in Applied Microbiology and Biochemistry**. Cambridge: Academic Press, 2019. p. 147–159. DOI 10.1016/B978-0-12-816328-3.00011-8. Available at: <https://www.sciencedirect.com/science/article/pii/B9780128163283000118>. Accessed on: 1 Jun. 2020.

CHANDRA, R. P.; ESTEGHLALIAN, A. R.; SADDLER, J. N. Assessing Substrate Accessibility to Enzymatic Hydrolysis by Cellulases. In: HU, T. Q. (ed.). **Characterization of Lignocellulosic Materials**. Hoboken: Wiley Online Library, 2008. p. 60–80. DOI 10.1002/9781444305425.ch4. Available at: <https://onlinelibrary.wiley.com/doi/abs/10.1002/9781444305425.ch4>. Accessed on: 1 May 2020.

CHELLAPANDI, P.; JANI, H. M. Production of endoglucanase by the native strains of *Streptomyces* isolates in submerged fermentation. **Brazilian Journal of Microbiology**, São Paulo, vol. 39, no. 1, p. 122–127, 2008. DOI 10.1590/S1517-83822008000100026. Available at: <https://www.scielo.br/bjm/a/b33ptnHZZgvgv8MmF4MqPHb/?lang=en#>. Accessed on: 1 May 2020.

CHEN, B.; LUO, Z.; CHEN, H.; CHEN, C.; CAI, D.; QIN, P.; CAO, H.; TAN, T. Wood Plastic Composites from the Waste Lignocellulosic Biomass Fibers of Bio-Fuels Processes: A Comparative Study on Mechanical Properties and Weathering Effects. **Waste and Biomass Valorization**, Berlin, vol. 11, no. 5, p. 1701–1710, 2020. DOI 10.1007/s12649-018-0413-8. Available at: <https://link.springer.com/article/10.1007/s12649-018-0413-8>. Accessed on: 10 May 2020.

CHEN, H.-Z.; LIU, Z.-H. Enzymatic hydrolysis of lignocellulosic biomass from low to high solids loading. **Engineering in Life Sciences**, Hoboken, vol. 17, no. 5, p. 489–499, 1 May 2017. DOI 10.1002/elsc.201600102. Available at: <https://link.springer.com/article/10.1007/s10924-019-01621-w>. Accessed on: 19 May 2020.

CHEN, J.-H.; WANG, K.; XU, F.; SUN, R.-C. Effect of hemicellulose removal on the structural and mechanical properties of regenerated fibers from bamboo. **Cellulose**, Amsterdam, vol. 22, no. 1, p. 63–72, 2015. DOI 10.1007/s10570-014-0488-8. Available at: <https://link.springer.com/article/10.1007/s10570-014-0488-8>. Accessed on: 1 May 2020.

CHUAN WEI, K. S.; TEOH, T. C.; KOSHY, P.; SALMAH, I.; ZAINUDIN, A. Cloning, expression and characterization of the

endoglucanase gene from *Bacillus subtilis* UMC7 isolated from the gut of the indigenous termite *Macrotermes malaccensis* in *Escherichia coli*. **Electronic Journal of Biotechnology**, Amsterdam, vol. 18, no. 2, p. 103–109, 2015. DOI 10.1016/j.ejbt.2014.12.007. Available at: <https://www.sciencedirect.com/science/article/pii/S0717345814001523>. Accessed on: 1 Jun. 2020.

CORAL MEDINA, J. D.; WOICIECHOWSKI, A.; ZANDONA FILHO, A.; NOSEDA, M. D.; KAUR, B. S.; SOCCOL, C. R. Lignin preparation from oil palm empty fruit bunches by sequential acid/alkaline treatment – A biorefinery approach. **Bioresource Technology**, Amsterdam, vol. 194, p. 172–178, 1 Oct. 2015. DOI 10.1016/J.BIORTECH.2015.07.018. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852415009712>. Accessed on: 6 Feb. 2018.

COSGROVE, D. J. Growth of the plant cell wall. **Nature Reviews Molecular Cell Biology**, Berlin, vol. 6, no. 11, p. 850–861, 2005. DOI 10.1038/nrm1746. Available at: <https://www.nature.com/articles/nrm1746>. Accessed on: 1 May 2020.

CREUZET, N.; BERENGER, J.-F.; FRIXON, C. Characterization of exoglucanase and synergistic hydrolysis of cellulose in *Clostridium stercorarium*. **FEMS Microbiology Letters**, Oxford, vol. 20, no. 3, p. 347–350, 1 Nov. 1983. DOI 10.1111/j.1574-6968.1983.tb00145.x. Available at: <https://academic.oup.com/femsle/article/20/3/347/475537>. Accessed on: 2 May 2020.

CROWE, J. D.; ZARGER, R. A.; HODGE, D. B. Relating Nanoscale Accessibility within Plant Cell Walls to Improved Enzyme Hydrolysis Yields in Corn Stover Subjected to Diverse Pretreatments. **Journal of Agricultural and Food Chemistry**, Washington, vol. 65, no. 39, p. 8652–8662, 4 Oct. 2017. DOI 10.1021/acs.jafc.7b03240. Available at: <https://pubs.acs.org/doi/10.1021/acs.jafc.7b03240>. Accessed on: 1 May 2020.

CYPRIANO, Z. D.; DA SILVA, L. L.; BOHÓRQUEZ, A. M.; LJUBICA, T. A Biomassa da Laranja e seus Subprodutos. **Revista Virtual de Química**, Niterói, vol. 9, no. 1, p. 176–191, 2017. DOI 10.21577/1984-6835.20170014. Available at: <http://static.sites.s bq.org.br/rvq.s bq.org.br/pdf/v9n1a14.pdf>. Accessed on: 4 May 2020.

DA SILVA, A. S.; ESPINHEIRA, R. P.; TEIXEIRA, R. S. S.; DE SOUZA, M. F.; FERREIRA-LEITÃO, V.; BON, E. P. S. Constraints and advances in high-solids enzymatic hydrolysis of lignocellulosic biomass: a critical review. **Biotechnology for Biofuels**, London, vol. 13, no. 1, p. 1–28, 2020. <https://doi.org/10.1186/s13068-020-01697-w>.

DA SILVA, A. S.; TEIXEIRA, R. S. S.; MOUTTA, R. de O.; FERREIRA-LEITÃO, V. S.; BARROS, R. da R. O. de; ELBA, M. A. F. and; BON, P. da S. Sugarcane and Woody Biomass Pretreatments for Ethanol Production. In: CHANDEL, A. K.; DA SILVA, S. S. (eds.). **Sustainable Degradation of Lignocellulosic Biomass**. London: IntechOpen, 2013. p. 47–88. DOI 10.5772/53378. Available at: <https://www.intechopen.com/chapters/44366>. Accessed on: 1 May 2020.

DASHNYAM, P.; MUDUDUDDLA, R.; HSIEH, T.-J.; LIN, T.-C.; LIN, H.-Y.; CHEN, P.-Y.; HSU, C.-Y.; LIN, C.-H. β -Glucuronidases of opportunistic bacteria are the major contributors to xenobiotic-induced toxicity in the gut. **Scientific Reports**, Berlin, vol. 8, no. 1, p. 1–12, 2018. DOI 10.1038/s41598-018-34678-z. Available at: <https://www.nature.com/articles/s41598-018-34678-z>. Accessed on: 15 May 2020.

DHEERAN, P.; NANDHAGOPAL, N.; KUMAR, S.; JAISWAL, Y. K.; ADHIKARI, D. K. A novel thermostable xylanase of *Paenibacillus macerans* IIPSP3 isolated from the termite gut. **Journal of Industrial Microbiology and Biotechnology**, Oxford, vol. 39, no. 6, p. 851–860, 1 Jun. 2012. DOI 10.1007/s10295-012-1093-1. Available at: <https://academic.oup.com/jimb/article/39/6/851/5994504>. Accessed on: 3 May 2020.

DIMAROGONA, M.; TOPAKAS, E. Chapter 12 - Regulation and Heterologous Expression of Lignocellulosic Enzymes in *Aspergillus*. In: GUPTA, V. K. B. T.-N. and F. D. in M. B. and B. (ed.). **New and Future Developments in Microbial Biotechnology and Bioengineering**. Amsterdam: Elsevier, 2016. p. 171–190. DOI 10.1016/B978-0-444-63505-1.00012-9. Available at: <https://www.sciencedirect.com/science/article/pii/B9780444635051000129>. Accessed on: 3 May 2020.

DU, B.; SHARMA, L. N.; BECKER, C.; CHEN, S.-F.; MOWERY, R. A.; WALSUM, G. P. van; CHAMBLISS, C. K. Effect of varying feedstock–pretreatment chemistry combinations on the formation and accumulation of potentially inhibitory degradation products in biomass hydrolysates. **Biotechnology and Bioengineering**, Hoboken, vol. 107, no. 3, p. 430–440, 2010. DOI 10.1002/bit.22829. Available at: <https://onlinelibrary.wiley.com/doi/10.1002/bit.22829>. Accessed on: 1 May 2020.

DUEDU, K. O.; FRENCH, C. E. Characterization of a *Cellulomonas fimi* exoglucanase/xylanase-endoglucanase gene fusion which improves microbial degradation of cellulosic biomass. **Enzyme and Microbial Technology**, Amsterdam, vol. 93–94, p. 113–121, 2016. DOI 10.1016/j.enzmitec.2016.08.005. Available at: <https://www.sciencedirect.com/science/article/pii/S0141022916301491>. Accessed on: 2 May 2020.

DULIE, N. W.; WOLDEYES, B.; DEMSASH, H. D.; JABASINGH, A. S. An Insight into the Valorization of Hemicellulose Fraction of Biomass into Furfural: Catalytic Conversion and Product Separation. **Waste and Biomass Valorization**, Berlin, 2020. DOI 10.1007/s12649-020-00946-1. Available at: <https://link.springer.com/article/10.1007/s12649-020-00946-1>. Accessed on: 5 May 2020.

DUONG-LY, K. C.; GABELLI, S. B. Chapter Seven - Salting out of Proteins Using Ammonium Sulfate Precipitation. In: LORSCH, J. B. T.-M. in E. (ed.). **Laboratory Methods in Enzymology: Protein Part C**. Cambridge: Academic Press, 2014. vol. 541, p. 85–94. DOI 10.1016/B978-0-12-420119-4.00007-0. Available at: <https://www.sciencedirect.com/science/article/pii/B9780124201194000070>. Accessed on: 1 Jun. 2020.

EBRINGEROVÁ, A. Structural Diversity and Application Potential of Hemicelluloses. **Macromolecular Symposia**, Hoboken, vol. 232, no. 1, p. 1–12, 2006. DOI 10.1002/masy.200551401. Available at: <https://onlinelibrary.wiley.com/doi/10.1002/masy.200551401>. Accessed on: 3 May 2020.

EK, R.; ALDERBORN, G.; NYSTRÖM, C. Particle analysis of microcrystalline cellulose: Differentiation between individual particles and their agglomerates. **International Journal of Pharmaceutics**, Amsterdam, vol. 111, no. 1, p. 43–50, 1994. DOI 10.1016/0378-5173(94)90400-6. Available at: <https://www.sciencedirect.com/science/article/pii/0378517394904006>. Accessed on: 1 May 2020.

ELSHAFEI A., M.; HUSSEIN, F.; EL-MAHALAWY, A.; KAHIL, T.; MOSTAFA ENAS, M.; ABD-ELTAWAB, B. Upgrading of Exoglucanase Production By *Trichoderma reesei* NRC 210. **International Journal of Engineering Research and Development**, [S.l.], vol. 10, no. 8, p. 68–73, 2014. Available at: http://www.ijerd.com/paper/vol10-issue8/Version_3/J1086873.pdf. Accessed on: 2 May 2020.

ERSSON, B.; RYDÉN, L.; JANSON, J.-C. Introduction to Protein Purification. In: JANSON, J.-C. (ed.). **Protein Purification: Principles, High Resolution Methods, and Applications**. 3rd ed. Hoboken: Wiley Online Library, 2011. p. 3–22. DOI 10.1002/9780470939932.ch1. Available at: <https://onlinelibrary.wiley.com/doi/10.1002/9780470939932.ch1>. Accessed on: 3 May 2020.

EVANGELISTA, J.; SUTTNAR, J. Separation used for purification of recombinant proteins. **Journal of Chromatography B: Biomedical Sciences and Applications**, Amsterdam, vol. 699, no. 1, p. 383–401, 1997. DOI 10.1016/S0378-4347(97)00201-6. Available at: <https://www.sciencedirect.com/science/article/pii/S0378434797002016>. Accessed on: 1 Jun. 2020.

EXCOFFIER, G.; TOUSSAINT, B.; VIGNON, M. R. Saccharification of steam-exploded poplar wood. **Biotechnology and Bioengineering**, Hoboken, vol. 38, no. 11, p. 1308–1317, 1991. DOI 10.1002/bit.260381108. Available at: <https://onlinelibrary.wiley.com/doi/10.1002/bit.260381108>. Accessed on: 1 May 2020.

FARINAS, C. S.; SCARPELINI, L. M.; MIRANDA, E. A.; BERTUCCI NETO, V. Evaluation of operational parameters on the precipitation of endoglucanase and xylanase produced by solid state fermentation of *Aspergillus niger*. **Brazilian Journal of Chemical Engineering**, São Paulo, vol. 28, no. 1, p. 17–26, 2011. DOI 10.1590/S0104-66322011000100003. Available at: <https://www.scielo.br/bjce/a/7bmDY3gM5syf6mW4zDmVskS/?lang=en>. Accessed on: 1 Jun. 2020.

FAUTH, U.; ROMANIEC, M. P. M.; KOBAYASHI, T.; DEMAİN, A. L. Purification and characterization of endoglucanase Ss from *Clostridium thermocellum*. **Biochemical Journal**, London, vol. 279, no. 1, p. 67–73, 1 Oct. 1991. DOI 10.1042/bj2790067. Available at: <https://portlandpress.com/biochemj/article-abstract/279/1/67/37152/Purification-and-characterization-of-endoglucanase?redirectedFrom=fulltext>. Accessed on: 2 May 2020.

FERNÁNDEZ-ESPINAR, M.; PIÑAGA, F.; DE GRAAFF, L.; VISSER, J.; RAMÓN, D.; VALLÉS, S. Purification, characterization and regulation of the synthesis of an *Aspergillus nidulans* acidic xylanase. **Applied Microbiology and Biotechnology**, Berlin, vol. 42, no. 4, p. 555–562, 1994. DOI 10.1007/BF00173920. Available at: <https://link.springer.com/article/10.1007/BF00173920>. Accessed on: 2 May 2020.

FU, L.-H.; JIANG, N.; LI, C.-X.; LUO, X.-M.; ZHAO, S.; FENG, J.-X. Purification and characterization of an endo-xylanase from *Trichoderma* sp., with xylobiose as the main product from xylan hydrolysis. **World Journal of Microbiology and Biotechnology**, Berlin, vol. 35, no. 11, p. 171, 2019. DOI 10.1007/s11274-019-2747-1. Available at: <https://link.springer.com/article/10.1007/s11274-019-2747-1#citeas>. Accessed on: 15 May 2020.

GANDINI, A.; BELGACEM, M. N. Chapter 1 - The State of the Art. In: BELGACEM, M. N.; GANDINI POLYMERS AND COMPOSITES FROM RENEWABLE RESOURCES, A. B. T.-M. (eds.). **Monomers, Polymers and Composites from Renewable Resources**. Amsterdam: Elsevier, 2008. p. 1–16. DOI 10.1016/B978-0-08-045316-3.00001-6. Available at: <http://www.sciencedirect.com/science/article/pii/B9780080453163000016>. Accessed on: 5 May 2020.

GARCÍA-KIRCHNER, O.; SEGURA-GRANADOS, M.; RODRÍGUEZ-PASCUAL, P. Effect of Media Composition and Growth Conditions on Production of β -Glucosidase by *Aspergillus niger* C-6. In: DAVISON, B. H.; EVANS, B. R.; FINKELSTEIN, M.; MCMILLAN, J. D. (eds.). **Twenty-Sixth Symposium on Biotechnology for Fuels and Chemicals**. Totowa: Humana Press, 2005. p. 347–359. DOI 10.1007/978-1-59259-991-2_30. Available at: https://link.springer.com/chapter/10.1007/978-1-59259-991-2_30. Accessed on: 2 May 2020.

GASPAR, A.; COSSCH, T.; ROQUES, C.; THONART, P. Study on the production of a xylanolytic complex from *penicillium canescens* 10-10c. **Applied Biochemistry and Biotechnology**, Berlin, vol. 67, no. 1, p. 45, 1997. DOI 10.1007/BF02787840. Available at: <https://link.springer.com/article/10.1007/BF02787840>. Accessed on: 2 May 2020.

GAUR, R.; TIWARI, S. Isolation, production, purification and characterization of an organic-solvent-thermostable alkalophilic cellulase from *Bacillus vallismortis* RG-07. **BMC Biotechnology**, Berlin, vol. 15, no. 1, p. 19, 2015. DOI 10.1186/s12896-015-0129-9. Available at: <https://bmcbiotechnol.biomedcentral.com/articles/10.1186/s12896-015-0129-9>. Accessed on: 2 May 2020.

GEANKOPLIS, C. **Transport Processes and Unit Operations**. 3rd ed. Englewood Cliffs: Prentice-Hall International, 1993.

GENÇ, B.; NADAROĞLU, H.; ADIGUZEL, A.; BALTACI, M. Purification and characterization of an extracellular Cellulase from *Anoxybacillus gonensis* O9 isolated from geothermal area in Turkey. **Journal of Environmental Biology**, Lucknow, vol. 36, no. 6, p. 1319–1324, 20 Mar. 2014. Available at: http://www.jeb.co.in/index.php?page=abstract&issue=201511_nov15&number=11. Accessed on: 2 May 2020.

GENERAL ELECTRIC HEALTHCARE. **Strategies for Protein Purification Handbook**, n. 28-9833–31 AA. Uppsala: General Electric, 2010. Available at: <https://www.gelifesciences.com/protein-purification>. Accessed on: 2 May 2020.

GEORGE, J.; SABAPATHI, S. Cellulose nanocrystals: synthesis, functional properties, and applications. **Nanotechnology, Science and Applications**, London, vol. 2015, no. 8, p. 45–54, 2015. DOI 10.2147/NSA.S64386. Available at: <https://www.dovepress.com/getfile.php?fileID=27858>. Accessed on: 1 May 2020.

GOEL, N.; PATRA, R.; VERMA, S. K.; SHARMA, P. C. Purification and characterization of cellulase from *Pseudomonas* sp. isolated from waste dumping site soil. **Applied Biotechnology & Bioengineering**, Edmond, vol. 6, no. 3, p. 118.124, 2019. DOI 10.15406/jabb.2019.06.00183. Available at: <https://medcraveonline.com/JABB/JABB-06-00183.pdf>. Accessed on: 1 May 2020.

GOSWAMI, G. K.; PATHAK, R. R. Microbial xylanases and their biomedical applications: a review. **International Journal of Basic & Clinical Pharmacology**, Ahmedabad, vol. 2, no. 3, p. 237–246, 2013. DOI 10.5455/2319-2003.ijbcp20130602. Available at: <https://www.ijbcp.com/index.php/ijbcp/article/view/1248>. Accessed on: 2 May 2020.

GOUFO, E. F. D.; MUGISHA, S. Complex harmonic poles in the evolution of macromolecules depolymerization. **Journal of Computational Analysis and Applications**, Cordova, vol. 25, no. 8, p. 1490–1503, 2018. Available at: <http://www.eudoxuspress.com/images/JOCAAA-2018-Vol-25-8.pdf>. Accessed on: 1 May 2020.

GUEDES, F.; SZKLO, A.; ROCHEDO, P.; LANTZ, F.; MAGALAR, L.; ARROYO, E. M. V. Climate-energy-water nexus in Brazilian oil refineries. **International Journal of Greenhouse Gas Control**, Amsterdam, vol. 90, p. 1–11, 2019. DOI 10.1016/j.ijggc.2019.102815. Available at: <http://www.sciencedirect.com/science/article/pii/S1750583618306844>. Accessed on: 1 Feb. 2021.

HAMDAN, N.; JASIM, H. Purification and Characterization of Cellulase enzyme from *Trichoderma longibrachiatum* isolated in Iraqi soil. **Journal of Biotechnology and Biochemistry**, Gurugram, vol. 4, no. 1, p. 32–41, 2018. DOI 10.9790/264X-04013241. Available at: <https://www.iosrjournals.org/iosr-jbb/pages/vol-4issue-2.html>. Accessed on: 1 May 2020.

HANIF, M.; ZAMAN, M. Thiolation of arabinoxylan and its application in the fabrication of controlled release mucoadhesive oral films. **DARU Journal of Pharmaceutical Sciences**, Berlin, vol. 25, no. 1, p. 6, 2017. DOI 10.1186/s40199-017-0172-2. Available at: <https://link.springer.com/article/10.1186/s40199-017-0172-2>. Accessed on: 2 May 2020.

HARMOKO, C.; INDRAWANSUCIPTO, K.; RETNONINGTYAS, E.; HARTONO, S. B. Vinyl functionalized cubic mesoporous silica nanoparticles as supporting material to enhance cellulase enzyme stability. **ARPN Journal of Engineering and Applied Sciences**, Islamabad, vol. 11, no. 5, p. 2981–2992, 2016. Available at: http://www.arpnjournals.com/jeas/volume_05_2016.htm. Accessed on: 1 May 2020.

HENDRIKS, A. T. W. M.; ZEEMAN, G. Pretreatments to enhance the digestibility of lignocellulosic biomass. **Bioresource Technology**, Amsterdam, vol. 100, no. 1, p. 10–18, 2009. DOI 10.1016/j.biortech.2008.05.027. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852408004574>. Accessed on: 1 May 2020.

HERRERA, A.; TÉLLEZ-LUIS, S. J.; RAMÍREZ, J. A.; VÁZQUEZ, M. Production of Xylose from Sorghum Straw Using Hydrochloric Acid. **Journal of Cereal Science**, Amsterdam, vol. 37, no. 3, p. 267–274, 2003. DOI 10.1006/jcrs.2002.0510. Available at: <https://www.sciencedirect.com/science/article/pii/S0733521002905102>. Accessed on: 1 May 2020.

HIDENO, A.; INOUE, H.; TSUKAHARA, K.; FUJIMOTO, S.; MINOWA, T.; INOUE, S.; ENDO, T.; SAWAYAMA, S. Wet disk milling pretreatment without sulfuric acid for enzymatic hydrolysis of rice straw. **Bioresource Technology**, Amsterdam, vol. 100, no. 10, p. 2706–2711, 2009. DOI 10.1016/j.biortech.2008.12.057. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852408011371>. Accessed on: 1 May 2020.

HIRASAWA, K.; UCHIMURA, K.; KASHIWA, M.; GRANT, W. D.; ITO, S.; KOBAYASHI, T.; HORIKOSHI, K. Salt-activated endoglucanase of a strain of alkaliphilic *Bacillus agaradhaerens*. **Antonie van Leeuwenhoek**, Berlin, vol. 89, no. 2, p. 211–219,

2006. DOI 10.1007/s10482-005-9023-0. Available at: <https://link.springer.com/article/10.1007/s10482-005-9023-0>. Accessed on: 2 May 2020.

HIRUNSUPACHOTE, S.; CHAVALPARIT, O. Predicting the Biomethanation Potential of Some Lignocellulosic Feedstocks using Linear Regression Models: The Effect of Pretreatment. **KSCE Journal of Civil Engineering**, Berlin, vol. 23, no. 4, p. 1501–1512, 2019. DOI 10.1007/s12205-019-1589-6. Available at: <https://link.springer.com/article/10.1007/s12205-019-1589-6>. Accessed on: 23 Sep. 2021.

HOEGH-GULDBERG, O.; JACOB, D.; TAYLOR, M. Impacts of 1.5°C of Global Warming on Natural and Human Systems. In: MARENGO, J. A.; PEREIRA, J.; SHERSTYUKOV, B. (eds.). **Global Warming of 1.5°C**. Geneva: United Nations Environment Programme & World Meteorological Organization, 2018. p. 175–311. Available at: https://www.ipcc.ch/site/assets/uploads/sites/2/2019/06/SR15_Chapter3_Low_Res.pdf. Accessed on: 1 Feb. 2021.

HOLWERDA, E. K.; WORTHEN, R. S.; KOTHARI, N.; LASKY, R. C.; DAVIDSON, B. H.; FU, C.; WANG, Z.-Y.; DIXON, R. A.; BISWAL, A. K.; MOHNEN, D.; NELSON, R. S.; BAXTER, H. L.; MAZAREI, M.; STEWART, C. N.; MUCHERO, W.; TUSKAN, G. A.; CAI, C. M.; GJERSING, E. E.; DAVIS, M. F.; ... LYND, L. R. Multiple levers for overcoming the recalcitrance of lignocellulosic biomass. **Biotechnology for Biofuels**, Berlin, vol. 12, no. 1, p. 15, 2019. DOI 10.1186/s13068-019-1353-7. Available at: <https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/s13068-019-1353-7>. Accessed on: 12 May 2020.

HSIEH, C. C.; CANNELLA, D.; JØRGENSEN, H.; FELBY, C.; THYGESEN, L. G. Cellulase Inhibition by High Concentrations of Monosaccharides. **Journal of Agricultural and Food Chemistry**, Washington, vol. 62, no. 17, p. 3800–3805, 30 Apr. 2014. DOI 10.1021/jf5012962. Available at: <https://pubs.acs.org/doi/full/10.1021/jf5012962>. Accessed on: 13 May 2020.

HU, B.; ZHU, S.; FANG, S.; HUO, M.; LI, Y.; YU, Y.; ZHU, M. Optimization and Scale-Up of Enzymatic Hydrolysis of Wood Pulp for Cellulosic Sugar Production. **BioResources**, Raleigh, vol. 11, no. 3, p. 7242–7257, 2016. DOI 10.15376/biores.11.3.7242-7257. Available at: <https://bioresources.cnr.ncsu.edu/resources/optimization-and-scale-up-of-enzymatic-hydrolysis-of-wood-pulp-for-cellulosic-sugar-production/>. Accessed on: 19 May 2020.

HU, J.; ARANTES, V.; SADDLER, J. N. The enhancement of enzymatic hydrolysis of lignocellulosic substrates by the addition of accessory enzymes such as xylanase: is it an additive or synergistic effect? **Biotechnology for Biofuels**, London, vol. 4, no. 1, p. 36, 2011. DOI 10.1186/1754-6834-4-36. Available at: <https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/1754-6834-4-36>. Accessed on: 1 Jun. 2020.

HUANG, R.; SU, R.; QI, W.; HE, Z. Understanding the key factors for enzymatic conversion of pretreated lignocellulose by partial least square analysis. **Biotechnology Progress**, Hoboken, vol. 26, no. 2, p. 384–392, 2010. DOI 10.1002/btpr.324. Available at: <https://aiche.onlinelibrary.wiley.com/doi/10.1002/btpr.324>. Accessed on: 1 May 2020.

IRFAN, M.; NADEEM, M.; SYED, Q. Influence of Nutritional Conditions for Endoglucanase Production by *Trichoderma viride* in SSF. **Global Journal of Biotechnology & Biochemistry**, Dubai, vol. 7, no. 1, p. 7–12, 2012. DOI 10.5829/idosi.gjbb.2012.7.1.6210. Available at: [https://www.idosi.org/gjbb/gjbb7\(1\)12.htm](https://www.idosi.org/gjbb/gjbb7(1)12.htm). Accessed on: 1 May 2020.

ISLAM, F.; ROY, N. Screening, purification and characterization of cellulase from cellulase producing bacteria in molasses. **BMC Research Notes**, Berlin, vol. 11, no. 1, p. 445, 2018. DOI 10.1186/s13104-018-3558-4. Available at: <https://bmcresearchnotes.biomedcentral.com/articles/10.1186/s13104-018-3558-4>. Accessed on: 2 May 2020.

JAHAVALI, G.; PRASHANTHI, G. S.; SRAVANTHI, K.; RAO, L. V. Status of availability of lignocellulosic feed stocks in India: Biotechnological strategies involved in the production of Bioethanol. **Renewable and Sustainable Energy Reviews**, Amsterdam, vol. 73, p. 798–820, 2017. DOI 10.1016/j.rser.2017.02.018. Available at: <http://www.sciencedirect.com/science/article/pii/S1364032117302198>. Accessed on: 17 May 2020.

JHA, A.; KUMAR, A. Biobased technologies for the efficient extraction of biopolymers from waste biomass. **Bioprocess and Biosystems Engineering**, Berlin, vol. 42, no. 12, p. 1893–1901, 2019. DOI 10.1007/s00449-019-02199-2. Available at: <https://link.springer.com/article/10.1007/s00449-019-02199-2>. Accessed on: 9 May 2020.

JUHÁSZ, T.; EGYHÁZI, A.; RÉCZEY, K. β -Glucosidase Production by *Trichoderma reesei*. In: DAVIDSON, B. H.; EVANS, B. R.; FINKELSTEIN, M.; MCMILLAN, J. D. (eds.). **Twenty-Sixth Symposium on Biotechnology for Fuels and Chemicals**. Totowa: Humana Press, 2005. p. 243–254. DOI 10.1007/978-1-59259-991-2_21. Available at: https://link.springer.com/chapter/10.1007/978-1-59259-991-2_21. Accessed on: 2 May 2020.

KADIĆ, A. **The effects of mixing on the enzymatic hydrolysis of lignocellulosic biomass**. 2017. 73 f. Lund University, 2017. Available at: <https://portal.research.lu.se/portal/files/25199689/Spikfil.pdf>. Accessed on: 20 May 2020.

KALOGIERIS, E.; CHRISTAKOPOULOS, P.; VRŠANSKÁ, M.; KEKOS, D.; BIELY, P.; MACRIS, B. J. Catalytic properties of the endoxylanase I from *Thermoascus aurantiacus*. **Journal of Molecular Catalysis B: Enzymatic**, Amsterdam, vol. 11, no. 4, p. 491–501, 2001. DOI 10.1016/S1381-1177(00)00178-8. Available at: <https://www.sciencedirect.com/science/article/pii/S1381117700001788>. Accessed on: 2 May 2020.

KARIMI, K.; TAHERZADEH, M. J. A critical review on analysis in pretreatment of lignocelluloses: Degree of polymerization, adsorption/desorption, and accessibility. **Bioresource Technology**, Amsterdam, vol. 203, p. 348–356, 2016. DOI 10.1016/j.biortech.2015.12.035. Available at: <https://www.sciencedirect.com/science/article/pii/S096085241501665X>. Accessed on: 1 May 2020.

KHOSRAVI, C.; BENOCCI, T.; BATTAGLIA, E.; BENOIT, I.; DE VRIES, R. P. Chapter One - Sugar Catabolism in *Aspergillus* and Other Fungi Related to the Utilization of Plant Biomass. In: SARIASLANI, S.; GADD, G. (eds.). **Advances in Applied Microbiology**. Amsterdam: Academic Press, 2015. vol. 90, p. 1–28. DOI 10.1016/bs.aambs.2014.09.005. Available at: <http://www.sciencedirect.com/science/article/pii/S0065216414000069>. Accessed on: 15 May 2020.

KIM, H.-B.; PARK, M.-J.; YANG, H.-C.; AN, D.-S.; JIN, H.-Z.; YANG, D.-C. *Burkholderia ginsengisoli* sp. nov., a β -glucosidase-producing bacterium isolated from soil of a ginseng field. **International Journal of Systematic and Evolutionary Microbiology**, London, vol. 56, no. 11, p. 2529–2533, 2006. DOI 10.1099/ijs.0.64387-0. Available at: <https://www.microbiologyresearch.org/content/journal/ijsem/10.1099/ijs.0.64387-0>. Accessed on: 2 May 2020.

KIM, H. S.; OH, Y. H.; JANG, Y.-A.; KANG, K. H.; DAVID, Y.; YU, J. H.; SONG, B. K.; CHOI, J.; CHANG, Y. K.; JOO, J. C.; PARK, S. J. Recombinant *Ralstonia eutropha* engineered to utilize xylose and its use for the production of poly(3-hydroxybutyrate) from sunflower stalk hydrolysate solution. **Microbial Cell Factories**, Berlin, vol. 15, no. 1, p. 95, 2016. DOI 10.1186/s12934-016-0495-6. Available at: <https://microbialcellfactories.biomedcentral.com/articles/10.1186/s12934-016-0495-6#citeas>. Accessed on: 10 May 2020.

KIM, M. K.; SATHIYARAJ, S.; PULLA, R. K.; YANG, D.-C. *Brevibacillus panacihumi* sp. nov., a β -glucosidase-producing bacterium. **International Journal of Systematic and Evolutionary Microbiology**, London, vol. 59, no. 5, p. 1227–1231, 2009. DOI 10.1099/ijs.0.001248-0. Available at: <https://www.microbiologyresearch.org/content/journal/ijsem/10.1099/ijs.0.001248-0>. Accessed on: 2 May 2020.

KISS, A. A. Reactive Separation Processes BT - Process Intensification Technologies for Biodiesel Production: Reactive Separation Processes. In: KISS, A. A. (ed.). **Process Intensification Technologies for Biodiesel Production**. Cham: Springer International Publishing, 2014. p. 25–33. DOI 10.1007/978-3-319-03554-3_3. Available at: https://doi.org/10.1007/978-3-319-03554-3_3. Accessed on: 2 May 2020.

https://link.springer.com/chapter/10.1007/978-3-319-03554-3_3. Accessed on: 20 May 2020.

KOTAKA, A.; BANDO, H.; KAYA, M.; KATO-MURAI, M.; KURODA, K.; SAHARA, H.; HATA, Y.; KONDO, A.; UEDA, M. Direct ethanol production from barley β -glucan by sake yeast displaying *Aspergillus oryzae* β -glucosidase and endoglucanase. **Journal of Bioscience and Bioengineering**, Amsterdam, vol. 105, no. 6, p. 622–627, 2008. DOI 10.1263/jbb.105.622. Available at: <https://www.sciencedirect.com/science/article/pii/S1389172308701191>. Accessed on: 1 May 2020.

KUCHARSKA, K.; SŁUPEK, E.; CIEŚLIŃSKI, H.; KAMIŃSKI, M. Advantageous conditions of saccharification of lignocellulosic biomass for biofuels generation via fermentation processes. **Chemical Papers**, Berlin, vol. 74, no. 4, p. 1199–1209, 2020. DOI 10.1007/s11696-019-00960-1. Available at: <https://link.springer.com/article/10.1007/s11696-019-00960-1#citeas>. Accessed on: 10 May 2020.

KUMAR, A.; NAKACHEW, M.; YILKAL, B.; YADAV, M. A review of factors affecting enzymatic hydrolysis of pretreated lignocellulosic Biomass. **Research Journal of Chemistry and Environment**, Indore, vol. 22, no. 7, p. 62–67, 2018. Available at: [https://worldresearchersassociations.com/Archives/RJCE/Vol\(22\)2018/July2018.aspx](https://worldresearchersassociations.com/Archives/RJCE/Vol(22)2018/July2018.aspx). Accessed on: 18 May 2020.

KUMAR, L.; ARANTES, V.; CHANDRA, R.; SADDLER, J. The lignin present in steam pretreated softwood binds enzymes and limits cellulose accessibility. **Bioresource Technology**, Amsterdam, vol. 103, no. 1, p. 201–208, 2012. DOI 10.1016/j.biortech.2011.09.091. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852411013794>. Accessed on: 1 Jun. 2020.

KUMAR, R.; MAGO, G.; BALAN, V.; WYMAN, C. E. Physical and chemical characterizations of corn stover and poplar solids resulting from leading pretreatment technologies. **Bioresource Technology**, Amsterdam, vol. 100, no. 17, p. 3948–3962, 2009. DOI 10.1016/j.biortech.2009.01.075. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852409001552>. Accessed on: 1 May 2020.

KUMAR, V.; SATYANARAYANA, T. Applicability of thermo-alkali-stable and cellulase-free xylanase from a novel thermo-halo-alkaliphilic *Bacillus halodurans* in producing xylooligosaccharides. **Biotechnology Letters**, Berlin, vol. 33, no. 11, p. 2279, 2011. DOI 10.1007/s10529-011-0698-1. Available at: <https://link.springer.com/article/10.1007/s10529-011-0698-1>. Accessed on: 2 May 2020.

LEU, S.-Y.; ZHU, J. Y. Substrate-Related Factors Affecting Enzymatic Saccharification of Lignocelluloses: Our Recent Understanding. **BioEnergy Research**, Berlin, vol. 6, no. 2, p. 405–415, 2013. DOI 10.1007/s12155-012-9276-1. Available at: <https://link.springer.com/article/10.1007/s12155-012-9276-1>. Accessed on: 1 May 2020.

LI, C.; KUMAR, S. Preparation of activated carbon from un-hydrolyzed biomass residue. **Biomass Conversion and Biorefinery**, Berlin, vol. 6, no. 4, p. 407–419, 2016. DOI 10.1007/s13399-016-0197-7. Available at: <https://link.springer.com/article/10.1007/s13399-016-0197-7>. Accessed on: 5 May 2020.

LIANG, Z.-Q.; SRINIVASAN, S.; KIM, Y.-J.; KIM, H.-B.; WANG, H.-T.; YANG, D.-C. *Lactobacillus kimchicus* sp. nov., a β -glucosidase-producing bacterium isolated from kimchi. **International Journal of Systematic and Evolutionary Microbiology**, London, vol. 61, no. 4, p. 894–897, 2011. DOI 10.1099/ijs.0.017418-0. Available at: <https://www.microbiologyresearch.org/content/journal/ijsem/10.1099/ijs.0.017418-0>. Accessed on: 2 May 2020.

LIN, Z.; HUANG, H.; ZHANG, H.; ZHANG, L.; YAN, L.; CHEN, J. Ball Milling Pretreatment of Corn Stover for Enhancing the Efficiency of Enzymatic Hydrolysis. **Applied Biochemistry and Biotechnology**, Berlin, vol. 162, no. 7, p. 1872–1880, 2010. DOI 10.1007/s12010-010-8965-5. Available at: <https://link.springer.com/article/10.1007/s12010-010-8965-5>. Accessed on: 1 May 2020.

LIU, W.; SHI, P.; CHEN, Q.; YANG, P.; WANG, G.; WANG, Y.; LUO, H.; YAO, B. Gene Cloning, Overexpression, and Characterization of a Xylanase from *Penicillium* sp. CGMCC 1669. **Applied Biochemistry and Biotechnology**, Berlin, vol. 162, no. 1, p. 1–12, 2010. DOI 10.1007/s12010-009-8719-4. Available at: <https://link.springer.com/article/10.1007/s12010-009-8719-4>. Accessed on: 2 May 2020.

LOOW, Y.-L.; WU, T. Y.; MD. JAHIM, J.; MOHAMMAD, A. W.; TEOH, W. H. Typical conversion of lignocellulosic biomass into reducing sugars using dilute acid hydrolysis and alkaline pretreatment. **Cellulose**, Berlin, vol. 23, no. 3, p. 1491–1520, 2016. DOI 10.1007/s10570-016-0936-8. Available at: <https://link.springer.com/article/10.1007/s10570-016-0936-8>. Accessed on: 5 May 2020.

LOPES, A. M.; FERREIRA FILHO, E. X.; MOREIRA, L. R. S. An update on enzymatic cocktails for lignocellulose breakdown. **Journal of Applied Microbiology**, Hoboken, vol. 125, no. 3, p. 632–645, 1 Sep. 2018. DOI 10.1111/jam.13923. Available at: <https://sfamjournals.onlinelibrary.wiley.com/action/showCitFormats?doi=10.1111%2Fjam.13923>. Accessed on: 19 May 2020.

LUO, X.; ZHU, J. Y. Effects of drying-induced fiber hornification on enzymatic saccharification of lignocelluloses. **Enzyme and Microbial Technology**, Amsterdam, vol. 48, no. 1, p. 92–99, 2011. DOI 10.1016/j.enzmictec.2010.09.014. Available at: <https://www.sciencedirect.com/science/article/pii/S0141022910002073>. Accessed on: 1 May 2020.

MAHMOOD, R. T.; ASAD, M. J.; MEHBOOB, N.; MUSHTAQ, M.; GULFRAZ, M.; ASGHER, M.; MINHAS, N. M.; HADRI, S. H. Production, Purification, and Characterization of Exoglucanase by *Aspergillus fumigatus*. **Applied Biochemistry and Biotechnology**, Berlin, vol. 170, no. 4, p. 895–908, 2013. DOI 10.1007/s12010-013-0227-x. Available at: <https://link.springer.com/article/10.1007/s12010-013-0227-x>. Accessed on: 1 May 2020.

MAHMOODI, S.; POURHASSAN-MOGHADDAM, M.; WOOD, D. W.; MAJDI, H.; ZARGHAMI, N. Current affinity approaches for purification of recombinant proteins. **Cogent Biology**, London, vol. 5, no. 1, p. 1–15, 1 Jan. 2019. DOI 10.1080/23312025.2019.1665406. Available at: <https://www.tandfonline.com/doi/full/10.1080/23312025.2019.1665406>. Accessed on: 1 Jun. 2020.

MALGAS, S.; MAFA, M. S.; MKABAYI, L.; PLETSCHKE, B. I. A mini review of xylanolytic enzymes with regards to their synergistic interactions during hetero-xylan degradation. **World Journal of Microbiology and Biotechnology**, Amsterdam, vol. 35, no. 12, p. 187, 2019. DOI 10.1007/s11274-019-2765-z. Available at: <https://link.springer.com/article/10.1007/s11274-019-2765-z>. Accessed on: 15 May 2020.

MANAVALAN, T.; MANAVALAN, A.; HEESE, K. Characterization of Lignocellulolytic Enzymes from White-Rot Fungi. **Current Microbiology**, Berlin, vol. 70, no. 4, p. 485–498, 2015. DOI 10.1007/s00284-014-0743-0. Available at: <https://link.springer.com/article/10.1007/s00284-014-0743-0>. Accessed on: 10 May 2020.

MARTÍN, C.; GALBE, M.; NILVEBRANT, N.-O.; JÖNSSON, L. J. Comparison of the fermentability of enzymatic hydrolyzates of sugarcane bagasse pretreated by steam explosion using different impregnating agents. **Applied Biochemistry and Biotechnology**, Berlin, vol. 98, no. 1, p. 699–716, 2002. DOI 10.1385/ABAB:98-100:1-9:699. Available at: <https://link.springer.com/article/10.1385/ABAB:98-100:1-9:699>. Accessed on: 1 May 2020.

MATHEW, S.; ABRAHAM, T. E. Ferulic Acid: An Antioxidant Found Naturally in Plant Cell Walls and Feruloyl Esterases Involved in its Release and Their Applications. **Critical Reviews in Biotechnology**, London, vol. 24, no. 2–3, p. 59–83, 1 Jan. 2004. DOI 10.1080/07388550490491467. Available at: <https://www.tandfonline.com/doi/full/10.1080/07388550490491467>. Accessed on: 2 May 2020.

MCCABE, W.; SMITH, J.; HARRIOT, P. **Operaciones Unitarias en Ingeniería Química**. 7th ed. Mexico: McGraw-Hill, 2007.

MEDINA, J. D. C.; WOICIECHOWSKI, A.; FILHO, A. Z.; NIGAM, P. S.; RAMOS, L. P.; SOCCOL, C. R. Steam explosion pretreatment of oil palm empty fruit bunches (EFB) using autocatalytic hydrolysis: A biorefinery approach. **Bioresource Technology**, Amsterdam, vol. 199, p. 173–180, Jan. 2016. DOI 10.1016/j.biortech.2015.08.126. Available at: <http://www.sciencedirect.com/science/article/pii/S0960852415012328>. Accessed on: 12 May 2020.

MEGHA; MARAGATHAVALLI, S.; BRINDHA, S.; ANNADURAI, V.; GANGWAR, S. K.; V, K. Isolation and purification of cellulase. **International Journal of Natural Sciences**, [S.l.], vol. 6, no. 3, p. 474–479, 2 Mar. 2015. Available at: [http://www.scienceandnature.org/IJSN/IJSN_Vol6\(3\)J2015/IJSN-VOL6\(3\)15-28.pdf](http://www.scienceandnature.org/IJSN/IJSN_Vol6(3)J2015/IJSN-VOL6(3)15-28.pdf). Accessed on: 1 May 2020.

MELATI, R. B.; SHIMIZU, F. L.; OLIVEIRA, G.; PAGNOCCA, F. C.; DE SOUZA, W.; SANT'ANNA, C.; BRIENZO, M. Key Factors Affecting the Recalcitrance and Conversion Process of Biomass. **BioEnergy Research**, Berlin, vol. 12, no. 1, p. 1–20, 2019. DOI 10.1007/s12155-018-9941-0. Available at: <https://link.springer.com/article/10.1007/s12155-018-9941-0#citeas>. Accessed on: 1 Jun. 2020.

MENG, X.; PU, Y.; YOO, C. G.; LI, M.; BALI, G.; PARK, D.-Y.; GJERSING, E.; DAVIS, M. F.; MUCHERO, W.; TUSKAN, G. A.; TSCHAPLINSKI, T. J.; RAGAUSKAS, A. J. An In-Depth Understanding of Biomass Recalcitrance Using Natural Poplar Variants as the Feedstock. **ChemSusChem**, Hoboken, vol. 10, no. 1, p. 139–150, 2017. DOI 10.1002/cssc.201601303. Available at: <https://chemistry-europe.onlinelibrary.wiley.com/doi/10.1002/cssc.201601303>. Accessed on: 1 May 2020.

MESA, L.; GONZÁLEZ, E.; CARA, C.; CASTRO, E.; MUSSATTO, S. I. An approach to cellulase recovery from enzymatic hydrolysis of pretreated sugarcane bagasse with high lignin content. **Biocatalysis and Biotransformation**, Milton Park, vol. 33, no. 5–6, p. 287–297, 2 Nov. 2016. DOI 10.3109/10242422.2016.1168816. Available at: <https://www.tandfonline.com/doi/abs/10.3109/10242422.2016.1168816?journalCode=ibab20>. Accessed on: 20 May 2020.

MISHRA, C.; FORRESTER, I. T.; KELLEY, B. D.; BURGESS, R. R.; LEATHAM, G. F. Characterization of a major xylanase purified from *Lentinula edodes* cultures grown on a commercial solid lignocellulosic substrate. **Applied Microbiology and Biotechnology**, Berlin, vol. 33, no. 2, p. 226–232, 1990. DOI 10.1007/BF00176530. Available at: <https://link.springer.com/article/10.1007/BF00176530>. Accessed on: 2 May 2020.

MONTE, J. R.; BRIENZO, M.; MILAGRES, A. M. F. Utilization of pineapple stem juice to enhance enzyme-hydrolytic efficiency for sugarcane bagasse after an optimized pre-treatment with alkaline peroxide. **Applied Energy**, Amsterdam, vol. 88, no. 1, p. 403–408, 2011. DOI 10.1016/j.apenergy.2010.08.009. Available at: <https://www.sciencedirect.com/science/article/pii/S0306261910003259>. Accessed on: 1 May 2020.

MOONEY, C. A.; MANSFIELD, S. D.; TOUHY, M. G.; SADDLER, J. N. The effect of initial pore volume and lignin content on the enzymatic hydrolysis of softwoods. **Bioresource Technology**, Amsterdam, vol. 64, no. 2, p. 113–119, 1998. DOI 10.1016/S0960-8524(97)00181-8. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852497001818>. Accessed on: 1 May 2020.

MUKHERJEE, S.; KARMAKAR, M.; RAY, R. R. Production of Extra Cellular Exoglucanase by *Rhizopus oryzae* from Submerged Fermentation of Agro Wastes. **Recent Research in Science and Technology**, Bangalore, vol. 3, no. 3, p. 69–75, 2 Mar. 2011. Available at: <https://updatepublishing.com/journal/index.php/rirst/article/view/635>. Accessed on: 1 May 2020.

MURPHY, L.; BOHLIN, C.; BAUMANN, M. J.; OLSEN, S. N.; SØRENSEN, T. H.; ANDERSON, L.; BORCH, K.; WESTH, P. Product inhibition of five *Hypocrea jecorina* cellulases. **Enzyme and Microbial Technology**, Amsterdam, vol. 52, no. 3, p. 163–169, 2013. DOI 10.1016/j.enzmictec.2013.01.002. Available at: <https://www.sciencedirect.com/science/article/abs/pii/S0141022913000069>. Accessed on: 13 May 2020.

NAIKA, G. S.; TIKU, P. K. Influence of Ethylenediaminetetraacetic Acid (EDTA) on the Structural Stability of Endoglucanase from *Aspergillus aculeatus*. **Journal of Agricultural and Food Chemistry**, Washington, vol. 59, no. 13, p. 7341–7345, 13 Jul. 2011. DOI 10.1021/jf103889m. Available at: <https://pubs.acs.org/doi/10.1021/jf103889m>. Accessed on: 1 Jun. 2020.

NANDA, S.; AZARGOHAR, R.; KOZINSKI, J. A.; DALAI, A. K. Characteristic Studies on the Pyrolysis Products from Hydrolyzed Canadian Lignocellulosic Feedstocks. **BioEnergy Research**, Berlin, vol. 7, no. 1, p. 174–191, 2014. DOI 10.1007/s12155-013-9359-7. Available at: <https://link.springer.com/article/10.1007/s12155-013-9359-7>. Accessed on: 5 May 2020.

NARRA, M.; DIXIT, G.; DIVECHA, J.; KUMAR, K.; MADAMWAR, D.; SHAH, A. R. Production, purification and characterization of a novel GH 12 family endoglucanase from *Aspergillus terreus* and its application in enzymatic degradation of delignified rice straw. **International Biodegradation & Biodegradation**, Amsterdam, vol. 88, p. 150–161, 2014. DOI 10.1016/j.ibiod.2013.12.016. Available at: <https://www.sciencedirect.com/science/article/pii/S0964830513004563>. Accessed on: 1 May 2020.

NI, J.; TAKEHARA, M.; WATANABE, H. Heterologous Overexpression of a Mutant Termite Cellulase Gene in *Escherichia coli* by DNA Shuffling of Four Orthologous Parental cDNAs. **Bioscience, Biotechnology, and Biochemistry**, Oxford, vol. 69, no. 9, p. 1711–1720, 1 Jan. 2005. DOI 10.1271/bbb.69.1711. Available at: <https://academic.oup.com/bbb/article/69/9/1711/5952855>. Accessed on: 1 Jun. 2020.

NORDWALD, E. M.; BRUNECKY, R.; HIMMEL, M. E.; BECKHAM, G. T.; KAAR, J. L. Charge engineering of cellulases improves ionic liquid tolerance and reduces lignin inhibition. **Biotechnology and Bioengineering**, Hoboken, vol. 111, no. 8, p. 1541–1549, 2014. DOI 10.1002/bit.25216. Available at: <https://onlinelibrary.wiley.com/doi/10.1002/bit.25216>. Accessed on: 1 Jun. 2020.

NURUL ADELA, B.; LOH, S. K.; NASRIN, A. B. The improvement on enzymatic hydrolysis of oil palm (*Elaeis guineensis*) empty fruit bunch lignocellulose. **Malaysian Applied Biology Journal**, Selangor, vol. 44, no. 1, p. 95–100, 2015. Available at: <https://www.cabdirect.org/cabdirect/abstract/20153192842>. Accessed on: 1 Jun. 2020.

OHGREN, K.; GALBE, M.; ZACCHI, G. Optimization of steam pretreatment of SO₂-impregnated corn stover for fuel ethanol production. **Applied Biochemistry and Biotechnology**, Totowa, vol. 124, no. 1–3, p. 1055–1067, 2005. DOI 10.1385/abab:124:1-3:1055. Available at: <https://pubmed.ncbi.nlm.nih.gov/15930581/>. Accessed on: 1 May 2020.

OKAFOR, U.; OKOCHI, V.; ONYEGEME-OKERENTA, B.; NWODO-CHINEDU, S. Xylanase production by *Aspergillus niger* ANL 301 using agrowastes. **African Journal of Biotechnology**, Lagos, vol. 6, no. 14, p. 1710–1714, 2007. Available at: <https://www.ajol.info/index.php/ajb/article/view/57761>. Accessed on: 2 May 2020.

ONOJA, E.; CHANDREN, S.; ABDUL RAZAK, F. I.; MAHAT, N. A.; WAHAB, R. A. Oil Palm (*Elaeis guineensis*) Biomass in Malaysia: The Present and Future Prospects. **Waste and Biomass Valorization**, Berlin, vol. 10, no. 8, p. 2099–2117, 2018. DOI 10.1007/s12649-018-0258-1. Available at: <https://link.springer.com/article/10.1007/s12649-018-0258-1>. Accessed on: 3 May 2020.

ORTIZ, L.; DORTA, E.; GLORIA LOBO, M.; GONZÁLEZ-MENDOZA, L. A.; DÍAZ, C.; GONZÁLEZ, M. Use of Banana (*Musa acuminata* Colla AAA) Peel Extract as an Antioxidant Source in Orange Juices. **Plant Foods for Human Nutrition**, Berlin, vol. 72, no. 1, p. 60–66, 2017. DOI 10.1007/s11130-016-0591-0. Available at: <https://link.springer.com/article/10.1007/s11130-016-0591-0>. Accessed on: 4 May 2020.

ÖZSIN, G.; PÜTÜN, A. E.; PÜTÜN, E. Investigating the interactions between lignocellulosic biomass and synthetic polymers during co-pyrolysis by simultaneous thermal and spectroscopic methods. **Biomass Conversion and Biorefinery**, Berlin, vol. 9, no. 3, p. 593–608, 2019. DOI 10.1007/s13399-019-00390-9. Available at: <https://link.springer.com/article/10.1007/s13399-019-00390-9>. Accessed on: 20 May 2020.

PARK, M.-J.; KIM, M. K.; KIM, H.-B.; IM, W.-T.; YI, T.-H.; KIM, S.-Y.; SOUNG, N.-K.; YANG, D.-C. *Microbacterium ginsengisoli* sp. nov., a -glucosidase-producing bacterium isolated from soil of a ginseng field. **International Journal of Systematic and Evolutionary Microbiology**, London, vol. 58, no. 2, p. 429–433, 2008. DOI 10.1099/ij.s.0.65226-0. Available at: <https://www.microbiologyresearch.org/content/journal/ijsem/10.1099/ij.s.0.65226-0>. Accessed on: 2 May 2020.

PAWAR, P. M.-A.; DERBA-MACELUCH, M.; CHONG, S.-L.; GÓMEZ, L. D.; MIEDES, E.; BANASIAK, A.; RATKE, C.; GAERTNER, C.; MOUILLE, G.; MCQUEEN-MASON, S. J.; MOLINA, A.; SELLSTEDT, A.; TENKANEN, M.; MELLEROWICZ, E. J. Expression of fungal acetyl xylan esterase in *Arabidopsis thaliana* improves saccharification of stem lignocellulose. **Plant Biotechnology Journal**, Hoboken, vol. 14, no. 1, p. 387–397, 1 Jan. 2016. DOI 10.1111/pbi.12393. Available at: <https://onlinelibrary.wiley.com/doi/full/10.1111/pbi.12393>. Accessed on: 15 May 2020.

PEREIRA, J. de C.; GIESE, E. C.; MORETTI, M. M. de S.; GOMES, A. C. dos S.; PERRONE, O. M.; BOSCOLO, M.; SILVA, R. da; GOMES, E.; MARTINS, D. A. B. Effect of Metal Ions, Chemical Agents and Organic Compounds on Lignocellulolytic Enzymes Activities. In: ŞENTÜRK, M. (ed.). **Enzyme Inhibitors and Activators**. London: IntechOpen, 2017. p. 139–164. DOI 10.5772/65934. Available at: <https://www.intechopen.com/chapters/52798>. Accessed on: 1 Jun. 2020.

PERVEZ, S.; AMAN, A.; IQBAL, S.; SIDDIQUI, N. N.; UL QADER, S. A. Saccharification and liquefaction of cassava starch: an alternative source for the production of bioethanol using amylolytic enzymes by double fermentation process. **BMC Biotechnology**, Berlin, vol. 14, no. 49, p. 1–10, 2014. DOI 10.1186/1472-6750-14-49. Available at: <https://link.springer.com/article/10.1186/1472-6750-14-49>. Accessed on: 8 May 2020.

PINA, A. S.; LOWE, C. R.; ROQUE, A. C. A. Challenges and opportunities in the purification of recombinant tagged proteins. **Biotechnology Advances**, Amsterdam, vol. 32, no. 2, p. 366–381, 2014. DOI 10.1016/j.biotechadv.2013.12.001. Available at: <https://www.sciencedirect.com/science/article/pii/S0734975013002176>. Accessed on: 1 Jun. 2020.

PINO, M. S.; RODRÍGUEZ-JASSO, R. M.; MICHELIN, M.; FLORES-GALLEGOS, A. C.; MORALES-RODRIGUEZ, R.; TEIXEIRA, J. A.; RUIZ, H. A. Bioreactor design for enzymatic hydrolysis of biomass under the biorefinery concept. **Chemical Engineering Journal**, Amsterdam, vol. 347, p. 119–136, 2018. DOI 10.1016/j.cej.2018.04.057. Available at: <https://www.sciencedirect.com/science/article/pii/S1385894718306235>. Accessed on: 1 May 2020.

POCAN, P.; BAHCEGUL, E.; OZTOP, M. H.; HAMAMCI, H. Enzymatic Hydrolysis of Fruit Peels and Other Lignocellulosic Biomass as a Source of Sugar. **Waste and Biomass Valorization**, Berlin, vol. 9, no. 6, p. 929–937, 2018. DOI 10.1007/s12649-017-9875-3. Available at: <https://link.springer.com/article/10.1007/s12649-017-9875-3>. Accessed on: 20 May 2020.

PUCHART, V.; FRAŇOVÁ, L.; MØRKEBERG KROGH, K. B. R.; HOFF, T.; BIELY, P. Action of different types of endoxylanases on eucalyptus xylan in situ. **Applied Microbiology and Biotechnology**, Amsterdam, vol. 102, no. 4, p. 1725–1736, 2018. DOI 10.1007/s00253-017-8722-6. Available at: <https://link.springer.com/article/10.1007/s00253-017-8722-6>. Accessed on: 15 May 2020.

QIN, L.; LI, W.-C.; LIU, L.; ZHU, J.-Q.; LI, X.; LI, B.-Z.; YUAN, Y.-J. Inhibition of lignin-derived phenolic compounds to cellulase. **Biotechnology for Biofuels**, London, vol. 9, no. 1, p. 70, 2016. DOI 10.1186/s13068-016-0485-2. Available at: <https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/s13068-016-0485-2>. Accessed on: 1 Jun. 2020.

RAMANI, G.; MEERA, B.; VANITHA, C.; RAO, M.; GUNASEKARAN, P. Production, Purification, and Characterization of a β -Glucosidase of *Penicillium funiculosum* NCL1. **Applied Biochemistry and Biotechnology**, Berlin, vol. 167, no. 5, p. 959–972, 2012. DOI 10.1007/s12010-012-9645-4. Available at: <https://link.springer.com/article/10.1007/s12010-012-9645-4>. Accessed on: 2 May 2020.

RAZA, A.; POTHULA, R.; ABDELGAFFAR, H.; BASHIR, S.; JURAT-FUENTES, J. L. Identification and functional characterization of a β -glucosidase from *Bacillus tequelensis* BD69 expressed in bacterial and yeast heterologous systems. **PeerJ**, San Diego, vol. 8, p. 1–22, 2020. DOI 10.7717/peerj.8792. Available at: <https://peerj.com/articles/8792/>. Accessed on: 1 Jun. 2020.

RAZEQ, F. M.; JURAK, E.; STOGIOS, P. J.; YAN, R.; TENKANEN, M.; KABEL, M. A.; WANG, W.; MASTER, E. R. A novel acetyl xylan esterase enabling complete deacetylation of substituted xyans. **Biotechnology for Biofuels**, London, vol. 11, no. 1, p. 1–12, 2018. DOI 10.1186/s13068-018-1074-3. Available at: <https://link.springer.com/article/10.1186/s13068-018-1074-3>. Accessed on: 15 May 2020.

REDDY, S. S.; KRISHNAN, C. Production of high-pure xylooligosaccharides from sugarcane bagasse using crude β -xylosidase-free xylanase of *Bacillus subtilis* KCX006 and their bifidogenic function. **LWT - Food Science and Technology**, Amsterdam, vol. 65, p. 237–245, 2016. DOI 10.1016/j.lwt.2015.08.013. Available at: <https://www.sciencedirect.com/science/article/pii/S0023643815301079>. Accessed on: 2 May 2020.

RENTIZELAS, A. A. 6 - Biomass storage. In: HOLM-NIELSEN, J. B.; EHIMEN, E. A. (eds.). **Biomass Supply Chains for Bioenergy and Biorefining**. Amsterdam: Woodhead Publishing, 2016. p. 127–146. DOI 10.1016/B978-1-78242-366-9.00006-X. Available at: <http://www.sciencedirect.com/science/article/pii/B978178242366900006X>. Accessed on: 3 May 2020.

REVERBEL-LEROY, C.; PAGES, S.; BELAICH, A.; BELAICH, J. P.; TARDIF, C. The processive endocellulase CelF, a major component of the *Clostridium cellulolyticum* cellulosome: purification and characterization of the recombinant form. **Journal of Bacteriology**, Washington, vol. 179, no. 1, p. 46–52, 1 Jan. 1997. DOI 10.1128/jb.179.1.46-52.1997. Available at: <https://journals.asm.org/doi/10.1128/jb.179.1.46-52.1997>. Accessed on: 1 Jun. 2020.

REVILLA, G.; ZARRA, I. Fisiología vegetal. Introducción a las células de las plantas: membranas y pared. In: AZCÓN-BIETO, J.; TALÓN, M. (eds.). **Fundamentos de Fisiología Vegetal**. 2da ed. Madrid: McGraw-Hill, 2013. p. 651.

RODIONOVA, N. A.; DUBOVAYA, N. V.; ENEISKAYA, E. V.; MARTINOVICH, L. I.; GRACHEVA, I. M.; BEZBORODOV, A. M. Purification and Characterization of endo-(1 $\rightarrow$ 4)- β -xylanase from *Geotrichum candidum* 3C. **Applied Biochemistry and Microbiology**, Berlin, vol. 36, no. 5, p. 460–465, 2000. DOI 10.1007/BF02731889. Available at: <https://link.springer.com/article/10.1007/BF02731889>. Accessed on: 2 May 2020.

ROMANIEC, M. P. M.; FAUTH, U.; KOBAYASHI, T.; HUSKISSON, N. S.; BARKER, P. J.; DEMAIN, A. L. Purification and characterization of a new endoglucanase from *Clostridium thermocellum*. **Biochemical Journal**, London, vol. 283, no. 1, p. 69–73, 1 Apr. 1992. DOI 10.1042/bj2830069. Available at: <https://portlandpress.com/biochemj/article-abstract/283/1/69/27096/Purification-and-characterization-of-a-new?redirectedFrom=fulltext>. Accessed on: 2 May 2020.

ROSALES-CALDERON, O.; ARANTES, V. A review on commercial-scale high-value products that can be produced alongside cellulosic ethanol. **Biotechnology for Biofuels**, Berlin, vol. 12, no. 1, p. 240, 2019. DOI 10.1186/s13068-019-1529-1. Available at: <https://link.springer.com/article/10.1186/s13068-019-1529-1>. Accessed on: 5 May 2020.

ROSEN, Y.; MAMANE, H.; GERCHMAN, Y. Short Ozonation of Lignocellulosic Waste as Energetically Favorable Pretreatment. **BioEnergy Research**, Berlin, vol. 12, no. 2, p. 292–301, 2019. DOI 10.1007/s12155-019-9962-3. Available at: <https://link.springer.com/article/10.1007/s12155-019-9962-3>. Accessed on: 11 May 2020.

RYAN, S. E.; NOLAN, K.; THOMPSON, R.; GUBITZ, G. M.; SAVAGE, A. V.; TUOHY, M. G. Purification and characterization of a new low molecular weight endoxylanase from *Penicillium capsulatum*. **Enzyme and Microbial Technology**, Amsterdam, vol. 33, no. 6, p. 775–785, 2003. DOI 10.1016/S0141-0229(03)00176-5. Available at: <https://www.sciencedirect.com/science/article/pii/S0141022903001765>. Accessed on: 2 May 2020.

SAINI, J. K.; PATEL, A. K.; ADSUL, M.; SINGHANIA, R. R. Cellulase adsorption on lignin: A roadblock for economic hydrolysis of biomass. **Renewable Energy**, Amsterdam, vol. 98, p. 29–42, 2016. DOI 10.1016/j.renene.2016.03.089. Available at: <https://www.sciencedirect.com/science/article/pii/S0960148116302737>. Accessed on: 1 Jun. 2020.

SCHMATZ, A. A.; TYHODA, L.; BRIENZO, M. Sugarcane biomass conversion influenced by lignin. **Biofuels, Bioproducts and Biorefining**, Hoboken, vol. 14, no. 2, p. 469–480, 2020. DOI 10.1002/bbb.2070. Available at: <https://onlinelibrary.wiley.com/doi/10.1002/bbb.2070>. Accessed on: 1 May 2020.

SEADER, J. D.; HENLEY, E.; ROPER, K. **Separation Process Principles Chemical and Biochemical Operations**. 3th ed. Hoboken: John Wiley & Sons, Ltd, 2011.

SEIDEL, Z. P.; LEE, C. T. Enhanced Activity of the Cellulase Enzyme β -Glucosidase upon Addition of an Azobenzene-Based Surfactant. **ACS Sustainable Chemistry & Engineering**, Washington, vol. 8, no. 4, p. 1751–1761, 3 Feb. 2020. DOI 10.1021/acssuschemeng.9b05240. Available at: <https://pubs.acs.org/doi/10.1021/acssuschemeng.9b05240>. Accessed on: 1 Jun. 2020.

SELIG, M. J.; VIAMAJALA, S.; DECKER, S. R.; TUCKER, M. P.; HIMMEL, M. E.; VINZANT, T. B. Deposition of Lignin Droplets Produced During Dilute Acid Pretreatment of Maize Stems Retards Enzymatic Hydrolysis of Cellulose. **Biotechnology Progress**, Hoboken, vol. 23, no. 6, p. 1333–1339, 2007. DOI 10.1021/bp0702018. Available at: <https://aiche.onlinelibrary.wiley.com/doi/full/10.1021/bp0702018>. Accessed on: 1 May 2020.

SHAHZADI, T.; ANWAR, Z.; IQBAL, Z.; ANJUM, A.; AQIL, T.; BAKHTAWAR, AFZAL, A.; KAMRAN, M.; MEHMOOD, S.; IRSHAD, M. Induced Production of Exoglucanase, and β -Glucosidase from Fungal Co-Culture of *T. viride* and *G. lucidum*. **Advances in Bioscience and Biotechnology**, Wuhan, vol. 5, no. 5, p. 426–433, 2014. DOI 10.4236/abb.2014.55051. Available at: <https://www.scirp.org/journal/paperinformation.aspx?paperid=44482>. Accessed on: 2 May 2020.

SHARMA, H. K.; XU, C.; QIN, W. Biological Pretreatment of Lignocellulosic Biomass for Biofuels and Bioproducts: An Overview. **Waste and Biomass Valorization**, Berlin, vol. 10, no. 2, p. 235–251, 2019. DOI 10.1007/s12649-017-0059-y. Available at: <https://link.springer.com/article/10.1007/s12649-017-0059-y>. Accessed on: 12 Aug. 2020.

SHELDON, R. A. Green and sustainable manufacture of chemicals from biomass: state of the art. **Green Chemistry**, Cambridge, vol. 16, no. 3, p. 950–963, 2014. DOI 10.1039/C3GC41935E. Available at: <https://pubs.rsc.org/en/content/articlelanding/2014/GC/C3GC41935E>. Accessed on: 1 Jun. 2020.

SHI, R.; LI, Z.; YE, Q.; XU, J.; LIU, Y. Heterologous expression and characterization of a novel thermo-halotolerant endoglucanase Cel5H from *Dictyoglomus thermophilum*. **Bioresource Technology**, Amsterdam, vol. 142, p. 338–344, 2013. DOI 10.1016/j.biortech.2013.05.037. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852413007979>. Accessed on: 1 Jun. 2020.

SHIMIZU, F. L.; DE AZEVEDO, G. O.; COELHO, L. F.; PAGNOCCA, F. C.; BRIENZO, M. Minimum Lignin and Xylan Removal to Improve Cellulose Accessibility. **BioEnergy Research**, Berlin, vol. 13, no. 3, p. 775–785, 2020. DOI 10.1007/s12155-020-10120-z. Available at: <https://link.springer.com/article/10.1007/s12155-020-10120-z>. Accessed on: 1 May 2020.

SHIMIZU, F. L.; ZAMORA, H. D. Z.; SCHMATZ, A. A.; MELATI, R. B.; BUENO, D.; BRIENZO, M. Biofuels Generation Based on Technical Process and Biomass Quality. In: SRIVASTAVA, N.; SRIVASTAVA, M.; MISHRA, P. K.; GUPTA, V. K. (eds.). **Biofuel Production Technologies: Critical Analysis for Sustainability**. Singapore: Springer Singapore, 2020. p. 37–64. DOI 10.1007/978-981-13-8637-4_2. Available at: https://link.springer.com/chapter/10.1007/978-981-13-8637-4_2. Accessed on: 5 Jul. 2022.

SILVA, T.; ZAMORA, H.; VARÃO, L.; PRADO, N.; BAFFI, M.; PASQUINI, D. Effect of Steam Explosion Pretreatment Catalysed by Organic Acid and Alkali on Chemical and Structural Properties and Enzymatic Hydrolysis of Sugarcane Bagasse. **Waste and Biomass Valorization**, Berlin, vol. 9, no. 11, p. 2191–2201, 2018. DOI 10.1007/s12649-017-9989-7. Available at: <https://link.springer.com/article/10.1007/s12649-017-9989-7>. Accessed on: 23 Sep. 2021.

SINGHANIA, R. R.; PATEL, A. K.; PANDEY, A.; GANANSOUNOU, E. Genetic modification: A tool for enhancing beta-glucosidase production for biofuel application. **Bioresource Technology**, Amsterdam, vol. 245, p. 1352–1361, 2017. DOI 10.1016/j.biortech.2017.05.126. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852417307940>. Accessed on: 2 May 2020.

SINGHVI, M. S.; GOKHALE, D. V. Lignocellulosic biomass: Hurdles and challenges in its valorization. **Applied Microbiology and Biotechnology**, Berlin, vol. 103, no. 23, p. 9305–9320, 2019. DOI 10.1007/s00253-019-10212-7. Available at: <https://link.springer.com/article/10.1007/s00253-019-10212-7>. Accessed on: 17 May 2020.

SIPPONEN, M. H.; RAHIKAINEN, J.; LESKINEN, T.; PIHLAJANIEMI, V.; MATTINEN, M.-L.; LANGE, H.; CRESTINI, C.; ÖSTERBERG, M. Ö. Structural changes of lignin in biorefinery pretreatments and consequences to enzyme-lignin interactions. **Nordic Pulp & Paper Research Journal**, Berlin, vol. 32, no. 4, p. 550–571, 2017. DOI 10.3183/npprj-2017-32-04_p550-571_sipponen. Available at: https://www.degruyter.com/document/doi/10.3183/npprj-2017-32-04_p550-571_sipponen/html. Accessed on: 1 Jun. 2020.

SIQUEIRA, G.; ARANTES, V.; SADDLER, J. N.; FERRAZ, A.; MILAGRES, A. M. F. Limitation of cellulose accessibility and unproductive binding of cellulases by pretreated sugarcane bagasse lignin. **Biotechnology for Biofuels**, London, vol. 10, no. 1, p. 176, 2017. DOI 10.1186/s13068-017-0860-7. Available at: <https://doi.org/10.1186/s13068-017-0860-7>. Accessed on: 1 Jun. 2020.

SØRENSEN, A.; ANDERSEN, J. J.; AHRING, B. K.; TELLER, P. J.; LÜBECK, M. Screening of carbon sources for beta-glucosidase production by *Aspergillus saccharolyticus*. **International Biodeterioration & Biodegradation**, Amsterdam, vol. 93, p. 78–83, 2014. DOI 10.1016/j.ibiod.2014.05.011. Available at: <https://www.sciencedirect.com/science/article/pii/S0964830514001462>. Accessed on: 1 May 2020.

SOROKINA, K. N.; SAMOYLOVA, Y. V.; PILIGAEV, A. V.; SIVAKUMAR, U.; PARMON, V. N. New methods for the one-pot processing of polysaccharide components (cellulose and hemicelluloses) of lignocellulose biomass into valuable products. Part 3: Products synthesized via the biotechnological conversion of poly- and monosaccharides of biomass. **Catalysis in Industry**, Berlin, vol. 9, no. 3, p. 270–276, 2017. DOI 10.1134/S2070050417030138. Available at: <https://link.springer.com/article/10.1134/S2070050417030138>. Accessed on: 5 May 2020.

STATISTA. Average annual Brent crude oil price from 1976 to 2020. 2020. Available at: <https://www.statista.com/statistics/262860/uk-brent-crude-oil-price-changes-since-1976/>. Accessed on: 1 May 2020.

STEINBACH, D.; KRUSE, A.; SAUER, J. Pretreatment technologies of lignocellulosic biomass in water in view of furfural and 5-hydroxymethylfurfural production- A review. **Biomass Conversion and Biorefinery**, Berlin, vol. 7, no. 2, p. 247–274, 2017. DOI 10.1007/s13399-017-0243-0. Available at: <https://link.springer.com/article/10.1007/s13399-017-0243-0>. Accessed on: 5 May 2020.

SUBHEDAR, P. B.; GOGATE, P. R. Enhancing the activity of cellulase enzyme using ultrasonic irradiations. **Journal of Molecular Catalysis B: Enzymatic**, Amsterdam, vol. 101, p. 108–114, 2014. DOI 10.1016/j.molcatb.2014.01.002. Available at: <https://www.sciencedirect.com/science/article/pii/S1381117714000046>. Accessed on: 1 Jun. 2020.

SUBRAMANIYAN, S. Isolation, Purification and Characterisation of Low Molecular Weight Xylanase from *Bacillus pumilus* SSP-34. **Applied Biochemistry and Biotechnology**, Berlin, vol. 166, no. 7, p. 1831–1842, 2012. DOI 10.1007/s12010-012-9600-4. Available at: <https://link.springer.com/article/10.1007/s12010-012-9600-4>. Accessed on: 3 May 2020.

SUMMERS, S. R.; SPRENGER, K. G.; PFAENDTNER, J.; MARCHANT, J.; SUMMERS, M. F.; KAAR, J. L. Mechanism of Competitive Inhibition and Destabilization of *Acidothermus cellulolyticus* Endoglucanase 1 by Ionic Liquids. **The Journal of Physical Chemistry B**, Washington, vol. 121, no. 48, p. 10793–10803, 7 Dec. 2017. DOI 10.1021/acs.jpcc.7b08435. Available at: <https://pubs.acs.org/doi/pdf/10.1021/acs.jpcc.7b08435>. Accessed on: 13 May 2020.

TAO, Y.-M.; ZHU, X.-Z.; HUANG, J.-Z.; MA, S.-J.; WU, X.-B.; LONG, M.-N.; CHEN, Q.-X. Purification and Properties of Endoglucanase from a Sugar Cane Bagasse Hydrolyzing Strain, *Aspergillus glaucus* XC9. **Journal of Agricultural and Food Chemistry**, Washington, vol. 58, no. 10, p. 6126–6130, 26 May 2010. DOI 10.1021/jf1003896. Available at: <https://pubs.acs.org/doi/10.1021/jf1003896>. Accessed on: 1 Jun. 2020.

TAYI, L.; KUMAR, S.; NATHAWAT, R.; HAQUE, A. S.; MAKU, R. V.; PATEL, H. K.; SANKARANARAYANAN, R.; SONTI, R. V. A mutation in an exoglucanase of *Xanthomonas oryzae* pv. *oryzae*, which confers an endo mode of activity, affects bacterial virulence, but not the induction of immune responses, in rice. **Molecular Plant Pathology**, Hoboken, vol. 19, no. 6, p. 1364–1376, 2017. DOI 10.1111/mpp.12620. Available at: <https://bsppjournals.onlinelibrary.wiley.com/doi/10.1111/mpp.12620>. Accessed on: 2 May 2020.

TEIXEIRA DA SILVA, V. de C.; DE SOUZA COTO, A. L.; DE CARVALHO SOUZA, R.; BERTOLDI SANCHEZ NEVES, M.; GOMES, E.; BONILLA-RODRIGUEZ, G. O. Effect of pH, Temperature, and Chemicals on the Endoglucanases and β -Glucosidases from the Thermophilic Fungus *Myceliophthora heterothallica* F.2.1.4. Obtained by Solid-State and Submerged Cultivation. **Biochemistry Research International**, London, vol. 2016, p. 1–9, 2016. DOI 10.1155/2016/9781216. Available at: <https://www.hindawi.com/journals/bri/2016/9781216/>. Accessed on: 12 May 2020.

TENKANEN, M.; PULS, J.; POUTANEN, K. Two major xylanases of *Trichoderma reesei*. **Enzyme and Microbial Technology**, Amsterdam, vol. 14, no. 7, p. 566–574, 1992. DOI 10.1016/0141-0229(92)90128-B. Available at: <https://www.sciencedirect.com/science/article/pii/014102299290128B>. Accessed on: 2 May 2020.

TERRONE, C. C.; MONTESINO DE FREITAS NASCIMENTO, J.; FANCHINI TERRASAN, C. R.; BRIENZO, M.; CARMONA, E. C. Salt-tolerant α -arabinofuranosidase from a new specie *Aspergillus hortai* CRM1919: Production in acid conditions, purification, characterization and application on xylan hydrolysis. **Biocatalysis and Agricultural Biotechnology**, Amsterdam, vol. 23, p. 101460, 2020. DOI 10.1016/j.bcab.2019.101460. Available at: <https://www.sciencedirect.com/science/article/pii/S1878818119312575>. Accessed on: 1 May 2020.

TRAJANO, H. L.; WYMAN, C. E. Fundamentals of Biomass Pretreatment at Low pH. In: WYMAN, C. E. (ed.). **Aqueous Pretreatment of Plant Biomass for Biological and Chemical Conversion to Fuels and Chemicals**. Hoboken: Wiley Online Library, 2013. p. 103–128. DOI 10.1002/9780470975831.ch6. Available at: <https://onlinelibrary.wiley.com/doi/10.1002/9780470975831.ch6>. Accessed on: 2 May 2020.

TROLLOPE, K.; NEL, D. W.; VOLSCHENK, H. The heterologous expression potential of an acid-tolerant *Talaromyces pinophilus* β -glucosidase in *Saccharomyces cerevisiae*. **Folia Microbiologica**, Amsterdam, vol. 63, no. 6, p. 725–734, 2018. DOI 10.1007/s12223-018-0613-4. Available at: <https://link.springer.com/article/10.1007/s12223-018-0613-4>. Accessed on: 1 Jun. 2020.

U.S. ENERGY INFORMATION ADMINISTRATION. Crude oil distillation and the definition of refinery capacity. 2012. Available at: <https://www.eia.gov/todayinenergy/detail.php?id=6970>. Accessed on: 1 Feb. 2021.

VASCONCELLOS, V. M.; TARDIOLI, P. W.; GIORDANO, R. L. C.; FARINAS, C. S. Addition of metal ions to a (hemi)cellulolytic enzymatic cocktail produced in-house improves its activity, thermostability, and efficiency in the saccharification of pretreated sugarcane bagasse. **New Biotechnology**, Amsterdam, vol. 33, no. 3, p. 331–337, 2016. DOI 10.1016/j.nbt.2015.12.002. Available at: <https://www.sciencedirect.com/science/article/pii/S1871678415002708>. Accessed on: 1 Jun. 2020.

VASSILEVA, P. S.; RADOYKOVA, T. H.; DETCHEVA, A. K.; AVRAMOVA, I. A.; ALEKSIEVA, K. I.; NENKOVA, S. K.; VALCHEV, I. V.; MEHANDJIEV, D. R. Adsorption of Ag⁺ ions on hydrolyzed lignocellulosic materials based on willow, paulownia, wheat straw and maize stalks. **International Journal of Environmental Science and Technology**, Berlin, vol. 13, no. 5, p. 1319–1328, 2016. DOI 10.1007/s13762-016-0970-y. Available at: <https://link.springer.com/article/10.1007/s13762-016-0970-y>. Accessed on: 5 May 2020.

VIANNA BERNARDI, A.; KIMIE YONAMINE, D.; AKIRA UYEMURA, S.; MAGNANI DINAMARCO, T. A Thermostable *Aspergillus fumigatus* GH7 Endoglucanase Over-Expressed in *Pichia pastoris* Stimulates Lignocellulosic Biomass Hydrolysis. **International Journal of Molecular Sciences**, Basel, vol. 20, no. 9, p. 2261, 7 May 2019. DOI 10.3390/ijms20092261. Available at: <https://www.mdpi.com/1422-0067/20/9/2261>. Accessed on: 16 May 2020.

VINUSELVI, P. Heterologous Expression and Extracellular Secretion of Cellulases in Recombinant Microbes. In: LIMA, A. P. (ed.). **Bioethanol**. London: IntechOpen, 2012. p. 239–252. DOI 10.5772/22268. Available at: <https://www.intechopen.com/chapters/27359>. Accessed on: 1 Jun. 2020.

VIRTANEN, S.; CHOWREDDY, R. R.; IRMAK, S.; HONKAPÄÄ, K.; ISOM, L. Food Industry Co-streams: Potential Raw Materials for Biodegradable Mulch Film Applications. **Journal of Polymers and the Environment**, Berlin, vol. 25, no. 4, p. 1110–1130, 2017. DOI 10.1007/s10924-016-0888-y. Available at: <https://link.springer.com/article/10.1007/s10924-016-0888-y>. Accessed on: 5 May 2020.

WALIA, A.; MEHTA, P.; CHAUHAN, A.; KULSHRESTHA, S.; SHIRKOT, C. K. Purification and characterization of cellulase-free low molecular weight endo β -1,4 xylanase from an alkalophilic *Cellulosimicrobium cellulans* CKMX1 isolated from mushroom compost. **World Journal of Microbiology and Biotechnology**, Berlin, vol. 30, no. 10, p. 2597–2608, 2014. DOI 10.1007/s11274-014-1683-3. Available at: <https://link.springer.com/article/10.1007/s11274-014-1683-3>. Accessed on: 2 May 2020.

WALKER, L. P.; WILSON, D. B. Enzymatic hydrolysis of cellulose: An overview. **Bioresource Technology**, Amsterdam, vol. 36, no. 1, p. 3–14, 1991. DOI 10.1016/0960-8524(91)90095-2. Available at: <https://www.sciencedirect.com/science/article/pii/0960852491900952>. Accessed on: 1 May 2020.

WALLACE, J.; BRIENZO, M.; GARCÍA-APARICIO, M. P.; GÖRGENS, J. F. Lignin enrichment and enzyme deactivation as the root cause of enzymatic hydrolysis slowdown of steam pretreated sugarcane bagasse. **New Biotechnology**, Amsterdam, vol. 33, no. 3, p. 361–371, 2016. DOI 10.1016/j.nbt.2016.01.004. Available at: <https://www.sciencedirect.com/science/article/pii/S1871678416000078>. Accessed on: 1 May 2020.

WANG, Q. Q.; HE, Z.; ZHU, Z.; ZHANG, Y.-H. P.; NI, Y.; LUO, X. L.; ZHU, J. Y. Evaluations of cellulose accessibilities of lignocelluloses by solute exclusion and protein adsorption techniques. **Biotechnology and Bioengineering**, Hoboken, vol. 109, no. 2, p. 381–389, 2012. DOI 10.1002/bit.23330. Available at: <https://onlinelibrary.wiley.com/doi/10.1002/bit.23330>. Accessed on: 1 May 2020.

WANG, W.; ZHUANG, X.; TAN, X.; WANG, Q.; CHEN, X.; YU, Q.; QI, W.; WANG, Z.; YUAN, Z. Dual Effect of Nonionic Surfactants on Improving the Enzymatic Hydrolysis of Lignocellulose. **Energy & Fuels**, Washington, vol. 32, no. 5, p. 5951–5959, 17 May

2018. DOI 10.1021/acs.energyfuels.8b00225. Available at: <https://pubs.acs.org/doi/10.1021/acs.energyfuels.8b00225>. Accessed on: 20 May 2020.

WINGFIELD, P. T. Protein Precipitation Using Ammonium Sulfate. **Current Protocols in Protein Science**, Hoboken, vol. 84, no. 1, p. A.3F.1–A.3F.9, 2016. DOI 10.1002/0471140864.psa03fs84. Available at: <https://currentprotocols.onlinelibrary.wiley.com/doi/10.1002/0471140864.psa03fs84>. Accessed on: 2 May 2020.

WONG, K. K. Y.; DEVERELL, K. F.; MACKIE, K. L.; CLARK, T. A.; DONALDSON, L. A. The relationship between fiber-porosity and cellulose digestibility in steam-exploded *Pinus radiata*. **Biotechnology and Bioengineering**, Hoboken, vol. 31, no. 5, p. 447–456, 1988. DOI 10.1002/bit.260310509. Available at: <https://onlinelibrary.wiley.com/doi/10.1002/bit.260310509>. Accessed on: 2 May 2020.

XIMENES, E.; KIM, Y.; MOSIER, N.; DIEN, B.; LADISCH, M. Deactivation of cellulases by phenols. **Enzyme and Microbial Technology**, Amsterdam, vol. 48, no. 1, p. 54–60, 2011. DOI 10.1016/j.enzmictec.2010.09.006. Available at: <https://www.sciencedirect.com/science/article/pii/S0141022910001997>. Accessed on: 1 May 2020.

YANG, L.; XU, F.; GE, X.; LI, Y. Challenges and strategies for solid-state anaerobic digestion of lignocellulosic biomass. **Renewable and Sustainable Energy Reviews**, Amsterdam, vol. 44, p. 824–834, 2015. DOI 10.1016/j.rser.2015.01.002. Available at: <https://www.sciencedirect.com/science/article/pii/S136403211500012X>. Accessed on: 1 May 2020.

YANG, Y.; ZHU, N.; YANG, J.; LIN, Y.; LIU, J.; WANG, R.; WANG, F.; YUAN, H. A novel bifunctional acetyl xylan esterase/arabinofuranosidase from *Penicillium chrysogenum* P33 enhances enzymatic hydrolysis of lignocellulose. **Microbial Cell Factories**, London, vol. 16, no. 1, p. 166, 2017. DOI 10.1186/s12934-017-0777-7. Available at: <https://microbialcellfactories.biomedcentral.com/articles/10.1186/s12934-017-0777-7>. Accessed on: 3 May 2020.

YEOMAN, C. J.; HAN, Y.; DODD, D.; SCHROEDER, C. M.; MACKIE, R. I.; CANN, I. K. O. B. T.-A. in A. M. Chapter 1 - Thermotable Enzymes as Biocatalysts in the Biofuel Industry. In: LASKIN, A. I.; SARIASLANI, S.; GADD, G. M. (eds.). **Advances in Applied Microbiology**. Berlin: Academic Press, 2010. vol. 70, p. 1–55. DOI 10.1016/S0065-2164(10)70001-0. Available at: <http://www.sciencedirect.com/science/article/pii/S0065216410700010>. Accessed on: 15 May 2020.

YIMER, D.; TILAHUN, A. Microbial biotechnology review in microbial enzyme production methods, assay techniques and protein separation and purifications. **Nutritional Health & Food Engineering**, Edmond, vol. 8, no. 1, p. 1–7, 2018. DOI 10.15406/jnhfe.2018.08.00249. Available at: <https://medcraveonline.com/JNHFE/JNHFE-08-00249.pdf>. Accessed on: 1 May 2020.

YING, W.; SHI, Z.; YANG, H.; XU, G.; ZHENG, Z.; YANG, J. Effect of alkaline lignin modification on cellulase–lignin interactions and enzymatic saccharification yield. **Biotechnology for Biofuels**, Amsterdam, vol. 11, no. 1, p. 214, 2018. DOI 10.1186/s13068-018-1217-6. Available at: <https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/s13068-018-1217-6>. Accessed on: 1 Jun. 2020.

YÜCEL, Y.; GÖYCINCIK, S. Optimization and Modelling of Process Conditions Using Response Surface Methodology (RSM) for Enzymatic Saccharification of Spent Tea Waste (STW). **Waste and Biomass Valorization**, Berlin, vol. 6, no. 6, p. 1077–1084, 2015. DOI 10.1007/s12649-015-9395-y. Available at: <https://link.springer.com/article/10.1007/s12649-015-9395-y>. Accessed on: 1 May 2020.

YUSUF, A. A. I. S.; KRESNOWATI, M. T. A. P. Evaluation of surfactant addition to enzymatic hydrolysis of oil palm empty fruit bunch (OPEFB). **AIP Conference Proceedings**, College Park, vol. 2085, no. 1, p. 1–8, 21 Mar. 2019. DOI 10.1063/1.5094988. Available at: <https://aip.scitation.org/doi/abs/10.1063/1.5094988>. Accessed on: 20 May 2020.

ZANELATO, A. I.; SHIOTA, V. M.; GOMES, E.; SILVA, R. da; THOMÉO, J. C. Endoglucanase production with the newly isolated *Myceliophthora* sp. i-1d3b in a packed bed solid state fermentor. **Brazilian Journal of Microbiology**, São Paulo, vol. 43, no. 4, p. 1536–1544, 2012. DOI 10.1590/S1517-83822012000400038. Available at: <https://www.scielo.br/bjm/a/GPwFXWmTbwwc7DKdfKJCyJM/?lang=en#>. Accessed on: 1 May 2020.

ZEESHAN, N.; NAZ, S.; NAZ, S.; AFROZ, A.; ZAHUR, M.; ZIA, S. Heterologous expression and enhanced production of β -1,4-glucanase of *Bacillus halodurans* C-125 in *Escherichia coli*. **Electronic Journal of Biotechnology**, Amsterdam, vol. 34, p. 29–36, 2018. DOI 10.1016/j.ejbt.2018.05.001. Available at: <https://www.sciencedirect.com/science/article/pii/S0717345818300186>. Accessed on: 1 Jun. 2020.

ZENG, R.; HU, Q.; YIN, X.-Y.; HUANG, H.; YAN, J.-B.; GONG, Z.-W.; YANG, Z.-H. Cloning a novel endo-1,4- β -d-glucanase gene from *Trichoderma virens* and heterologous expression in *E. coli*. **AMB Express**, Berlin, vol. 6, no. 1, p. 108, 2016. DOI 10.1186/s13568-016-0282-0. Available at: <https://amb-express.springeropen.com/articles/10.1186/s13568-016-0282-0>. Accessed on: 1 Jun. 2020.

ZHAI, R.; HU, J.; SADDLER, J. N. What Are the Major Components in Steam Pretreated Lignocellulosic Biomass That Inhibit the Efficacy of Cellulase Enzyme Mixtures? **ACS Sustainable Chemistry & Engineering**, Washington, vol. 4, no. 6, p. 3429–3436, 6 Jun. 2016. DOI 10.1021/acssuschemeng.6b00481. Available at: <https://pubs.acs.org/doi/10.1021/acssuschemeng.6b00481>. Accessed on: 13 May 2020.

ZHANG, M.; BUEKENS, A.; LI, X. Dioxins from Biomass Combustion: An Overview. **Waste and Biomass Valorization**, Berlin, vol. 8, p. 1–20, 2016. DOI 10.1007/s12649-016-9744-5. Available at: <https://doi.org/10.1007/s12649-016-9744-5>. Accessed on: 3 May 2020.

ZHANG, P.; YUAN, X.; DU, Y.; LI, J.-J. Heterologous expression and biochemical characterization of a GHF9 endoglucanase from the termite *Reticulitermes speratus* in *Pichia pastoris*. **BMC Biotechnology**, Berlin, vol. 18, no. 1, p. 35, 2018. DOI 10.1186/s12896-018-0432-3. Available at: <https://bmcbiotechnol.biomedcentral.com/articles/10.1186/s12896-018-0432-3>. Accessed on: 1 Jun. 2020.

ZHANG, X.; WANG, H.; LIU, C.; ZHANG, A.; REN, J. Synthesis of Thermoplastic Xylan-Lactide Copolymer with Amidine-Mediated Organocatalyst in Ionic Liquid. **Scientific Reports**, Berlin, vol. 7, no. 1, p. 551, 2017. DOI 10.1038/s41598-017-00464-6. Available at: <https://www.nature.com/articles/s41598-017-00464-6>. Accessed on: 2 May 2020.

ZHAO, X.; LI, S.; WU, R.; LIU, D. Organosolv fractionating pre-treatment of lignocellulosic biomass for efficient enzymatic saccharification: chemistry, kinetics, and substrate structures. **Biofuels, Bioproducts and Biorefining**, Hoboken, vol. 11, no. 3, p. 567–590, 1 May 2017. DOI 10.1002/bbb.1768. Available at: <https://onlinelibrary.wiley.com/doi/10.1002/bbb.1768>. Accessed on: 27 Sep. 2020.

ZHAO, X.; ZHANG, L.; LIU, D. Biomass recalcitrance. Part I: the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose. **Biofuels, Bioproducts and Biorefining**, Hoboken, vol. 6, no. 4, p. 465–482, 2012. DOI 10.1002/bbb.1331. Available at: <https://onlinelibrary.wiley.com/doi/10.1002/bbb.1331>. Accessed on: 1 May 2020.

ZHENG, H.; SUN, M.; MENG, L.; PEI, H.; ZHANG, X.; YAN, Z.; ZENG, W.; ZHANG, J.; HU, J.; LU, F.; SUN, J. Purification and Characterization of a Thermotable Xylanase from *Paenibacillus* sp. NF1 and its Application in Xylooligosaccharides Production. **Journal of Microbiology and Biotechnology**, Seoul, vol. 24, no. 4 ed. 2014/04/28, p. 489–496, Apr. 2014. DOI 10.4014/jmb.1312.12072. Available at: <https://www.jmb.or.kr/journal/view.html?doi=10.4014/jmb.1312.12072>. Accessed on: 2 May 2020.

ZHOU, Y.; YANG, J.; LUO, C.; YANG, B.; LIU, C.; XU, B. Effect of metal ions and surfactants on the enzymatic hydrolysis of pretreated lignocellulose. **BioResources**, Raleigh, vol. 14, no. 1, p. 1653–1667, 2019. DOI 10.15376/biores.14.1.1653-1667. Available at: <https://bioresources.cnr.ncsu.edu/resources/effect-of-metal-ions-and-surfactants-on-the-enzymatic-hydrolysis-of-pretreated-lignocellulose/>. Accessed on: 20 May 2020.

ZHU, J. Y.; PAN, X.; ZALESNY, R. S. Pretreatment of woody biomass for biofuel production: energy efficiency, technologies, and recalcitrance. **Applied Microbiology and Biotechnology**, Berlin, vol. 87, no. 3, p. 847–857, 2010. DOI 10.1007/s00253-010-2654-8. Available at: <https://link.springer.com/article/10.1007/s00253-010-2654-8>. Accessed on: 1 May 2020.

CHAPTER 2 - Hemicelluloses Role in Biorefinery Systems of Cellulosic Bioethanol, Particleboard, and Pulp and Paper Industries

ZAMORA ZAMORA, H. D.; OLAYIWOLA, H. O.; JACOBUS, A. P.; GROSS, J.; TYHODA, L.; BRIENZO, M. Hemicelluloses Role in Biorefinery Systems of Cellulosic Bioethanol, Particleboard, and Pulp and Paper Industries. In: BRIENZO, M. (ed.). **Hemicellulose Biorefinery: A Sustainable Solution for Value Addition to Bio-Based Products and Bioenergy**. Singapore: Springer Nature Singapore, 2022. p. 1–37. DOI 10.1007/978-981-16-3682-0_1. Available at: https://link.springer.com/chapter/10.1007/978-981-16-3682-0_1. Accessed on: 5 Jul. 2022.

Abstract

Hemicelluloses are located in the walls of vegetal cells and are the second most abundant macromolecules in the world. Thanks to the inherent chemical composition, hemicelluloses can be directly used as substrates for enzymes synthesis and in film or polymer manufacture, or turned into several products, which include among others biofuels, biopolymers, organic acids, sweeteners, and intermediate bioproducts. However, for the generation of such value-added bioproducts as mentioned above, to succeed, there is a requirement for the development of appropriate conversion paths and processes from hemicelluloses under the realm of the biorefinery concept. One of the major challenges in the conversion of hemicelluloses to bioproducts is due to the inherent links that exist between hemicelluloses and such cell wall components as lignin and cellulose. Due to these links, it is difficult to liberate hemicelluloses from biomass with most processes, most notably the pulping process, without incurring any losses or degradation of structural properties. With the selection and application of appropriate processing routes, most of the hemicelluloses can be recovered, and can serve as precursors or raw materials for the development of value-added products in a biorefinery scheme. The production processes for cellulosic bioethanol, particleboard and, pulp and paper are potential avenues where hemicelluloses can be recovered and upgraded without significant effects on the main process or product. The most common techniques for hemicelluloses extraction include chemical paths, which are characterized by the use of hydroxides, organic solvents, acids and peroxides. In the realm of a biorefinery, these techniques can be applied prior to cellulosic bioethanol, pulp and paper or particleboard production to recover hemicelluloses in a much purer form and high structural integrity in order to be functionalized or converted into

bioproducts integrated. In this context, the chapter presents potential avenues for hemicelluloses recovery and application of such recovered hemicelluloses for bioproducts development. The chapter further details specific applications and challenges related to such applications.

Keywords

Biomass utilization, Hemicelluloses, Biorefinery, Renewable energy

2.1. Introduction

The biorefinery (BRF) concept is of great interest as promising route for bioproducts obtaining from biomass (BS). The central characteristic of the concept is the efficient use of a diverse array of bio-based precursor materials, such as BS, to obtain several classes of value-added bioproducts under an integrated platform or platforms, or simultaneous processes. The BS that is most used under this scheme is the vegetal (VB), which can be gathered from agroindustrial crops or residues from VB processing (SHARMA; SAINI, 2020a).

The current dynamics of the global energy consumption points to crude oil as the central and the main source that supports the world energy and chemical supply matrix (in 2019 oil shared 33.1%) (BRITISH PETROLEUM, 2020). This is largely, due to the fact that oil can be processed through a refinery system that allows production of several chemical products (U.S. ENERGY INFORMATION ADMINISTRATION, 2012). However, the drawbacks associated with the use of crude oil including its extraction, refining, and the use of its derivatives are associated with the generation of greenhouse gas emissions, which have huge negative effects to human health and the environment (GUEDES *et al.*, 2019; HOEGH-GULDBERG; JACOB; TAYLOR, 2018). BS, especially from vegetal or animal waste, has become in an alternative raw material to replace oil and other non-renewable resources like coal and natural gas (FELIPUCI *et al.*, 2021). One of the challenges concerning the use of BS as an alternative to crude oil is the lack of similar processing routes as those of crude oil due mainly to the dissimilarities of the two materials respect to structure and properties. The biorefinery concept was therefore born out of this need to develop bioproducts using BS as an alternative feedstock to crude oil, taking advantage of its renewable nature and wide availability (ZAMORA *et al.*, 2021).

The biorefinery concept has received tremendous attention in research recently owing to the need to reduce greenhouse emissions from oil-based products. Further, such BS utilized in industries as the pulp and paper, saw milling, board production, sugar processing has adopted this concept so that they can add value to such BS residue which do not lead to product, as black liquor, saw dust, wood chipping rejects, and bagasse respectively. BS has a distinct chemical structure, which is principally constituted of water and predominantly of cellulose (CL), hemicelluloses (HC) and lignin (LG) (BELLO *et al.*, 2021; GE *et al.*, 2018; YOUSUF; PIROZZI; SANNINO, 2020). BS composition widely varies due to the vast nature and number of plant species and growing conditions that these plants are subjected to on the planet. Such differences can be seen in Table 2-1 below, which indicates the proportions of each of the major macromolecules i.e., CL, HC and LG of several BS processing residues material on a dry basis.

Table 2-1 - Different mass proportions of Cellulose (CL), hemicelluloses (HC) and lignin (LG) in selected waste vegetal biomass (VB)

Vegetal Biomass	CL (%)	HC (%)	LG (%)	Reference
MD2 pineapple leaves	30	37	22	Mansor <i>et al.</i> , 2019
MD2 pineapple stem	37	34	20	
MD2 pineapple root	42	32	19	
Coffee cut-stems	40.4	34	10.1	García <i>et al.</i> , 2018
Rice husk	35	33	23	Hafemann <i>et al.</i> , 2020
Tamarind husk	14.9	33.4	25.8	Alves <i>et al.</i> , 2020
Tamarind seed	20.9	15.8	28.8	
Sugar bagasse	42.8	27.6	24.2	Silva <i>et al.</i> , 2018
Waste triticale	31.9	25.4	18.4	Kucharska <i>et al.</i> , 2020
Meadow grass	27.1	22.6	16.8	
Beech	38.6	19.9	28.3	
Empty fruit bunches	30.4	19.4	33.3	Coral <i>et al.</i> , 2016
Corn straw	36.2	28.1	21.9	Cutrim <i>et al.</i> , 2019
Corn cob	28	26.7	21.7	
Rice peel	30.6	14.5	29.7	
Soy peel	22.4	13	17.5	

Cassava peel	24.2	4.9	50.7	
Wheat straw	37.5	23.8	20.5	
Pine bark	21.9	18.3	40.7	Díez <i>et al.</i> , 2020
Spruce bark	29.7	13.9	45.1	
Orange bagasse pellet	5.4	13.6	6.2	Da Silva <i>et al.</i> , 2020
Coffee silver skin	20.9	7.7	30.5	Niglio <i>et al.</i> , 2020

CL: Cellulose; HC: Hemicelluloses; LG: Lignin

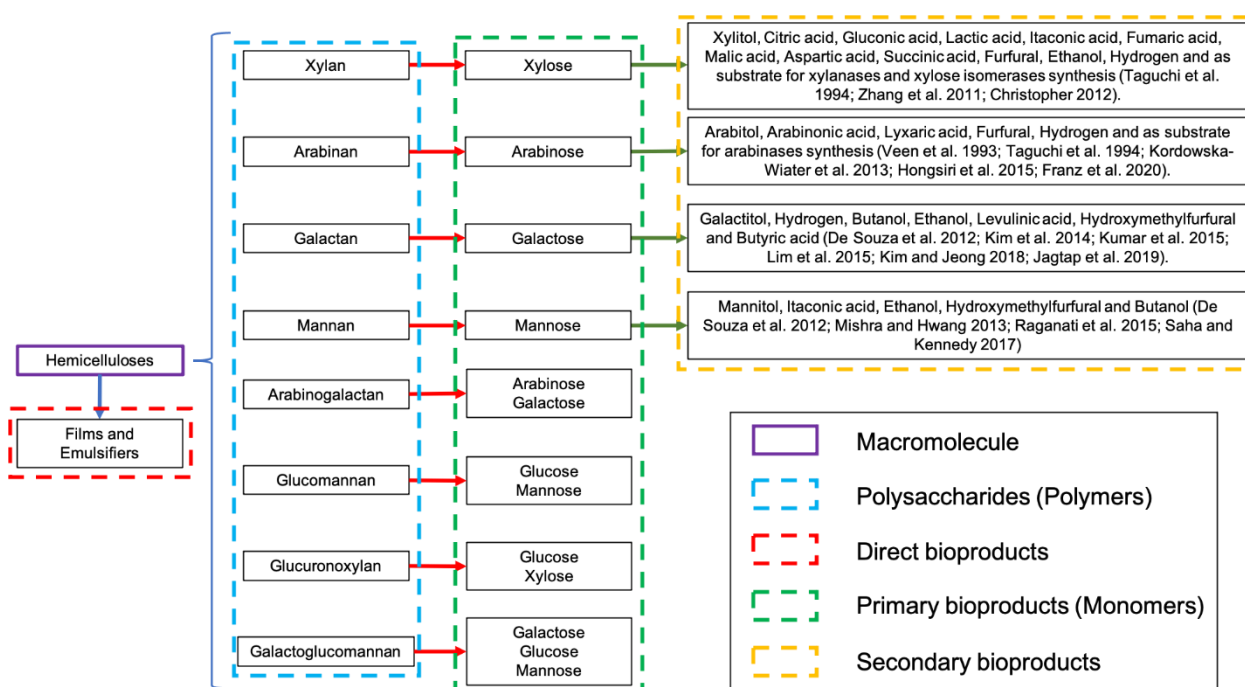
Source: By author

Although the chemical compositions of LC materials are dissimilar, they share similar functions. The principal constituents (CL, HC and LG) are located into walls of vegetal cells and have particular tasks, that is to protect vegetal cells against fungal and insect attacks as well degradation by UV ray. Further, they facilitate the transport water, nutrients and minerals. (ALBERTS *et al.*, 2014). From a biological point of view, the LC configuration resulting from its intricate structure allows it to suitably perform its role as discussed above. However, the very same intricate structure is a huge challenge to the processing or separation of the BS components due to the intimate association between CL, HC and LG, which results in immense resistance to processing (FÜLÖP; ECKER, 2020). The strong association of BS individual components is responsible of its recalcitrance. Therefore, the primary aim of any BS-processing endeavor should first be the establishment of suitable processing procedures to overcome its recalcitrance so that individual components can be more accessible and obtained in high yield and quality. These could then be used as feedstocks to different bioproducts (ZOGHLAMI; PAËS, 2019a).

The biorefinery system is mainly comprised of the sugar and the LG platforms. The sugar platform focusses on the development of biobased products from CL and HC. The LG platform on the other hand focuses on the valorization of LG into various bioproducts. The biorefinery system in both platforms include two main stages, namely the primary and the secondary biorefinery stages. The primary biorefinery stage emphasises on such initial processing steps as pretreatment to change the recalcitrant structure so that it is amenable to further processing via chemicals or enzymes. Alternatively, it is comprised of a fractionation step aimed at separating the individual components so that they can be used as feedstocks downstream. The secondary biorefinery stage is comprised mainly of the conversion of the three main BS

macromolecules i.e. CL, HC and LG into intermediate and final bioproducts. (ZAMORA *et al.*, 2021). The mass yields in each stage largely depends on the VB composition and among the three main components, HC has received special attention due to its potential as a raw material for various bioproducts (DELGADO-ARCAÑO *et al.*, 2021; HILL; ALTGEN; RAUTKARI, 2021). The Figure 2.1 shows the most important direct, primary and secondary bioproducts that can be obtained from HC utilizing a BRF system.

Figure 2.1 - Main bioproducts developed from hemicelluloses (HC)



Source: By author

HC are natural macromolecules constituted by different polymers (mainly xylan, arabinan, mannan, galactan, arabinogalactan, glucomannan and galactoglucomannan), which in turn, are composed predominantly by monomers of hexoses and pentoses like xylose (XY), arabinose (AR), mannose, galactose and glucose (BENAIMECHE *et al.*, 2020). HC composition (dry basis) varies in each plant species and therefore in its respective byproducts. In VB, HC content can range from 5% to 40% (DÍEZ *et al.*, 2020; MANSOR *et al.*, 2019). Extracted raw HC (polymers mixture), can be mostly used as gum or emulsification agent and for film production. However, broadening the scope of its application, requires the establishment of

suitable processing conditions to tailor make its composition of constituent polymers for such applications (ABE; BRANCIFORTI; BRIENZO, 2021a; OLORUNSOLA, 2018).

2.2. Hemicelluloses valorization in the cellulosic bioethanol production

In a BRF system of LC biomass, the primary biorefinery, which includes, pretreatment as well as the secondary biorefinery step comprising mainly hydrolysis are key stages in the production of intermediate or final bioproducts depending on the processing conditions developed (SHARMA; SAINI, 2020a). Generally, physical, chemical, biological or combined methods are performed with the objective of enhancing access to the LC structural components through the removal or structural alteration of specific macromolecules contained in the LC biomass (FELIPUCI *et al.*, 2021; SHIMIZU *et al.*, 2020; SILVA *et al.*, 2018). For example, in 2nd generation (2G) bioethanol production various pretreatment techniques are applied whose focus is to remove LG and HC are applied. The presence of these components creates a protective barrier to CL, the main precursor macromolecule from which fermentable hexoses are obtained (NARESH *et al.*, 2019). Although very good results have been obtained with regards to the hydrolysis of CL into hexoses, current works have also focused on the development of new or better techniques for HC conversion as well, thus improving total sugar conversion efficiency and improving global yields in 2G bioethanol production (GUIGOU *et al.*, 2017).

As discussed previously and also illustrated in Figure 2.1, HC are considered copolymers constituted by different types of polymers, among them are xylan, arabinan, galactan, mannan, arabinogalactan, glucomannan, glucuronoxylan and galactoglucomannan (BENAIMECHE *et al.*, 2020). These polymers are formed through polymerization of monomeric sugar units. These monomers can be the first bioproducts obtained through a BRF system. However, for an efficient conversion of the polymers to monomers to occur, the LC biomass must undergo some sort of physical, chemical or enzymatic treatment (MELATI *et al.*, 2019). Based on HC chemical composition, there are two possible ways for improving global yields in 2G bioethanol production. The first one is related to the valorization of HC through direct use in materials or products with emulsifying features. The second pathway is geared towards the conversion of HC through supplementary processes (pretreatment and hydrolysis) to obtain additional bioproducts (DELGADO-ARCAÑO *et al.*, 2021;

OLORUNSOLA, 2018; ZHAO *et al.*, 2020a). In both cases (direct uses and supplementary processes), it is necessary to implement a delignification stage, which can be performed prior to or simultaneously with HC extraction (MARTINO *et al.*, 2017).

The one-pot processing i.e., direct HC extraction without a separate delignification stage, using an alkaline-peroxide pretreatment, has been shown to efficiently remove LG while extracting HC in one single step. The resulting LC residue has shown good enzymatic digestibility for its subsequent hydrolysis (ALVES *et al.*, 2021; MELATI *et al.*, 2021). On the other hand, when delignification and HC extraction are performed as separate processes, it is common to complete LG removal first. This can be achieved through alkaline-organosolv delignification at 75 °C, using 30% solvent and 70% alkali concentrations (FARHAT *et al.*, 2017a). After delignification, HC can be extracted with alkali using low hydroxide concentrations (4% to 20% at moderate temperatures) and washings with 96% ethanol to precipitate HC (FLÓREZ-PARDO; GONZÁLEZ-CÓRDOBA; LÓPEZ-GALÁN, 2018).

Whether one pot or separate treatment process is applied for HC extraction and LG removal, several options of procedures are available for obtaining monosaccharides (xylose, arabinose, mannose, galactose and glucose). The extraction of XY for instance, often follows the use of inorganic acid solutions (around to 50% of concentration) and low solid loadings in a solid/liquid ratios of 2 to 8% m/v (SANTOS, C *et al.*, 2020). Nevertheless, the acid route is not the only route by which XY can be produced from HC (especially xylan). Enzymatic procedures that break down HC into XY through xylanases treatment of BS have also been successfully applied (DE FREITAS *et al.*, 2021; FELIPUCI *et al.*, 2021; FORSAN *et al.*, 2021). When such enzymatic routes for HC hydrolysis are incorporated in a BRF system, this normally follows two routes; the first one is related to the use of synthesized xylanases from specific LC substrates and specific microorganisms and the second one is based on the use of commercial cocktails of xylanases (BHARDWAJ; KUMAR; VERMA, 2019).

Once free XY is obtained through any or combination of the aforementioned processes, it can then be turned into bioethanol through fermentation processes using different strains of *Candida shehatae*, *Pichia (Scheffersomyces) stipitis*, *Spathaspora passalidarum*, *Pachysolen tannophilus* and genetically engineered *Saccharomyces cerevisiae* (MILESSI *et al.*, 2020; ROBAK; BALCEREK, 2018; RODRUSAMEE;

SATTAYAWAT; YAMADA, 2018). However, this procedure has shown several issues related to operational conditions, including the microorganism selected and these have an impact of bioethanol yield.

2.2.1. Hemicelluloses recovery in 2G bioethanol process

According to diverse studies, 2G bioethanol is not cost-effective yet compared with 1st generation (1G) bioethanol or electricity production (LOSORDO *et al.*, 2016). This is because 2G production exhibits low profitability when only 2G biofuel is produced. This relates mainly to such factors as the high enzymes cost, costs attributed to energy consumption in different stages (mainly pretreatment and hydrolysis) as well loss of potential revenue as a result of non-utilization of byproducts with high potential to be turned into products (ZHANG, 2019). In order to deal with these financial drawbacks, economic models can be used to predict business favorable scenarios when 2G processes are incorporated to 1G processes, or when other processes aimed at adding value to side streams are incorporated (CORAL; MAGALHAES, 2020; JUNQUEIRA *et al.*, 2017).

In the 2G bioethanol framework, one of the main objectives is to deconstruct LC biomass and to try to remove LG and HC through different techniques. However, from the BRF point of view both LG and HC can have value as raw materials for obtaining several value-added bioproducts using suitable procedures (SHARMA; SAINI, 2020a). In the HC case, it is possible to obtain xylitol, citric acid, gluconic acid, lactic acid, itaconic acid, fumaric acid, malic acid, aspartic acid, succinic acid, furfural, bioethanol and hydrogen from XY (CHRISTOPHER, 2012; TAGUCHI *et al.*, 1994; ZHANG; TU; PAICE, 2011). Arabitol, arabinonic acid, lyxaric acid, furfural, hydrogen from AR (FRANZ *et al.*, 2020; HONGSIRI; DANON; DE JONG, 2015; KORDOWSKA-WIATER; KUBIK-KOMAR; TARGOŃSKI, 2013; TAGUCHI *et al.*, 1994; VEEN *et al.*, 1993). Galactitol, hydrogen, butanol, bioethanol, levulinic acid, hydroxymethylfurfural and butyric acid can be obtained from galactose (DE SOUZA *et al.*, 2012; JAGTAP *et al.*, 2019; KIM; JEONG, 2018; KIM *et al.*, 2014; KUMAR *et al.*, 2015; LIM; LIM; JUNG, 2015). Mannitol, itaconic acid, bioethanol, hydroxymethylfurfural and butanol can be obtained from mannose (DE SOUZA *et al.*, 2012; MISHRA; HWANG, 2013; RAGANATI *et al.*, 2015; SAHA; KENNEDY, 2017).

Continuing with BRF context, some works have analyzed several methods for simultaneous breaking down of HC and CL into pentoses and hexoses using different kind of pretreatments (physical, chemical, biological or combined). Classical acid pretreatments using diluted conditions (0.1 – 10%) at mild temperatures (100 – 250 °C) allow for production of sugar monomers while reduce fermentation inhibitors from forming (BARUAH *et al.*, 2018). Modified Fenton pretreatment (peroxide + chloride + solvent), at 130 °C, 1.5 bar and short reaction times (0.5 to 4 h) exhibits high yields of carbohydrates recovery (94%) in saccharide solution (that subsequently is driven to hydrolysis and fermentation) and allows a separation of up to 87% of LG by simply filtration. Steam explosion pretreatment catalyzed by acid catalysts is of particular importance as it contributes to maximize HC solubilization in water and its subsequent conversion into hexoses and pentoses through hydrolysis of glycosidic bonds in CL and HC (mainly in HC), which improves efficiency of polysaccharides recovery/separation (ROBAK; BALCEREK, 2018). Its common configurations are of high pressure (0.5 – 5 MPa), moderate temperature (150 – 270 °C) and low retention times (1 – 10 min) to prevent inhibitors formation. (YU *et al.*, 2018).

Finally, it is important to mention that depending on chemical pretreatments performed for taking advantage HC, HC-based products can have different characteristics. For instance, if diluted acid methods are applied, then the main obtained bioproducts will be XY and xylooligosaccharides. If on the other hand alkali procedures are performed, then the polymeric form of XY will be extracted (BRIENZO *et al.*, 2016).

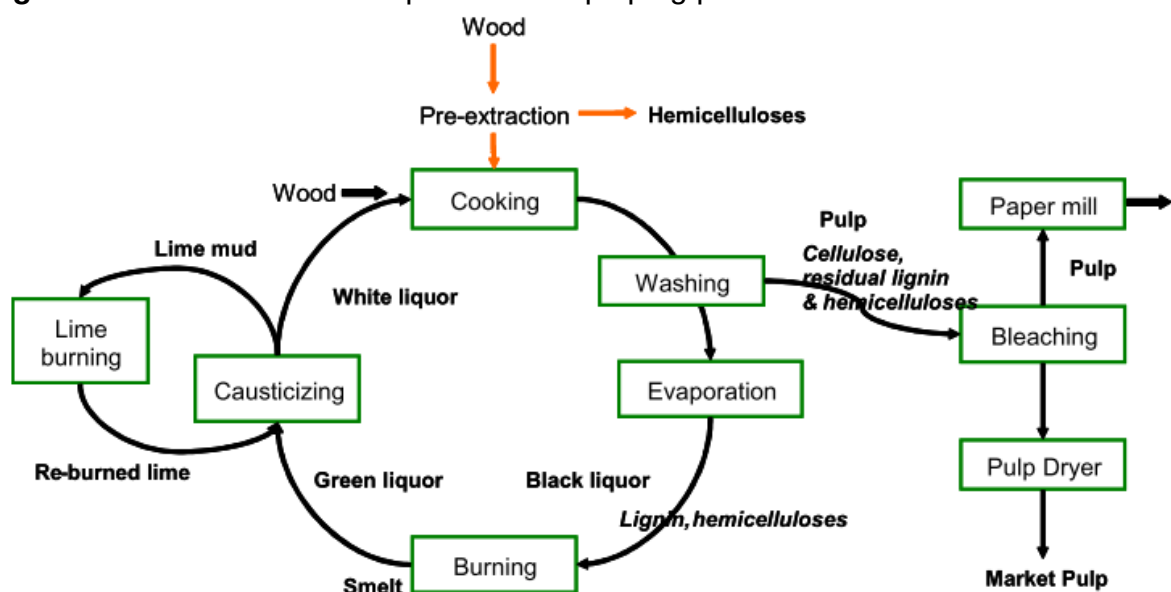
2.3. Hemicelluloses valorization in the Pulp and Paper Industry

The increasing demand of products from virgin feedstocks and the resultant pressure on the environment has expanded the concept of biorefinery which is based on exhaustive utilization of feedstocks. The implementation of biorefinery concepts in the pulp mills leads to sustainable co-production of paper, fuels, power and valuable chemicals from different LC materials (VENA, 2013). In the standard kraft pulping process a significant quantity of HC which is equivalent to about 15% of the feedstock is dissolved into the black liquor (CHRISTOPHER, 2013). The black liquor is recycled back to the reboiler for combustion due to the high heating value of dissolved LG (about 26.9 MJ/kg), but the low heating value of HC (about 13.6 MJ/kg) makes it underutilized

when incinerated in the boiler. Hence, pre-extraction of HC prior to pulping entails important economic benefits by offering more valuable alternative in generating additional streams of high value products (UM; VAN WALSUM, 2010). They have excellent potentials in food and pharmaceutical industries (JOUBERT; CHIMPHANGO; GÖRGENS, 2016).

The extraction of HC from the feedstock has both advantages and disadvantages on the final pulp and paper, depending on the extraction methods and the pulping process. Common extraction methods include steam, hot water, dilute acid and alkaline treatments (AL-DAJANI; TSCHIRNER; JENSEN, 2009; KÄMPPI *et al.*, 2010; LEI *et al.*, 2010). Hot water and acid methods produce higher HC yields, but the severity of the treatments may reduce the final pulp yield and paper quality (MENDES *et al.*, 2011; VENA, 2013). HC recovery vary considerably during alkaline extraction, but the effect on the pulp yield reduction is minimal (AL-DAJANI; TSCHIRNER; JENSEN, 2009; JUN; TSCHIRNER; TAUER, 2012; VENA, 2013). Pre-extraction prior to pulping increases delignification which causes reduction in chemical consumption during cooking and bleaching stages (KAUTTO; SAUKKONEN; HENRICSON, 2010; YOON; TUNC; HEININGEN, 2011). Despite this benefit, its removal could have detrimental effects as it contributes to pulp quality. HC has a high affinity for water molecules which improves pulp beatability (BAN *et al.*, 2011). It also improves the tensile, burst and tear strength of paper products by serving as an inter-fiber binding agent (LIMA; OLIVEIRA; BUCKERIDGE, 2003; SCHÖNBERG *et al.*, 2001).

Figure 2.2 - Extraction of HC prior to kraft pulping process



Source: Vena (2013)

As discussed in the above section, HC extraction can be integrated into the pulping process (JOUBERT; CHIMPHANGO; GÖRGENS, 2016; VENA, 2013). According to Postma & Chimphango (2014), the extracted HC can be functionalized into a product which can be used as an additive in paper making. The authors extracted 4-O-methyl glucuronoxylan (Me-GluX) from *Eucalyptus grandis*, under mild alkaline conditions, which was cationized and added to *E. grandis* bleached kraft pulp before refining. This resulted in improved tensile, burst and tear strength when compared to cationic starch. Therefore, in order to fully harness the potentials HC pre-extraction and pulping, a combined approach requiring optimization of pre-extraction conditions and pulping process with minimal degradation of the CL component should be prioritized (VENA, 2013).

2.4. Hemicelluloses valorization in particleboard fabrication

Wood fibers are incorporated into polymeric resins, thermoplastics, and inorganic binders in a bid to produce low cost, sustainable building components, or as a means of adding values to secondary materials. Their unique attributes such as low cost, biodegradability, sustainability, low density, and good mechanical properties make them suitable alternatives to synthetic reinforcements in composite panel construction. The cell wall of wood is composed of CL, HC, LG, extractives, and minerals in varying compositions. The influence of the components is dependent on the type of the binder. In polymeric and thermoplastic matrices, the susceptibility of wood to water uptake and fungal decay is the major challenge. HC are the most hydrophilic wood components and are responsible for high water affinity in thermoplastic binder (PELAEZ-SAMANIEGO *et al.*, 2014; PEREIRA FERRAZ *et al.*, 2016). Moisture uptake causes swelling which stresses the interfacial fiber-plastic bonds (PELAEZ-SAMANIEGO *et al.*, 2013). In inorganic matrices, the HC and extractives inhibit setting and are well-known causes of wood-cement incompatibility. They promote the formation of impermeable shields around non-hydrated cement grains, depriving them access to water, thereby impairing fiber-matrix bond (QUIROGA; MARZOCCHI; RINTOUL, 2016).

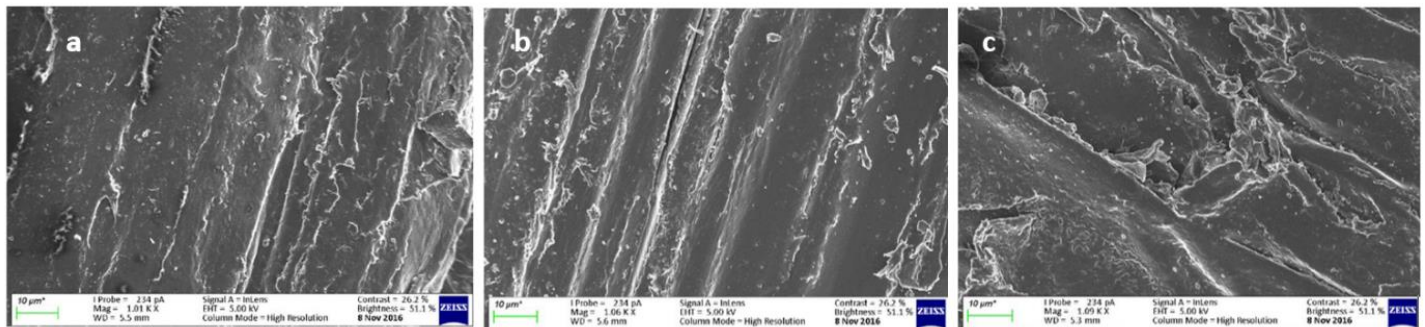
Pretreatment of wood to remove or modify the hydrophilic and inhibitory components becomes mandatory to improve their performance in the different matrices. The treatments include thermal and chemical or a combination of both processes. Thermal treatment involving aqueous hot water extraction removes sugars, and other inhibitory water soluble compounds (PELAEZ-SAMANIEGO *et al.*, 2013; QUIROGA; MARZOCCHI; RINTOUL, 2016). It reduces the hygroscopicity, improved the crystallinity of CL, enhance the dimensional stability and resistance to degradation (PELAEZ-SAMANIEGO *et al.*, 2013). Hot water extraction is the most environmentally treatment as it does not require additional chemicals (GARROTE; DOMÍNGUEZ; PARAJO, 1999). Chemical treatments include alkalization, acetylation, methylation, cyanoethylation, and sulfuric acid treatment (BLEDZKI *et al.*, 2008; OLADELE; OLAJIDE; OGUNBADEJO, 2015). Alkalization delignifies the sample and degrades the HC into non-inhibitory substances (QUIROGA; MARZOCCHI; RINTOUL, 2016). Other chemical treatments such as acetylation and methylation modify the BS components to become more hydrophobic, thereby improving their durability and dimensional stability. Acetylation causes the hydroxyl groups in amorphous CL, LG and HC with acetyl group, thereby reducing the available sorption sites on the fiber surface (BLEDZKI *et al.*, 2008).

Pelaez-Samaniego *et al.*, (2014) incorporated hot water extracted hardwood and softwood chips in polymeric resin (Urea Formaldehyde) matrix and concluded that the removal of HC improved the dimensional stability of the boards. Although the effect was not significant on the mechanical properties, the removal of HC promoted easier compression and flexibility of the fiber. A similar observation was reported in a separate study where hot water treated pine particles were encapsulated in PP and HDPE matrices (PELAEZ-SAMANIEGO *et al.*, 2013). The treatment had significant effect on the moisture resistance of the boards and had an overall positive impact on the board quality. Extraction of HC from anaerobic digested dairy fibers incorporated into HDPE reduced the hydrophilicity of the fibers and improved the stiffness and mechanical strength of the boards by 30% and 36%, respectively (PEREIRA FERRAZ *et al.*, 2016).

Enhancement of properties of inorganic-bonded particleboards have also been reported. Amiandamhen *et al.* (2018) incorporated pretreated natural fibers into phosphate-based inorganic binder. The treatment influenced the fiber properties and improvement was observed in all the properties evaluated. It was recommended that a mild alkaline treatment is sufficient to improve the properties of the boards.

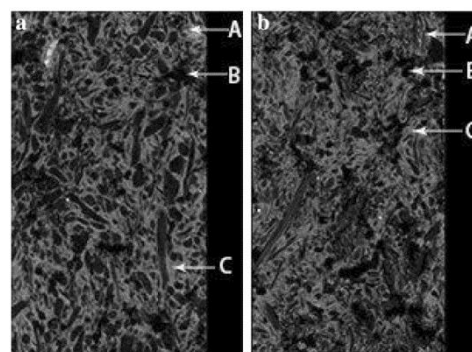
As shown in the SEM images in Figure 2.3, the authors posited that apart from altering the chemical compositions of the fiber, treatment methods removed other impurities such as waxes and pectin. The 2D segmentation image shown in Figure 2.4 indicated that the removal of the impurities, as a result of the pretreatment, enhanced the bonding between the fibers and the binder. The modulus of rupture of untreated bagasse boards increased from 150MPa to 175MPa after alkali treatment. This also caused a reduction in the water absorption potential of the boards by 33%. It was recommended that a mild alkaline treatment is sufficient to improve the properties of the boards.

Figure 2.3 - SEM micrograph of (a) untreated (b) hot water extracted and (c) alkalinized bagasse fibers



Source: Amiandamhen; Meincken; Tyhoda (2018)

Figure 2.4 - 2D segmentation of bagasse panel untreated (a), alkali treated (b) (A fibers, B voids, C matrix)

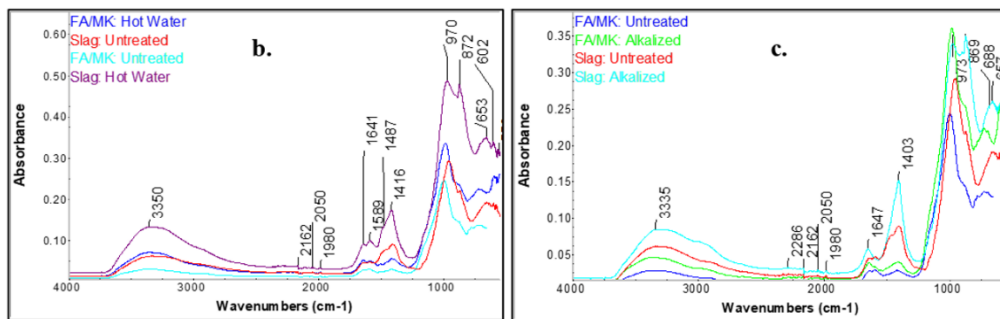


Source: Amiandamhen; Meincken; Tyhoda (2018)

A similar observation has been reported in geopolymer bonded wood composites (ASANTE, 2020; OLAYIWOLA, 2021). Olayiwola et al. (2021) reported that unlike in ordinary Portland cement, the presence of HC did not prevent geopolymer

setting, but lowered the reaction kinetics. The reaction improved after pretreatment. The FTIR spectra shown in Figure 2.5 indicated formation of new geopolymeric products, which confirms the observation that pretreatment enhanced the geopolymerization reactions. Mild alkali treatment of *A. mearnsii* particles incorporated in a 100% slag based geopolymer increased the modulus of rupture of the panels by 23.94% (OLAYIWOLA, 2021).

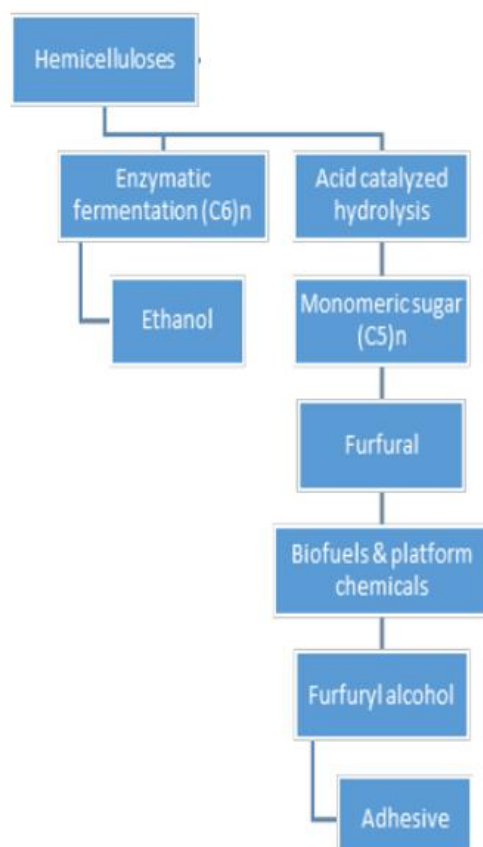
Figure 2.5 - FTIR spectra of untreated bagasse fibers, hot water extracted (a) and mild alkali treated (b) in fly ash-metakaolin and slag based geopolymer matrix



Source: Olayiwola (2021)

While the discussion above focuses solely on the removal of HC to improve fiber properties, a few studies are now reporting on value addition on such extracted HC. Soludongwe (2020) examined the co-production of furfural from extracted XY and wood composites from the remaining wood residue in a manner resembling a process called value prior pulping (VPP) in the pulp industry. Through this approach, HC was extracted and then used a raw material to produce furfural. The furfural would then be functionalized to produce a furfural-based binder, which then could be used to bind BS particles during composite product production. This shows that pretreatment, in addition to improving BS properties, could also be used to produce raw materials such as furfural which finds wide application in foundry and furfural-based binding derivatives (BINDER *et al.*, 2010; ORLOVA, 2016). Orlova (2016) developed a furfural-based binder which had good polymerization, polycondensation and hardening properties. The binders could find applications in producing caulking compound, putties and chemically resistant gaskets and joints. Figure 2.6 shows the different pathways of bioconversion of HC.

Figure 2.6 - Different bioconversion pathways of HC (Adapted from Soludongwe, 2020).



Source: By author

2.5. Challenges of Biological Aspects of Pentoses Fermentation of Lignocellulosic Hydrolysates using *Saccharomyces cerevisiae*

The biofuel industry in Brazil stands as one of the most successful in the world with regards to cost-effective bioethanol generation from sugarcane. The bioethanol (first generation) is based on fermentation of sugars (mainly sucrose) derived from the soluble fraction of processed sugarcane plants using *Saccharomyces cerevisiae* (BASSO *et al.*, 2013; DOS SANTOS *et al.*, 2016a). Despite the easy access to available hexoses, there is a fraction of sugars that still are unavailable for conversion in the sugarcane BS, which if converted would lead to an increase in bioethanol yield. These sugars are not soluble, but being part of a complex structure, as glycoside molecules, on the plant cell wall (known as lignocellulose) (BASSO *et al.*, 2013; DOS SANTOS *et al.*, 2016a; ISIKGOR; BECER, 2015). Typical plant BS contains approximately 40–50% CL, 20–40% HC and 20–35% LG (LIAO *et al.*, 2016).

According to Nunes *et al.*, (2020), sugarcane bagasse is, on average, made up of 42.2%±1.93 CL, 27.6%±0.88 HC, 21.6%±1.67 LG, 5.63%±2.31 extractive compounds (sugars), and 2.84%±1.22 ash. Considering that 140 kg of dried bagasse is produced per ton of processed sugarcane in the 2020/2021 sugarcane season, in Brazil, were harvested 665.105 millions of tons of sugarcane producing 93.114 millions of tons of dry bagasse (CONAB, 2020; PEREIRA *et al.*, 2015). In addition, only 32 millions of liters from cellulosic bioethanol (2G) were estimated to be produced in 2020 contrasting with 31.35 billion liters from 1G bioethanol in Brazil (USDA, 2020).

There is still a huge opportunity to grow on cellulosic bioethanol production. However, challenges relating to economic and technical factors still need to be resolved. Among the technical challenges is the realization of efficient plant BS deconstruction techniques with cost-effective enzymatic treatments as well as an efficient implementation of large-scale fermentations in industrial settings (BASSO *et al.*, 2013; DOS SANTOS *et al.*, 2016a). Another important limitation is that *S. cerevisiae* does not have the metabolic arsenal to convert pentoses, which comprise an economically relevant fraction of sugars released from cellulosic BS (BASSO *et al.*, 2013; DOS SANTOS *et al.*, 2016a). In the LC biomass the amounts of D-XY and L-AR, derived from HC and pectin polymers in plant BS, account for 10–25% and 2–3%, respectively, of the carbohydrate content of LC feedstocks (LYND, 1996). That problem has been addressed by genetically engineering yeasts for efficient conversion of XY and AR into bioethanol (DEMEKE *et al.*, 2013; DOS SANTOS *et al.*, 2016b) that will be shortly described below. Nevertheless, one of the remaining complications for implementation of a 2G bioethanol production is the significant inhibition of yeast fermentation by toxic compounds derived from lignocellulose polymers (BASSO *et al.*, 2013; DOS SANTOS, Leandro Vieira; CARAZZOLLE; *et al.*, 2016; ISIKGOR; BECER, 2015).

2.5.1. Yeast Engineering For 2nd Generation Bioethanol

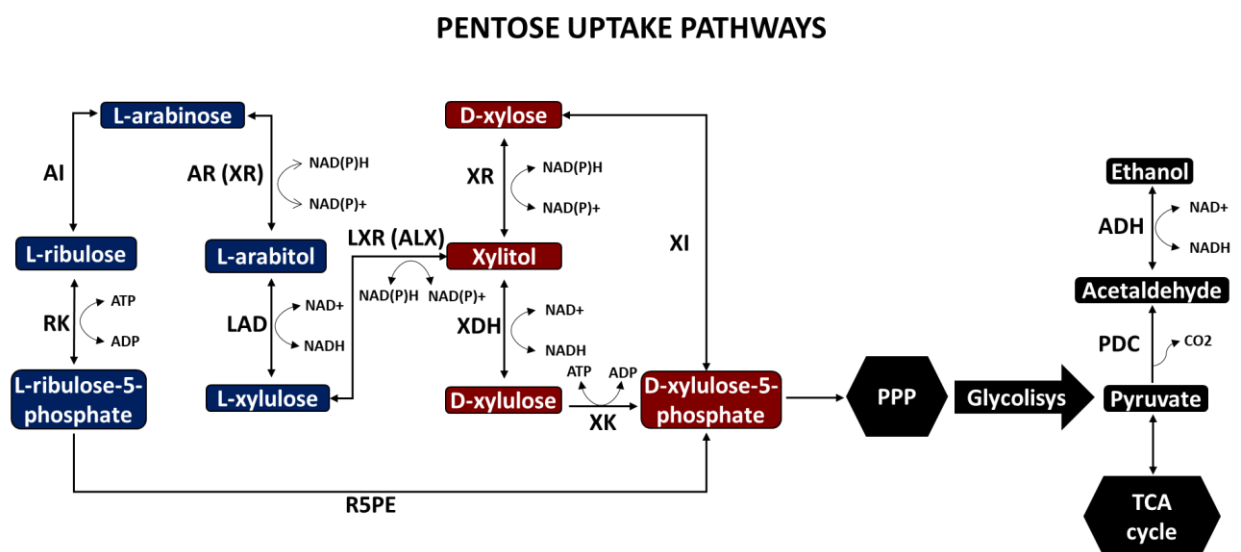
For many decades, researchers have been exploring ways to increase the efficiency of pentose fermentation/ethanol production using *Saccharomyces cerevisiae*. Recently, this quest has benefitted from the availability of such advanced tools as genomics (whole genome sequencing, QTL analysis), genome editing, evolutionary engineering (metabolic engineering, experimental evolution) and protein

engineering (JANSEN *et al.*, 2017). The strategy to build yeast strains that are capable to consume XY and AR addresses is based in to link the sugars up taking with glycolysis producing bioethanol economically worthwhile.

2.5.1.1. Xylose Fermentation

For D-XY, researchers described that two modifications are necessary (JEFFRIES; JIN, 2004; VAN MARIS *et al.*, 2007). The first involves the conversion of XY into D-xylulose through a heterologous pathway. The second focuses on the modification of *Saccharomyces cerevisiae* xylulokinase and non-oxidative pentose-phosphate pathway (PPP). For conversion of XY to D-xylulose two strategies were developed; i.e. one that relies on simultaneous expression of a heterologous xylose reductase (XR) and xylitol dehydrogenase (XDH). This is the strategy found in some yeast and filamentous fungi. The other approach is related to the expression of a heterologous xylose isomerase (XI), mechanism from procaryotes, mainly (HAHN-HÄGERDAL *et al.*, 2001) (Figure 2.7).

Figure 2.7 - Representative scheme of pentose uptake/fermentation pathways. The figure shows metabolic oxireductase and isomerase pathways for Arabinose (blue) and Xylose (red) in fungi and bacteria, and central metabolism (black).



AR(XR); Aldose reductase, LAD; L-arabitol-4-dehydrogenase, LXR(ALX); L-xylulose reductase, XDH; D-xylulose reductase, XK; Xylulokinase, AI; L-arabinose isomerase, RK; L-Ribulokinase, R5PE; L-

Ribulose-5-P-4-epimerase; PDC, pyruvate decarboxylase; ADH alcohol dehydrogenase; PPP, Phospho Pentose Pathway, TCA cycle; tricarboxylic acid cycle

Source: By author

The first attempt was to engineer XR and XDH focused on using those enzymes from xylose-metabolizing yeast *Scheffersomyces stipitis* (KÖTTER; CIRIACY, 1993). Having *S. stipitis* as the donor organism and based on its capability to utilize NADH in the XY reduction step. However, the recombinant *S. cerevisiae* expressing heterologous XR/XDH have resulted in low bioethanol yield and xylitol by-product formation (undesirable). This was found to be partially related to unfavorable thermodynamic properties of the reactions and that the worth nothing that first reaction (XR) preferably consumes NADPH, whereas the second reaction (XDH) exclusively produces NADH, which is not regenerate in anaerobic conditions. When less NADH is consumed in the XR reaction, then less NAD⁺ is available for the XDH reaction. If the amount of NAD⁺ is insufficient, xylitol is increased (BRUINENBERG *et al.*, 1983; RIZZI *et al.*, 1989).

Many strategies were developed to counterpart this setback. Among them was introducing a change in activity ratios for XR and XDH compensating the equilibrium constants. With this goal yeast strains with higher XDH activity than XR which works, when the high activity of XDH less xylitol was formed were built (WALFRIDSSON *et al.*, 1997). To this equation another enzyme was added. A third step (xylulose kinase) XK, the enzyme which phosphorylates xylulose to xylulose 5-phosphate, and was determined that the optimal XR:XDH:XK ratio was 1:10:4 for minimal xylitol formation and confirmed that a decreasing XR:XDH ratio correspondingly decreased xylitol and acetate formation and increased bioethanol production. Overproduction of XK also enhanced the specific XY consumption (HAHN-HÄGERDAL *et al.*, 2001).

Other approaches such as, Fusion Protein or Site-Specific Mutagenesis, but without greater success were used. On top of that, *S. cerevisiae* have a gene *GRE3*, that encodes a NADPH-dependent aldose reductase that can convert XY to xylitol (KUHN *et al.*, 1995). The activity of this enzyme could impair the redox balance, accumulating more xylitol resulting in a decrease in XY fermentation. To overcome that, the removal of *GRE3*, and genetic modifications aimed at increasing cofactor regeneration resulted in a decrease in xylitol accumulation while increasing bioethanol

production (COSTA *et al.*, 2017; CUNHA *et al.*, 2019; ROMANÍ *et al.*, 2015; TRÄFF *et al.*, 2001; VERHO *et al.*, 2003).

The second strategy to link XY with glycolysis is to use XI enzyme. This approach has the advantage of being a one-step reaction that directly converts XY to xylulose without cofactor requirement (KWAK; JIN, 2017; ZHOU *et al.*, 2012). The XI catalyzes the reaction in a reversible way involving the isomerization of XY and D-glucose to D-xylulose and D-fructose, respectively. Previous study of to express xylose isomerase from bacteria from *Actinoplanes missouriensis* (AMORE; WILHELM; HOLLENBERG, 1989) and *Clostridium thermosulfurogenes* (MOES; PRETORIUS; VAN ZYL, 1996) did not show expression of the protein despite the mRNA's being detected. Other attempts based on the use of XI from *E. coli* (HO, 1989; SARTHY *et al.*, 1987) and the *Bacillus subtilis* (AMORE; WILHELM; HOLLENBERG, 1989), heterologous expression of the gene directed synthesis of mostly insoluble protein, catalytically inactive. *Thermus thermophilus* XI was expressed successfully in *S. cerevisiae* but at low levels and xylitol by-product accumulation was observed (WALFRIDSSON *et al.*, 1996). The problem was solved with expression of a xylose isomerase from the fungus *Piromyces sp* leading to an efficient XY fermentation (KUYPER *et al.*, 2003).

The xylose isomerase from *Lachnoclostridium phytofermentans* expressed with highly efficient in *S. cerevisiae* (BRAT; BOLES; WIEDEMANN, 2009), among others such as from *Orpinomyces sp. ukk1*, *Ruminococcus flavefaciens*, *Prevotella ruminicola TC2-24*, *Burkholderia cenocepacia J2315*, *Ruminiclostridium cellulolyticum H10* and *Streptomyces rubiginosus*, as reviewed and tested by Seike *et al* (2019). The development of XI strains was completed with extensive mutagenesis and evolutionary engineering (DEMEKE *et al.*, 2013). Shortly, combination between expression of XI from fungi and overexpression of *S. cerevisiae* genes encoding xylulokinase and non-oxidative PPP enzymes enabled anaerobic yeast growth on XY (HARHANGI *et al.*, 2003). The deletion of *GRE3* gene and encoding an aldose reductase resulted in the reduction of xylitol formation and thereby increasing bioethanol yields on XY fermentation (KUYPER *et al.*, 2005). Using multicopy XI expression cassettes, with optimum codon usage and mutagenesis improved the efficiency of XY fermentation (LEE; JELLISON; ALPER, 2012). Mutations on *PHO13* phosphatase resulted in increased fermentation efficiency on XI and XR/XDH which are related to upregulation of PPP genes (XU, Haiqing *et al.*, 2016). Likewise, there are the identification of other

relevant mutations in evolved strains, in genes associated in iron–sulfur cluster assembly, in the mitogen-activated protein kinase signaling pathway and identified mutations affecting intracellular homeostasis of Mn^{2+} (DOS SANTOS *et al.*, 2016b; SATO *et al.*, 2016; VERHOEVEN *et al.*, 2017).

2.5.1.2. Arabinose Fermentation

L-arabinose is a five-carbon sugar found more abundantly in nature as an L isomer, which differentiates it from the other sugars which are most commonly in D form (YE; KIM; KIM, 2019). While L-arabinose from LC biomass derived from HC and pectin correspond at 2–3% of the carbohydrate content of LC feedstocks (LYND, 1996) and approximately 0.2%, g/g dry weight, L-arabinose content in various fruits and vegetables ranges from 3.3 to 21.6 g/L (YE; KIM; KIM, 2019). Fruit processing wastes are high in pectin (12-35%, g/g dry weight) with much less LG content (approximately 2%, g/g dry weight) than LC biomass (EDWARDS; DORAN-PETERSON, 2012), showing an important niche of wastes as biofuel or biomaterial sources research. Likewise, is stated that in corn fiber hydrolysates and in sugar beet pulp, the amount of L-arabinose is 26% of the total sugar content (GROHMANN; BOTHAST, 1997; LYND, 1996).

Arabinose metabolic pathways of native fungal and bacterial strains can be divided into the oxidoreductase and isomerase pathways, respectively (Figure 2.6). In these two pathways, L-arabinose is converted into D-xylulose-5-phosphate and then metabolized by the non-oxidative PPP (HAHN-HÄGERDAL *et al.*, 2007), or by the phospho- ketolase pathway (SERVINSKY *et al.*, 2012).

Fungal L-arabinose and D-xylose pathways share three enzymes: AR (L-arabinose specific XR), XDH and XK. However, the conversion of arabinose into D-Xylulose, different from D-xylose, needs two reduction and two oxidation reactions i.e., NAD(P)H-specific aldose reductase (AR or XR), ii. NAD^+ -specific L-arabitol-4-dehydrogenase (LAD), iii. NAD(P)H-specific L-xylose reductase (LXR or ALX), and iv. NAD^+ -specific xylitol dehydrogenase (XDH) (HAHN-HÄGERDAL *et al.*, 2007). The AR reductase enzyme usually prefers NADPH, while the dehydrogenases strictly use NAD^+ (with variation among other fungal species). However, in anaerobioses the production of NAD^+ is limited, depending on oxygen (mitochondrial electron chain) and thus impairing bioethanol production (DIEN *et al.*, 1996; KURTZMAN; DIEN, 1998).

Because of this difficulty related to redox balance in fungal oxireductase pathway, the strategy to produce a L-arabinose fermenting strain relies on the bacterial pathway that converts L-arabinose into xylulose-5-phosphate, including L-arabinose isomerase (AraA), L-ribulokinase (AraB), and L-ribulose-5-phosphate-4-epimerase (AraD) (LEE, N *et al.*, 1986). This pathway, followed by the non-oxidative PPP and glycolysis allows the L-arabinose fermentation and subsequently ethanol production with equilibrium or neutral redox balance. These enzymes are heterologously expressed in *S. cerevisiae* and could be from different origins such as araA gene from *Bacillus subtilis* (BECKER; BOLES, 2003), *Bacillus licheniformis* (WANG *et al.*, 2019; WIEDEMANN; BOLES, 2008) and *Lactobacillus plantarum* (WANG *et al.*, 2013; WISSELINK *et al.*, 2007). The araB and araD genes can be from *Escherichia coli* (WANG, Xin *et al.*, 2019; WIEDEMANN; BOLES, 2008) or *L. plantarum* (WANG *et al.*, 2013; WISSELINK *et al.*, 2007). Other approaches to improve L-arabinose fermentation have been tested. These include the overexpression of the non-oxidative PPP genes (*TAL1*, *TKL1*, *RPE1* and *RK11*), co-expression of L-arabinose fermenting enzymes in *S. cerevisiae* with araA from *E. coli* with the groEL and groES chaperonins and adaptive evolution strategies (BECKER; BOLES, 2003; WIEDEMANN; BOLES, 2008; WISSELINK *et al.*, 2007).

2.5.1.3. Engineering Pentose transports in *S. cerevisiae* strains

In yeasts, the main monosaccharides transporters are the major facilitator superfamily (MFS). These facilitation diffusion permeases are classified as uniport, symport (coupled to an ion transport) and antiport, and consist in two sets of six hydrophobic transmembrane-spanning α -helices connected by a hydrophilic loop (NIJLAND; DRIESSEN, 2020). There are no specific pentose transporters in *S. cerevisiae*. The pentoses can be internalized using the native hexoses transporters but with much less efficiency than hexoses. In *S. cerevisiae* are 18 Hxt (hexoses) transporters (Hxt1–17 and Gal2) in which the Hxt1p, Hxt2p, Hxt4p, Hxt5p, Hxt7p and Gal2p can also transport D-xylose (BUENO *et al.*, 2020). Meanwhile the *K_m* values for these pentoses are one to two orders of magnitude higher than for glucose (FARWICK *et al.*, 2014). The galactose transporter Gal2 also has a higher *K_m* for L-arabinose than for glucose (SUBTIL; BOLES, 2011).

All this together means that cells might only start transporting D-xylose and L-arabinose once D-glucose is decreased outside the cells. This feature not suitable for an industrial bioethanol process based on co-consumption of the sugars available to ferment. With the high K_m values for Hxt transporters for pentoses, the sugar fermentation is slowed down during the pentose-fermentation phase called “Tailing effect”. This sums up with the accumulation of ethanol and the reduction of inhibitor tolerance in *S. cerevisiae* at low sugar fermentation rates. This describes the difficulties for the whole scenario for pentose transport in *S. cerevisiae* naturally (JANSEN *et al.*, 2017).

In order to improve the co-consumption and transport of pentoses during the fermentation, several studies aimed at developing a specific pentose transporter from a heterologous source *S. cerevisiae* with have been embarked on (NIJLAND; DRIESSEN, 2020). As reviewed by Jansen *et al.*, (2017) and Nijland and Driessen (2020) low-, moderate-, and high-affinity pentose transporters from pentose-metabolizing filamentous fungi or non-*Saccharomyces* yeasts have been functionally expressed in *S. cerevisiae*, and their expression and activity were improved by direct evolution or evolutionary engineering.

In heterologous expression of xylose transporters in *S. cerevisiae*, some processes do result in an increase in xylose uptake. These include the Gxf1 from *Candida intermedia* which has a 2-fold lower K_m value for D-xylose transport, which increases xylose consumption, but only in low concentrations of the sugar (4 g/L) (RUNQUIST, D *et al.*, 2009). From *Arabidopsis thaliana*, a xylose transporter homolog based on a sequence similar to xylose-H⁺ symporter At5g59250 shows a slight improvement in the D-xylose uptake kinetics (RUNQUIST; HAHN-HÄGERDAL; RÅDSTRÖM, 2010). In addition, several transporters were studied (Sut1, Sut2, Sut3, Xut1, Xut3, Hxt2.6, Qup2) from *Scheffersomyces stipitis*, a well-known xylose fermenting organism. The Sut1, Sut2, and Sut3 encoding glucose transporters that showed lower K_m (at mM range) for xylose compared to glucose in *hxt* null *S. cerevisiae* strains and Sut1 also demonstrated increasing the D-xylose uptake ability and ethanol yield during D-xylose fermentation similar to glucose (KATAHIRA *et al.*, 2008). Xut1 and Xut3 transporters did uptake xylose alone and in co-fermentation with glucose and in a *S. cerevisiae* strain transformed with a genomic DNA library from *S. stipitis* were identified Xut1, Hxt2.6 and Qup2 as promising xylose transporters in an

experiment in which the strains were screened in sustaining growth on D-xylose (DE SALES *et al.*, 2015).

An arabinose:H⁺ symporter (BsAraE) from the *Bacillus subtilis* was expressed in a null *hxt* *S. cerevisiae* resulting into a 4 fold D-xylose consumption increase. However, xylitol production was also observed. These strains also consumed xylose with all Hxt transporters in place (KIM *et al.*, 2015). Buneo *et al.* (2020) have searched in diverse microbiomes, such as the digestive tract of plague insects, and isolated several yeast species capable of using xylose. In *Candida sojae* they found potential high-affinity transporters, of which the Cs4130 was able to consume xylose in high concentration (50 g/L). However, glucose fermentation inhibition also occurred by competition, a known fact for these transporters. In co-fermentation xylose/glucose had demonstrated the same profile as the previously described, using glucose (first 10 h first) then xylose fermentation.

In the case of L-arabinose uptake, less is known probably because it has less economic impact so far. As it is described above, Gal2 (galactose permease) is the arabinose preferential transporter in *S. cerevisiae*. If the Gal2 is deleted, the cell was found to abolish the L-arabinose transport (WISSELINK *et al.*, 2007), and with the overexpression of Gal2 this transport was increased (BECKER; BOLES, 2003). The L-arabinose uptake is also inhibited by competition with glucose that is preferentially transported by Gal2. Some strategies were applied to improve the flux of L-arabinose through known transporters, for example mutation on Gal2 substrate binding site (F85G). This F85G mutation was associated with an increase in the L-arabinose transport activity and on the counterpoint reduction on specificity of the transporter for D-glucose and D-xylose occurred (WANG *et al.*, 2017a). A mutation found in a genome sequencing of evolved D-glucose-phosphorylation-negative *S. cerevisiae* cells growing in xylose/glucose /arabinose, N376 of the Gal2, was found to decrease the affinity and *V*_{max} for glucose, while it also resulted in an increase in L-arabinose transport (WISSELINK *et al.*, 2014). Subtil and Boles (2011) demonstrated two arabinose transporters with high transport affinity derived from *Scheffersomyces stipitis* (AraT) and from *Arabidopsis thaliana* (Stp2). Both transporters could mediate the entrance for only L-arabinose but no other sugars such as glucose. They found that *S. cerevisiae* with all Hxt transporters deleted and with bacterial L-arabinose pathway expressed could no longer grow with arabinose being the only carbon source. This was possible only when other glucose transporters (Gal2, Hxt9 e Hxt10) were

expressed and with supplemented media such as maltose. They suggested that together with Gal2, this set of transporters can be used for industrial purposes.

Other heterologous arabinose transporters have been identified from *Neurospora crassa* and *Myceliophthora thermophila* (LI *et al.*, 2015), and *Penicillium chrysogenum* (BRACHER *et al.*, 2018). However, but it was established that glucose/xylose competition on those transporters favored the hexoses and still no heterologous pentose transporter was reported to either improve L- arabinose fermentation or allow arabinose and glucose simultaneous fermentation (YE; KIM; KIM, 2019).

2.6. Yield loss through the formation of inhibitors during the hydrolysis of lignocellulose materials

The key polymers comprising the lignocellulose fraction of plants are represented by the CL (D-glucose units condensed through $\beta(1\rightarrow4)$ -glycosidic bonds), the HC (xylan, galactomannan, glucuronoxylan, arabinoxylan, glucomannan and xyloglucan) and the LG (originated from the phenylpropanoid units p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol) (ISIKGOR; BECER, 2015; KUMAR *et al.*, 2009).

CL represents half of the organic carbon in the biosphere, while HC is the second most abundant biopolymer in nature (ISIKGOR; BECER, 2015). Such amounts highlight the great potential that lignocellulose may have in our future renewable energy matrix. Despite being found in vast quantities, the monomeric saccharide units from the lignocellulose are not readily available for microbial fermentation, and their release is dependent on hydrolysis of the constituting polysaccharides. Since *S. cerevisiae* cannot hydrolyze CL or HC, 2G bioethanol production must be preceded by a chemical/physical pretreatment, followed by chemical or enzymatic hydrolysis (BASSO *et al.*, 2013; DOS SANTOS *et al.*, 2016a; ISIKGOR; BECER, 2015).

The initial fractionation of lignocellulose into LG, CL, and HC is achieved by basically four types of pretreatments, such as 1) chemical (acid, alkaline, oxidative delignification, and organosolv), 2) physical (milling, pyrolysis, and microwave), 3) physicochemical (steam explosion, ammonia fiber explosion, and CO₂ explosion), and 4) biological (biopulping via fungi, bacteria, and specific enzymes) (DOS SANTOS *et al.*, 2016a; KUMAR *et al.*, 2009). Following pretreatment, hydrolysis can be obtained by chemical methods (e.g., dilute or concentrated acid hydrolysis) or enzymatically by

cocktails of hydrolases (BASSO *et al.*, 2013; DOS SANTOS *et al.*, 2016a). Hydrolysis ensures that CL is broken down into its glucose units, while HC is decomposed into its C5 (xylose and arabinose) and C6 (galactose, mannose, rhamnose and glucose) monomeric sugars (BASSO *et al.*, 2013; DOS SANTOS *et al.*, 2016a; ISIKGOR; BECER, 2015; PALMQVIST; HAHN-HÄGERDAL, 2000).

The resulting liquor, referred to as lignocellulosic hydrolysate (LCH), is the actual substrate for downstream fermentations. Unfortunately, along with precious sugars, the hydrolysis also produced many undesirable byproducts toxic to microorganisms. For example, dehydration of xylose and arabinose results in furfural, while 5-hydroxy methylfurfural (HMF) is formed by dehydration of hexoses (glucose, mannose, and galactose) (JÖNSSON; ALRIKSSON; NILVEBRANT, 2013; PALMQVIST; HAHN-HÄGERDAL, 2000). Further, furfural can generate formic acid, while HMF can be broken down into formic and levulinic acids. Acetic acid is released upon HC hydrolysis and consists in one of the most abundant inhibitors present in LCHs.

The LG-derived phenolic compounds such as 4- hydroxybenzoic acid, vanillin, catechol, ferulic acid, and coumaric acid, complete the catalog of top-ranking inhibitors. It should be noticed that the full list of LCH inhibitors spreads beyond those cited above, and their identity and concentration in a given LCH depends on each feedstock used and on the pretreatment method applied (JÖNSSON; ALRIKSSON; NILVEBRANT, 2013). The fermentation efficiency of *S. cerevisiae* in LCHs is heavily compromised by those inhibitors, which cause overall prolongation of lag phases, slower growth rates, reduced BS formation, and decreased bioethanol productivity. Yeast responses to LCH toxicity generally involve ATP and NAD(P)H expending processes to detoxify inhibitors and repair their damage (PIOTROWSKI *et al.*, 2014). For instance, acidification occurs when weak organic acids (chiefly acetic, but also formic and levulinic acids) are generated upon lignocellulose hydrolysis at low pH, weak acids shift to the undissociated form that easily permeates yeast cell membranes. Inside the near-neutral cytosolic pH, acids dissociate and decrease the intracellular pH, triggering the actions of plasma membrane and vacuolar ATPases to clear protons from the cytosol at the expense of ATP hydrolysis (DOS SANTOS *et al.*, 2016a; JÖNSSON; ALRIKSSON; NILVEBRANT, 2013; PALMQVIST; HAHN-HÄGERDAL, 2000).

Besides promoting futile waste of energy, decreased intracellular pH further interferes with many enzymatic activities (e.g., glycolytic enzymes) (PALMQVIST;

HAHN-HÄGERDAL, 2000). On the other hand, the anionic form of weak acids is also toxic by increasing the intracellular osmolarity, which impairs the synthesis of macromolecules and DNA replication, and causes the formation of reactive oxygen species (ROS) (JUNG; KIM, 2015). In this regard, extreme acetic acid toxicity can lead to programmed cell death (GIANNATTASIO *et al.*, 2013). In compensating manner, yeast cells extrude anionic acids, while lowering intracellular accumulation of several amino acids (JUNG; KIM, 2015). Generally, acetic acid, with higher pKa (4.76) and abundance than levulinic and formic acids, champions the toxic effects associated with the organic acids contained in LCHs (DOS SANTOS *et al.*, 2016a; JÖNSSON; ALRIKSSON; NILVEBRANT, 2013; PALMQVIST; HAHN-HÄGERDAL, 2000).

As for the inhibitors furfural and HMF, their toxicity is primarily exerted by direct inhibition of enzymes controlling the central carbon and energy metabolism, and by depletion of NAD(P)H pools during detoxification through a cellular pool of oxidoreductase enzymes (DOS SANTOS *et al.*, 2016a; HEER; SAUER, 2008; JÖNSSON; ALRIKSSON; NILVEBRANT, 2013; PALMQVIST; HAHN-HÄGERDAL, 2000). Such redox reactions may also account for ROS formation as byproducts (KIM; HAHN, 2013). Detoxification of furfurals may also occur at the expense of ATP, possibly via their cellular extrusion by efflux pumps (DOS SANTOS *et al.*, 2016a; JÖNSSON; ALRIKSSON; NILVEBRANT, 2013; PALMQVIST; HAHN-HÄGERDAL, 2000).

Within the LG-derived inhibitors, higher toxicity has been associated with lower molecular weight phenolic compounds, although the mechanisms of action have not yet been elucidated. In general, the lipophilic nature of phenols accounts for pronounced toxic effects at level of the plasma membrane, although phenols can also cause systemic damages, such as denaturation of proteins, oxidative stress, decrease in intracellular pH, depletion of ATP, translation inhibition, and DNA mutagenesis. Overall, the toxic effects of LCH inhibitors comprise a major obstacle that must be removed to enable cost-effective industrial 2G fermentations. To achieve this goal, many physical and/or chemical treatments, either applied during or after the hydrolysis, have been proposed to minimize the production, or to detoxify the inhibitors (KUMAR *et al.*, 2009). Similarly, many chemical treatments applied during fermentations have been tested to ameliorate LCH toxicity and/or to improve overall fermentation performance (JÖNSSON; ALRIKSSON; NILVEBRANT, 2013). However, most of these methods demand processes or chemical additives that impact final production

costs. A promising alternative is to increase/discover the intrinsic (genetic) capability of *S. cerevisiae* to withstand toxicity of LCH inhibitors.

2.6.1. Genetic advances to improve yeast tolerance to lignocellulosic hydrolysates

The advance of knowledge of traits linked to yeast tolerance to LCH inhibitors has mostly benefited from novel approaches such as experimental/adaptive evolution protocols, genome wide studies and biology synthetic tools available lately. Adaptive laboratory evolution protocols have been developed followed by genome sequencing, transcriptomic analysis, proteomic and their combinations. In the resulting evolved strains, molecular mechanisms resulting in growth rate improvement under various stress conditions, including LC inhibitors factors were established. (FLETCHER; BAETZ, 2020).

As it is known that yeast LCH inhibitors tolerance is a multigenic complex trait (DE WITT *et al.*, 2019), advances in systems biology approaches have transformed the way by which cells respond to inhibitor toxicity. Genome-wide approaches has given insight into how yeasts develop tolerance, enabling the identification of genetic and metabolic targets that can be engineered to improve tolerance to fermentation inhibitors as well as revealed optimal yeast genetic backgrounds with insights about how the combination of genes might alter the response towards stresses in the nature.

Prospecting data from transcriptomic studies with *S. cerevisiae* have provided a rational basis for engineering master regulators to elicit global cell responses to toxic LCH inhibitors. For instance, overexpression of the transcription factors (TFs) Msn2 (general stress response) (SASANO *et al.*, 2012) and Yap1 (oxidative stress regulator) (KIM; HAHN, 2013), resulted in improved yeast resistance to furfural. Similarly, overexpression of the Haa1 demonstrated systemic adaptation to acetic acid (SWINNEN *et al.*, 2017), whereas raised levels of Sfp1 and Ace2 improved fermentation exposed to acetic acid and furfural (CHEN *et al.*, 2016). Besides TFs, higher tolerance to acetic acid and other stresses was also attained through overexpression of global regulators that instead acted at a post-transcriptional (Rtc3) translational (Anb1) (HASUNUMA; SAKAMOTO; KONDO, 2016), or even at a post-translational level (Whi2) (CHEN, Yingying; STABRYLA; WEI, 2016). In contrast, the modifying particular metabolic pathways have been proven by overexpression of

genes related to aldehyde reduction (HEER; HEINE; SAUER, 2009), spermidine synthesis (KIM *et al.*, 2015), and the pentose phosphate pathway (HASUNUMA *et al.*, 2014), resulting in higher tolerance to furan aldehydes. Similarly, the resistance to phenolic inhibitors could be improved by synergic expression of heterologous Lcc2 (laccase) and overexpression of endogenous Sso2 (a t-SNARE protein that controls protein traffic to the plasma membrane) (LARSSON; CASSLAND; JÖNSSON, 2001). Other specific metabolic modifications, such as overexpression of Acetyl-CoA synthetase (DING; HOLZWARTH; BRADFORD; *et al.*, 2015), or the Pep3 protein (required for vacuolar biogenesis) (DING; HOLZWARTH; PENNER; *et al.*, 2015), could also enhance yeast tolerance to acetic acid.

Playing with known pathways related to stress regulation also can help to manage the yeast response, such as over or under expressing enzymes. In this regard, it has been reported that glutathione (GSH) intracellular levels can cope with stress responses elicited by toxic compounds found in LCH. It is known that glutathione (GSH), can protect the cell from reactive oxygen species (ROS) and free radicals, and GSH is oxidized to Glutathione disulfide GSSG by reactions with free radicals. Inhibitors present in the LCH, furfural and HMF can act as thiol reactive electrophiles oxidating GSH in *Saccharomyces cerevisiae*. Ouyang *et al.* (2021) have identified *YMR152W*, a novel aldehyde reductase with catalytic functions for reduction of at least six aldehydes, including two furan aldehydes (furfural and 5-hydroxymethylfurfural), three aliphatic aldehydes (acetaldehyde, glycolaldehyde, and 3-methylbutanal), and an aromatic aldehyde (benzaldehyde) with NADH or NADPH as the co-factor. The *S. cerevisiae* Ymr152wp was classified as the quinone oxidoreductase and is promising for detoxification of aldehyde inhibitors derived from LC hydrolysis. In this sense, has been shown that increasing levels of GSH through overexpressing enzymes on its biosynthetic pathway (GSH1 and GLR1) lead to an increase on *S. cerevisiae* tolerance towards LCH furfural and HMF (KIM; HAHN, 2013). Raghavendran *et al.* (2020) described that native Gsh1p enzyme have regulation by negative feedback and has a threshold because of the inhibition, however they overexpressed two bacterial enzymes that lacks this kind of regulation, *StgshF* from *Streptococcus thermophilus* and *LmgshF* from *Listeria monocytogenes* which show increasing the glutathione (GSH) content in yeasts confers tolerance towards lignocellulose inhibitors. Subsequently increasing the bioethanol titers.

Another tool available is the use of yeast deletion mutant library (reviewed by Giaever and Nislow (2014)), that comprise more than 21000 mutant strains that carry precise start-to-stop deletions of approximately 6000 open reading frames. This collection includes heterozygous and homozygous diploids, and haploids of both *MATa* and *MATα* mating types. The researchers applied yeast deletion mutant library in an agar-based array screening using sub-lethal concentrations of the compound(s) being tested (LCH, mostly phenolics compounds) the researchers were able to find correspondence with tolerance with specific compounds and identified several targets. One example of a suppressor gene is *BNA7* which was found to enable yeast growth, when deleted, in media containing ferulic acid (FLETCHER *et al.*, 2019). Likewise, were found several genes clustered into biological processes related to tolerance against ferulic acid and vanillin, mainly associated with protein transport (COG6, COG7, and ARL1), chromatin modification and transcription (SWC3, ARP6, YAF9, HTZ1) (ENDO *et al.*, 2008; FLETCHER *et al.*, 2019). These information help as scaffold to created possible metabolic engineering on those pathways. Other genes were found using this deletion libraries, using wheat straw hydrolysate and synthetic miscanthus hydrolysate tolerance, were genes involved in oxidative stress response (*SOD1*, *SOD2*), protein synthesis (*RPL13B*, *RPL13A*) and ergosterol biosynthesis (*ERG2*) (PEREIRA *et al.*, 2014; SKERKER *et al.*, 2013).

Using adaptive laboratory evolution followed by whole-genome sequencing technologies is possible to access evolution in “real time”. Process of evolution by natural selection, in a sense that microorganisms are challenged to propagate for hundreds or thousands of generations under a given selective pressure to evolve towards adapted phenotypes (DRAGOSITS; MATTANOVICH, 2013). Selective pressure (stress) ensures that more adapted genotypes in which benefic mutations occurred, outcompete non-adapted cells in a population. These will finally dominate the entire culture after prolonged cultivation A mutant allele can be tested by reverse engineered into the parental (progenitor) background, and may (or not) partially recapitulate the adaptive phenotype gained during the evolution experiment (BARRICK; LENSKI, 2013). Under this perspective, adaptive evolution experiments are excellent in pointing out candidate alleles for metabolic engineering of industrial yeast strains.

There are few experiments so far, mostly using one or two inhibitors isolated for LHC tolerance. For example, the zinc finger transcription factor, *YRR1* acquired a

frameshift mutation resulting in a loss of function which improved yeast growth in vanillin (WANG *et al.*, 2017b), In another experiment, nonsense mutations acquired by *MUK1* and *MRS4* resulted in tolerance to coniferyl aldehyde (HACISALIHOĞLU *et al.*, 2019).

Using genome shuffling, a technique that is based on performing mutagenesis or followed by adaptive evolution experiment on haploid yeasts of both mating types (α and a), selecting for desirable stress factor in tolerant haploids and mating them to obtain diploid strains (FLETCHER; BAETZ, 2020). Genes including *NRG1*, *GSH1*, and *GDH1* were identified as important by yeast for improved tolerance to LCH (BIOT-PELLETIER *et al.*, 2018), this approach allowed to discovery of positive epistasis and the accumulation of beneficial mutations. Cheng *et al.* (2015) described that, using genome shuffling after adaptive evolution experiments, they re-sequenced several of these adaptive mutants after genome shuffling and identified a total of eight intergenic mutations and nine non-synonymous single nucleotide polymorphisms (SNPs) in coding regions. The main mutation found was *SMF1*, as different alleles in four recombinants. *SMF1* is known to be involved in transport of divalent ions.

Quantitative trait loci (QTL) mapping is another technique that can be used to select traits from promising backgrounds (wild or industrial), especially when the response is multigenic. Using whole genome sequencing on F1+n segregating population, originated from crosses of stress-tolerant versus stress-sensible haploids, allows a genome-wide scanning for DNA variations (SNPs/InDels) that correlate with the tolerance phenotype (MEIJNEN *et al.*, 2016). A statistically significant co-segregation of a given DNA variant with a stress-tolerance trait indicates that both are genetic linked and hence co-localized at a chromosomal level. Thereby, QTL candidates can be observed at a chromosomal location nearby the scored DNA variant. Chosen QTL candidates are then validated if their genetic introduction into the stress-sensible parental strain can recapitulate part of the phenotype associated with the stress-tolerant parental (MEIJNEN *et al.*, 2016; PAIS; FOULQUIÉ-MORENO; THEVELEIN, 2014). Studies related to the QTL mapping of F1 segregants identified an acetic acid-adaptive allele related to the *HAA1* gene, and further analysis of F7 segregants allowed isolation of novel causal alleles of the genes *GLO1*, *DOT5*, *CUP2*, and confirm the previously identified allele of *VMA7* (MEIJNEN *et al.*, 2016).

Synthetic biology is emerging as a cutting-edge tool, especially after the discovery of gene editing technologies, ultimately by CRISPR/Cas technologies. The

CRISPR-based knock-out, interference and activation systems have offering genetic screens with broader phenotypic scope (GUTMANN *et al.*, 2021) and might unveiled genotype–phenotype relations via precise transcriptional repression. Gutmann *et al.* (2021) tested yeast growth in the presence of lignocellulose hydrolysate and a supplementing inhibitor cocktail with a plasmid-based CRISPRi system that allows for continuous expression of a nuclease-deactivated (d)Cas9 protein fused to the potent Mxi1 repressor domain, and gRNA's inducible by anhydrotetracycline. They build a library of 1573 gRNAs (up to six gRNA locations per gene target) to repress 161 transcription factors and 129 protein kinases, which are highly involved in the regulation of growth adaptation and cellular signaling. They measured, fitness under stress (LCH plus cocktail), competition assays, bioethanol production and confirmed their results using Chromatin Immuno-Precipitation on chip (ChIP-chip) data. They found that hydrolysate-stress adaptation is related to modulation of oxidative (Yap1, Stb5), osmotic (Hog1, Pbs2, Sko1), acidic (Haa1) and general stress (Dot6, Ume6), and show the CRIPRi may identify genetic elements underlying complex environmental conditions encountered by cells in industrial bioprocesses.

2.7. Concluding Remarks

The use of 2G bioethanol is worthwhile once might produces biofuels and other biomaterials from sources before neglected or used as burning material. Studies on yeast copying with stresses from LCH open a wide way in order to understand the steps that will have to take to advance on the knowledge and strategies in this issue. Many approaches had been developed and used in this sense, and with those new technologies, mining genes and metabolic engineering of the desirable traits, together with better LCH ways to produce a less toxic hydrolysate. Hopefully, soon enough we can contribute with environment, less GHG emissions and sustainable way to produce energy.

Acknowledgments: The authors acknowledge the São Paulo Research Foundation (FAPESP) for the financial support (process number 2019/12997-6; 2017/22401-8 and 2017/24453-5 to APJ).

References

- ABE, M. M.; BRANCIFORTI, M. C.; BRIENZO, M. Biodegradation of Hemicellulose-Cellulose-Starch-Based Bioplastics and Microbial Polyesters. **Recycling**, Basel, vol. 6, no. 1, p. 1–21, 2021. DOI 10.3390/recycling6010022. Available at: <https://www.mdpi.com/2313-4321/6/1/22>. Accessed on: 14 Feb. 2021.
- AL-DAJANI, W. W.; TSCHIRNER, U. W.; JENSEN, T. Pre-extraction of hemicelluloses and subsequent kraft pulping Part II: Acid and autohydrolysis. **Tappi Journal**, Peachtree Corners, vol. 8, no. 9, p. 30–37, 2009. Available at: <https://imisrise.tappi.org/TAPPI/Products/09/SEP/09SEP30.aspx>. Accessed on: 14 Feb. 2021.
- ALBERTS, B.; BRAY, D.; HPKIN, K.; JOHNSON, A.; LEWIS, J.; RAFF, M.; ROBERTS, K.; WALTER, P. **Essential Cell Biology**. 4th ed. New York: Garland Science, 2014.
- ALVES, J. L. F.; DA SILVA, J. C. G.; MUMBACH, G. D.; DOMENICO, M. Di; DE SENA, R. F.; MACHADO, R. A. F.; MARANGONI, C. Demonstrating the Suitability of Tamarind Residues to Bioenergy Exploitation Via Combustion Through Physicochemical Properties, Performance Indexes, and Emission Characteristics. **BioEnergy Research**, Berlin, vol. 13, no. 4, p. 1308–1320, 2020. DOI 10.1007/s12155-020-10158-z. Available at: <https://link.springer.com/article/10.1007/s12155-020-10158-z>. Accessed on: 6 Feb. 2021.
- ALVES, R. C.; MELATI, R. B.; CASAGRANDE, G. M. S.; CONTIERO, J.; PAGNOCCA, F. C.; BRIENZO, M. Sieving process selects sugarcane bagasse with lower recalcitrance to xylan solubilization. **Journal of Chemical Technology & Biotechnology**, Hoboken, vol. 96, no. 2, p. 327–334, 1 Feb. 2021. DOI 10.1002/jctb.6541. Available at: <https://onlinelibrary.wiley.com/doi/abs/10.1002/jctb.6541>. Accessed on: 15 Feb. 2021.
- AMANDAMHEN, S. O.; MEINCKEN, M.; TYHODA, L. The effect of chemical treatments of natural fibres on the properties of phosphate-bonded composite products. **Wood Science and Technology**, Berlin, vol. 52, no. 3, p. 653–675, 2018. DOI 10.1007/s00226-018-0999-9. Available at: <https://link.springer.com/article/10.1007/s00226-018-0999-9>. Accessed on: 10 Feb. 2021.
- AMORE, R.; WILHELM, M.; HOLLENBERG, C. P. The fermentation of xylose —an analysis of the expression of Bacillus and Actinoplanes xylose isomerase genes in yeast. **Applied Microbiology and Biotechnology**, Berlin, vol. 30, no. 4, p. 351–357, 1989. DOI 10.1007/BF00296623. Available at: <https://link.springer.com/article/10.1007/BF00296623>. Accessed on: 14 Feb. 2021.
- ASANTE, B. **Geopolymer bonded wood composites**. South Africa: BioHome at UHH, 2020.
- BAN, W.; CHEN, X.; ANDREWS, G.; HEININGEN, A. Van. Influence of hemicelluloses pre-extraction and re-adsorption on pulp physical strength II. beatability and strength study. **Cellulose Chemistry and Technology**, Iași, vol. 45, no. 9–10, p. 633–641, 2011. Available at: [https://www.cellulosechemtechnol.ro/pdf/CCT45,9-10\(2011\)/p.633-641.pdf](https://www.cellulosechemtechnol.ro/pdf/CCT45,9-10(2011)/p.633-641.pdf). Accessed on: 15 Feb. 2021.
- BARRICK, J. E.; LENSKE, R. E. Genome dynamics during experimental evolution. **Nature Reviews Genetics**, Berlin, vol. 14, no. 12, p. 827–839, 2013. DOI 10.1038/nrg3564. Available at: <https://www.nature.com/articles/nrg3564>. Accessed on: 10 Feb. 2021.
- BARUAH, J.; NATH, B. K.; SHARMA, R.; KUMAR, S.; DEKA, R. C.; BARUAH, D. C.; KALITA, E. Recent Trends in the Pretreatment of Lignocellulosic Biomass for Value-Added Products. **Frontiers in Energy Research**, Lausanne, vol. 6, p. 1–19, 2018. Available at: <https://www.frontiersin.org/article/10.3389/fenrg.2018.00141>. Accessed on: 16 Feb. 2021.
- BASSO, T. P.; BASSO, T. O.; GALLO, C. R.; BASSO, L. C. Towards the Production of Second Generation Ethanol from Sugarcane Bagasse in Brazil. In: MATOVIC, M. D. (ed.). **Biomass Now - Cultivation and Utilization**. London: IntechOpen, 2013. p. 347–354. DOI 10.5772/54179. Available at: <https://api.intechopen.com/chapter/pdf-preview/44400>. Accessed on: 16 Feb. 2021.
- BECKER, J.; BOLES, E. A Modified Saccharomyces cerevisiae Strain That Consumes L-Arabinose and Produces Ethanol. **Applied and Environmental Microbiology**, Washington, vol. 69, no. 7, p. 4144–4150, 1 Jul. 2003. DOI 10.1128/AEM.69.7.4144-4150.2003. Available at: <https://aem.asm.org/content/69/7/4144>. Accessed on: 16 Feb. 2021.
- BELLO, S.; SALIM, I.; FEIJOO, G.; MOREIRA, M. T. Inventory review and environmental evaluation of first- and second-generation sugars through life cycle assessment. **Environmental Science and Pollution Research**, Berlin, 2021. DOI 10.1007/s11356-021-12405-y. Available at: <https://link.springer.com/article/10.1007/s11356-021-12405-y>. Accessed on: 6 Feb. 2021.
- BENAIMECHE, O.; SEGHIR, N. T.; SADOWSKI, Ł.; MELLAS, M. The Utilization of Vegetable Fibers in Cementitious Materials. In: HASHMI, S.; CHOUDHURY, I. A. B. T.-E. of R. and S. M. (eds.). **Encyclopedia of Renewable and Sustainable Materials**. Oxford: Elsevier, 2020. vol. 2, p. 649–662. DOI 10.1016/B978-0-12-803581-8.11596-6. Available at: <https://www.sciencedirect.com/science/article/pii/B9780128035818115966>. Accessed on: 10 Feb. 2021.
- BHARDWAJ, N.; KUMAR, B.; VERMA, P. A detailed overview of xylanases: an emerging biomolecule for current and future prospective. **Bioresources and Bioprocessing**, Amsterdam, vol. 6, no. 1, p. 1–36, 2019. DOI 10.1186/s40643-019-0276-2. Available at: <https://link.springer.com/article/10.1186/s40643-019-0276-2>. Accessed on: 12 Feb. 2021.
- BINDER, J.; BLANK, J.; CEFALI, A.; RAINES, R. Synthesis of Furfural from Xylose and Xylan. **ChemSusChem**, Hoboken, vol. 3, no. 11, p. 1268–1272, 2010. DOI 10.1002/cssc.201000181. Available at: <https://chemistry-europe.onlinelibrary.wiley.com/doi/10.1002/cssc.201000181>. Accessed on: 2 Feb. 2021.
- BIOT-PELLETIER, D.; PINEL, D.; LARUE, K.; MARTIN, V. J. J. Determinants of selection in yeast evolved by genome shuffling. **Biotechnology for Biofuels**, Berlin, vol. 11, no. 1, p. 282, 2018. DOI 10.1186/s13068-018-1283-9. Available at: <https://link.springer.com/article/10.1186/s13068-018-1283-9>. Accessed on: 16 Feb. 2021.
- BLEDZKI, A. K.; MAMUN, A. A.; LUCKA-GABOR, M.; GUTOWSKI, V. S. The effects of acetylation on properties of flax fibre and its polypropylene composites. **EXPRESS Polymer Letters**, Budapest, vol. 2, no. 6, p. 413–422, 2008. DOI 10.3144/expresspolymlett.2008.50. Available at: [w.doi.org/10.3144/expresspolymlett.2008.50](https://doi.org/10.3144/expresspolymlett.2008.50). Accessed on: 10 Feb. 2021.
- BRACHER, J. M.; VERHOEVEN, M. D.; WISSELINK, H. W.; CRIMI, B.; NIJLAND, J. G.; DRIESSEN, A. J. M.; KLAASSEN, P.; VAN MARIS, A. J. A.; DARAN, J.-M. G.; PRONK, J. T. The Penicillium chrysogenum transporter PcAraT enables high-affinity, glucose-insensitive L-arabinose transport in Saccharomyces cerevisiae. **Biotechnology for Biofuels**, Berlin, vol. 11, no. 1, p. 63, 2018. DOI 10.1186/s13068-018-1047-6. Available at: <https://link.springer.com/article/10.1186/s13068-018-1047-6>. Accessed on: 10 Feb. 2021.
- BRAT, D.; BOLES, E.; WIEDEMANN, B. Functional Expression of a Bacterial Xylose Isomerase in Saccharomyces cerevisiae. **Applied and Environmental Microbiology**, Washington, vol. 75, no. 8, p. 2304 LP – 2311, 15 Apr. 2009. DOI 10.1128/AEM.02522-08. Available at: <https://aem.asm.org/content/75/8/2304>. Accessed on: 10 Feb. 2021.
- BRIENZO, M.; AZEVEDO CARVALHO, A. F.; DE FIGUEIREDO, F. C.; NETO, P. de O. Sugarcane bagasse hemicellulose properties, extraction technologies and xylooligosaccharides production. In: RILEY, G. L. (ed.). **Food Waste: Practices, Management and Challenges**. Hauppauge: Nova Science Publishers, 2016. p. 155–188. Available at: <https://novapublishers.com/wp-content/uploads/2019/01/Sugarcane-Bagasse-Hemicellulose-Properties.pdf>. Accessed on: 17 Feb. 2022.

BRITISH PETROLEUM. **Statistical Review of World Energy**. London: Pureprint Group Limited, 2020. Available at: <https://www.bp.com/content/dam/bp/business-sites/en/global/corporate/pdfs/energy-economics/statistical-review/bp-stats-review-2020-full-report.pdf>. Accessed on: 3 Aug. 2020.

BRUINENBERG, P. M.; DE BOT, P. H. M.; VAN DIJKEN, J. P.; SCHEFFERS, W. A. The role of redox balances in the anaerobic fermentation of xylose by yeasts. **European journal of applied microbiology and biotechnology**, Berlin, vol. 18, no. 5, p. 287–292, 1983. DOI 10.1007/BF00500493. Available at: <https://link.springer.com/article/10.1007/BF00500493>. Accessed on: 13 Feb. 2021.

BUENO, J. G. R.; BORELLI, G.; CORRÊA, T. L. R.; FIAMENGHI, M. B.; JOSÉ, J.; DE CARVALHO, M.; DE OLIVEIRA, L. C.; PEREIRA, G. A. G.; DOS SANTOS, L. V. Novel xylose transporter Cs4130 expands the sugar uptake repertoire in recombinant *Saccharomyces cerevisiae* strains at high xylose concentrations. **Biotechnology for Biofuels**, Berlin, vol. 13, no. 1, p. 145, 2020. DOI 10.1186/s13068-020-01782-0. Available at: <https://link.springer.com/article/10.1186/s13068-020-01782-0>. Accessed on: 1 Feb. 2021.

CHEN, Y.; SHENG, J.; JIANG, T.; STEVENS, J.; FENG, X.; WEI, N. Transcriptional profiling reveals molecular basis and novel genetic targets for improved resistance to multiple fermentation inhibitors in *Saccharomyces cerevisiae*. **Biotechnology for Biofuels**, Berlin, vol. 9, no. 1, p. 9, 2016. DOI 10.1186/s13068-015-0418-5. Available at: <https://link.springer.com/article/10.1186/s13068-015-0418-5>. Accessed on: 1 Feb. 2021.

CHEN, Y.; STABRYLA, L.; WEI, N. Improved Acetic Acid Resistance in *Saccharomyces cerevisiae* by Overexpression of the WHI2 Gene Identified through Inverse Metabolic Engineering. **Applied and Environmental Microbiology**, Washington, vol. 82, no. 7, p. 2156 LP – 2166, 1 Apr. 2016. DOI 10.1128/AEM.03718-15. Available at: <https://aem.asm.org/content/82/7/2156>. Accessed on: 2 Feb. 2021.

CHENG, C.; ALMARIO, M. P.; KAO, K. C. Genome shuffling to generate recombinant yeasts for tolerance to inhibitors present in lignocellulosic hydrolysates. **Biotechnology Letters**, Berlin, vol. 37, no. 11, p. 2193–2200, 2015. DOI 10.1007/s10529-015-1895-0. Available at: <https://link.springer.com/article/10.1007/s10529-015-1895-0>. Accessed on: 10 Feb. 2021.

CHRISTOPHER, L. Adding Value Prior to Pulping: Bioproducts from Hemicellulose. **Global Perspectives on Sustainable Forest Management**. London: IntechOpen, 2012. p. 225–246. DOI 10.5772/36849. Available at: <https://www.intechopen.com/books/global-perspectives-on-sustainable-forest-management/adding-value-prior-to-pulping-biofuels-and-bioproducts-from-hemicellulose>. Accessed on: 10 Feb. 2021.

CHRISTOPHER, L. P. Integrated Forest Biorefineries: Current State and Development Potential. In: CHRISTOPHER, L. P. (ed.). **Integrated Forest Biorefineries: Challenges and Opportunities**. Cambridge: Royal Society of Chemistry, 2013. p. 1–66. DOI 10.1039/9781849735063-00001. Available at: <https://pubs.rsc.org/en/content/ebook/978-1-84973-321-2>. Accessed on: 10 Feb. 2021.

CONAB. Produção da safra atual de cana-de-açúcar deve crescer 3,5%, diz Conab. 2020. Available at: <https://agenciabrasil.ebc.com.br/economia/noticia/2020-12/producao-da-safra-atual-de-cana-de-acucar-deve-crescer-35-diz-conab>. Accessed on: 2 Feb. 2021.

CORAL MEDINA, Jesús David; MAGALHAES, A. I. J. Ethanol Production, Current Facts, Future Scenarios, and Techno-Economic Assessment of Different Biorefinery Configurations. In: INAMBAO, F. L. (ed.). **Bioethanol Technologies**. London: IntechOpen, 2020. p. 1–14. DOI 10.5772/intechopen.95081. Available at: <https://www.intechopen.com/chapters/74288>. Accessed on: 6 Jul. 2022.

CORAL MEDINA, Jesus David; WOJCIECHOWSKI, A. L.; ZANDONA FILHO, A.; BISSOQUI, L.; NOSEDA, M. D.; DE SOUZA VANDENBERGHE, L. P.; ZAWADZKI, S. F.; SOCCOL, C. R. Biological activities and thermal behavior of lignin from oil palm empty fruit bunches as potential source of chemicals of added value. **Industrial Crops and Products**, Amsterdam, vol. 94, p. 630–637, 2016. DOI 10.1016/j.indcrop.2016.09.046. Available at: <https://www.sciencedirect.com/science/article/pii/S0926669016306264>. Accessed on: 6 Feb. 2021.

COSTA, C. E.; ROMANÍ, A.; CUNHA, J. T.; JOHANSSON, B.; DOMINGUES, L. Integrated approach for selecting efficient *Saccharomyces cerevisiae* for industrial lignocellulosic fermentations: Importance of yeast chassis linked to process conditions. **Bioresource Technology**, Amsterdam, vol. 227, p. 24–34, 2017. DOI 10.1016/j.biortech.2016.12.016. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852416316674>. Accessed on: 10 Feb. 2021.

CUNHA, J. T.; SOARES, P. O.; ROMANÍ, A.; THEVELEIN, J. M.; DOMINGUES, L. Xylose fermentation efficiency of industrial *Saccharomyces cerevisiae* yeast with separate or combined xylose reductase/xylitol dehydrogenase and xylose isomerase pathways. **Biotechnology for Biofuels**, Berlin, vol. 12, no. 1, p. 20, 2019. DOI 10.1186/s13068-019-1360-8. Available at: <https://link.springer.com/article/10.1186/s13068-019-1360-8>. Accessed on: 10 Feb. 2021.

CUTRIM, F. M.; RAMOS, E. C. S. S.; ABREU, M. C. C.; GODINHO, A. S.; MACIEL, A. P.; MENDONÇA, C. J. S.; CAVALCANTE, K. S. B. A Study of Chemical Composition and Enzymatic Hydrolysis of Solid Organic Waste from Agrosilvopastoral Systems. **Journal of the Brazilian Chemical Society**, Campinas, vol. 30, no. 9, p. 1955–1963, 2019. DOI 10.21577/0103-5053.20190110. Available at: https://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-50532019000901955. Accessed on: 6 Feb. 2021.

DA SILVA, G. F.; MATHIAS, S. L.; DE MENEZES, A. J.; VICENTE, J. G. P.; DELFORNO, T. P.; VARESCHE, M. B. A.; DUARTE, I. C. S. Orange Bagasse Pellets as a Carbon Source for Biobutanol Production. **Current Microbiology**, Berlin, vol. 77, no. 12, p. 4053–4062, 2020. DOI 10.1007/s00284-020-02245-3. Available at: <https://link.springer.com/article/10.1007/s00284-020-02245-3>. Accessed on: 6 Feb. 2021.

DE FREITAS, C.; TERRONE, C. C.; MASARIN, F.; CARMONA, E. C.; BRIENZO, M. In vitro study of the effect of xylooligosaccharides obtained from banana pseudostem xylan by enzymatic hydrolysis on probiotic bacteria. **Biocatalysis and Agricultural Biotechnology**, vol. 33, p. 101973, 2021. DOI 10.1016/j.bcab.2021.101973. Available at: <https://www.sciencedirect.com/science/article/pii/S1878818121000694>. Accessed on: 2 Jan. 2021.

DE SALES, B. B.; SCHEID, B.; GONÇALVES, D. L.; KNYCHALA, M. M.; MATSUSHIKA, A.; BON, E. P. S.; STAMBUK, B. U. Cloning novel sugar transporters from *Scheffersomyces (Pichia) stipitis* allowing d-xylose fermentation by recombinant *Saccharomyces cerevisiae*. **Biotechnology Letters**, Berlin, vol. 37, no. 10, p. 1973–1982, 2015. DOI 10.1007/s10529-015-1893-2. Available at: <https://link.springer.com/article/10.1007/s10529-015-1893-2>. Accessed on: 14 Feb. 2021.

DE SOUZA, R. L.; YU, H.; RATABOUL, F.; ESSAYEM, N. 5-Hydroxymethylfurfural (5-HMF) Production from Hexoses: Limits of Heterogeneous Catalysis in Hydrothermal Conditions and Potential of Concentrated Aqueous Organic Acids as Reactive Solvent System. **Challenges**, Basel, vol. 3, no. 2, p. 212–232, 2012. DOI 10.3390/challe3020212. Available at: <https://www.mdpi.com/2078-1547/3/2/212>. Accessed on: 10 Feb. 2021.

DE WITT, R. N.; KROUKAMP, H.; VAN ZYL, W. H.; PAULSEN, I. T.; VOLSCHENK, H. QTL analysis of natural *Saccharomyces cerevisiae* isolates reveals unique alleles involved in lignocellulosic inhibitor tolerance. **FEMS Yeast Research**, Oxford, vol. 19, no. 5, p. 1–14, 1 Aug. 2019. DOI 10.1093/femsyr/foz047. Available at: <https://academic.oup.com/femsyr/article/19/5/foz047/5528620>. Accessed on: 10 Feb. 2021.

DELGADO-ARCAÑO, Y.; MANDELLI, D.; CARVALHO, W. A.; PONTES, L. A. M. Valorization of Corn cob by Hydrolysis-

Hydrogenation to Obtain Xylitol Under Mild Conditions. **Waste and Biomass Valorization**, Berlin, 2021. DOI 10.1007/s12649-021-01348-7. Available at: <https://link.springer.com/article/10.1007/s12649-021-01348-7>. Accessed on: 8 Feb. 2021.

DEMEKE, M. M.; DIETZ, H.; LI, Y.; FOULQUIÉ-MORENO, M. R.; MUTTURI, S.; DEPREZ, S.; DEN ABT, T.; BONINI, B. M.; LIDEN, G.; DUMORTIER, F.; VERPLAETSE, A.; BOLES, E.; THEVELEIN, J. M. Development of a D-xylose fermenting and inhibitor tolerant industrial *Saccharomyces cerevisiae* strain with high performance in lignocellulose hydrolysates using metabolic and evolutionary engineering. **Biotechnology for Biofuels**, Berlin, vol. 6, no. 1, p. 89, 2013. DOI 10.1186/1754-6834-6-89. Available at: <https://link.springer.com/article/10.1186/1754-6834-6-89>. Accessed on: 10 Feb. 2021.

DIEN, B. S.; KURTZMAN, C. P.; SAHA, B. C.; BOTHAST, R. J. Screening for arabinose fermenting yeasts. **Applied Biochemistry and Biotechnology**, Berlin, vol. 57, no. 1, p. 233–242, 1996. DOI 10.1007/BF02941704. Available at: <https://link.springer.com/article/10.1007/BF02941704>. Accessed on: 10 Feb. 2021.

DÍEZ, D.; URUEÑA, A.; PIÑERO, R.; BARRIO, A.; TAMMINEN, T. Determination of Hemicellulose, Cellulose, and Lignin Content in Different Types of Biomasses by Thermogravimetric Analysis and Pseudocomponent Kinetic Model (TGA-PKM Method). **Processes**, Basel, vol. 8, no. 9, p. 1–21, 2020. DOI 10.3390/pr8091048. Available at: <https://www.mdpi.com/2227-9717/8/9/1048/htm>. Accessed on: 8 Feb. 2021.

DING, J.; HOLZWARTH, G.; BRADFORD, C. S.; COOLEY, B.; YOSHINAGA, A. S.; PATTON-VOGT, J.; ABELIOVICH, H.; PENNER, M. H.; BAKALINSKY, A. T. PEP3 overexpression shortens lag phase but does not alter growth rate in *Saccharomyces cerevisiae* exposed to acetic acid stress. **Applied Microbiology and Biotechnology**, Berlin, vol. 99, no. 20, p. 8667–8680, 2015. DOI 10.1007/s00253-015-6708-9. Available at: <https://link.springer.com/article/10.1007/s00253-015-6708-9>. Accessed on: 10 Feb. 2021.

DING, J.; HOLZWARTH, G.; PENNER, M. H.; PATTON-VOGT, J.; BAKALINSKY, A. T. Overexpression of acetyl-CoA synthetase in *Saccharomyces cerevisiae* increases acetic acid tolerance. **FEMS Microbiology Letters**, Oxford, vol. 362, no. 3, p. 1–7, 1 Feb. 2015. DOI 10.1093/femsle/fnu042. Available at: <https://academic.oup.com/femsle/article/362/3/1/1803244>. Accessed on: 14 Feb. 2021.

DOS SANTOS, L. V.; CARAZZOLLE, M. F.; NAGAMATSU, S. T.; SAMPAIO, N. M. V.; ALMEIDA, L. D.; PIROLA, R. A. S.; BORELLI, G.; CORRÊA, T. L. R.; ARGUESO, J. L.; PEREIRA, G. A. G. Unraveling the genetic basis of xylose consumption in engineered *Saccharomyces cerevisiae* strains. **Scientific Reports**, Berlin, vol. 6, no. 1, p. 1–14, 2016. DOI 10.1038/srep38676. Available at: <https://www.nature.com/articles/srep38676>. Accessed on: 10 Feb. 2021.

DOS SANTOS, L. V.; DE BARROS GRASSI, M. C.; GALLARDO, J. C. M.; PIROLA, R. A. S.; CALDERÓN, L. L.; DE CARVALHO-NETTO, O. V.; PARREIRAS, L. S.; CAMARGO, E. L. O.; DREZZA, A. L.; MISSAWA, S. K.; TEIXEIRA, G. S.; LUNARDI, I.; BRESSIANI, J.; PEREIRA, G. A. G. Second-Generation Ethanol: The Need is Becoming a Reality. **Industrial Biotechnology**, New Rochelle, vol. 12, no. 1, p. 40–57, 1 Feb. 2016. DOI 10.1089/ind.2015.0017. Available at: <https://www.liebertpub.com/doi/10.1089/ind.2015.0017>. Accessed on: 10 Feb. 2021.

DRAGOSITS, M.; MATTANOVICH, D. Adaptive laboratory evolution – principles and applications for biotechnology. **Microbial Cell Factories**, Berlin, vol. 12, no. 1, p. 1–17, 2013. DOI 10.1186/1475-2859-12-64. Available at: <https://link.springer.com/article/10.1186/1475-2859-12-64>. Accessed on: 12 Feb. 2021.

EDWARDS, M. C.; DORAN-PETERSON, J. Pectin-rich biomass as feedstock for fuel ethanol production. **Applied Microbiology and Biotechnology**, Berlin, vol. 95, no. 3, p. 565–575, 2012. DOI 10.1007/s00253-012-4173-2. Available at: <https://link.springer.com/article/10.1007/s00253-012-4173-2>. Accessed on: 12 Feb. 2021.

ENDO, A.; NAKAMURA, T.; ANDO, A.; TOKUYASU, K.; SHIMA, J. Genome-wide screening of the genes required for tolerance to vanillin, which is a potential inhibitor of bioethanol fermentation, in *Saccharomyces cerevisiae*. **Biotechnology for Biofuels**, Berlin, vol. 1, no. 1, p. 1–7, 2008. DOI 10.1186/1754-6834-1-3. Available at: <https://link.springer.com/article/10.1186/1754-6834-1-3>. Accessed on: 12 Feb. 2021.

FARHAT, W.; VENDITTI, R.; QUICK, A.; TAHA, M.; MIGNARD, N.; BECQUART, F.; AYOUB, A. Hemicellulose extraction and characterization for applications in paper coatings and adhesives. **Industrial Crops and Products**, Amsterdam, vol. 107, p. 370–377, 2017. DOI 10.1016/j.indcrop.2017.05.055. Available at: <https://www.sciencedirect.com/science/article/pii/S0926669017303758>. Accessed on: 11 Feb. 2021.

FARWICK, A.; BRUDER, S.; SCHADEWEG, V.; OREB, M.; BOLES, E. Engineering of yeast hexose transporters to transport d-xylose without inhibition by d-glucose. **Proceedings of the National Academy of Sciences**, Washington, vol. 111, no. 14, p. 5159–5164, 8 Apr. 2014. DOI 10.1073/pnas.1323464111. Available at: <http://www.pnas.org/content/111/14/5159.abstract>. Accessed on: 12 Feb. 2021.

FELIPUCI, J. P.; DE FREITAS, C.; ZAMORA ZAMORA, H. D.; ANGELIS, D. A.; BRIENZO, M. Biotechnological Aspects of Microbial Pretreatment of Lignocellulosic Biomass. In: VERMA, P. (ed.). **Biorefineries: A Step Towards Renewable and Clean Energy**. Singapore: Springer Singapore, 2021. p. 121–150. DOI 10.1007/978-981-15-9593-6_6. Available at: https://link.springer.com/chapter/10.1007/978-981-15-9593-6_6. Accessed on: 23 Sep. 2021.

FLETCHER, E.; BAETZ, K. Multi-Faceted Systems Biology Approaches Present a Cellular Landscape of Phenolic Compound Inhibition in *Saccharomyces cerevisiae*. **Frontiers in Bioengineering and Biotechnology**, Lausanne, vol. 8, p. 1–13, 2020. DOI 10.3389/fbioe.2020.539902. Available at: <https://www.frontiersin.org/article/10.3389/fbioe.2020.539902>. Accessed on: 12 Jan. 2021.

FLETCHER, E.; GAO, K.; MERCURIO, K.; ALI, M.; BAETZ, K. Yeast chemogenomic screen identifies distinct metabolic pathways required to tolerate exposure to phenolic fermentation inhibitors ferulic acid, 4-hydroxybenzoic acid and coniferyl aldehyde. **Metabolic Engineering**, Amsterdam, vol. 52, p. 98–109, 2019. DOI 10.1016/j.ymben.2018.11.010. Available at: <https://www.sciencedirect.com/science/article/pii/S1096717618303240>. Accessed on: 14 Feb. 2021.

FLÓREZ-PARDO, L. M.; GONZÁLEZ-CÓRDOBA, A.; LÓPEZ-GALÁN, J. E. Evaluation of different methods for efficient extraction of hemicelluloses leaves and tops of sugarcane. **DYNA**, Medellín, vol. 85, no. 204, p. 18–27, 2018. DOI 10.15446/dyna.v85n204.66626. Available at: http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0012-73532018000100018. Accessed on: 11 Feb. 2021.

FORSAN, C. F.; DE FREITAS, C.; MASARIN, F.; BRIENZO, M. Xylooligosaccharide production from sugarcane bagasse and leaf using *Aspergillus versicolor* endoxylanase and diluted acid. **Biomass Conversion and Biorefinery**, Berlin, , p. 1–16, 2021. DOI 10.1007/s13399-021-01403-2. Available at: <https://link.springer.com/article/10.1007/s13399-021-01403-2>. Accessed on: 2 Jan. 2021.

FRANZ, S.; SHCHERBAN, N. D.; SIMAKOVA, I. L.; PEURLA, M.; ERÄNEN, K.; WÄRNÄ, J.; SALMI, T.; MURZIN, D. Y. Oxidation of glucose and arabinose mixtures over Au/Al₂O₃. **Reaction Kinetics, Mechanisms and Catalysis**, Berlin, 2020. DOI 10.1007/s11144-020-01901-w. Available at: <https://link.springer.com/article/10.1007/s11144-020-01901-w>. Accessed on: 10 Feb. 2021.

FÜLÖP, L.; ECKER, J. An overview of biomass conversion: exploring new opportunities. **PeerJ - Life and Environment**, San Diego, vol. 8, p. 1–21, 2020. DOI 10.7717/peerj.9586. Available at: <https://peerj.com/articles/9586>. Accessed on: 8 Feb. 2021.

GARCÍA, C. A.; PEÑA, Á.; BETANCOURT, R.; CARDONA, C. A. Energetic and environmental assessment of thermochemical and biochemical ways for producing energy from agricultural solid residues: Coffee Cut-Stems case. **Journal of Environmental Management**, Amsterdam, vol. 216, p. 160–168, 2018. DOI 10.1016/j.jenvman.2017.04.029. Available at: <https://www.sciencedirect.com/science/article/pii/S0301479717303705>. Accessed on: 6 Feb. 2021.

GARROTE, G.; DOMÍNGUEZ, H.; PARAJO, J. C. Hydrothermal processing of lignocellulosic materials. **Holz als Roh- und Werkstoff**, Berlin, vol. 57, no. 3, p. 191–202, 1999. DOI 10.1007/s001070050039. Available at: <https://link.springer.com/article/10.1007/s001070050039>. Accessed on: 10 Feb. 2021.

GE, X.; CHANG, C.; ZHANG, L.; CUI, S.; LUO, X.; HU, S.; QIN, Y.; LI, Y. Chapter Five - Conversion of Lignocellulosic Biomass Into Platform Chemicals for Biobased Polyurethane Application. In: LI, Y.; GE, X. B. T.-A. In B. (eds.). **Advances in Bioenergy**. Amsterdam: Elsevier, 2018. vol. 3, p. 161–213. DOI 10.1016/bs.aibe.2018.03.002. Available at: <https://www.sciencedirect.com/science/article/abs/pii/S2468012518300051>. Accessed on: 6 Feb. 2021.

GIAEVER, G.; NISLOW, C. The Yeast Deletion Collection: A Decade of Functional Genomics. **Genetics**, Rockville, vol. 197, no. 2, p. 451–465, 1 Jun. 2014. DOI 10.1534/genetics.114.161620. Available at: <http://www.genetics.org/content/197/2/451.abstract>. Accessed on: 14 Feb. 2021.

GIANNATTASIO, S.; GUARAGNELLA, N.; ŽDRALEVIĆ, M.; MARRA, E. Molecular mechanisms of *Saccharomyces cerevisiae* stress adaptation and programmed cell death in response to acetic acid. **Frontiers in Microbiology**, Lausanne, vol. 4, p. 1–7, 2013. DOI 10.3389/fmicb.2013.00033. Available at: <https://www.frontiersin.org/article/10.3389/fmicb.2013.00033>. Accessed on: 12 Feb. 2021.

GROHMANN, K.; BOTHAST, R. J. Saccharification of corn fibre by combined treatment with dilute sulphuric acid and enzymes. **Process Biochemistry**, Amsterdam, vol. 32, no. 5, p. 405–415, 1997. DOI 10.1016/S0032-9592(96)00095-7. Available at: <https://www.sciencedirect.com/science/article/pii/S0032959296000957>. Accessed on: 13 Feb. 2021.

GUEDES, F.; SZKLO, A.; ROCHEDO, P.; LANTZ, F.; MAGALAR, L.; ARROYO, E. M. V. Climate-energy-water nexus in Brazilian oil refineries. **International Journal of Greenhouse Gas Control**, Amsterdam, vol. 90, p. 1–11, 2019. DOI 10.1016/j.ijggc.2019.102815. Available at: <http://www.sciencedirect.com/science/article/pii/S1750583618306844>. Accessed on: 1 Feb. 2021.

GUIGOU, M. D.; CEBREIROS, F.; CABRERA, M. N.; FERRARI, M. D.; LAREO, C. Bioethanol production from *Eucalyptus grandis* hemicellulose recovered before kraft pulping using an integrated biorefinery concept. **Biomass Conversion and Biorefinery**, Berlin, vol. 7, no. 2, p. 191–197, 2017. DOI 10.1007/s13399-016-0218-6. Available at: <https://link.springer.com/article/10.1007/s13399-016-0218-6>. Accessed on: 11 Feb. 2021.

GUTMANN, F.; JANN, C.; PEREIRA, F.; JOHANSSON, A.; STEINMETZ, L. M.; PATIL, K. R. CRISPRi screens reveal genes modulating yeast growth in lignocellulose hydrolysate. **Biotechnology for Biofuels**, Berlin, vol. 14, no. 1, p. 41, 2021. DOI 10.1186/s13068-021-01880-7. Available at: <https://link.springer.com/article/10.1186/s13068-021-01880-7>. Accessed on: 14 Feb. 2021.

HACISALIHOĞLU, B.; HOLYAVKIN, C.; TOPALOĞLU, A.; KISAKESEN, H. İ.; ÇAKAR, Z. P. Genomic and transcriptomic analysis of a coniferyl aldehyde-resistant *Saccharomyces cerevisiae* strain obtained by evolutionary engineering. **FEMS Yeast Research**, Oxford, vol. 19, no. 3, p. 1, 1 May 2019. DOI 10.1093/femsyr/foz021. Available at: <https://academic.oup.com/femsyr/article-abstract/19/3/foz021/5369625>. Accessed on: 12 Feb. 2021.

HAFEMANN, E.; BATTISTI, R.; BRESOLIN, D.; MARANGONI, C.; MACHADO, R. A. F. Enhancing Chlorine-Free Purification Routes of Rice Husk Biomass Waste to Obtain Cellulose Nanocrystals. **Waste and Biomass Valorization**, Berlin, vol. 11, no. 12, p. 6595–6611, 2020. DOI 10.1007/s12649-020-00937-2. Available at: <https://link.springer.com/article/10.1007/s12649-020-00937-2>. Accessed on: 6 Feb. 2021.

HAHN-HÄGERDAL, B.; KARHUMAA, K.; FONSECA, C.; SPENCER-MARTINS, I.; GORWA-GRAUSLUND, M. F. Towards industrial pentose-fermenting yeast strains. **Applied Microbiology and Biotechnology**, Berlin, vol. 74, no. 5, p. 937–953, 2007. DOI 10.1007/s00253-006-0827-2. Available at: <https://link.springer.com/article/10.1007/s00253-006-0827-2>. Accessed on: 12 Feb. 2021.

HAHN-HÄGERDAL, B.; WAHLBOM, C. F.; GÁRDONYI, M.; VAN ZYL, W. H.; OTERO, R. R. C.; JÖNSSON, L. J. Metabolic Engineering of *Saccharomyces cerevisiae* for Xylose Utilization. In: NIELSEN, J.; EGGELING, L.; DYNESSEN, J.; GÁRDONYI, M.; GILL, R. T.; DE GRAAF, A. A.; HAHN-HÄGERDAL, B.; JÖNSSON, L. J.; KHOSLA, C.; LICARI, R.; MCDANIEL, R.; MCINTYRE, M.; MILLER, C.; NIELSEN, J.; CORDERO OTERO, R. R.; SAHM, H.; SAUER, U.; STAFFORD, D. E.; STEPHANOPOULOS, G.; ... VAN ZYL, W. H. (eds.). **Metabolic Engineering**. Berlin: Springer Berlin Heidelberg, 2001. p. 53–84. DOI 10.1007/3-540-45300-8_4. Available at: https://link.springer.com/chapter/10.1007/3-540-45300-8_4. Accessed on: 14 Feb. 2021.

HARHANGI, H. R.; AKHMANOVA, A. S.; EMMENS, R.; VAN DER DRIFT, C.; DE LAAT, W. T. A. M.; VAN DIJKEN, J. P.; JETTEN, M. S. M.; PRONK, J. T.; OP DEN CAMP, H. J. M. Xylose metabolism in the anaerobic fungus *Piromyces* sp. strain E2 follows the bacterial pathway. **Archives of Microbiology**, Berlin, vol. 180, no. 2, p. 134–141, 2003. DOI 10.1007/s00203-003-0565-0. Available at: <https://link.springer.com/article/10.1007/s00203-003-0565-0>.

HASUNUMA, T.; ISMAIL, K. S. K.; NAMBU, Y.; KONDO, A. Co-expression of TAL1 and ADH1 in recombinant xylose-fermenting *Saccharomyces cerevisiae* improves ethanol production from lignocellulosic hydrolysates in the presence of furfural. **Journal of Bioscience and Bioengineering**, Amsterdam, vol. 117, no. 2, p. 165–169, 2014. DOI 10.1016/j.jbiosc.2013.07.007. Available at: <https://www.sciencedirect.com/science/article/pii/S1389172313002697>. Accessed on: 13 Feb. 2021.

HASUNUMA, T.; SAKAMOTO, T.; KONDO, A. Inverse metabolic engineering based on transient acclimation of yeast improves acid-containing xylose fermentation and tolerance to formic and acetic acids. **Applied Microbiology and Biotechnology**, Berlin, vol. 100, no. 2, p. 1027–1038, 2016. DOI 10.1007/s00253-015-7094-z. Available at: <https://link.springer.com/article/10.1007/s00253-015-7094-z>. Accessed on: 14 Feb. 2021.

HEER, D.; HEINE, D.; SAUER, U. Resistance of *Saccharomyces cerevisiae* to High Concentrations of Furfural Is Based on NADPH-Dependent Reduction by at Least Two Oxidoreductases. **Applied and Environmental Microbiology**, Washington, vol. 75, no. 24, p. 7631–7638, 15 Dec. 2009. DOI 10.1128/AEM.01649-09. Available at: <http://aem.asm.org/content/75/24/7631.abstract>. Accessed on: 14 Feb. 2021.

HEER, D.; SAUER, U. Identification of furfural as a key toxin in lignocellulosic hydrolysates and evolution of a tolerant yeast strain. **Microbial Biotechnology**, Hoboken, vol. 1, no. 6, p. 497–506, 1 Nov. 2008. DOI 10.1111/j.1751-7915.2008.00050.x. Available at: <https://sfamjournals.onlinelibrary.wiley.com/doi/10.1111/j.1751-7915.2008.00050.x>. Accessed on: 12 Feb. 2021.

HILL, C.; ALTGEN, M.; RAUTKARI, L. Thermal modification of wood—a review: chemical changes and hygroscopicity. **Journal of Materials Science**, Berlin, vol. 56, no. 11, p. 6581–6614, 2021. DOI 10.1007/s10853-020-05722-z. Available at: <https://link.springer.com/article/10.1007/s10853-020-05722-z>. Accessed on: 8 Feb. 2021.

HO, N. W. Y. Cloning and Over-Expression of the *E. coli* D-Xylose Isomerase Gene in Various Microorganisms. 1989. **Bioproducts and Bioprocesses** [...]. Berlin: Springer Berlin Heidelberg, 1989. p. 181–188. DOI 10.1007/978-3-642-74227-9_15. Available at: https://link.springer.com/chapter/10.1007/978-3-642-74227-9_15. Accessed on: 14 Feb. 2021.

HOEGH-GULDBERG, O.; JACOB, D.; TAYLOR, M. Impacts of 1.5°C of Global Warming on Natural and Human Systems. In: MARENGO, J. A.; PEREIRA, J.; SHERSTYUKOV, B. (eds.). **Global Warming of 1.5°C**. Geneva: United Nations Environment Programme & World Meteorological Organization, 2018. p. 175–311. Available at: https://www.ipcc.ch/site/assets/uploads/sites/2/2019/06/SR15_Chapter3_Low_Res.pdf. Accessed on: 1 Feb. 2021.

HONGSIRI, W.; DANON, B.; DE JONG, W. The effects of combined catalysis of oxalic acid and seawater on the kinetics of xylose and arabinose dehydration to furfural. **International Journal of Energy and Environmental Engineering**, Berlin, vol. 6, no. 1, p. 21–30, 2015. DOI 10.1007/s40095-014-0146-9. Available at: <https://link.springer.com/article/10.1007/s40095-014-0146-9>. Accessed on: 10 Feb. 2021.

ISIKGOR, F. H.; BECER, C. R. Lignocellulosic biomass: a sustainable platform for the production of bio-based chemicals and polymers. **Polymer Chemistry**, London, vol. 6, no. 25, p. 4497–4559, 2015. DOI 10.1039/C5PY00263J. Available at: <https://pubs.rsc.org/en/content/articlelanding/2015/py/c5py00263j#divAbstract>. Accessed on: 14 Feb. 2021.

JAGTAP, S. S.; BEDEKAR, A. A.; LIU, J.-J.; JIN, Y.-S.; RAO, C. V. Production of galactitol from galactose by the oleaginous yeast *Rhodospiridium toruloides* IFO0880. **Biotechnology for Biofuels**, Berlin, vol. 12, no. 1, p. 250, 2019. DOI 10.1186/s13068-019-1586-5. Available at: <https://link.springer.com/article/10.1186/s13068-019-1586-5>. Accessed on: 10 Feb. 2021.

JANSEN, M. L. A.; BRACHER, J. M.; PAPAPETRIDIS, I.; VERHOEVEN, M. D.; DE BRUIJN, H.; DE WAAL, P. P.; VAN MARIS, A. J. A.; KLAASSEN, P.; PRONK, J. T. Saccharomyces cerevisiae strains for second-generation ethanol production: from academic exploration to industrial implementation. **FEMS Yeast Research**, Oxford, vol. 17, no. 5, p. 1–8, 1 Aug. 2017. DOI 10.1093/femsyr/fox044. Available at: <https://academic.oup.com/femsyr/article/17/5/fox044/3868933>. Accessed on: 13 Feb. 2021.

JEFFRIES, T. W.; JIN, Y.-S. Metabolic engineering for improved fermentation of pentoses by yeasts. **Applied Microbiology and Biotechnology**, Berlin, vol. 63, no. 5, p. 495–509, 2004. DOI 10.1007/s00253-003-1450-0. Available at: <https://link.springer.com/article/10.1007/s00253-003-1450-0>.

JÖNSSON, L. J.; ALRIKSSON, B.; NILVEBRANT, N.-O. Bioconversion of lignocellulose: inhibitors and detoxification. **Biotechnology for Biofuels**, Berlin, vol. 6, no. 1, p. 16, 2013. DOI 10.1186/1754-6834-6-16. Available at: <https://link.springer.com/article/10.1186/1754-6834-6-16>. Accessed on: 14 Feb. 2021.

JOUBERT, A. J.; CHIMPHANGO, A. F. A.; GÖRGES, J. F. Effect of integrating xylan extraction from *E. grandis* into the kraft pulping process on pulp yield and chemical balance. **BioResources**, Raleigh, vol. 11, no. 1, p. 2417–2437, 2016. DOI 10.15376/BIORES.11.1.2417-2437. Available at: <https://bioresources.cnr.ncsu.edu/resources/effect-of-integrating-xylan-extraction-from-e-grandis-into-the-kraft-pulping-process-on-pulp-yield-and-chemical-balance/>. Accessed on: 10 Feb. 2021.

JUN, A.; TSCHIRNER, U. W.; TAUER, Z. Hemicellulose extraction from aspen chips prior to kraft pulping utilizing kraft white liquor. **Biomass and Bioenergy**, Amsterdam, vol. 37, p. 229–236, 2012. DOI 10.1016/j.biombioe.2011.12.008. Available at: <https://www.sciencedirect.com/science/article/pii/S0961953411006155>. Accessed on: 16 Feb. 2021.

JUNG, Y. H.; KIM, K. H. Chapter 3 - Acidic Pretreatment. In: PANDEY, A.; NEGI, S.; BINOD, P.; LARROCHE, C. B. T.-P. of B. (eds.). **Pretreatment of Biomass**. Amsterdam: Elsevier, 2015. p. 27–50. DOI 10.1016/B978-0-12-800080-9.00003-7. Available at: <https://www.sciencedirect.com/science/article/pii/B9780128000809000037>. Accessed on: 12 Feb. 2021.

JUNQUEIRA, T. L.; CHAGAS, M. F.; GOUVEIA, V. L. R.; REZENDE, M. C. A. F.; WATANABE, M. D. B.; JESUS, C. D. F.; CAVALETT, O.; MILANEZ, A. Y.; BONOMI, A. Techno-economic analysis and climate change impacts of sugarcane biorefineries considering different time horizons. **Biotechnology for Biofuels**, Berlin, vol. 10, no. 1, p. 50, 2017. DOI 10.1186/s13068-017-0722-3. Available at: <https://link.springer.com/article/10.1186/s13068-017-0722-3>. Accessed on: 16 Feb. 2021.

KÄMPPI, R.; HÖRHAMMER, H.; LEPONIEMI, A.; VAN HEININGEN, A. Pre-extraction and PSAQ pulping of Siberian larch. **Nordic Pulp & Paper Research Journal**, Berlin, vol. 25, no. 2, p. 243–248, 2010. DOI 10.3183/npprj-2010-25-02-p243-248. Available at: <https://www.degruyter.com/document/doi/10.3183/npprj-2010-25-02-p243-248/html>. Accessed on: 15 Feb. 2021.

KATAHIRA, S.; ITO, M.; TAKEMA, H.; FUJITA, Y.; TANINO, T.; TANAKA, T.; FUKUDA, H.; KONDO, A. Improvement of ethanol productivity during xylose and glucose co-fermentation by xylose-assimilating *S. cerevisiae* via expression of glucose transporter Sut1. **Enzyme and Microbial Technology**, Amsterdam, vol. 43, no. 2, p. 115–119, 2008. DOI 10.1016/j.enzmictec.2008.03.001. Available at: <https://www.sciencedirect.com/science/article/pii/S0141022908000847>. Accessed on: 13 Feb. 2021.

KAUTTO, J.; SAUKKONEN, E.; HENRICSON, K. Digestibility and paper-making properties of prehydrolyzed softwood chips. **BioResources**, Raleigh, vol. 5, no. 4, p. 2502–2519, 2010. Available at: <https://bioresources.cnr.ncsu.edu/resources/digestibility-and-paper-making-properties-of-prehydrolyzed-softwood-chips/>. Accessed on: 14 Feb. 2021.

KIM, D.; HAHN, J.-S. Roles of the Yap1 Transcription Factor and Antioxidants in *Saccharomyces cerevisiae*'s Tolerance to Furfural and 5-Hydroxymethylfurfural, Which Function as Thiol-Reactive Electrophiles G. **Applied and Environmental Microbiology**, Washington, vol. 79, no. 16, p. 5069–5077, 15 Aug. 2013. DOI 10.1128/AEM.00643-13. Available at: <http://aem.asm.org/content/79/16/5069.abstract>. Accessed on: 12 Feb. 2021.

KIM, H. S.; JEONG, G.-T. Valorization of galactose into levulinic acid via acid catalysis. **Korean Journal of Chemical Engineering**, Berlin, vol. 35, no. 11, p. 2232–2240, 2018. DOI 10.1007/s11814-018-0126-5. Available at: <https://link.springer.com/article/10.1007/s11814-018-0126-5>. Accessed on: 10 Feb. 2021.

KIM, J. H.; RYU, J.; HUH, I. Y.; HONG, S.-K.; KANG, H. A.; CHANG, Y. K. Ethanol production from galactose by a newly isolated *Saccharomyces cerevisiae* KL17. **Bioprocess and Biosystems Engineering**, Berlin, vol. 37, no. 9, p. 1871–1878, 2014. <https://doi.org/https://doi.org/10.1007/s00449-014-1161-1>.

KIM, S.-K.; JIN, Y.-S.; CHOI, I.-G.; PARK, Y.-C.; SEO, J.-H. Enhanced tolerance of *Saccharomyces cerevisiae* to multiple lignocellulose-derived inhibitors through modulation of spermidine contents. **Metabolic Engineering**, Amsterdam, vol. 29, p. 46–55, 2015. DOI 10.1016/j.ymben.2015.02.004. Available at: <https://www.sciencedirect.com/science/article/pii/S1096717615000154>. Accessed on: 12 Feb. 2021.

KORDOWSKA-WIATER, M.; KUBIK-KOMAR, A.; TARGONSKI, Z. Application of response surface methodology for the optimization of arabinose biotransformation to arabinol by *Candida parapsilosis*. **Central European Journal of Biology**, Berlin, vol. 8, no. 9, p. 835–842, 2013. DOI 10.2478/s11535-013-0196-z. Available at: <https://link.springer.com/article/10.2478/s11535-013-0196-z>. Accessed on: 10 Feb. 2021.

KÖTTER, P.; CIRIACY, M. Xylose fermentation by *Saccharomyces cerevisiae*. **Applied Microbiology and Biotechnology**, Berlin, vol. 38, no. 6, p. 776–783, 1993. DOI 10.1007/BF00167144. Available at: <https://link.springer.com/article/10.1007/BF00167144>. Accessed on: 14 Feb. 2021.

KUCHARSKA, K.; SŁUPEK, E.; CIEŚLIŃSKI, H.; KAMIŃSKI, M. Advantageous conditions of saccharification of lignocellulosic biomass for biofuels generation via fermentation processes. **Chemical Papers**, Berlin, vol. 74, no. 4, p. 1199–1209, 2020. DOI 10.1007/s11696-019-00960-1. Available at: <https://link.springer.com/article/10.1007/s11696-019-00960-1#citeas>. Accessed on: 10 May 2020.

KUHN, A.; VAN ZYL, C.; VAN TONDER, A.; PRIOR, B. A. Purification and partial characterization of an aldo-keto reductase from *Saccharomyces cerevisiae*. **Applied and Environmental Microbiology**, Washington, vol. 61, no. 4, p. 1580–1585, 1 Apr. 1995. Available at: <http://aem.asm.org/content/61/4/1580.abstract>.

KUMAR, G.; SIVAGURUNATHAN, P.; PARK, J.-H.; KIM, S.-H. Improved Hydrogen Production from Galactose Via Immobilized Mixed Consortia. **Arabian Journal for Science and Engineering**, Berlin, vol. 40, no. 8, p. 2117–2122, 2015. DOI 10.1007/s13369-015-1729-3. Available at: <https://link.springer.com/article/10.1007/s13369-015-1729-3>. Accessed on: 10 Feb. 2021.

KUMAR, P.; BARRETT, D. M.; DELWICHE, M. J.; STROEVE, P. Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. **Industrial & Engineering Chemistry Research**, Washington, vol. 48, no. 8, p. 3713–3729, Apr. 2009. DOI 10.1021/ie801542g. Available at: <https://pubs.acs.org/doi/10.1021/ie801542g#>. Accessed on: 13 Feb. 2021.

KURTZMAN, C. P.; DIEN, B. S. Candida arabinofermentans, a new L-arabinose fermenting yeast. **Antonie van Leeuwenhoek**, Berlin, vol. 74, no. 4, p. 237–243, 1998. DOI 10.1023/A:1001799607871. Available at: <https://link.springer.com/article/10.1023/A:1001799607871>. Accessed on: 14 Feb. 2021.

KUYPER, M.; HARHANGI, H. R.; STAVE, A. K.; WINKLER, A. A.; JETTEN, M. S. M.; DE LAAT, W. T. A. M.; DEN RIDDER, J. J. J.; OP DEN CAMP, H. J. M.; VAN DIJKEN, J. P.; PRONK, J. T. High-level functional expression of a fungal xylose isomerase: the key to efficient ethanolic fermentation of xylose by *Saccharomyces cerevisiae*? **FEMS Yeast Research**, Oxford, vol. 4, no. 1, p. 69–78, 1 Oct. 2003. DOI 10.1016/S1567-1356(03)00141-7. Available at: <https://academic.oup.com/femsyr/article/4/1/69/623131>. Accessed on: 14 Feb. 2021.

KUYPER, M.; HARTOG, M. M. P.; TOIRKENS, M. J.; ALMERING, M. J. H.; WINKLER, A. A.; VAN DIJKEN, J. P.; PRONK, J. T. Metabolic engineering of a xylose-isomerase-expressing *Saccharomyces cerevisiae* strain for rapid anaerobic xylose fermentation. **FEMS Yeast Research**, Oxford, vol. 5, no. 4–5, p. 399–409, Feb. 2005. DOI 10.1016/j.femsyr.2004.09.010. Available at: <https://academic.oup.com/femsyr/article/5/4-5/399/580715>. Accessed on: 4 Feb. 2021.

KWAK, S.; JIN, Y.-S. Production of fuels and chemicals from xylose by engineered *Saccharomyces cerevisiae*: a review and perspective. **Microbial Cell Factories**, Berlin, vol. 16, no. 1, p. 1–15, 2017. DOI 10.1186/s12934-017-0694-9. Available at: <https://link.springer.com/article/10.1186/s12934-017-0694-9>. Accessed on: 1 Feb. 2021.

LARSSON, S.; CASSLAND, P.; JÖNSSON, L. J. Development of a *Saccharomyces cerevisiae* Strain with Enhanced Resistance to Phenolic Fermentation Inhibitors in Lignocellulose Hydrolysates by Heterologous Expression of Laccase. **Applied and Environmental Microbiology**, Washington, vol. 67, no. 3, p. 1163–1170, 1 Mar. 2001. DOI 10.1128/AEM.67.3.1163-1170.2001. Available at: <http://aem.asm.org/content/67/3/1163.abstract>.

LEE, N.; GIELOW, W.; MARTIN, R.; HAMILTON, E.; FOWLER, A. The organization of the araBAD operon of *Escherichia coli*. **Gene**, Amsterdam, vol. 47, no. 2, p. 231–244, 1986. DOI 10.1016/0378-1119(86)90067-3. Available at: <https://www.sciencedirect.com/science/article/pii/0378111986900673>. Accessed on: 14 Feb. 2021.

LEE, S.-M.; JELLISON, T.; ALPER, H. S. Directed Evolution of Xylose Isomerase for Improved Xylose Catabolism and Fermentation in the Yeast *Saccharomyces cerevisiae*. **Applied and Environmental Microbiology**, Washington, vol. 78, no. 16, p. 5708–5716, 15 Aug. 2012. DOI 10.1128/AEM.01419-12. Available at: <http://aem.asm.org/content/78/16/5708.abstract>. Accessed on: 1 Feb. 2021.

LEI, Y.; LIU, S.; LI, J.; SUN, R. Effect of hot water extraction on alkaline pulping of bagasse. **Biotechnology Advances**, vol. 28, p. 609–612, 2010.

LI, J.; XU, J.; CAI, P.; WANG, B.; MA, Y.; BENZ, J. P.; TIAN, C. Functional Analysis of Two L-Arabinose Transporters from Filamentous Fungi Reveals Promising Characteristics for Improved Pentose Utilization in *Saccharomyces cerevisiae*. **Applied and Environmental Microbiology**, Washington, vol. 81, no. 12, p. 4062–4070, 15 Jun. 2015. DOI 10.1128/AEM.00165-15. Available at: <http://aem.asm.org/content/81/12/4062.abstract>.

LIAO, J. C.; MI, L.; PONTRELLI, S.; LUO, S. Fuelling the future: microbial engineering for the production of sustainable biofuels. **Nature Reviews Microbiology**, Berlin, vol. 14, no. 5, p. 288–304, 2016. DOI 10.1038/nrmicro.2016.32. Available at: <https://www.nature.com/articles/nrmicro.2016.32>. Accessed on: 14 Feb. 2021.

LIM, H. G.; LIM, J. H.; JUNG, G. Y. Modular design of metabolic network for robust production of n-butanol from galactose–glucose mixtures. **Biotechnology for Biofuels**, Berlin, vol. 8, no. 1, p. 137, 2015. DOI 10.1186/s13068-015-0327-7. Available at: <https://link.springer.com/article/10.1186/s13068-015-0327-7>. Accessed on: 10 Feb. 2021.

LIMA, D. U.; OLIVEIRA, R. C.; BUCKERIDGE, M. S. Seed storage hemicelluloses as wet-end additives in papermaking. **Carbohydrate Polymers**, Amsterdam, vol. 52, no. 4, p. 367–373, 2003. DOI 10.1016/S0144-8617(03)00008-0. Available at: <https://www.sciencedirect.com/science/article/pii/S0144861703000080>. Accessed on: 15 Feb. 2021.

LOSORDO, Z.; MCBRIDE, J.; ROOYEN, J. Van; WENGER, K.; WILLIES, D.; FROELICH, A.; MACEDO, I.; LYND, L. Cost competitive second-generation ethanol production from hemicellulose in a Brazilian sugarcane biorefinery. **Biofuels, Bioproducts and Biorefining**, Hoboken, vol. 10, no. 5, p. 589–602, 1 Sep. 2016. DOI 10.1002/bbb.1663. Available at: <https://onlinelibrary.wiley.com/doi/10.1002/bbb.1663>. Accessed on: 16 Feb. 2021.

LYND, L. R. Overview and evaluation of fuel ethanol from cellulosic biomass: technology, economics, the environment, and policy. **Annual Review of Energy and the Environment**, Palo Alto, vol. 21, no. 1, p. 403–465, 1 Nov. 1996. DOI 10.1146/annurev.energy.21.1.403. Available at: <https://www.annualreviews.org/doi/abs/10.1146/annurev.energy.21.1.403>. Accessed on: 13 Jan. 2021.

MANSOR, A. M.; LIM, J. S.; ANI, F. N.; HASHIM, H.; HO, W. S. Characteristics of Cellulose, Hemicellulose and Lignin of MD2 Pineapple Biomass. **Chemical Engineering Transactions**, Milan, vol. 72, p. 79–84, 31 Jan. 2019. DOI 10.3303/CET1972014. Available at: <https://www.cetjournal.it/index.php/cet/article/view/CET1972014>. Accessed on: 8 Feb. 2021.

MARTINO, D. C.; COLODETTE, J. L.; CHANDRA, R.; SADDLER, J. Steam explosion pretreatment used to remove hemicellulose to enhance the production of a eucalyptus organosolv dissolving pulp. **Wood Science and Technology**, Berlin, vol. 51, no. 3, p. 557–569, 2017. DOI 10.1007/s00226-016-0889-y. Available at: <https://link.springer.com/article/10.1007/s00226-016-0889-y>. Accessed on: 15 Feb. 2021.

MEIJNEN, J.-P.; RANDAZZO, P.; FOULQUIÉ-MORENO, M. R.; VAN DEN BRINK, J.; VANDECRUYS, P.; STOJILJKOVIC, M.; DUMORTIER, F.; ZALAR, P.; BOEKHOUT, T.; GUNDE-CIMERMAN, N.; KOKOŠAR, J.; ŠTAJDOHAR, M.; CURK, T.; PETROVIĆ, U.; THEVELEIN, J. M. Polygenic analysis and targeted improvement of the complex trait of high acetic acid tolerance in the yeast *Saccharomyces cerevisiae*. **Biotechnology for Biofuels**, Berlin, vol. 9, no. 1, p. 1–18, 2016. DOI 10.1186/s13068-015-0421-x. Available at: <https://link.springer.com/article/10.1186/s13068-015-0421-x>. Accessed on: 12 Feb. 2021.

MELATI, R. B.; SASS, D. C.; PAGNOCCA, F. C.; BRIENZO, M. Anatomic influence of sugarcane biomass on xylan solubilization. **Industrial Crops and Products**, Amsterdam, vol. 164, p. 113357, 2021. DOI 10.1016/j.indcrop.2021.113357. Available at: <https://www.sciencedirect.com/science/article/pii/S0926669021001217>. Accessed on: 1 Feb. 2021.

MELATI, R. B.; SHIMIZU, F. L.; OLIVEIRA, G.; PAGNOCCA, F. C.; DE SOUZA, W.; SANT'ANNA, C.; BRIENZO, M. Key Factors Affecting the Recalcitrance and Conversion Process of Biomass. **BioEnergy Research**, Berlin, vol. 12, no. 1, p. 1–20, 2019. DOI 10.1007/s12155-018-9941-0. Available at: <https://link.springer.com/article/10.1007/s12155-018-9941-0#citeas>. Accessed on: 1 Jun. 2020.

MENDES, C. V. T.; ROCHA, J. M. S.; SOUSA, G. D. A.; CARVALHO, G. V. S. Extraction of hemicelluloses prior to kraft cooking:

A step for an integrated biorefinery in the pulp mill. **O Papel**, vol. 72, no. 9, p. 79–83, 2011. .

MILESSI, T. S.; ZANGIROLAMI, T. C.; PEREZ, C. L.; SANDRI, J. P.; CORRADINI, F. A. S.; FOULQUIÉ-MORENO, M. R.; THEVELEIN, J. M.; GIORDANO, R. C.; GIORDANO, R. L. C. Bioethanol Production from Xylose-Rich Hydrolysate by Immobilized Recombinant *Saccharomyces cerevisiae* in Fixed-Bed Reactor. **Industrial Biotechnology**, New Rochelle, vol. 16, no. 2, p. 75–80, 2020. DOI 10.1089/ind.2020.29198.tsm. Available at: <https://www.liebertpub.com/doi/abs/10.1089/ind.2020.29198.tsm?journalCode=ind>. Accessed on: 12 Jan. 2021.

MISHRA, D. K.; HWANG, J.-S. Selective hydrogenation of d-mannose to d-mannitol using NiO-modified TiO₂ (NiO-TiO₂) supported ruthenium catalyst. **Applied Catalysis A: General**, Amsterdam, vol. 453, p. 13–19, 2013. DOI 10.1016/j.apcata.2012.11.042. Available at: <https://www.sciencedirect.com/science/article/pii/S0926860X12007600>. Accessed on: 10 Feb. 2021.

MOES, C. J.; PRETORIUS, I. S.; VAN ZYL, W. H. Cloning and expression of the *Clostridium thermosulfurogenes* D-xylose isomerase gene (xyIA) in *Saccharomyces cerevisiae*. **Biotechnology Letters**, Berlin, vol. 18, no. 3, p. 269–274, 1996. DOI 10.1007/BF00142943. Available at: <https://link.springer.com/article/10.1007/BF00142943>. Accessed on: 12 Feb. 2021.

NARESH KUMAR, M.; RAVIKUMAR, R.; THENMOZHI, S.; RANJITH KUMAR, M.; KIRUPA SHANKAR, M. Choice of Pretreatment Technology for Sustainable Production of Bioethanol from Lignocellulosic Biomass: Bottle Necks and Recommendations. **Waste and Biomass Valorization**, Berlin, vol. 10, p. 1693–1709, 2019. DOI 10.1007/s12649-017-0177-6. Available at: <https://link.springer.com/article/10.1007/s12649-017-0177-6>. Accessed on: 11 Feb. 2021.

NIGLIO, S.; PROCENTESE, A.; RUSSO, M. E.; SANNIA, G.; MARZOCHELLA, A. Combined pretreatments of coffee silverskin to enhance fermentable sugar yield. **Biomass Conversion and Biorefinery**, Berlin, vol. 10, no. 4, p. 1237–1249, 2020. DOI 10.1007/s13399-019-00498-y. Available at: <https://link.springer.com/article/10.1007/s13399-019-00498-y>. Accessed on: 6 Feb. 2021.

NIJLAND, J. G.; DRIESSEN, A. J. M. Engineering of Pentose Transport in *Saccharomyces cerevisiae* for Biotechnological Applications. **Frontiers in Bioengineering and Biotechnology**, Lausanne, vol. 7, p. 1–13, 2020. DOI 10.3389/fbioe.2019.00464. Available at: <https://www.frontiersin.org/article/10.3389/fbioe.2019.00464>. Accessed on: 10 Feb. 2021.

NUNES, L. J. R.; LOUREIRO, L. M. E. F.; SÁ, L. C. R.; SILVA, H. F. C. Sugarcane Industry Waste Recovery: A Case Study Using Thermochemical Conversion Technologies to Increase Sustainability. **Applied Sciences**, Basel, vol. 10, no. 18, p. 1–17, 2020. DOI 10.3390/app10186481. Available at: <https://www.mdpi.com/2076-3417/10/18/6481>.

OLADELE, I. O.; OLAJIDE, J. L.; OGUNBADEJO, A. S. The Influence of Chemical Treatment on the Mechanical Behaviour of Animal Fibre-Reinforced High Density Polyethylene Composites. **American Journal of Engineering Research**, vol. 4, no. 2, p. 19–26, 2015. Available at: <http://citeseerx.ist.psu.edu/viewdoc/summary?doi=10.1.1.684.2375>. Accessed on: 10 Feb. 2021.

OLAYIWOLA, H. . **Geopolymer bonded wood composites**. 2021. Stellenbosch University, South Africa, 2021.

OLAYIWOLA, H. .; AMIANDAMHEN, S. .; TYHODA, L.; MEINCKEN, M. Investigating the suitability of fly ash / metakaolin _based geopolymers reinforced with South African alien invasive wood and sugarcane bagasse residues for use in outdoor conditions. **European Journal of Wood and Wood Products**, no. 0123456789, 2021. <https://doi.org/10.1007/s00107-020-01636-4>.

OLORUNSOLA, E. O. Emulsifying Properties of Hemicelluloses. In: AKPABIO, E. I. (ed.). **Science and Technology Behind Nanoemulsions**. London: IntechOpen, 2018. p. 29–42. DOI 10.5772/intechopen.74473. Available at: <https://www.intechopen.com/books/science-and-technology-behind-nanoemulsions/emulsifying-properties-of-hemicelluloses>. Accessed on: 10 Feb. 2021.

ORLOVA, A. M. Furfural-based Binder for Composite Construction Materials. **Procedia Engineering**, vol. 153, p. 527–530, 2016. <https://doi.org/10.1016/j.proeng.2016.08.178>.

OUYANG, Y.; LI, Q.; KUANG, X.; WANG, H.; WU, J.; AYEPA, E.; CHEN, H.; ABRHA, G. T.; ZHANG, Z.; LI, X.; MA, M. YMR152W from *Saccharomyces cerevisiae* encoding a novel aldehyde reductase for detoxification of aldehydes derived from lignocellulosic biomass. **Journal of Bioscience and Bioengineering**, Amsterdam, vol. 131, no. 1, p. 39–46, 2021. DOI 10.1016/j.jbiosc.2020.09.004. Available at: <https://www.sciencedirect.com/science/article/pii/S1389172320303364>. Accessed on: 12 Feb. 2021.

PAIS, T. M.; FOULQUIÉ-MORENO, M. R.; THEVELEIN, J. M. QTL Mapping by Pooled-Segregant Whole-Genome Sequencing in Yeast. In: MAPELLI, V. (ed.). **Yeast Metabolic Engineering: Methods and Protocols**. New York: Springer New York, 2014. p. 251–266. DOI 10.1007/978-1-4939-0563-8_15. Available at: https://link.springer.com/protocol/10.1007/978-1-4939-0563-8_15. Accessed on: 15 Feb. 2021.

PALMQVIST, E.; HAHN-HÄGERDAL, B. Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. **Bioresource Technology**, Amsterdam, vol. 74, no. 1, p. 25–33, 2000. DOI 10.1016/S0960-8524(99)00161-3. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852499001613>. Accessed on: 14 Feb. 2021.

PELAEZ-SAMANIEGO, M. R.; YADAMA, V.; GARCIA-PEREZ, T.; LOWELL, E.; AMIDON, T. Effect of hot water extracted hardwood and softwood chips on particleboard properties. **Holzforschung**, Berlin, vol. 68, no. 7, p. 807–815, 2014. DOI 10.1515/hf-2013-0150. Available at: <https://www.degruyter.com/document/doi/10.1515/hf-2013-0150/html>. Accessed on: 8 Feb. 2021.

PELAEZ-SAMANIEGO, M. R.; YADAMA, V.; LOWELL, E.; AMIDON, T. E.; CHAFFEE, T. L. Hot water extracted wood fiber for production of wood plastic composites (WPCs). **Holzforschung**, Berlin, vol. 67, no. 2, p. 193–200, 2013. DOI 10.1515/hf-2012-0071. Available at: <https://www.degruyter.com/document/doi/10.1515/hf-2012-0071/html>. Accessed on: 10 Feb. 2021.

PEREIRA, F. B.; TEIXEIRA, M. C.; MIRA, N. P.; SÁ-CORREIA, I.; DOMINGUES, L. Genome-wide screening of *Saccharomyces cerevisiae* genes required to foster tolerance towards industrial wheat straw hydrolysates. **Journal of Industrial Microbiology & Biotechnology**, Berlin, vol. 41, no. 12, p. 1753–1761, 2014. DOI 10.1007/s10295-014-1519-z. Available at: <https://link.springer.com/article/10.1007/s10295-014-1519-z>. Accessed on: 15 Feb. 2021.

PEREIRA FRERAZ, G.; FREAR, C.; PELAEZ-SAMANIEGO, M. R.; ENGLUND, K.; GARCIA-PEREZ, M. Hot Water Extraction of Anaerobic Digested Dairy Fiber for Wood Plastic Composite Manufacturing. **BioResources**, Raleigh, vol. 11, no. 4, p. 8139–8154, 2016. DOI 10.15376/biores.11.4.8139-8154. Available at: https://ojs.cnr.ncsu.edu/index.php/BioRes/article/view/BioRes_11_4_8139_Pereira_Hot_Water_Extraction_Dairy_Fiber. Accessed on: 10 Jan. 2021.

PEREIRA, L. G.; DIAS, M. O. S.; MARIANO, A. P.; MACIEL FILHO, R.; BONOMI, A. Economic and environmental assessment of n-butanol production in an integrated first and second generation sugarcane biorefinery: Fermentative versus catalytic routes. **Applied Energy**, Amsterdam, vol. 160, p. 120–131, 2015. DOI 10.1016/j.apenergy.2015.09.063. Available at: <https://www.sciencedirect.com/science/article/pii/S0306261915011708>. Accessed on: 12 Feb. 2021.

PIOTROWSKI, J.; ZHANG, Y.; SATO, T.; ONG, I.; KEATING, D.; BATES, D.; LANDICK, R. Death by a thousand cuts: the challenges and diverse landscape of lignocellulosic hydrolysate inhibitors. **Frontiers in Microbiology**, Lausanne, vol. 5, p. 1–8, 2014. DOI 10.3389/fmicb.2014.00090. Available at: <https://www.frontiersin.org/article/10.3389/fmicb.2014.00090>. Accessed on: 14 Feb. 2021.

POSTMA, D.; CHIMPHANGO, A. F. A. Cationization of Eucalyptus grandis 4- O -methyl glucuronoxylan for application as a wet-end additive in a papermaking process. vol. 68, no. 5, p. 519–527, 2014. <https://doi.org/10.1515/hf-2013-0100>.

QUIROGA, A.; MARZOCCHI, V.; RINTOUL, I. Influence of wood treatments on mechanical properties of wood–cement composites and of Populus Euroamericana wood fibers. **Composites Part B: Engineering**, Amsterdam, vol. 84, p. 25–32, 2016. DOI 10.1016/j.compositesb.2015.08.069. Available at: <https://www.sciencedirect.com/science/article/pii/S1359836815005090>. Accessed on: 10 Feb. 2021.

RAGANATI, F.; PROCENTESE, A.; OLIVIERI, G.; GÖTZ, P.; SALATINO, P.; MARZOCHELLA, A. Kinetic study of butanol production from various sugars by Clostridium acetobutylicum using a dynamic model. **Biochemical Engineering Journal**, Amsterdam, vol. 99, p. 156–166, 2015. DOI 10.1016/j.bej.2015.03.001. Available at: <https://www.sciencedirect.com/science/article/pii/S1369703X15000832>. Accessed on: 10 Feb. 2021.

RAGHAVENDRAN, V.; MARX, C.; OLSSON, L.; BETTIGA, M. The protective role of intracellular glutathione in Saccharomyces cerevisiae during lignocellulosic ethanol production. **AMB Express**, Berlin, vol. 10, no. 1, p. 219, 2020. DOI 10.1186/s13568-020-01148-7. Available at: <https://link.springer.com/article/10.1186/s13568-020-01148-7>. Accessed on: 12 Feb. 2021.

RIZZI, M.; HARWART, K.; BUI-THANH, N.-A.; DELLWEG, H. A kinetic study of the NAD⁺-xylitol-dehydrogenase from the yeast Pichia stipitis. **Journal of Fermentation and Bioengineering**, Amsterdam, vol. 67, no. 1, p. 25–30, 1989. DOI 10.1016/0922-338X(89)90081-0. Available at: <https://www.sciencedirect.com/science/article/pii/0922338X89900810>. Accessed on: 14 Feb. 2021.

ROBAK, K.; BALCEREK, M. Review of Second Generation Bioethanol Production from Residual Biomass. **Food technology and biotechnology**, Bethesda, vol. 56, no. 2, p. 174–187, Jun. 2018. DOI 10.17113/ftb.56.02.18.5428. Available at: <https://pubmed.ncbi.nlm.nih.gov/30228792>. Accessed on: 15 Feb. 2021.

RODRUSSAMEE, N.; SATTAYAWAT, P.; YAMADA, M. Highly efficient conversion of xylose to ethanol without glucose repression by newly isolated thermotolerant Spathaspora passalidarum CMUWF1–2. **BMC Microbiology**, London, vol. 18, no. 1, p. 73, 2018. DOI 10.1186/s12866-018-1218-4. Available at: <https://bmcmicrobiol.biomedcentral.com/articles/10.1186/s12866-018-1218-4>. Accessed on: 12 Feb. 2021.

ROMANÍ, A.; PEREIRA, F.; JOHANSSON, B.; DOMINGUES, L. Metabolic engineering of Saccharomyces cerevisiae ethanol strains PE-2 and CAT-1 for efficient lignocellulosic fermentation. **Bioresource Technology**, Amsterdam, vol. 179, p. 150–158, 2015. DOI 10.1016/j.biortech.2014.12.020. Available at: <https://www.sciencedirect.com/science/article/pii/S096085241401757X>. Accessed on: 15 Feb. 2021.

RUNQUIST, D.; FONSECA, C.; RÄDSTRÖM, P.; SPENCER-MARTINS, I.; HAHN-HÄGERDAL, B. Expression of the Gxf1 transporter from Candida intermedia improves fermentation performance in recombinant xylose-utilizing Saccharomyces cerevisiae. **Applied Microbiology and Biotechnology**, Berlin, vol. 82, no. 1, p. 123–130, 2009. DOI 10.1007/s00253-008-1773-y. Available at: <https://link.springer.com/article/10.1007/s00253-008-1773-y>. Accessed on: 10 Feb. 2021.

RUNQUIST, David; HAHN-HÄGERDAL, B.; RÄDSTRÖM, P. Comparison of heterologous xylose transporters in recombinant Saccharomyces cerevisiae. **Biotechnology for Biofuels**, Berlin, vol. 3, no. 1, p. 1–7, 2010. DOI 10.1186/1754-6834-3-5. Available at: <https://link.springer.com/article/10.1186/1754-6834-3-5>. Accessed on: 10 Feb. 2021.

SAHA, B. C.; KENNEDY, G. J. Mannose and galactose as substrates for production of itaconic acid by Aspergillus terreus. **Letters in Applied Microbiology**, Hoboken, vol. 65, no. 6, p. 527–533, 1 Dec. 2017. DOI 10.1111/lam.12810. Available at: <https://sfamjournals.onlinelibrary.wiley.com/doi/full/10.1111/lam.12810>. Accessed on: 10 Feb. 2021.

SANTOS, C.; BUENO, D.; SANT'ANNA, C.; BRIENZO, M. High xylose yield from stem and external fraction of sugarcane biomass by diluted acid pretreatment. **Biomass Conversion and Biorefinery**, Berlin, 2020. DOI 10.1007/s13399-020-01088-z. Available at: <https://link.springer.com/article/10.1007/s13399-020-01088-z>. Accessed on: 11 Feb. 2021.

SARTHY, A. V.; MCCONAUGHY, B. L.; LOBO, Z.; SUNDSTROM, J. A.; FURLONG, C. E.; HALL, B. D. Expression of the Escherichia coli xylose isomerase gene in Saccharomyces cerevisiae. **Applied and Environmental Microbiology**, Washington, vol. 53, no. 9, p. 1996–2000, 1 Sep. 1987. Available at: <http://aem.asm.org/content/53/9/1996.abstract>. Accessed on: 13 Feb. 2021.

SASANO, Y.; WATANABE, D.; UKIBE, K.; INAI, T.; OHTSU, I.; SHIMO, H.; TAKAGI, H. Overexpression of the yeast transcription activator Msn2 confers furfural resistance and increases the initial fermentation rate in ethanol production. **Journal of Bioscience and Bioengineering**, Amsterdam, vol. 113, no. 4, p. 451–455, 2012. DOI 10.1016/j.jbiosc.2011.11.017. Available at: <https://www.sciencedirect.com/science/article/pii/S138917231100483X>. Accessed on: 2 Feb. 2021.

SATO, T. K.; TREMAINE, M.; PARREIRAS, L. S.; HERBERT, A. S.; MYERS, K. S.; HIGBEE, A. J.; SARDI, M.; MCILWAIN, S. J.; ONG, I. M.; BREUER, R. J.; AVANASI NARASIMHAN, R.; MCGEE, M. A.; DICKINSON, Q.; LA REAU, A.; XIE, D.; TIAN, M.; REED, J. L.; ZHANG, Y.; COON, J. J.; ... LANDICK, R. Directed Evolution Reveals Unexpected Epistatic Interactions That Alter Metabolic Regulation and Enable Anaerobic Xylose Use by Saccharomyces cerevisiae. **PLoS genetics**, San Francisco, vol. 12, no. 10, p. e1006372–e1006372, 14 Oct. 2016. DOI 10.1371/journal.pgen.1006372. Available at: <https://pubmed.ncbi.nlm.nih.gov/27741250>. Accessed on: 1 Feb. 2021.

SCHÖNBERG, C.; OKSANEN, T.; SUURNÄKKI, A.; KETTUNEN, H.; BUCHERT, J. The Importance of Xylan for the Strength Properties of Spruce Kraft Pulp Fibres. **Holzforschung**, Berlin, vol. 55, no. 6, p. 639–644, 2001. DOI 10.1515/HF.2001.104. Available at: <https://www.degruyter.com/document/doi/10.1515/HF.2001.104/html>. Accessed on: 14 Feb. 2021.

SERVINSKY, M. D.; GERMANE, K. L.; LIU, S.; KIEL, J. T.; CLARK, A. M.; SHANKAR, J.; SUND, C. J. Arabinose is metabolized via a phosphoketolase pathway in Clostridium acetobutylicum ATCC 824. **Journal of Industrial Microbiology and Biotechnology**, Oxford, vol. 39, no. 12, p. 1859–1867, 1 Dec. 2012. DOI 10.1007/s10295-012-1186-x. Available at: <https://academic.oup.com/jimb/article/39/12/1859/5994581>. Accessed on: 1 Feb. 2021.

SHARMA, D.; SAINI, A. Lignocellulosic Waste Valorization and Biorefineries Concept. In: SHARMA, D.; SAINI, A. (eds.). **Lignocellulosic Ethanol Production from a Biorefinery Perspective: Sustainable Valorization of Waste**. Singapore: Springer Singapore, 2020. p. 195–215. DOI 10.1007/978-981-15-4573-3_7. Available at: https://link.springer.com/chapter/10.1007/978-981-15-4573-3_7. Accessed on: 1 Jul. 2022.

SHIMIZU, F. L.; ZAMORA, H. D. Z.; SCHMATZ, A. A.; MELATI, R. B.; BUENO, D.; BRIENZO, M. Biofuels Generation Based on Technical Process and Biomass Quality. In: SRIVASTAVA, N.; SRIVASTAVA, M.; MISHRA, P. K.; GUPTA, V. K. (eds.). **Biofuel Production Technologies: Critical Analysis for Sustainability**. Singapore: Springer Singapore, 2020. p. 37–64. DOI 10.1007/978-981-13-8637-4_2. Available at: https://link.springer.com/chapter/10.1007/978-981-13-8637-4_2. Accessed on: 5 Jul. 2022.

SILVA, T.; ZAMORA, H.; VARÃO, L.; PRADO, N.; BAFFI, M.; PASQUINI, D. Effect of Steam Explosion Pretreatment Catalysed by Organic Acid and Alkali on Chemical and Structural Properties and Enzymatic Hydrolysis of Sugarcane Bagasse. **Waste and Biomass Valorization**, Berlin, vol. 9, no. 11, p. 2191–2201, 2018. DOI 10.1007/s12649-017-9989-7. Available at: <https://link.springer.com/article/10.1007/s12649-017-9989-7>. Accessed on: 23 Sep. 2021.

SKERKER, J. M.; LEON, D.; PRICE, M. N.; MAR, J. S.; TARJAN, D. R.; WETMORE, K. M.; DEUTSCHBAUER, A. M.; BAUMOHL,

J. K.; BAUER, S.; IBÁÑEZ, A. B.; MITCHELL, V. D.; WU, C. H.; HU, P.; HAZEN, T.; ARKIN, A. P. Dissecting a complex chemical stress: chemogenomic profiling of plant hydrolysates. **Molecular Systems Biology**, Heidelberg, vol. 9, no. 1, p. 1–21, 1 Jan. 2013. DOI 10.1038/msb.2013.30. Available at: <https://www.embopress.org/doi/full/10.1038/msb.2013.30>. Accessed on: 10 Feb. 2021.

SOLUDONGWE, S. M. **Co-production of furfural and wood composite products from bio-based processing residues**. 2020. Stellenbosch University, 2020.

SUBTIL, T.; BOLES, E. Competition between pentoses and glucose during uptake and catabolism in recombinant *Saccharomyces cerevisiae*. **Biotechnology for Biofuels**, Berlin, vol. 5, no. 1, p. 1–12, 2011. DOI 10.1186/1754-6834-5-14. Available at: <https://link.springer.com/article/10.1186/1754-6834-5-14>. Accessed on: 1 Feb. 2021.

SWINNEN, S.; HENRIQUES, S. F.; SHRESTHA, R.; HO, P.-W.; SÁ-CORREIA, I.; NEVOIGT, E. Improvement of yeast tolerance to acetic acid through Haa1 transcription factor engineering: towards the underlying mechanisms. **Microbial Cell Factories**, Berlin, vol. 16, no. 1, p. 1–15, 2017. DOI 10.1186/s12934-016-0621-5. Available at: <https://link.springer.com/article/10.1186/s12934-016-0621-5>. Accessed on: 1 Feb. 2021.

TAGUCHI, F.; MIZUKAMI, N.; HASEGAWA, K.; SAITO-TAKI, T. Microbial conversion of arabinose and xylose to hydrogen by a newly isolated *Clostridium* sp. No. 2. **Canadian Journal of Microbiology**, Otatwa, vol. 40, no. 3, p. 228–233, 1 Mar. 1994. DOI 10.1139/m94-037. Available at: <https://cdnsiencepub.com/doi/pdf/10.1139/m94-037>.

TRÄFF, K. L.; OTERO CORDERO, R. R.; VAN ZYL, W. H.; HAHN-HÄGERDAL, B. Deletion of the GRE3 Aldose Reductase Gene and Its Influence on Xylose Metabolism in Recombinant Strains of *Saccharomyces cerevisiae* Expressing the xylA and XKS1 Genes. **Applied and Environmental Microbiology**, Washington, vol. 67, no. 12, p. 5668–5674, 1 Dec. 2001. DOI 10.1128/AEM.67.12.5668-5674.2001. Available at: <http://aem.asm.org/content/67/12/5668.abstract>. Accessed on: 1 Feb. 2021.

U.S. ENERGY INFORMATION ADMINISTRATION. Crude oil distillation and the definition of refinery capacity. 2012. Available at: <https://www.eia.gov/todayinenergy/detail.php?id=6970>. Accessed on: 1 Feb. 2021.

UM, B.-H.; VAN WALSUM, G. P. Mass balance on green liquor pre-pulping extraction of northeast mixed hardwood. **Bioresource Technology**, Amsterdam, vol. 101, no. 15, p. 5978–5987, 2010. DOI 10.1016/j.biortech.2010.03.004. Available at: <https://www.sciencedirect.com/science/article/pii/S096085241000458X>. Accessed on: 11 Feb. 2021.

USDA. **Brazil: Biofuels Annual**, n. BR2020-0032. Washington: United States Department of Agriculture, 2020. Available at: <https://www.fas.usda.gov/data/brazil-biofuels-annual-6>. Accessed on: 1 Feb. 2021.

VAN MARIS, A. J. A.; WINKLER, A. A.; KUYPER, M.; DE LAAT, W. T. A. M.; VAN DIJKEN, J. P.; PRONK, J. T. Development of Efficient Xylose Fermentation in *Saccharomyces cerevisiae*: Xylose Isomerase as a Key Component. In: OLSSON, L. (ed.). **Biofuels**. Berlin: Springer Berlin Heidelberg, 2007. p. 179–204. DOI 10.1007/10_2007_057. Available at: https://link.springer.com/chapter/10.1007/10_2007_057. Accessed on: 10 Feb. 2021.

VEEN, P. v. d.; FLIPPIN, M. J. A.; VORAGEN, A. G. J.; VISSER, J. Induction of extracellular arabinases on monomeric substrates in *Aspergillus niger*. **Archives of Microbiology**, Berlin, vol. 159, no. 1, p. 66–71, 1993. DOI 10.1007/BF00244266. Available at: <https://link.springer.com/article/10.1007%2FBF00244266>. Accessed on: 10 Feb. 2021.

VENA, P. F. **Integration of xylan extraction prior to kraft and sodaAQ pulping from South African grown Eucalyptus grandis, giant bamboo and sugarcane bagasse to produce paper pulps, value added biopolymers and fermentable sugars**. 2013. 251 f. Stellenbosch University, 2013. Available at: <https://scholar.sun.ac.za/handle/10019.1/80116>. Accessed on: 10 Feb. 2021.

VERHO, R.; LONDESBOROUGH, J.; PENTTILÄ, M.; RICHARD, P. Engineering Redox Cofactor Regeneration for Improved Pentose Fermentation in *Saccharomyces cerevisiae*. **Applied and Environmental Microbiology**, Washington, vol. 69, no. 10, p. 5892–5897, 1 Oct. 2003. DOI 10.1128/AEM.69.10.5892-5897.2003. Available at: <http://aem.asm.org/content/69/10/5892.abstract>. Accessed on: 1 Feb. 2021.

VERHOEVEN, M. D.; LEE, M.; KAMOEN, L.; VAN DEN BROEK, M.; JANSSEN, D. B.; DARAN, J.-M. G.; VAN MARIS, A. J. A.; PRONK, J. T. Mutations in PMR1 stimulate xylose isomerase activity and anaerobic growth on xylose of engineered *Saccharomyces cerevisiae* by influencing manganese homeostasis. **Scientific Reports**, Berlin, vol. 7, no. 1, p. 1–11, 2017. DOI 10.1038/srep46155. Available at: <https://www.nature.com/articles/srep46155>. Accessed on: 1 Feb. 2021.

WALFRIDSSON, M.; ANDERLUND, M.; BAO, X.; HAHN-HÄGERDAL, B. Expression of different levels of enzymes from the *Pichia stipitis* XYL1 and XYL2 genes in *Saccharomyces cerevisiae* and its effects on product formation during xylose utilisation. **Applied Microbiology and Biotechnology**, Berlin, vol. 48, no. 2, p. 218–224, 1997. DOI 10.1007/s002530051041. Available at: <https://link.springer.com/article/10.1007%2Fs002530051041>. Accessed on: 1 Feb. 2021.

WALFRIDSSON, M.; BAO, X.; ANDERLUND, M.; LILIUS, G.; BÜLOW, L.; HAHN-HÄGERDAL, B. Ethanol fermentation of xylose with *Saccharomyces cerevisiae* harboring the *Thermus thermophilus* xylA gene, which expresses an active xylose (glucose) isomerase. **Applied and Environmental Microbiology**, Washington, vol. 62, no. 12, p. 4648–4651, 1 Dec. 1996. Available at: <http://aem.asm.org/content/62/12/4648.abstract>. Accessed on: 1 Feb. 2021.

WANG, C.; LI, Y.; QIU, C.; WANG, S.; MA, J.; SHEN, Y.; ZHANG, Q.; DU, B.; DING, Y.; BAO, X. Identification of Important Amino Acids in Gal2p for Improving the L-arabinose Transport and Metabolism in *Saccharomyces cerevisiae*. **Frontiers in Microbiology**, Lausanne, vol. 8, p. 1–11, 2017. DOI 10.3389/fmicb.2017.01391. Available at: <https://www.frontiersin.org/article/10.3389/fmicb.2017.01391>. Accessed on: 1 Feb. 2021.

WANG, C.; SHEN, Y.; ZHANG, Y.; SUO, F.; HOU, J.; BAO, X. Improvement of L-Arabinose Fermentation by Modifying the Metabolic Pathway and Transport in *Saccharomyces cerevisiae*. **BioMed Research International**, London, vol. 2013, p. 1–10, 2013. DOI 10.1155/2013/461204. Available at: <https://www.hindawi.com/journals/bmri/2013/461204/>. Accessed on: 10 Feb. 2021.

WANG, X.; YANG, J.; YANG, S.; JIANG, Y. Unraveling the genetic basis of fast L-arabinose consumption on top of recombinant xylose-fermenting *Saccharomyces cerevisiae*. **Biotechnology and Bioengineering**, Hoboken, vol. 116, no. 2, p. 283–293, 1 Feb. 2019. DOI 10.1002/bit.26827. Available at: <https://onlinelibrary.wiley.com/doi/abs/10.1002/bit.26827>. Accessed on: 1 Feb. 2021.

WANG, Xinning; LIANG, Z.; HOU, J.; SHEN, Y.; BAO, X. The Absence of the Transcription Factor Yrr1p, Identified from Comparative Genome Profiling, Increased Vanillin Tolerance Due to Enhancements of ABC Transporters Expressing, rRNA Processing and Ribosome Biogenesis in *Saccharomyces cerevisiae*. **Frontiers in Microbiology**, Lausanne, vol. 8, p. 1–12, 2017. DOI 10.3389/fmicb.2017.00367. Available at: <https://www.frontiersin.org/article/10.3389/fmicb.2017.00367>. Accessed on: 1 Feb. 2021.

WIEDEMANN, B.; BOLES, E. Codon-Optimized Bacterial Genes Improve L-Arabinose Fermentation in Recombinant *Saccharomyces cerevisiae*. **Applied and Environmental Microbiology**, Washington, vol. 74, no. 7, p. 2043–2050, 1 Apr. 2008. DOI 10.1128/AEM.02395-07. Available at: <http://aem.asm.org/content/74/7/2043.abstract>. Accessed on: 1 Feb. 2021.

WISSELINK, H. Wouter; TOIRKENS, M. J.; DEL ROSARIO FRANCO BERRIEL, M.; WINKLER, A. A.; VAN DIJKEN, J. P.; PRONK, J. T.; VAN MARIS, A. J. A. Engineering of *Saccharomyces cerevisiae* for Efficient Anaerobic Alcoholic Fermentation of L-Arabinose. **Applied and Environmental Microbiology**, Washington, vol. 73, no. 15, p. 4881–4891, 1 Aug. 2007. DOI

10.1128/AEM.00177-07. Available at: <http://aem.asm.org/content/73/15/4881.abstract>. Accessed on: 1 Feb. 2021.

WISSELINK, Hendrik Wouter; VAN MARIS, A. J. A.; PRONK, J. T.; KLAASSEN, P.; DE JONG, R. M. **Polypeptides with Permease Activity**. Netherlands: International Bureau, 2014. Available at: <https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2012049173&tab=PCTBIBLIO>.

XU, H.; KIM, S.; SOREK, H.; LEE, Y.; JEONG, D.; KIM, J.; OH, E. J.; YUN, E. J.; WEMMER, D. E.; KIM, K. H.; KIM, S. R.; JIN, Y.-S. PHO13 deletion-induced transcriptional activation prevents sedoheptulose accumulation during xylose metabolism in engineered *Saccharomyces cerevisiae*. **Metabolic Engineering**, Amsterdam, vol. 34, p. 88–96, 2016. DOI 10.1016/j.ymben.2015.12.007. Available at: <https://www.sciencedirect.com/science/article/pii/S109671761500169X>. Accessed on: 1 Feb. 2021.

YE, S.; KIM, J.; KIM, S. R. Metabolic Engineering for Improved Fermentation of L-Arabinose. **Journal of Microbiology and Biotechnology**, Seoul, vol. 29, no. 3 ed. 2019/03/28, p. 339–346, Mar. 2019. DOI 10.4014/jmb.1812.12015. Available at: <http://jmb.or.kr/journal/view.html?doi=10.4014/jmb.1812.12015>. Accessed on: 1 Feb. 2021.

YOON, S.-H.; TUNC, M. S.; HEININGEN, A. Van. Near-neutral pre-extraction of hemicelluloses and subsequent kraft pulping of southern mixed hardwoods. **Tappi Journal**, Peachtree Corners, vol. 10, no. 1, p. 7–15, 2011. Available at: <https://imisrise.tappi.org/TAPPI/Products/11/JAN/11JAN07.aspx>. Accessed on: 15 Feb. 2021.

YOUSUF, A.; PIROZZI, D.; SANNINO, F. Chapter 1 - Fundamentals of lignocellulosic biomass. In: YOUSUF, A.; PIROZZI, D.; SANNINO, F. B. T.-L. B. to L. B. (eds.). **Lignocellulosic Biomass to Liquid Biofuels**. Cambridge: Academic Press, 2020. p. 1–15. DOI 10.1016/B978-0-12-815936-1.00001-0. Available at: <https://www.sciencedirect.com/science/article/pii/B9780128159361000010>. Accessed on: 6 Feb. 2021.

YU, H.-T.; CHEN, B.-Y.; LI, B.-Y.; TSENG, M.-C.; HAN, C.-C.; SHYU, S.-G. Efficient pretreatment of lignocellulosic biomass with high recovery of solid lignin and fermentable sugars using Fenton reaction in a mixed solvent. **Biotechnology for Biofuels**, Berlin, vol. 11, no. 1, p. 287, 2018. DOI 10.1186/s13068-018-1288-4. Available at: <https://link.springer.com/article/10.1186/s13068-018-1288-4>.

ZAMORA, H. D. Z.; DE FREITAS, C.; BUENO, D.; SHIMIZU, F. L.; CONTIERO, J.; BRIENZO, M. Biomass Fractionation Based on Enzymatic Hydrolysis for Biorefinery Systems. In: VERMA, P. (ed.). **Biorefineries: A Step Towards Renewable and Clean Energy**. Singapore: Springer Singapore, 2021. p. 217–254. DOI 10.1007/978-981-15-9593-6_9. Available at: https://link.springer.com/chapter/10.1007/978-981-15-9593-6_9. Accessed on: 23 Sep. 2021.

ZHANG, C. Lignocellulosic Ethanol: Technology and Economics. In: YUN, Y. (ed.). **Alcohol Fuels - Current Technologies and Future Prospect**. London: IntechOpen, 2019. p. 1–21. DOI 10.5772/intechopen.86701. Available at: <https://www.intechopen.com/books/alcohol-fuels-current-technologies-and-future-prospect/lignocellulosic-ethanol-technology-and-economics>. Accessed on: 16 Feb. 2021.

ZHANG, X.; TU, M.; PAICE, M. G. Routes to Potential Bioproducts from Lignocellulosic Biomass Lignin and Hemicelluloses. **BioEnergy Research**, Berlin, vol. 4, no. 4, p. 246–257, 2011. DOI 10.1007/s12155-011-9147-1. Available at: <https://link.springer.com/article/10.1007/s12155-011-9147-1>. Accessed on: 10 Feb. 2021.

ZHAO, Y.; SUN, H.; YANG, B.; WENG, Y. Hemicellulose-Based Film: Potential Green Films for Food Packaging. **Polymers**, Basel, vol. 12, no. 8, p. 1–14, 2020. DOI 10.3390/polym12081775. Available at: <https://www.mdpi.com/2073-4360/12/8/1775/htm>. Accessed on: 15 Feb. 2021.

ZHOU, H.; CHENG, J.; WANG, B. L.; FINK, G. R.; STEPHANOPOULOS, G. Xylose isomerase overexpression along with engineering of the pentose phosphate pathway and evolutionary engineering enable rapid xylose utilization and ethanol production by *Saccharomyces cerevisiae*. **Metabolic Engineering**, Amsterdam, vol. 14, no. 6, p. 611–622, 2012. DOI 10.1016/j.ymben.2012.07.011. Available at: <https://www.sciencedirect.com/science/article/pii/S1096717612000821>. Accessed on: 1 Feb. 2021.

ZOGLAMI, A.; PAËS, G. Lignocellulosic Biomass: Understanding Recalcitrance and Predicting Hydrolysis. **Frontiers in Chemistry**, Lausanne, vol. 7, p. 1–11, 2019. DOI 10.3389/fchem.2019.00874/full. Available at: <https://www.frontiersin.org/article/10.3389/fchem.2019.00874>. Accessed on: 6 Feb. 2021.

CHAPTER 3 - Assessment of organosolv pretreatment catalyzed by alkali as fractionation strategy of banana plant pseudostem

Abstract

This study shows the impact of alkali ethanolic organosolv pretreatment on the fractionation of banana plant pseudostem aiming to concomitantly extract hemicelluloses and lignin. Hemicelluloses and lignin were extracted through the ethanol addition step, and solvent evaporation and acid precipitation, respectively. The global performance of the alkali ethanolic organosolv pretreatment was examined by digestibility of the pretreated biomass using enzymatic hydrolysis with Cellic® Ctec2. The evaluation of the alkali ethanolic organosolv pretreatment involved a central composite design 2^3 . The alkali concentration with respect to raw biomass, temperature, and ethanol aqueous solution concentration effects on mass yields of extracted hemicelluloses and lignin, and on cellulose conversion were studied. The alkali concentration and temperature had the most significant effects on the mass yield of extracted hemicelluloses, ethanol aqueous solution concentration and temperature for mass yield of extracted lignin, and ethanol aqueous solution concentration for cellulose conversion. The highest mass yield of extracted hemicelluloses (78%) was achieved at 170 °C, ethanol aqueous solution concentration of 30% and alkali concentration of 60%. For mass yield of extracted lignin (35.2%) at 170 °C, ethanol aqueous solution concentration of 30% and alkali concentration of 10%. And the maximum cellulose conversion (96.6%) was obtained at 120 °C, ethanol aqueous solution concentration of 70% and alkali concentration of 60%. The experimental conditions allowed to obtain high glucose release and hemicelluloses recovery.

Keywords: Fruit waste, hemicelluloses, lignin, enzymatic hydrolysis, biorefinery.

Acknowledgments

The authors are grateful to the Brazilian Improvement Coordination of Higher Education Personnel (CAPES) and São Paulo Research Foundation (FAPESP, process number 17/22401-8) for their financial support.

Statement of Novelty

Currently, the generation of value-added bioproducts from lignocellulosic (LC) biomass wastes through biorefineries is considered preponderant matter. Thus, this study is the first one accomplished in Brazil that assessed alterations in the chemical composition of *in natura* and pretreated banana pseudostem, and the influence of alkali ethanolic organosolv pretreatment (OHEOP) on LC structure fractionation aiming to extract hemicelluloses (HC) and lignin (LG). Ethanol addition to spent liquor provoked HC precipitation and therefore its recovery/extraction, and the solvent evaporation guaranteed LG precipitation. Additionally, enzymatic hydrolysis of pretreated biomass (PB) achieved values of cellulose conversion close to 100%. This means OHEOP improves the accessibility of macromolecules allowing that banana pseudostem is considered a potential source to obtain fermentable sugars. The HC, LG, and fermentable sugars production have an important role in different types of industries, due to those are considered as platform chemicals for biofuels and biopolymers production.

3.1. Introduction

Nowadays, it is well known that ecosystems are facing negative subjects about climate change caused by greenhouse gas emissions, which directly are related to frequent practices around the fossil sources such as exploration methods, refining procedures, and derivative uses. This situation has led to a continuous search for suitable options where renewable sources contribute either to reduce or eliminate the use of fossil sources. Among renewable raw materials, vegetal biomass is deemed as the best substitute for fossil sources, since from that can be obtained (in a similar way to petroleum refinery) different types of biofuels, biopolymers, biomaterials, carboxylic acids, pharmaceuticals, sweeteners, and platform products. The current trend in using vegetal biomass is centered on looking for LC wastes as precursors of diverse 2nd generation bio-based products (OREJUELA-ESCOBAR; LANDÁZURI; GOODELL, 2021).

Several experiences about the generation of bio-based products through biorefinery systems using LC wastes come from sugarcane (straw and bagasse), rice (spent grounds, husk, and straw), corn (cob and leaves), coffee (pulp and husk) and

fruit (peel, seeds, and vegetal plant by-products) have been completed around the world (MANHONGO *et al.*, 2022a). Among all countries, Brazil has remained in the spotlight due to its huge agricultural production, which in turn originates a vast quantity of LC wastes (CAVIGLIA-HARRIS *et al.*, 2021; USDA, 2022). Sugarcane bagasse is the Brazilian LC waste most generated and studied to produce bioproducts, however, other LC wastes such as fruit wastes deserve to be researched too. Among fruit wastes, banana plant pseudostem is a LC biomass that can be a potential feedstock in industries to obtain biofuels, biopolymers, and other bioproducts (PEREIRA *et al.*, 2022b).

In the 2020 harvest, Brazil produced close to 6.64 million tons of banana, from which it was estimated a generation of 19.92 million tons of fresh plant pseudostem (moisture around 92%) (IBGE, 2022; MA, Jun; SRZEDNICKI; ARCOT, 2017; SOUZA *et al.*, 2010). The pseudostem is the trunk of the banana plant, is composed of a soft center enfolded by leaf sheaths, and is generated after fruit harvesting (the fruit is bore only once in the banana plant lifetime) (BALDA *et al.*, 2021). The banana plant pseudostem is mainly constituted (dry basis) by cellulose, HC, and LG, which (after solubilization/recovery) have aroused special interest in the development of different industrial applications (PEREIRA *et al.*, 2022b). However, HC and LG extraction processes exhibit technical difficulties linked to the natural recalcitrance of LC structure caused by complex physical-chemical interactions among the three macromolecules (cellulose, HC and LG) (ZOGHLAMI; PAËS, 2019a).

To extract/recover individual HC and LG, it is necessary to implement (prior to extraction/recovery processes) a stage called pretreatment (SHIMIZU *et al.*, 2020a). The key objective of pretreatments is to beat recalcitrance in order to obtain a biomass (depending on biorefinery purposes) with looser LC structure, with increased porosity and surface area, improved digestibility, decreased cellulose crystallinity, diminished inhibitor formation (an important aspect for the subsequent enzymatic process), minimized cellulose and HC degradation, enhanced accessibility to cellulose, HC and LG, or in some cases, cellulose, HC or LG modification or removal, or cellulose and HC direct depolymerization (SHAH *et al.*, 2022; SHIMIZU *et al.*, 2020a).

Among the most favorable pretreatments used in biorefinery, organosolv process is advised since is considered a technique for LC biomass valorization, due to that process allows discriminatory separation of LC biomass into individual cellulose-rich, HC, and LG fractions (THORESEN *et al.*, 2020). This pretreatment is

distinguished by working with an organic solvent, where ethanol is the most employed because of its low toxicity, low commercial price, comes from renewable sources, can be recovered and recircled, and shows higher miscibility in water than others (ZHANG, Ke; PEI; WANG, 2016a). The ethanolic organosolv pretreatment can be performed either without catalyst addition at temperature conditions above 200 °C or by adding catalyst at intermediate temperatures (130 to 200 °C) (FERREIRA; TAHERZADEH, 2020). Nevertheless, from the biorefinery expectative, choosing processes over 200 °C is not advisable since cellulose can suffer chemical degradation, harming the objectives in succeeding stages (KLAMRASSAMEE *et al.*, 2013a). Thus, the most widely organosolv pretreatment developed and researched at industrial, pilot, and laboratory scales is the catalytic one with ethanol, which employed acids to enhance the HC and LG remotion, and the cellulose isolation (ZHANG *et al.*, 2020a).

Despite its advantages, the organosolv process catalyzed by acid addition provokes cellulose and HC depolymerization, and generation of inhibitory substances from hexoses and pentoses dehydration (issues for downstream biological processes) (SHIMIZU; *et al.*, 2020a; ZHANG *et al.*, 2020a). For these reasons, to research on other types of catalysts such as alkalis have generated great interest, especially when biological processes occur after pretreatment in biorefinery systems (WEI *et al.*, 2021a). In the present study, it was evaluated an alkali ethanolic organosolv process (OHEOP) with NaOH as a pretreatment strategy on banana plant pseudostem aiming to concomitantly produce HC and LG. The OHEOP global performance was examined by PB digestibility using enzymatic hydrolysis (Novozymes Cellic® CTec2). HC and LG were extracted/recovered by ethanol addition to spent liquor and sulfuric acid addition to the remaining liquid after HC precipitation/recovery, respectively. A central composite design 2³ was performed where alkali concentration (OHC), temperature (T), and ethanol aqueous solution concentration (EC) were the factors, and mass yield of extracted HC (HCy), mass yield of extracted LG (LGy), and cellulose conversion (CLX) were the response variables.

3.2. Material and methods

3.2.1. Lignocellulosic (LC) biomass

Banana plant pseudostem was collected (after fruit harvesting) from the Institute for Research in Bioenergy at São Paulo State University in Rio Claro - São Paulo state, Brazil - (-22° 23' 58.94" N -47° 34' 17.6" W). The biomass was naturally dried through sun exposition (5 days), and milled and sieved until to achieve an average particle size lesser than 0.841 mm (mesh 20). The milled and sieved biomass was oven-dried at 50 °C for 24 h, and its characterization was performed by quantification of cellulose, HC, LG, ash, and extractives (ethanol + water) following the National Renewable Energy Laboratory methodology (SLUITER *et al.*, 2012a; SLUITER; HAMES; *et al.*, 2008a; SLUITER; RUIZ; *et al.*, 2008a).

3.2.2. Alkali ethanolic organosolv pretreatment (OHEOP) for hemicelluloses (HC) and lignin (LG) production/extraction

The OHEOP ran in laboratory scale reactor made of stainless steel (nominal capacity: 75 mL) using 3 g of biomass and 50 mL of ethanolic aqueous solution (biomass loading: 6% m/v). The reaction time was fixed in 1 h and a previous soaking of 45 min was completed. The OHEOP efficiency on biomass fractionation was evaluated using a central composite design 2³ (Table 3-1) executed by duplicate, where the response variables were HCy, LGy and CLX. The factors (OHC, T and EC) influence was studied via StatSoft Statistica Enterprise v.12 software (confidence level of 95%).

Table 3-1 - Factor levels for central composite design 2³

Factor	Levels				
	-1	+1	-1.682	0	+1.682
T (°C)	120	170	103.0	145	187.0
EC (% m/m)	30	70	16.4	50	83.6
OHC (%)	10	60	0.0	35	77.0

T: Temperature; EC: Ethanol aqueous solution concentration; OHC: alkali concentration (respect to raw biomass)

Source: By author

After pretreatment, PB was obtained by filtration using filter paper and washing up to achieve the distilled water pH (pH~7.0). HC extraction/recovery was conducted through precipitation adding ethanol 92.8% to the spent liquor and decantation for at

least 18 h. The remnant liquid after HC extraction/recovery was boiled (80 °C) to guarantee ethanol evaporation. LG extraction/recovery was managed via precipitation adding sulfuric acid to the liquid after ethanol evaporation to reach pH below 2, followed of decantation for at least 18 h, separation (filter paper) and washing up to achieve the distilled water pH. Once extracted, HC and LG were oven-dried at 60 °C for 72 h. Using mass balances of HC and LG were calculated the extraction yields with respect to LC composition of the raw biomass.

3.2.3. Characterization of pretreated biomass (PB), and extracted hemicelluloses (HC) and lignin (LG)

PB characterization was performed by quantification of cellulose, HC, and LG following the National Renewable Energy Laboratory methodology (SLUITER *et al.*, 2012a). For characterization of the extracted/recovered HC and LG, Fourier Transform Infrared (FTIR) spectroscopy was carried out, samples were mixed with KBr using a mass ratio of 1:125, and spectra were obtained in the region of 4000 – 400 cm⁻¹ with a resolution of 4 cm⁻¹ and 32 scans (JAVIER-ASTETE; JIMENEZ-DAVALOS; ZOLLA, 2021a). Here, xylan from birchwood (XYN - Sigma Aldrich, Lot 067K0701) and kraft lignin (LGK - Sigma Aldrich, Lot MKCP5496) were used as FTIR spectra references to be compared with the HC and LG extracted from banana pseudostem, respectively. The spectra were normalized by maximum intensity method and baseline correction by the 3 points method. For complementary characterization of HC, the molecular weight was determined by Ultra Performance Liquid Chromatography (UPLC) on Waters according to De Freitas and Brienzo (2022) methodology.

3.2.4. Enzymatic hydrolysis

Enzymatic hydrolysis was completed in 50 mL falcon tubes with 0.1 g of PB (dry basis), 5 mL of sodium citrate buffer 50 mmol/L (pH 4.8) and 15 FPU/g of PB using Cellic® Ctec2 multi-enzyme blend (Novozymes). The tubes were tilted and shaken at 150 rpm during 24 h in an incubator (50 °C) (DE FREITAS; BRIENZO, 2022a). Afterward, the samples were centrifuged at 6000 rpm during 20 min and the supernatant was used to determine the concentration of monosaccharides by high performance liquid chromatography (HPLC). The HPLC was performed with Bio-Rad

Aminex HPX-87H (300 x 7.8 mm) column at 65 °C, refractive index detector Waters 2414, mobile phase of H₂SO₄ 0.005 mol/L and rate of 0.6 mL/min (SCHMATZ *et al.*, 2021a). The pretreated biomass digestibility was analyzed based on CLX achieved after hydrolysis.

3.3. Results and discussion

3.3.1. Raw biomass characterization

The chemical composition values revealed great similarity with the homologous ones employed in past works (Table 3-2). However, the LG value (25.7%) was higher than those reported in the other studies (12.2 – 17.6%). This variability behavior can be caused firstly, to the different methodologies employed to characterize the same LC biomasses, and secondly, each type of the same vegetal biomass, and therefore its by-products, exhibits different chemical compositions (YAN *et al.*, 2020a). Besides that, it is important to mention that the chemical composition of the same vegetal biomass depends on cultivation features and climatic conditions (KOUTSOUKIS *et al.*, 2019a). Concerning to ash content, it was observed a high value, which could provoke buffering effect affecting negatively the efficiency of pretreatments (HUANG *et al.*, 2017).

Table 3-2 - Chemical composition (% , dry basis) of banana plant pseudostem

CL	HC	LG	Ex	Ash	Reference
37.1±1.1	12.0±0.07	25.7±0.26	17.3±0.09	9.6±0.17	This work
42.1	12.2	17.6	--	7.14	Jayaprabha; Brahmakumar; Manilal, 2011*
34.5	25.6	12.7	13.5 ¹	13.9	Cordeiro <i>et al.</i> , 2004
31.3	15.0	15.1	4.5 ²	8.7	Bilba; Arsene; Ouensanga, 2007**

CL: Cellulose; HC: Hemicelluloses; LG: Lignin; Ex: Extractives

*7th leaf sheath, **pseudostem core, ¹ethanol-toluene mixture and water, ²ethanol-bencene mixture

Source: By author

Table 3-3 shows LC compositions of some agroindustrial wastes similar to banana pseudostem. Thus, it would be possible to suggest that banana plant pseudostem would be a potential biomass to obtain bioenergy and bioproducts.

Table 3-3 - LC composition (% in dry basis) of some agroindustrial wastes

Biomass	Goal	CL	HC	LG	Reference
Banana pseudostem	HC & LG extraction	37.1+1.1	12.0+0.07	25.7+0.26	This work
Pineapple stem	Bioenergy	37	34	20	Mansor <i>et al.</i> , 2019
Coffee cut-stem	Bioenergy	40.4	34	10.1	García <i>et al.</i> , 2018
Corn stalk	HC extraction	43.7	23.1	23.6	Puişel <i>et al.</i> , 2022
Tomato stem	Activated carbon	27.0	21.1	16.0	Tiryaki <i>et al.</i> , 2014

CL: Cellulose; HC: Hemicelluloses; LG: Lignin

Source: By author

3.3.2. Extraction, and characterization by FTIR spectroscopy of hemicelluloses (HC) and lignin (LG)

The analysis of T, EC and OHC (the factors) influences on HCy and LGy (response variables) was completed at 95% of significance using analysis of variance (ANOVA) (Table 3-4). The FTIR and characterization of the extracted/recovered HC and LG will be presented later on.

Table 3-4 - Extraction/recovery yields of HC and LG, and LC composition and CLX for pretreated banana pseudostem

Exp	Factors			Yield (%)			LC composition (%; dry basis)			CLX* (%)
	T (°C)	EC (%)	OHC (%)	PB	HCy	LGy	CL	HC	LG	
B1	120	30	10	67.2±0.24	2.0±0.08	20.8±0.21	43.5±0.11	9.5±0.30	33.9±1.11	77.3±0.50
B2	120	30	60	53.0±0.00	55.8±0.83	21.9±0.55	59.2±0.67	10.9±0.27	34.1±0.45	92.2±1.04
B3	120	70	10	74.5±0.23	20.8±0.71	18.7±0.86	44.5±0.12	15.5±0.05	30.2±1.42	42.6±0.78
B4	120	70	60	58.8±0.23	66.7±0.13	16.1±0.04	55.1±0.55	16.7±0.37	25.0±0.21	96.6±0.68
B5	170	30	10	55.5±0.23	56.2±0.79	35.2±0.60	54.5±0.06	10.8±0.24	32.5±1.38	78.9±0.72
B6	170	30	60	42.2±0.24	78.0±0.04	34.0±0.45	50.8±0.81	6.6±0.56	35.2±1.19	92.1±1.21
B7	170	70	10	61.3±0.47	27.6±0.12	19.7±0.26	46.9±0.44	15.1±0.92	33.4±0.15	78.3±1.06
B8	170	70	60	37.0±0.00	72.7±0.24	20.1±0.82	64.9±0.23	9.7±0.26	28.1±1.16	78.7±0.75
B9	103	50	35	68.3±0.94	28.7±1.71	16.7±0.88	49.3±0.71	14.0±0.76	30.3±1.38	75.5±1.39
B10	187	50	35	44.1±0.41	66.2±0.75	29.9±1.00	55.5±1.63	9.6±1.04	32.8±0.90	83.7±1.09
B11	145	16.4	35	53.3±0.97	48.4±1.09	31.1±1.13	51.7±1.15	7.8±1.12	35.6±0.83	88.9±0.96
B12	145	83.6	35	59.1±0.91	46.6±0.83	15.5±1.13	53.1±0.98	15.9±1.05	27.6±0.86	70.3±0.95
B13	145	50	0	68.0±1.00	18.3±0.86	23.7±1.11	45.3±1.06	13.1±1.09	32.9±1.01	65.2±0.91
B14	145	50	77	42.0±1.11	77.4±0.97	22.8±1.02	60.9±0.99	10.4±1.14	30.0±1.00	96.0±1.19
B15(c)	145	50	35	56.2	47.5	23.3	52.4	11.8	31.6	79.6
B16(c)	145	50	35	57.5	46.8	23.0	53.8	12.0	32.0	80.5
B17(c)	145	50	35	55.9	48.0	24.0	53	10.9	31.0	79.0

T: Temperature; EC: Ethanol aqueous solution concentration; OHC: Alkali concentration; PB: Yield of recovered pretreated biomass; HCy: Extraction yield of hemicelluloses; LGy: Extraction yield of lignin; CL: Cellulose; HC: Hemicelluloses; LG: Lignin; CLX: Cellulose conversion. * For untreated pseudostem: 8.5±0.05

Source: By author

The ANOVA (Table 3-5) shows the statistical analysis based on full experimental design 2^3 with central point, which established curvatures were not statistically significant. For confirming this matter about curvature, the statistical analysis was run once more but including axial points (central composite design), which showed again curvature did not have significance, therefore, a linear model of the effects of the factors was chosen (Eq 1).

$$y_{ijkl} = \mu + T_i + EC_j + OHC_k + (T \cdot EC)_{ij} + (T \cdot OHC)_{ik} + (EC \cdot OHC)_{jk} + (T \cdot EC \cdot OHC)_{ijk} + \varepsilon_{ijkl} \quad (1)$$

Here y_{ijkl} is the response variable (HCy or LGy), and μ , T_i , EC_j , OHC_k , $(T \cdot EC)_{ij}$, $(T \cdot OHC)_{ik}$, $(EC \cdot OHC)_{jk}$, $(T \cdot EC \cdot OHC)_{ijk}$ and ε_{ijkl} , correspond to the global average, the effect of i-th level of factor temperature (T_i), the effect of j-th level of factor ethanol aqueous solution concentration (EC_j), the effect of k-th level of factor alkali concentration (OHC_k), the interactions of the factors T_i , EC_j and OHC_k , and the random error, respectively (MONTGOMERY, 2001).

Table 3-5 - ANOVA for the effects of factors on response variables

<u>Response variable: HCy</u>			$R^2 = 0.999$		
Factor	SS	df	MS	F-value	p-value
Curvature	0.00	1	0.004	0.01	0.904781
T (°C)	1989.16	1	1989.160	7972.99	0.000000
EC (%)	4.62	1	4.623	18.53	0.000856
OHC (%)	6930.56	1	6930.563	27779.23	0.000000
T-EC	1011.24	1	1011.240	4053.27	0.000000
T-OHC	272.25	1	272.250	1091.24	0.000000
T-EC-OHC	58.52	1	58.523	234.57	0.000000
Error	246.49	1	246.490	987.99	0.000000
Total	3.24	13	0.249	--	--
<u>Response variable: LGy</u>			$R^2 = 0.995$		
Factor	SS	df	MS	F-value	p-value
Curvature	0.0928	1	0.0928	0.364	0.556611
T (°C)	244.9225	1	244.9225	960.964	0.000000
EC (%)	344.1025	1	344.1025	1350.100	0.000000
OHC (%)	0.0025	1	0.0025	0.010	0.922618
T-EC	113.4225	1	113.4225	445.018	0.000000
T-OHC	2.7225	1	2.7225	10.682	0.006109
T-EC-OHC	5.0625	1	5.0625	19.863	0.000647
Error	1.8225	1	1.8225	7.151	0.019116
Total	3.3133	13	0.2549	--	--

<u>Response variable: CLX</u>			$R^2 = 0.998$		
Factor	SS	df	MS	F-value	p-value
Curvature	0.044	1	0.044	0.067	0.799384
T (°C)	93.122	1	93.122	143.605	0.000000
EC (%)	490.623	1	490.623	756.595	0.000000
OHC (%)	1701.563	1	1701.563	2623.999	0.000000
T-EC	65.610	1	65.610	101.178	0.000000
T-OHC	756.250	1	756.250	1166.222	0.000000
T-EC-OHC	174.240	1	174.240	268.698	0.000000
Error	673.402	1	673.402	1038.462	0.000000
Total	8.430	13	0.648	--	--

T: Temperature; EC: Ethanol concentration; OHC: Alkali concentration; HCy: Extraction yield of hemicelluloses; LGy: Extraction yield of lignin; CLX: Cellulose conversion; SS: Sum of squares; df: Degrees of freedom; MS: Mean squares

Source: By author

Every model exhibited a correlation coefficient (R^2) upper than 0.99, which supposes that the association intensity between factors (T, EC, and OHC) and the response variables (HCy, LGy and CLX) was elevated (MONTGOMERY, 2001). Response surfaces are shown at supplementary information section.

3.3.3. Hemicelluloses (HC) extraction and characterization by FTIR

From every OHEOP experiment, HC could be extracted/recovered. The maximum HCy (78.0% with respect to HC content in biomass) happened when OHEOP was performed at 170 °C, EC 30%, and OHC 60%, and under this configuration the HC content of the PB (6.6%) was the lowest one obtained in the LC characterizations. This finding obeys to the incidence of ethanolic organosolv pretreatment on LC biomass involving HC solubilization (DE FREITAS *et al.*, 2021a). As this research is the first one concerning HC extraction/recovery from spent liquor from OHEOP, then the HCy discussion by comparison was done regarding other pretreatments commonly completed in similar conditions of biomass loading (6% m/v), time (1 h), T (170 °C) and OHC (60% = 0.9 mol/L = 3.6% m/v). The uppermost HCy in this study (9.4% with respect to biomass) was higher than the found one by a previous study developed with hot water on depectinized apple pomace (biomass loading of 5% m/v), in which the extracted/recovered HC yield reached 4.3% (respect to biomass) using identical reaction time (1 h) and alike temperature (155 °C) (WOLF *et al.*, 2022a). This same study also informed that the highest HCy was 8.4% (respect to biomass)

during 20 min at 125 °C, however, the outcome was inferior to the one observed in the present study. Gallina *et al.* (2018) reported 40.3% (respect to HC content in biomass) as the highest HCy from *Eucalyptus globulus* pruning using hot pressurized water in a batchwise operated cascade reactor at 160 °C and 9 bar between 40 and 80 min of reaction time. Here, the yield found one more time was lesser than the one reported herein.

To improve the hydrothermal route performance aiming to extract/recover HC, generally, alkalis have been incorporated in pretreatment systems (YAO *et al.*, 2015). This is the case of Rabetafika *et al.* (2014), who found the highest HCy (61.2% respect to HC content in biomass) by direct extraction with NaOH 4 mol/L from pear pomace (biomass loading: 5% m/v) at 60 °C during 18 h. Another example of alkali incorporation showed that the maximum HCy (62.0% respect to HC content in biomass) was achieved with NaOH 6% at 100 °C and 90 min using wheat straw as feedstock (PUIȚEL *et al.*, 2022). In both precedents the maximum HCy were inferior than one in the present study (78.0% respect to HC content in biomass).

So far, have been mentioned researches that reported HCy lower than one found in this study, however, some experiences confirmed that it is possible to get results around 95% (respect to HC content in biomass) by alkali route but including an additional step. Xu *et al.* (2018) obtained the highest HCy (95.2%) using KOH 5% at 50 °C during 3 h aided by 30 min of ultrasound, from *Eucalyptus grandis* delignified with Na(ClO₂) in acidic medium at 75 °C during 2 h. Singh *et al.* (2018) obtained HCy of 94.2% from areca nut husk using two sequential stages with 10% (m/v) of NaOH, the first one called incubation at 65 °C by 8 h, and the second one the hydrothermal process at 121 °C during 1 h. And Rabetafika *et al.* (2014) reported 94.5% as the best result for HCy using NaOH 2 mol/L at 60 °C during 18 h from pear pomace delignified with acidified Na(ClO₂) at 70 °C during 1 h.

Based on the statistical analysis supported in the ANOVA (Table 3-5), all 1st order effects were statistically significative. OHC was the factor with the highest effect on HCy, revealing a positive influence at NaOH 60% and the second highest one was T, which favored the HCy when OHEOP was performed at 170 °C. EC was significative too, but with intensity low, maintaining 30% let to the HCy to achieve the maximum. Besides that, every interaction effect among the factors was significative too. The OHC result is in concordance with other studies, which indicated that increasing loads of alkalis the HCy increases too (PUIȚEL *et al.*, 2022; YAO *et al.*, 2015). Alkali extraction

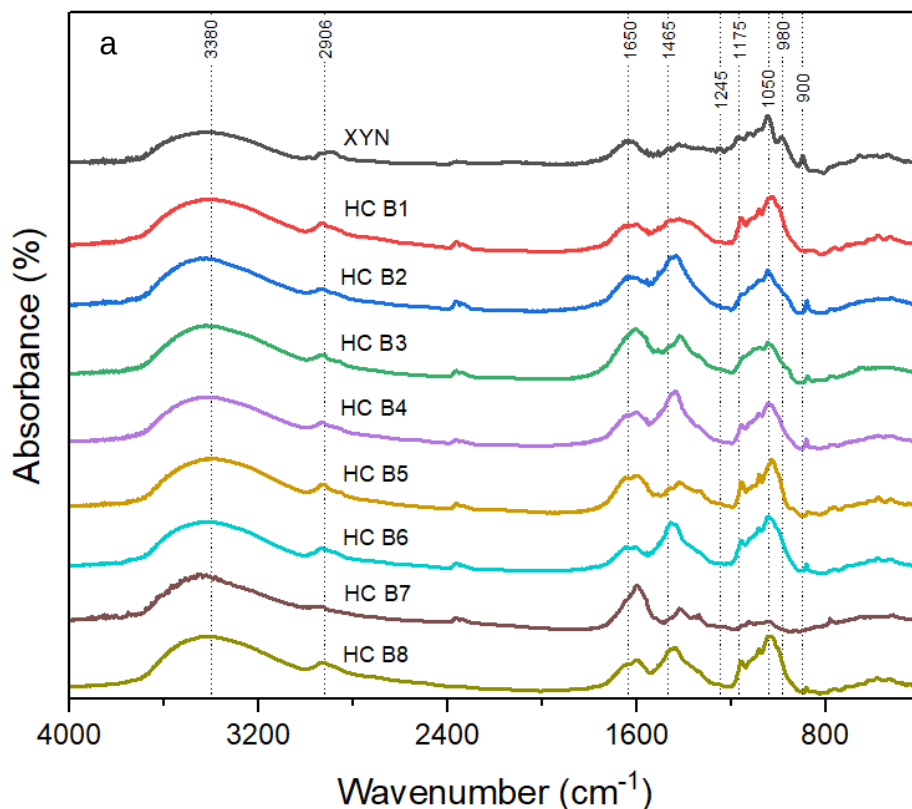
of HC occurs due to hydrogen bonds between HC and cellulose, and the ester linkages between HC and LG are attacked. In addition, cellulose suffers swelling, thereby HC can be solubilized and its extraction results in high purity (LU *et al.*, 2021). The finding about T effect was in agree with previous studies where T was the main variable to pretreat LC biomass by thermal routes; mild conditions (190 – 194 °C) were suitable to achieve the best performances to solubilize HC from LC wastes (HANIM *et al.*, 2012; MIHIRETU *et al.*, 2017). Reyes *et al.* (2013) also found T was a significative factor to solubilize HC; 170°C let the best performance to pretreat *Pinus radiata* wood chips by alkaline route. And, regarding the use of EC near to 30%, Li *et al.* (2020) reported 80.5% (respect HC content in biomass) of recovered xylan (estimated by mass balance) from the rind of sorghum stem through OHEOP using 2% m/v of NaOH as catalyst at 65 °C, EC 40% and 1 h of reaction time. Santos *et al.* (2022) found that the organosolv pretreatment was a good fractionation strategy of *Pinus radiata* to obtain HC solubilized in spent liquor, here the third best result (1.41 g/L) was reported with 33% of EC at 185 °C and 75 min of reaction time.

FTIR spectroscopy of the extracted/recovered HC (from every OHEOP setup of the 2³ design) was performed to evaluate its structural characteristics (Figure 3.1). Two distinguished regions of analysis (800 - 1700 cm⁻¹ and 2800 - 3800 cm⁻¹) were observed.

The band at 900 cm⁻¹ (C-O-C stretching) is reported as characteristic of β-glycosidic linkages between sugar units in HC (SUN; TOMKINSON, 2002). These stretchings were presented in some samples (B2, B3, B4, B6), where B2 showed the most intensity. Any HC sample shown the 980 cm⁻¹ band, which is related to the presence of arabinan in the HC structure (LIU *et al.*, 2021). Every HC sample (excepting B7) exhibited intensity and sharpness at 1050 cm⁻¹ band, which represents the C-O-C stretching in xylan (JU *et al.*, 2011). The 1175 cm⁻¹ signal is attributed to the presence of arabinosyl groups in the HC side chains, this band was found in the B1, B4, B5, B6, and B8 samples (BRIENZO, M; SIQUEIRA; MILAGRES, 2009). For any HC sample, the C-O stretching (1245 cm⁻¹) corresponding to acetyl group was not detected (LI *et al.*, 2012). Every HC sample exhibited C-H deformations and C=O (conjugated carbonyl) vibrations, which are represented by the 1465 and 1650 cm⁻¹ bands, respectively, indicating presence of aromatics released possibly from LG (SAMMONS *et al.*, 2013a). In closing, the bands at 2906 and 3380 cm⁻¹ are attributed

to O-H and C-H stretchings, respectively, which are characteristic of HC, however, it is important to mention cellulose exhibits it too (JU *et al.*, 2011).

Figure 3.1 - FTIR spectra of recovered hemicelluloses (HC) from banana pseudostem



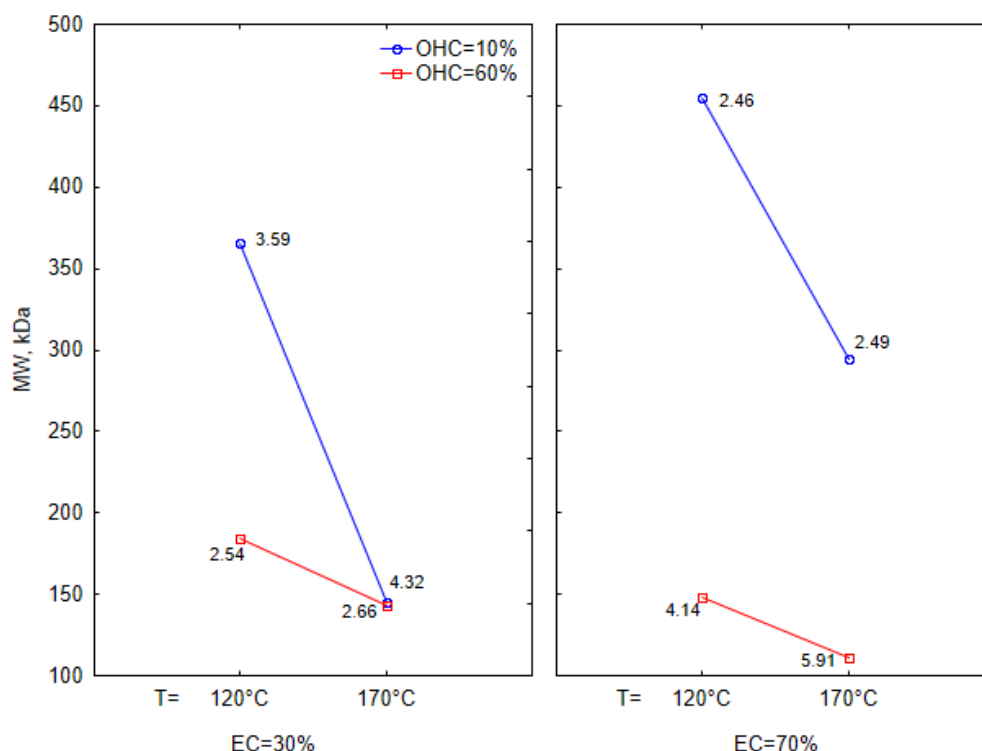
XYN: Birchwood xylan; HC B1: Recovered hemicelluloses from banana pseudostem in experiment 1

Source: By author

3.3.4. Hemicelluloses (HC) molecular weight

All samples of the extracted HC shown molecular weight and polydispersity higher than the commercial one (XYN: molecular weight=36.0 kDa and polydispersity=1.14) (Figure 3-2). This expected behavior is supported in the difference of chemical composition of each specimen (XYN and extracted HC); the commercial sample corresponds to one type of polymer found into HC, the xylans, and the HC extracted from OHEOP possibly includes another polysaccharides such as xyloglucan, mannans and β -linked glucans (SCHELLER; ULVSKOV, 2010). Besides that, the molecular weight either of HC (as group of polymers) or a specific polymer varies depending on the solubilization and recovery methods, and the biomass source (DE FREITAS; BRIENZO, 2022a).

Figure 3.2 - Average molecular weight (MW) and polydispersity of extracted HC



MW: Molecular weight; T: Temperature; EC: Ethanol aqueous solution concentration; OHC: alkali concentration

Source: By author

The highest molecular weight of HC extracted from banana pseudostem pretreated by OHEOP (170 °C, EC 70% and OHC 10%) was 455.1 kDa, that although seems high value, did not exceed the one cited for locust galactomannan (2000 kDa) (BARAK; MUDGIL, 2014). The extracted HC with high molecular weights were obtained when OHEOP was performed at OHC 10% (=0.2 mol/L) and 120 °C, in contrast, the HC showing low molecular weights were extracted from PB after OHEOP at OHC 60% (=0.9 mol/L) and 170 °C. The OHC behavior was in accordance with the found by Farhat et al. (2017), who observed an increasing of molecular weight (from 35 to 47.7 kDa) of HC extracted by alkali route from partially delignified switchgrass when OHC was reduced from 17% (4.25 mol/L) to 3% (0.75 mol/L) NaOH solution. The explication maybe is supported by the fact that low OHCs allow to neutralize the acids released from HC during pretreatment, therefore the HC autohydrolysis is minimized, which contributes to preserve HC. And in the T case, it is well known that increasing it, the molecular weights of extracted HC dropped (SUN *et al.*, 2014).

3.3.5. Lignin (LG) extraction and characterization by FTIR

According to results listed in the Table 3-4, from every OHEOP configuration it was possible to extract/recover LG. The greatest LGy (35.2% respect to LG content in biomass = 9% respect to biomass) was achieved at 170 °C, EC 30% and OHC 10%. Owhe et al. (2021) obtained similar values from coffee chaff (9.6% respect to biomass) but using deep eutectic solvents (lactic acid-chlorine chloride) at 155 °C for 2 h employing 10% m/m of biomass loading. In contrast, Başakçılardan and Tanış (2021) informed LGy of 42% using alkali (NaOH) organosolv pretreatment with glycerol from wood sawdust (biomass loading: 10% m/v) at identical T (170 °C) for 2 h. Zhong et al. (2021) extracted LG with almost 66.0% of mass yield using solid residue comes from xylose production (biomass loading: 5% m/v), through organosolv pretreatment catalyzed by diluted alkali (OHC 2%) at 100 °C during 1 h (same reaction time), stirring at 300 rpm and using methanolic solution of 65%. This high yield due to the pretreatment was performed using a raw material with very low HC content. Mondylaksita et al. (2020) reported 64.9% as the highest LGy from oil palm empty fruit bunches (unknown biomass loading) by organosolv pretreatment with ethanol at 210 °C for 90 min, biomass:solvent ratio of 1:10 and employing H₂SO₄ as catalyst (0.07%). This result of yield because the acid pretreatment is favorable to preserve and recover LG.

Considering the ANOVA (Table 3-5), among the 1st order effects, EC and T had the highest significance, respectively. EC showed a beneficial impact on LGy when the OHEOP was performed at 30% and T favorably influenced the same response variable at 170 °C. OHC did not indicate statistical significance, however, all interaction effects among factors were significative. The result concerning to T is in agreement with the Vaidya et al. (2022) review, in which is cited that T causes a high influence on efficiency of alkali organosolv pretreatment on LC biomass, being 150-200 °C the common range. The Suriyachai et al. (2018) research, a more specific study involving removal and recovery yield of LG through organosolv pretreatment, found that T effect was the most statistically significative, where 170 °C guaranteed the highest LGy (71.1 - 80.2%).

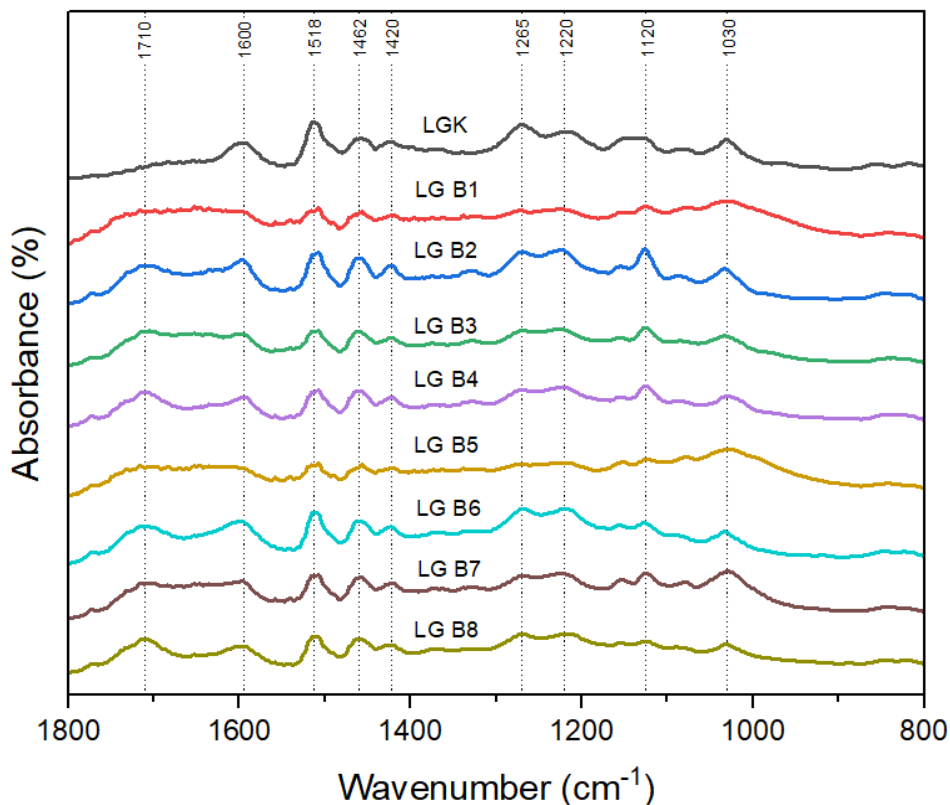
About the EC effect on LG extraction/recovery, it is well known that LG solubilization occurs in the organosolv pretreatment and is caused by the solvent

action and its concentration. In a previous research, concentrations between 25 and 75% of EC were employed to achieve deconstruction of the LC structure (BORAND; KARAOSMANOĞLU, 2018). Ethanol or methanol presence in organosolv pretreatments let to solubilize HC and LG through two main steps: firstly, the breaking of HC-LG linkages between 4-O-methylglucuronic acid esters of HC and the α -carbons of LG, and secondly the internal cleavage of LG linkages (α - and β -O-aryl ether bonds between LG units) (SANNIGRAHI; RAGAUSKAS, 2013a). The best LGy herein was above that the found one by Pereira *et al.* (2017), who reported yields lesser than 3% (respect to LG content in biomass) through acid organosolv pretreatment (in reflux during 24 h) of rice husks (biomass loading 5% m/v) using EC 25%. Maybe it is possible to suppose that a low LG content in raw biomass, such as banana pseudostem (25.7%) would need low EC (30%) to solubilize LG (and therefore recover it).

FTIR spectroscopy of the extracted LG from every OHEOP setup (2^3 design) is showed in the Figure 3.3, where the 800 – 1800 cm^{-1} region has been commonly used for this type of analysis. The band at 1030 cm^{-1} is attributed to C-O(H) and C-O(C) stretchings corresponding to C bonds with hydroxyl functional group (1st order aliphatic OH) present in primary alcohols and ether linkage, respectively (ABD *et al.*, 2022). The existence of this band means carbohydrate presence, which was observed in the LGK spectra and the sample with the lowest intensity was the B1. For every sample of extracted LG, the 1120 cm^{-1} band was present, however, the B5 and B8 samples shown the lowest absorbance, this band denotes the aromatic C-H deformation of syringyl units (SAMMONS *et al.*, 2013a). The 1220 cm^{-1} band is characteristic of C-C, C-O and C=O stretches in guaiacyl rings, its more visible absorbance occurred in the B2 and B6 samples (SAMMONS *et al.*, 2013a). The 1265 cm^{-1} signal marks the guaiacyl ring stretching vibration and its behavior in LG samples was the same than 1220 cm^{-1} (INKROD *et al.*, 2018). The band at 1420 cm^{-1} represents the C-C stretching in the aromatic skeletal of LG, all samples exhibited absorbance and its highest one was observed in the B2 sample (ABD *et al.*, 2022). Every LG sample shown C-H asymmetric deformation (in -CH₃ and -CH₂-) in LG and guaiacyl ring stretching, which are represented by 1462 and 1518 cm^{-1} bands, respectively (JOY *et al.*, 2021; SUN; TREVORAH; OTHMAN, 2018). In closing, the bands at 1600 and 1710 cm^{-1} are attributed to C=O stretching vibration in the aromatic ring and unconjugated C=O stretching in phenyl ester from LG (ABD *et al.*, 2022; INKROD *et al.*, 2018; SAMMONS

et al., 2013a). For LGK, B1, B5 and B7 the unconjugated C=O stretching was not detected.

Figure 3.3 - FTIR spectra of recovered lignin (LG) from banana pseudostem



LGK: Kraft lignin; LG B1: Recovered lignin from banana pseudostem in experiment 1

Source: By author

3.3.6. Enzymatic hydrolysis (Biomass digestibility)

CLX of PB obtained from every OHEOP configuration was higher than one corresponding to unpretreated biomass (Table 3-4). This result confirms that application of the pretreatment stage on LC biomass is necessary to beat recalcitrance in order to obtain subsequently bioproducts (ZAMORA *et al.*, 2021). CLX reached the maximum (96.6% = 512 mg/g) using biomass pretreated at 120 °C, EC 70%, and OHC 60% (= 0.9 mol/L = 3.6% m/v), and when PB shown the lowest LG content (25.0±0.21%). This behavior was found by Singh *et al.* (2022) too, who reported high performance of enzymatic hydrolysis when biomass was pretreated using alkaline route and the LG content of PB was low. Besides that, it is important to mention that

using NaOH in pretreatments is more effective on LG low-content biomasses, which influences the enzymatic hydrolysis performance on PB (KANDHOLA *et al.*, 2017a).

The maximum CLX, was higher than one reported (~460 mg/g) by Weerasai *et al.* (2021), who used the same commercial enzyme (Cellic® CTec2) with 10 FPU/g, PB loading 5% m/v at 50 °C during 72 h and 30 rpm (vertical rotation). The sugarcane bagasse was pretreated through alkaline organosolv (biomass loading 10% m/v) in methanol medium using identical T (120 °C) during 30 min and OHC 5% m/v. This same study also informed a CLX around 130 mg/g with the same hydrolysis and organosolv configurations, but using triethylamine instead of NaOH as catalyst. Li *et al.* (2020) reported 84.3% as the highest CLX from sorghum pith pretreated through OHEOP (unknown setup) with enzymatic hydrolysis carried out with PB loading 2% m/v, Cellic® CTec2 (15 FPU/g) aided by β -glucosidase, and 50 °C during 72 h. And Yuan *et al.* (2018) found around 90% of CLX from corn stover pretreated by methanolic (unknown solution concentration) organosolv catalyzed with NaOH (OHC 10%) during 1 h at 80 °C. The hydrolysis (PB loading: 5% m/v) was performed with Cellic® CTec2 (15 FPU/g) at 50 °C for 24 h.

Finally, according to statistical analysis (Table 3-5), OHC, EC, and T were significative on CLX and exhibited favorable effects when OHEOP was performed with 60% (=3.6% m/v), 30%, and 170 °C, respectively, and every interaction effect among factors was significative too. The result about high OHC utilization was in concordance with Raita *et al.* (2017), who employing high catalyst loading (NaOH 5% m/v) during OHEOP on rice straw improved the performance of enzymatic hydrolysis. Besides that, the yield of released sugars from PB after enzymatic hydrolysis is positively influenced by alkali catalysis during pretreatment since alkalis are considered selective on LG remotion/solubilization and avoid inhibitory formation (ZAMORA *et al.*, 2021; ZHANG *et al.*, 2020a). Concerning to use EC 30%, Lesar *et al.* (2016) reported that the highest released sugar yield (71.4% after enzymatic hydrolysis) was achieved from recycled wood pretreated by ethanolic organosolv (without catalyst use) at 220 °C for 3 h of reaction time and using EC 35%. The Bernal-Lugo *et al.* (2019) research was another example of good performance of enzymatic hydrolysis (CLX ~ 74% after 24 h) from *Pinus* spp. sawdust pretreated with organosolv at EC 45%, the pretreatment setup included NaOH 3% m/v at 165 °C during 2 h. Finally, the T finding herein agrees with the cited by the Vaidya *et al.* (2022) review, in which 170 °C marks a favorable trend

in sugar releasing yield after enzymatic hydrolysis from different kinds of LC biomasses pretreated by different types of process such as organosolv, alkaline, acidic and others.

3.4. Conclusions

Probably the ash elevated content did not let a good performance in OHEOP in terms of LG extraction/recovery, since ash high quantities in raw biomasses could provoke buffering effect causing disadvantages in the efficiency during pretreatment and therefore in the separation of LG, and its subsequent extraction. According to ANOVA, for all factors and response variables, every curvature effect did not show statistical significance, therefore, the linear models of factor effects were chosen. With 95% of significance level, the OHC and T had the greatest significative effects on HCy, EC and T for LGy, and OHC for CLX.

After every OHEOP experiment, the ethanol addition to the spent liquor provoked HC precipitation and the subsequent solvent evaporation guaranteed LG precipitation. Thus, HC and LG could be extracted/recovered from banana pseudostem. The best HCy occurred when HC content in the PB was the lowest obtained in the LC characterization. In conformity with the FTIR spectroscopy analysis, every extracted/recovered HC shown great correspondences with the commercial one (XYN). The best similarity was that the band describing acetyl group was not detected in any extracted HC and neither in the XYN. Likewise (according to FTIR study), every isolated LG shown great matches with the commercial LG, highlighting each extracted/recovered LG sample exhibited absorbances of aromatic C-H deformation in syringyl units and aromatic skeletal of LG. OHEOP incorporation as deconstruction strategy of banana pseudostem resulted in glucose high releasing by enzymatic hydrolysis, and this highest CLX was obtained when PB exhibited LG content lower than raw biomass.

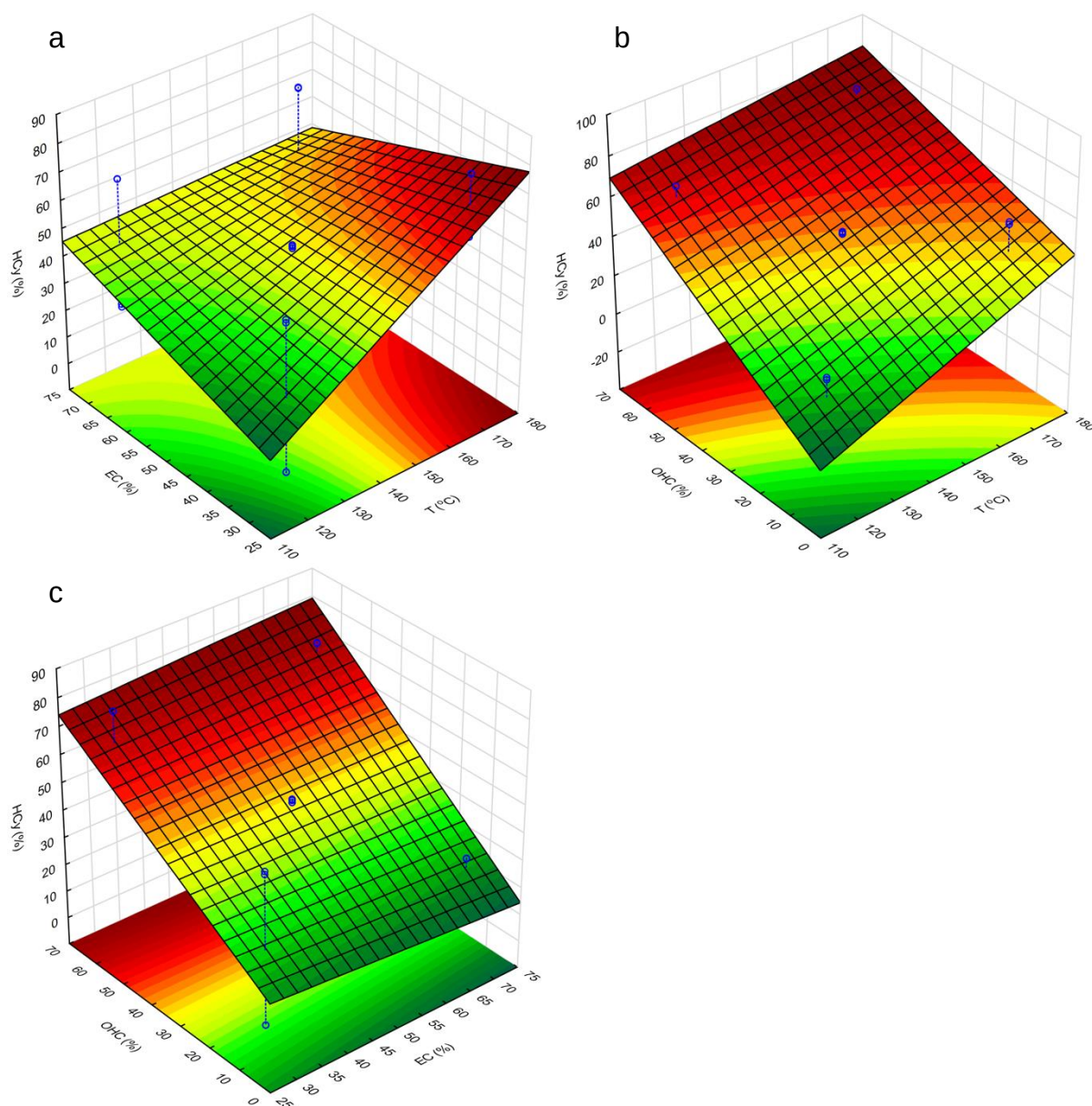
3.5. Supplementary Information

List of figures

Figure S3.1	Response surfaces for HCy at EC and T (a), OHC and T (b) and OHC and EC (c)
----------------	---

Figure S3.2	Response surfaces for LGy at EC and T (a), OHC and T (b) and OHC and EC (c)
Figure S3.3	Response surfaces for CLX at EC and T (a), OHC and T (b) and OHC and EC (c)

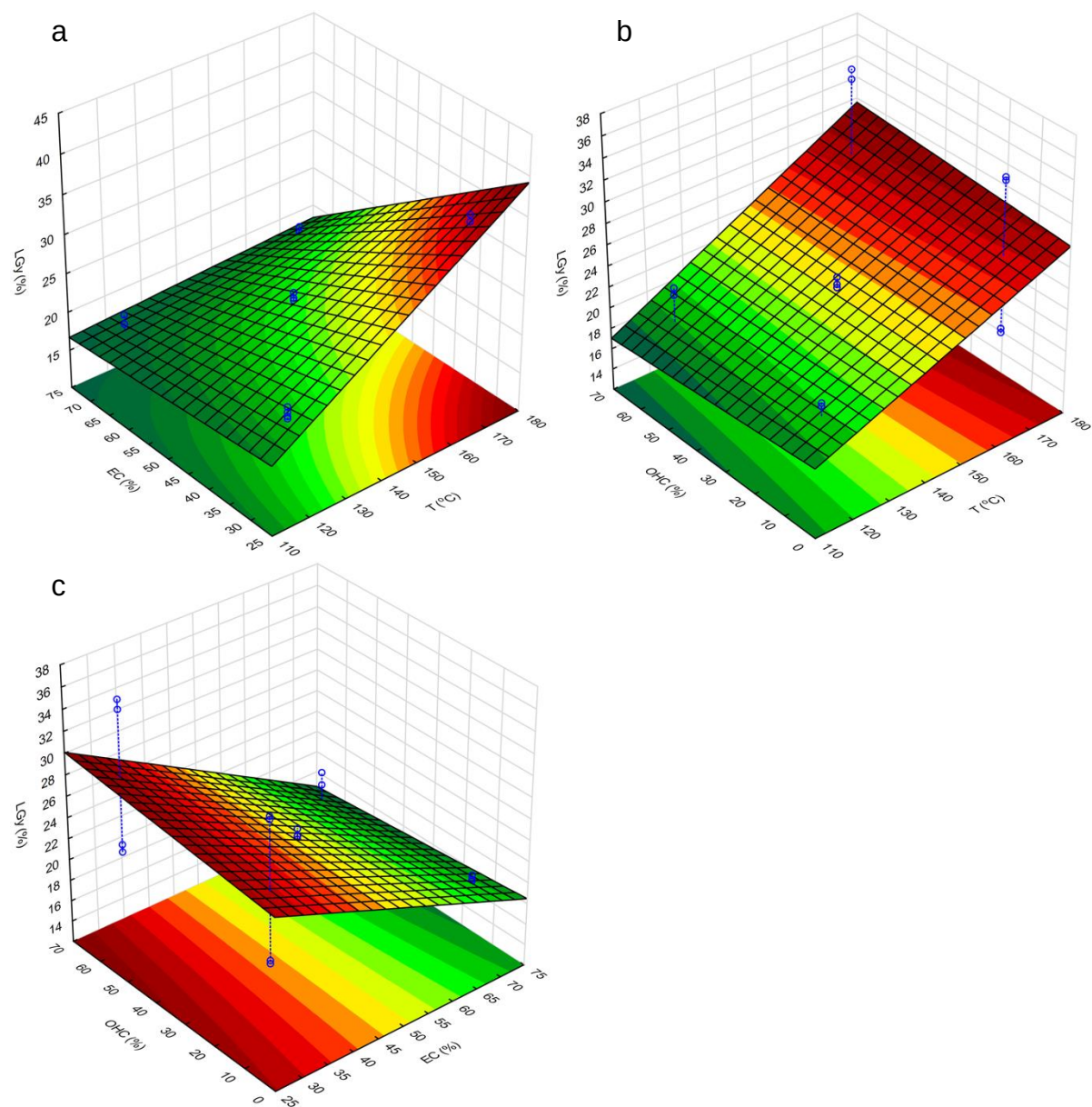
Figure S3.1 - Response surfaces for HCy at EC and T (a), OHC and T (b) and OHC and EC (c)



T: Temperature; EC: Ethanol aqueous solution concentration; OHC: Alkali concentration; HCy: Extraction yield of hemicelluloses

Source: By author

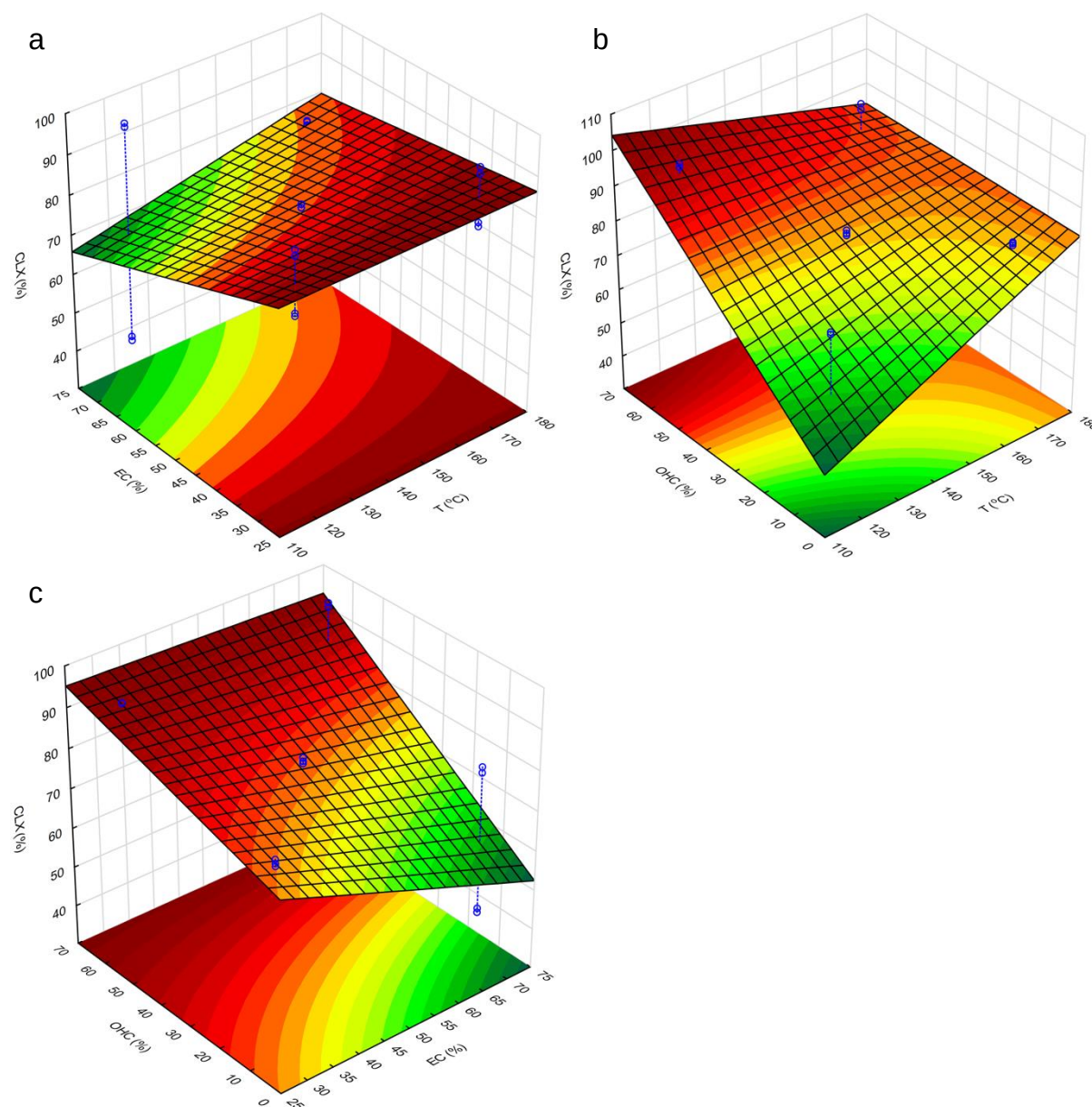
Figure S3.2 - Response surfaces for LGy at EC and T (a), OHC and T (b) and OHC and EC (c)



T: Temperature; EC: Ethanol aqueous solution concentration; OHC: Alkali concentration; LGy: Extraction yield of lignin

Source: By author

Figure S3.3 - Response surfaces for CLX at EC and T (a), OHC and T (b) and OHC and EC (c)



T: Temperature; EC: Ethanol aqueous solution concentration; OHC: Alkali concentration; CLX: Cellulose conversion.

Source: By author

References

- ABD, N. H.; BROSSE, N.; ZIEGLER-DEVIN, I.; CHRUSIEL, L.; HASHIM, R.; HUSSIN, M. H. A Comparison of Alkaline and Organosolv Lignin Extraction Methods from Coconut Husks as an Alternative Material for Green Applications. **BioResources**, Raleigh, vol. 17, no. 1, p. 469–491, 2022. DOI 10.15376/biores.17.1.469-491. Available at: <https://bioresources.cnr.ncsu.edu/issues/vol17-issue1/page/4/>. Accessed on: 17 Aug. 2022.
- BALDA, S.; SHARMA, A.; CAPALASH, N.; SHARMA, P. Banana fibre: a natural and sustainable bioresource for eco-friendly applications. **Clean Technologies and Environmental Policy**, vol. 23, no. 5, p. 1389–1401, 2021. <https://doi.org/10.1007/s10098-021-02041-y>.

BARAK, S.; MUDGIL, D. Locust bean gum: Processing, properties and food applications—A review. **International Journal of Biological Macromolecules**, Amsterdam, vol. 66, p. 74–80, 2014. <https://doi.org/10.1016/j.ijbiomac.2014.02.017>.

BAŞAKÇILARDAN KABAKCI, S.; TANIŞ, M. H. Pretreatment of lignocellulosic biomass at atmospheric conditions by using different organosolv liquors: a comparison of lignins. **Biomass Conversion and Biorefinery**, Berlin, vol. 11, no. 6, p. 2869–2880, 2021. DOI 10.1007/s13399-020-00677-2. Available at: <https://link.springer.com/article/10.1007/s13399-020-00677-2>. Accessed on: 8 Aug. 2022.

BERNAL-LUGO, I.; JACINTO-HERNANDEZ, C.; GIMENO, M.; MONTIEL, C.; RIVERO-CRUZ, F.; VELASCO, O. Highly efficient single-step pretreatment to remove lignin and hemicellulose from softwood. **BioResources**, Raleigh, vol. 14, no. 2, p. 3567–3577, 2019. DOI 10.15376/biores.14.2.3567-3577. Available at: <https://bioresources.cnr.ncsu.edu/issues/vol14-issue2/page/8/>. Accessed on: 23 Aug. 2022.

BILBA, K.; ARSENE, M.-A.; OUENSANGA, A. Study of banana and coconut fibers: Botanical composition, thermal degradation and textural observations. **Bioresource Technology**, Amsterdam, vol. 98, no. 1, p. 58–68, 2007. DOI 10.1016/j.biortech.2005.11.030. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852405005791>. Accessed on: 21 Jul. 2021.

BORAND, M. N.; KARAOSMANOĞLU, F. Effects of organosolv pretreatment conditions for lignocellulosic biomass in biorefinery applications: A review. **Journal of Renewable and Sustainable Energy**, Melville, vol. 10, no. 3, p. 1–22, 1 May 2018. DOI 10.1063/1.5025876. Available at: <https://aip.scitation.org/doi/10.1063/1.5025876>. Accessed on: 7 Jul. 2022.

BRIENZO, M.; SIQUEIRA, A. F.; MILAGRES, A. M. F. Search for optimum conditions of sugarcane bagasse hemicellulose extraction. **Biochemical Engineering Journal**, Amsterdam, vol. 46, no. 2, p. 199–204, 2009. DOI 10.1016/j.bej.2009.05.012. Available at: <https://www.sciencedirect.com/science/article/pii/S1369703X09001569>. Accessed on: 29 Sep. 2021.

CAVIGLIA-HARRIS, J.; BIGGS, T.; FERREIRA, E.; HARRIS, D. W.; MULLAN, K.; SILLS, E. O. The color of water: The contributions of green and blue water to agricultural productivity in the Western Brazilian Amazon. **World Development**, Amsterdam, vol. 146, p. 1–13, 2021. DOI 10.1016/j.worlddev.2021.105607. Available at: <https://www.sciencedirect.com/science/article/pii/S0305750X21002229>. Accessed on: 19 Jul. 2022.

CORDEIRO, N.; BELGACEM, M. N.; TORRES, I. C.; MOURA, J. C. V. Chemical composition and pulping of banana pseudostems. **Industrial Crops and Products**, Amsterdam, vol. 19, no. 2, p. 147–154, 2004. DOI 10.1016/j.indcrop.2003.09.001. Available at: <https://www.sciencedirect.com/science/article/pii/S0926669003001055>. Accessed on: 21 Jul. 2021.

DE FREITAS, C.; BRIENZO, M. Enzymatic Hydrolysis Applied to Banana Pseudostem Biomass Compared to Solubilized Xylan for Xylooligosaccharides Production with High Substrate Concentration. **BioEnergy Research**, Amsterdam, 2022. DOI 10.1007/s12155-022-10508-z. Available at: <https://doi.org/10.1007/s12155-022-10508-z>. Accessed on: 22 Mar. 2022.

DE FREITAS, E.; KHATRI, V.; WU, J.; TAKADA, M.; SCARCELLA, A. S. de A.; MARTINEZ, C. A.; SADDLER, J. N.; POLIZELI, M. de L. T. M. Structural and compositional changes induced by hydrothermal and organosolv pretreatments impacts enzymatic hydrolysis of a tropical forage grass grown under future climate conditions. **Industrial Crops and Products**, Amsterdam, vol. 171, p. 1–13, 2021. DOI 10.1016/j.indcrop.2021.113937. Available at: <https://www.sciencedirect.com/science/article/pii/S0926669021007019>. Accessed on: 3 Aug. 2022.

FARHAT, W.; VENDITTI, R.; QUICK, A.; TAHA, M.; MIGNARD, N.; BECQUART, F.; AYOUB, A. Hemicellulose extraction and characterization for applications in paper coatings and adhesives. **Industrial Crops and Products**, Amsterdam, vol. 107, p. 370–377, 2017. DOI 10.1016/j.indcrop.2017.05.055. Available at: <https://www.sciencedirect.com/science/article/pii/S0926669017303758>. Accessed on: 11 Feb. 2021.

FERREIRA, J. A.; TAHERZADEH, M. J. Improving the economy of lignocellulose-based biorefineries with organosolv pretreatment. **Bioresource Technology**, Amsterdam, vol. 299, p. 1–13, 2020. DOI 10.1016/j.biortech.2019.122695. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852419319248>. Accessed on: 2 Jul. 2022.

GALLINA, G.; CABEZA, Á.; GRÉNMAN, H.; BIASI, P.; GARCÍA-SERNA, J.; SALMI, T. Hemicellulose extraction by hot pressurized water pretreatment at 160°C for 10 different woods: Yield and molecular weight. **The Journal of Supercritical Fluids**, Amsterdam, vol. 133, no. Part 2, p. 716–725, 2018. DOI 10.1016/j.supflu.2017.10.001. Available at: <https://www.sciencedirect.com/science/article/pii/S0896844617303819>. Accessed on: 3 Aug. 2022.

GARCÍA, C. A.; PEÑA, Á.; BETANCOURT, R.; CARDONA, C. A. Energetic and environmental assessment of thermochemical and biochemical ways for producing energy from agricultural solid residues: Coffee Cut-Stems case. **Journal of Environmental Management**, Amsterdam, vol. 216, p. 160–168, 2018. DOI 10.1016/j.jenvman.2017.04.029. Available at: <https://www.sciencedirect.com/science/article/pii/S0301479717303705>. Accessed on: 6 Feb. 2021.

HANIM, S. S.; NORSYABILAH, R.; SUHAILA, M. H. N.; NORASHAH, A.; KARTINA, A. K. S. Effects of Temperature, Time and Pressure on the Hemicelluloses Yield Extracted Using Subcritical Water Extraction. **Procedia Engineering**, Amsterdam, vol. 42, p. 562–565, 2012. DOI 10.1016/j.proeng.2012.07.448. Available at: <https://www.sciencedirect.com/science/article/pii/S1877705812028482>. Accessed on: 5 Aug. 2022.

HUANG, C.; HUANG, C.; LAI, C.; WU, X.; HUANG, Y.; HE, J.; LI, X.; YONG, Q. Strategy to utilize the high ash content biomass feedstock for fermentable sugars. **BioResources**, Raleigh, vol. 12, no. 4, p. 8306–8319, 2017. DOI 10.15376/biores.12.4.8306-8319. Available at: <https://bioresources.cnr.ncsu.edu/issues/volume-12-issue-4/page/11/>. Accessed on: 25 Jul. 2022.

IBGE. Produção Agropecuária. 2022. Available at: <https://www.ibge.gov.br/explica/producao-agropecuaria/>. Accessed on: 8 Jul. 2022.

INKROD, C.; RAITA, M.; CHAMPREDA, V.; LAOSIROPOJANA, N. Characteristics of Lignin Extracted from Different Lignocellulosic Materials via Organosolv Fractionation. **BioEnergy Research**, Berlin, vol. 11, no. 2, p. 277–290, 2018. DOI 10.1007/s12155-018-9895-2. Available at: <https://link.springer.com/article/10.1007/s12155-018-9895-2>. Accessed on: 17 Aug. 2022.

JAVIER-ASTETE, R.; JIMENEZ-DAVALOS, J.; ZOLLA, G. Determination of hemicellulose, cellulose, holocellulose and lignin content using FTIR in Calycophyllum spruceanum (Benth.) K. Schum. and Guazuma crinita Lam. **PLOS ONE**, San Francisco, vol. 16, no. 10, p. 1–12, 27 Oct. 2021. DOI 10.1371/journal.pone.0256559. Available at: <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0256559>. Accessed on: 20 Jul. 2021.

JAYAPRABHA, J. S.; BRAHMAKUMAR, M.; MANILAL, V. B. Banana Pseudostem Characterization and Its Fiber Property Evaluation on Physical and Bioextraction. **Journal of Natural Fibers**, London, vol. 8, no. 3, p. 149–160, 1 Jul. 2011. DOI 10.1080/15440478.2011.601614. Available at: <https://www.tandfonline.com/doi/abs/10.1080/15440478.2011.601614>. Accessed on: 21 Jul. 2021.

JOY, S. P.; KUMAR, A. A.; GORTHY, S.; JAGANATHAN, J.; KUNAPPAREDDY, A.; GADDAMEEDI, A.; KRISHNAN, C. Variations in structure and saccharification efficiency of biomass of different sorghum varieties subjected to aqueous ammonia and glycerol pretreatments. **Industrial Crops and Products**, Amsterdam, vol. 159, p. 1–12, 2021. DOI 10.1016/j.indcrop.2020.113072. Available at: <https://www.sciencedirect.com/science/article/pii/S0926669020309894>. Accessed on: 20 Jul. 2021.

JU, Y.-H.; HUYNH, L.-H.; KASIM, N. S.; GUO, T.-J.; WANG, J.-H.; FAZARY, A. E. Analysis of soluble and insoluble fractions of

alkali and subcritical water treated sugarcane bagasse. **Carbohydrate Polymers**, Amsterdam, vol. 83, no. 2, p. 591–599, 2011. DOI 10.1016/j.carbpol.2010.08.022. Available at: <https://www.sciencedirect.com/science/article/pii/S0144861710006429>. Accessed on: 11 Aug. 2022.

KANDHOLA, G.; DJIOLEU, A.; CARRIER, D. J.; KIM, J.-W. Pretreatments for Enhanced Enzymatic Hydrolysis of Pinewood: a Review. **BioEnergy Research**, Berlin, vol. 10, no. 4, p. 1138–1154, 2017. DOI 10.1007/s12155-017-9862-3. Available at: <https://link.springer.com/article/10.1007/s12155-017-9862-3>. Accessed on: 22 Aug. 2022.

KLAMRASSAMEE, T.; CHAMPREDA, V.; REUNGLEK, V.; LAOSIROPOJANA, N. Comparison of homogeneous and heterogeneous acid promoters in single-step aqueous-organosolv fractionation of eucalyptus wood chips. **Bioresource Technology**, Amsterdam, vol. 147, p. 276–284, 2013. DOI 10.1016/j.biortech.2013.08.015. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852413012443>. Accessed on: 28 Sep. 2021.

KOUTSOUKIS, C.; ROUKOS, C.; DEMERTZIS, P. G.; KANDRELIS, S.; AKRIDA-DEMERTZI, K. The variation of the chemical composition of the main plant species in a subalpine grassland in northwestern Greece. **Legume Science**, Hoboken, vol. 1, no. 1, p. 1–11, 2019. DOI 10.1002/leg3.23. Available at: <https://onlinelibrary.wiley.com/doi/full/10.1002/leg3.23>. Accessed on: 21 Jul. 2022.

LESAR, B.; HUMAR, M.; HORA, G.; HACHMEISTER, P.; SCHMIEDL, D.; PINDEL, E.; SIIKA-AHO, M.; LIITIÄ, T. Utilization of recycled wood in biorefineries: preliminary results of steam explosion and ethanol/water organosolv pulping without a catalyst. **European Journal of Wood and Wood Products**, Berlin, vol. 74, no. 5, p. 711–723, 2016. DOI 10.1007/s00107-016-1064-8. Available at: <https://link.springer.com/article/10.1007/s00107-016-1064-8>. Accessed on: 23 Aug. 2022.

LI, D.; LONG, L.; DING, S. Alkaline organosolv pretreatment of different sorghum stem parts for enhancing the total reducing sugar yields and p-coumaric acid release. **Biotechnology for Biofuels**, Berlin, vol. 13, no. 1, p. 1–14, 2020. DOI 10.1186/s13068-020-01746-4. Available at: <https://link.springer.com/article/10.1186/s13068-020-01746-4>. Accessed on: 14 Aug. 2022.

LI, F.; HU, H.; YAO, R.; WANG, H.; LI, M. Structure and Saccharification of Rice Straw Pretreated with Microwave-Assisted Dilute Lye. **Industrial & Engineering Chemistry Research**, Washington, vol. 51, no. 17, p. 6270–6274, 2 May 2012. DOI 10.1021/ie202547w. Available at: <https://pubs.acs.org/doi/10.1021/ie202547w>. Accessed on: 11 Aug. 2022.

LIU, X.; RENARD, C. M. G. C.; BUREAU, S.; LE BOURVELLEC, C. Revisiting the contribution of ATR-FTIR spectroscopy to characterize plant cell wall polysaccharides. **Carbohydrate Polymers**, Amsterdam, vol. 262, p. 1–10, 2021. DOI 10.1016/j.carbpol.2021.117935. Available at: <https://www.sciencedirect.com/science/article/pii/S0144861721003222>. Accessed on: 11 Aug. 2022.

LU, Y.; HE, Q.; FAN, G.; CHENG, Q.; SONG, G. Extraction and modification of hemicellulose from lignocellulosic biomass: A review. **Green Processing and Synthesis**, Berlin, vol. 10, no. 1, p. 779–804, 2021. DOI 10.1515/gps-2021-0065. Available at: <https://www.degruyter.com/document/doi/10.1515/gps-2021-0065/html>. Accessed on: 5 Aug. 2022.

MA, J.; SRZEDNICKI, G.; ARCOT, J. Effects of Drying on Stability of Nutrients in Banana Pseudostem in Species *Musa balbisiana* and *Musa acuminata*. **Journal of Food Processing and Preservation**, Hoboken, vol. 41, no. 1, p. e12865, Feb. 2017. <https://doi.org/10.1111/jfpp.12865>.

MANHONGO, T. T.; CHIMPHANGO, A. F. A.; THORNLEY, P.; RÖDER, M. Current status and opportunities for fruit processing waste biorefineries. **Renewable and Sustainable Energy Reviews**, Amsterdam, vol. 155, p. 111823, 2022. DOI 10.1016/j.rser.2021.111823. Available at: <https://www.sciencedirect.com/science/article/pii/S1364032121010911>. Accessed on: 7 Jul. 2022.

MANSOR, A. M.; LIM, J. S.; ANI, F. N.; HASHIM, H.; HO, W. S. Characteristics of Cellulose, Hemicellulose and Lignin of MD2 Pineapple Biomass. **Chemical Engineering Transactions**, Milan, vol. 72, p. 79–84, 31 Jan. 2019. DOI 10.3303/CET1972014. Available at: <https://www.cetjournal.it/index.php/cet/article/view/CET1972014>. Accessed on: 8 Feb. 2021.

MIHIRETU, G. T.; BRODIN, M.; CHIMPHANGO, A. F.; ØYAS, K.; HOFF, B. H.; GÖRGENS, J. F. Single-step microwave-assisted hot water extraction of hemicelluloses from selected lignocellulosic materials – A biorefinery approach. **Bioresource Technology**, Amsterdam, vol. 241, p. 669–680, 2017. DOI 10.1016/j.biortech.2017.05.159. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852417308271>. Accessed on: 5 Aug. 2022.

MONDYLAKSITA, K.; FERREIRA, J. A.; MILLATI, R.; BUDHIJANTO, W.; NIKLASSON, C.; TAHERZADEH, M. J. Recovery of High Purity Lignin and Digestible Cellulose from Oil Palm Empty Fruit Bunch Using Low Acid-Catalyzed Organosolv Pretreatment. **Agronomy**, Basel, vol. 10, no. 5, p. 1–15, 2020. DOI 10.3390/agronomy10050674. Available at: <https://www.mdpi.com/2073-4395/10/5/674>. Accessed on: 31 Dec. 2023.

MONTGOMERY, D. **Design and Analysis of Experiments**. 5th ed. New York: John Wiley & Sons, Ltd, 2001.

OREJUELA-ESCOBAR, L. M.; LANDÁZURI, A. C.; GOODELL, B. Second generation biorefining in Ecuador: Circular bioeconomy, zero waste technology, environment and sustainable development: The nexus. **Journal of Bioresources and Bioproducts**, Amsterdam, vol. 6, no. 2, p. 83–107, 2021. DOI 10.1016/j.jobab.2021.01.004. Available at: <https://www.sciencedirect.com/science/article/pii/S2369969821000050>. Accessed on: 19 Jul. 2022.

OWHE, E. O.; KUMAR, N.; LYNAM, J. G. Lignin extraction from waste biomass with deep eutectic solvents: Molecular weight and heating value. **Biocatalysis and Agricultural Biotechnology**, Amsterdam, vol. 32, p. 1–7, 2021. DOI 10.1016/j.bcab.2021.101949. Available at: <https://www.sciencedirect.com/science/article/pii/S1878818121000451>. Accessed on: 9 Aug. 2022.

PEREIRA, B. S.; DE FREITAS, C.; VIEIRA, R. M.; BRIENZO, M. Brazilian banana, guava, and orange fruit and waste production as a potential biorefinery feedstock. **Journal of Material Cycles and Waste Management**, Berlin, vol. 24, no. 6, p. 2126–2140, 2022. DOI 10.1007/s10163-022-01495-6. Available at: <https://link.springer.com/article/10.1007/s10163-022-01495-6>. Accessed on: 6 Feb. 2022.

PEREIRA, M.; BECK, P. H.; GOMES, D.; MOREIRA, J. B.; SILVEIRA, J.; MUNARETTO, A. M. Extraction of Organosolv Lignin from Rice Husk under Reflux Conditions. **Biological and Chemical Research**, Delaware, vol. 4, no. 4, p. 87–98, 2017. Available at: <http://www.ss-pub.org/article/extraction-of-organosolv-lignin-from-rice-husk-under-reflux-conditions/>. Accessed on: 17 Aug. 2022.

PUITEL, A. C.; SUDITU, G. D.; DANU, M.; AILIESEI, G.-L.; NECHITA, M. T. An Experimental Study on the Hot Alkali Extraction of Xylan-Based Hemicelluloses from Wheat Straw and Corn Stalks and Optimization Methods. **Polymers**, Basel, vol. 14, no. 9, p. 1–17, 2022. DOI 10.3390/polym14091662. Available at: <https://www.mdpi.com/2073-4360/14/9/1662>. Accessed on: 22 Jul. 2022.

RABETAFIKA, H. N.; BCHIR, B.; BLECKER, C.; PAQUOT, M.; WATHELET, B. Comparative study of alkaline extraction process of hemicelluloses from pear pomace. **Biomass and Bioenergy**, Amsterdam, vol. 61, p. 254–264, 2014. DOI 10.1016/j.biombioe.2013.12.022. Available at: <https://www.sciencedirect.com/science/article/pii/S0961953413005357>. Accessed on: 3 Aug. 2022.

RAITA, M.; DENCHOKEPRAGUY, N.; CHAMPREDA, V.; LAOSIROPOJANA, N. Effects of alkaline catalysts on acetone-based organosolv pretreatment of rice straw. **3 Biotech**, Amsterdam, vol. 7, no. 5, p. 340, 2017. DOI 10.1007/s13205-017-0969-1.

Available at: <https://link.springer.com/article/10.1007/s13205-017-0969-1>. Accessed on: 24 Aug. 2022.

REYES, P.; TEIXEIRA MENDONÇA, R.; RODRÍGUEZ, J.; FARDIM, P.; VEGA, B. Characterization of the hemicellulosic fraction obtained after pre-hydrolysis of pinus radiata wood chips with hot-water at different initial pH. **Journal of the Chilean Chemical Society**, Concepción, vol. 58, no. 1, p. 1614–1618, 2013. DOI 10.4067/S0717-97072013000100024. Available at: <https://www.scielo.cl/pdf/jcchems/v58n1/art24.pdf>. Accessed on: 5 Aug. 2022.

SAMMONS, R. J.; HARPER, D. P.; LABBÉ, N.; BOZELL, J. J.; ELDER, T.; RIALS, T. G. Characterization of organosolv lignins using thermal and FT-IR spectroscopic analysis. **BioResources**, Raleigh, vol. 8, no. 2, p. 2752–2767, 2013. Available at: <https://bioresources.cnr.ncsu.edu/issues/vol8-issue2/page/11/>.

SANNIGRAHI, P.; RAGAUSKAS, A. J. Fundamentals of Biomass Pretreatment by Fractionation. In: WYMAN, C. E. (ed.). **Aqueous Pretreatment of Plant Biomass for Biological and Chemical Conversion to Fuels and Chemicals**. Wiley Online Books. Hoboken: John Wiley & Sons, 2013. p. 201–222. DOI 10.1002/9780470975831.ch10. Available at: <https://onlinelibrary.wiley.com/doi/10.1002/9780470975831.ch10>. Accessed on: 16 Aug. 2022.

SANTOS, T. M.; RIGUAL, V.; DOMÍNGUEZ, J. C.; ALONSO, M. V.; OLIET, M.; RODRÍGUEZ, F. Fractionation of Pinus radiata by ethanol-based organosolv process. **Biomass Conversion and Biorefinery**, Berlin, , p. 1–14, 2022. DOI 10.1007/s13399-022-02329-z. Available at: <https://link.springer.com/article/10.1007/s13399-022-02329-z>. Accessed on: 8 Aug. 2022.

SCHELLER, H. V.; ULVSKOV, P. Hemicelluloses. **Annual Review of Plant Biology**, San Mateo, vol. 61, no. 1, p. 263–289, May 2010. <https://doi.org/10.1146/annurev-arplant-042809-112315>.

SCHMATZ, A. A.; SALAZAR-BRYAM, A. M.; CONTIERO, J.; SANT'ANNA, C.; BRIENZO, M. Pseudo-Lignin Content Decreased with Hemicellulose and Lignin Removal, Improving Cellulose Accessibility, and Enzymatic Digestibility. **BioEnergy Research**, Berlin, vol. 14, no. 1, p. 106–121, 2021. DOI 10.1007/s12155-020-10187-8. Available at: <https://link.springer.com/article/10.1007/s12155-020-10187-8>. Accessed on: 23 Sep. 2022.

SHAH, A. A.; SEEHAR, T. H.; SHARMA, K.; TOOR, S. S. Chapter 7 - Biomass pretreatment technologies. In: MAITY, S. K.; GAYEN, K.; BHOWMICK, T. K. B. T.-H. B. (eds.). **Hydrocarbon Biorefinery: Sustainable Processing of Biomass for Hydrocarbon Biofuels**. Amsterdam: Elsevier, 2022. p. 203–228. DOI 10.1016/B978-0-12-823306-1.00014-5. Available at: <https://www.sciencedirect.com/science/article/pii/B9780128233061000145>. Accessed on: 17 Aug. 2022.

SHIMIZU, F. L.; ZAMORA, H. D. Z.; SCHMATZ, A. A.; MELATI, R. B.; BUENO, D.; BRIENZO, M. Biofuels Generation Based on Technical Process and Biomass Quality. In: SRIVASTAVA, N.; SRIVASTAVA, M.; MISHRA, P. K.; GUPTA, V. K. (eds.). **Biofuel Production Technologies: Critical Analysis for Sustainability**. Singapore: Springer Singapore, 2020. p. 37–64. DOI 10.1007/978-981-13-8637-4_2. Available at: https://link.springer.com/chapter/10.1007/978-981-13-8637-4_2. Accessed on: 5 Jul. 2022.

SINGH, R. D.; BANERJEE, J.; SASMAL, S.; MUIR, J.; ARORA, A. High xylan recovery using two stage alkali pre-treatment process from high lignin biomass and its valorisation to xylooligosaccharides of low degree of polymerisation. **Bioresource Technology**, Amsterdam, vol. 256, p. 110–117, 2018. DOI 10.1016/j.biortech.2018.02.009. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852418301834>. Accessed on: 5 Aug. 2022.

SINGH, S. K.; SAULNIER, B. K.; HODGE, D. B. Lignin properties and cell wall response to deconstruction by alkaline pretreatment and enzymatic hydrolysis in brown midrib sorghums. **Industrial Crops and Products**, Amsterdam, vol. 178, p. 1–8, 2022. DOI 10.1016/j.indcrop.2022.114566. Available at: <https://www.sciencedirect.com/science/article/pii/S0926669022000498>. Accessed on: 22 Aug. 2022.

SLUITER, A.; HAMES, B.; RUIZ, R.; SCARLATA, C.; SLUITER, J.; TEMPLETON, D. **Determination of Ash in Biomass**, n. NREL/TP-510-42622. Golden: U.S. Department of Energy, 2008. Available at: <https://www.nrel.gov/docs/gen/fy08/42622.pdf>. Accessed on: 20 Jul. 2021.

SLUITER, A.; HAMES, B.; RUIZ, R.; SCARLATA, C.; SLUITER, J.; TEMPLETON, D.; CROCKER, D. **Determination of structural carbohydrates and lignin in biomass**, n. NREL/TP-510-42618. Golden: U.S. Department of Energy, 2012. Available at: <https://www.nrel.gov/docs/gen/fy13/42618.pdf>. Accessed on: 28 Sep. 2021.

SLUITER, A.; RUIZ, R.; SCARLATA, C.; SLUITER, J.; TEMPLETON, D. **Determination of Extractives in Biomass**, n. NREL/TP-510-42619. Golden: U.S. Department of Energy, 2008. Available at: <https://www.nrel.gov/docs/gen/fy08/42619.pdf>. Accessed on: 20 Jul. 2021.

SOUZA, O.; FEDERIZZI, M.; COELHO, B.; WAGNER, T. M.; WISBECK, E. Biodegradação de resíduos lignocelulósicos gerados na bananicultura e sua valorização para a produção de biogás. **Revista Brasileira de Engenharia Agrícola e Ambiental**, Campina Grande, vol. 14, no. 4, p. 438–443, 2010. <https://doi.org/10.1590/S1415-43662010000400014>.

SUN, R. C.; TOMKINSON, J. Characterization of hemicelluloses obtained by classical and ultrasonically assisted extractions from wheat straw. **Carbohydrate Polymers**, Amsterdam, vol. 50, no. 3, p. 263–271, 2002. DOI 10.1016/S0144-8617(02)00037-1. Available at: <https://www.sciencedirect.com/science/article/pii/S0144861702000371>. Accessed on: 8 Aug. 2022.

SUN, S.-N.; CAO, X.-F.; LI, H.-Y.; XU, F.; SUN, R.-C. Structural characterization of residual hemicelluloses from hydrothermal pretreated Eucalyptus fiber. **International Journal of Biological Macromolecules**, Amsterdam, vol. 69, p. 158–164, 2014. <https://doi.org/10.1016/j.ijbiomac.2014.05.037>.

SUN, W.; TREVORAH, R.; OTHMAN, M. Z. Fractionation of spent liquor from organosolv-pretreatment using lignin-incompatible extraction. **Bioresource Technology**, Amsterdam, vol. 269, p. 255–261, 2018. DOI 10.1016/j.biortech.2018.08.097. Available at: <https://www.sciencedirect.com/science/article/pii/S096085241831201X>. Accessed on: 18 Aug. 2022.

SURIYACHAI, N.; CHAMPREDA, V.; KRAIKUL, N.; TECHANAN, W.; LAOSIRIPOJANA, N. Fractionation of lignocellulosic biopolymers from sugarcane bagasse using formic acid-catalyzed organosolv process. **3 Biotech**, Berlin, vol. 8, no. 5, p. 1–14, 2018. DOI 10.1007/s13205-018-1244-9. Available at: <https://link.springer.com/article/10.1007/s13205-018-1244-9>. Accessed on: 12 Aug. 2022.

THORESEN, P. P.; MATSAKAS, L.; ROVA, U.; CHRISTAKOPOULOS, P. Recent advances in organosolv fractionation: Towards biomass fractionation technology of the future. **Bioresource Technology**, Amsterdam, vol. 306, p. 1–10, 2020. DOI 10.1016/j.biortech.2020.123189. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852420304600>. Accessed on: 2 Jul. 2022.

TIRYAKI, B.; YAGMUR, E.; BANFORD, A.; AKTAS, Z. Comparison of activated carbon produced from natural biomass and equivalent chemical compositions. **Journal of Analytical and Applied Pyrolysis**, Amsterdam, vol. 105, p. 276–283, 2014. DOI 10.1016/j.jaap.2013.11.014. Available at: <https://www.sciencedirect.com/science/article/pii/S0165237013002532>. Accessed on: 22 Jul. 2022.

USDA. **World Agricultural Production**, n. WAP 7-22. Washington: U.S. Department of Agriculture, 2022. Available at: <https://www.fas.usda.gov/data/world-agricultural-production>. Accessed on: 19 Jul. 2022.

VAIDYA, A. A.; MURTON, K. D.; SMITH, D. A.; DEDUAL, G. A review on organosolv pretreatment of softwood with a focus on enzymatic hydrolysis of cellulose. **Biomass Conversion and Biorefinery**, Berlin, , p. 1–16, 2022. DOI 10.1007/s13399-022-02373-9. Available at: <https://link.springer.com/article/10.1007/s13399-022-02373-9>. Accessed on: 7 Jul. 2022.

WEERASAI, K.; LAOSIROPOJANA, N.; IMMAN, S.; KREETACHAT, T.; SURIYACHAI, N. Reusable alkaline catalyzed organosolv pretreatment and delignification of bagasse for sugar platform biorefinery. **Biomass Conversion and Biorefinery**, Berlin, , p. 1–11, 2021. DOI 10.1007/s13399-020-01269-w. Available at: <https://link.springer.com/article/10.1007/s13399-020-01269-w>. Accessed on: 23 Aug. 2022.

WEI, W.; WANG, B.; WANG, X.; LING, R.; JIN, Y. Comparison of acid and alkali catalyzed ethylene glycol organosolv pretreatment for sugar production from bagasse. **Bioresource Technology**, Amsterdam, vol. 320, no. A, p. 1–9, 2021. DOI 10.1016/j.biortech.2020.124293. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852420315674>. Accessed on: 20 Jul. 2022.

WOLF, M.; BERGER, F.; HANSTEIN, S.; WEIDENKAFF, A.; ENDRESS, H.-U.; OESTREICH, A. M.; EBRAHIMI, M.; CZERMAK, P. Hot-Water Hemicellulose Extraction from Fruit Processing Residues. **ACS Omega**, Washington, vol. 7, no. 16, p. 13436–13447, 26 Apr. 2022. DOI 10.1021/acsomega.1c06055. Available at: <https://pubs.acs.org/doi/10.1021/acsomega.1c06055>. Accessed on: 3 Aug. 2022.

XU, J.-Y.; YUAN, T.-Q.; XIAO, L.; SUN, R.-C. Effect of ultrasonic time on the structural and physico-chemical properties of hemicelluloses from Eucalyptus grandis. **Carbohydrate Polymers**, Amsterdam, vol. 195, p. 114–119, 2018. DOI 10.1016/j.carbpol.2018.04.067. Available at: <https://www.sciencedirect.com/science/article/pii/S0144861718304533>. Accessed on: 5 Aug. 2022.

YAN, J.; OYEDEJI, O.; LEAL, J. H.; DONOHOE, B. S.; SEMELSBERGER, T. A.; LI, C.; HOOVER, A. N.; WEBB, E.; BOSE, E. A.; ZENG, Y.; WILLIAMS, C. L.; SCHALLER, K. D.; SUN, N.; RAY, A. E.; TANJORE, D. Characterizing Variability in Lignocellulosic Biomass: A Review. **ACS Sustainable Chemistry & Engineering**, Washington, vol. 8, no. 22, p. 8059–8085, 8 Jun. 2020. DOI 10.1021/acssuschemeng.9b06263. Available at: <https://pubs.acs.org/doi/full/10.1021/acssuschemeng.9b06263>. Accessed on: 21 Jul. 2022.

YAO, S.; NIE, S.; YUAN, Y.; WANG, S.; QIN, C. Efficient extraction of bagasse hemicelluloses and characterization of solid remainder. **Bioresource Technology**, Amsterdam, vol. 185, p. 21–27, 2015. DOI 10.1016/j.biortech.2015.02.052. Available at: <https://www.sciencedirect.com/science/article/pii/S096085241500231X>. Accessed on: 3 Aug. 2022.

YUAN, W.; GONG, Z.; WANG, G.; ZHOU, W.; LIU, Y.; WANG, X.; ZHAO, M. Alkaline organosolv pretreatment of corn stover for enhancing the enzymatic digestibility. **Bioresource Technology**, Amsterdam, vol. 265, p. 464–470, 2018. DOI 10.1016/j.biortech.2018.06.038. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852418308174>. Accessed on: 23 Aug. 2022.

ZAMORA, Hernán Darío Zamora; DE FREITAS, C.; BUENO, D.; SHIMIZU, F. L.; CONTIERO, J.; BRIENZO, M. Biomass Fractionation Based on Enzymatic Hydrolysis for Biorefinery Systems. In: VERMA, P. (ed.). **Biorefineries: A Step Towards Renewable and Clean Energy**. Singapore: Springer Singapore, 2021. p. 217–254. DOI 10.1007/978-981-15-9593-6_9. Available at: https://link.springer.com/chapter/10.1007/978-981-15-9593-6_9. Accessed on: 23 Sep. 2021.

ZAMORA, Hernán Darío Zamora; SILVA, T. A. L.; VARÃO, L. H. R.; BAFFI, M. A.; PASQUINI, D. Simultaneous production of cellulases, hemicellulases, and reducing sugars by *Pleurotus ostreatus* growth in one-pot solid state fermentation using *Alstroemeria* sp. waste. **Biomass Conversion and Biorefinery**, Berlin, , p. 1–14, 2021. DOI 10.1007/s13399-021-01723-3. Available at: <https://link.springer.com/article/10.1007/s13399-021-01723-3>. Accessed on: 19 Aug. 2022.

ZHANG, J.; ZHOU, H.; LIU, D.; ZHAO, X. Chapter 2 - Pretreatment of lignocellulosic biomass for efficient enzymatic saccharification of cellulose. In: YOUSUF, A.; PIROZZI, D.; SANNINO, F. B. T.-L. B. to L. B. (eds.). **Lignocellulosic Biomass to Liquid Biofuels**. Cambridge: Academic Press, 2020. p. 17–65. DOI 10.1016/B978-0-12-815936-1.00002-2. Available at: <https://www.sciencedirect.com/science/article/pii/B9780128159361000022>. Accessed on: 27 Sep. 2021.

ZHANG, K.; PEI, Z.; WANG, D. Organic solvent pretreatment of lignocellulosic biomass for biofuels and biochemicals: A review. **Bioresource Technology**, Amsterdam, vol. 199, p. 21–33, 2016. DOI 10.1016/j.biortech.2015.08.102. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852415012080>. Accessed on: 27 Sep. 2021.

ZHONG, L.; WANG, C.; XU, M.; JI, X.; YANG, G.; CHEN, J.; JANASWAMY, S.; LYU, G. Alkali-Catalyzed Organosolv Pretreatment of Lignocellulose Enhances Enzymatic Hydrolysis and Results in Highly Antioxidative Lignin. **Energy & Fuels**, Washington, vol. 35, no. 6, p. 5039–5048, 18 Mar. 2021. DOI 10.1021/acs.energyfuels.1c00320. Available at: <https://pubs.acs.org/doi/10.1021/acs.energyfuels.1c00320>. Accessed on: 9 Aug. 2022.

ZOGLHAMI, A.; PAËS, G. Lignocellulosic Biomass: Understanding Recalcitrance and Predicting Hydrolysis. **Frontiers in Chemistry**, Lausanne, vol. 7, p. 1–11, 2019. DOI 10.3389/fchem.2019.00874/full. Available at: <https://www.frontiersin.org/article/10.3389/fchem.2019.00874>. Accessed on: 6 Feb. 2021.

CHAPTER 4 - Hemicelluloses added to chitosan-based bioplastic improved the tensile strength and allowed to control the material swelling

Abstract

In this study, bioplastics from commercial chitosan and hemicelluloses (extracted from banana plant pseudostem) mixtures in variable proportions were prepared and assessed. Bioplastics were produced by casting method and its characterizations included moisture content, solubility in water, opacity, water absorption (swelling), tensile tests, and thermogravimetric analysis. The formulations containing hemicelluloses exhibited higher moisture content and waster solubility than the bioplastic made only of chitosan; the maximum values of these properties (22.3 and 22.4%) were achieved adding 10 and 5% of hemicelluloses, respectively. The thickness varied from 0.083 to 0.110 mm, however, there were no significative statistical differences. A positive linear correlation ($r = 0.86$) between added hemicelluloses and opacity was found, and the formulation with the highest opacity (1.95 mm^{-1}) was for the formulation with the highest hemicelluloses incorporation. The bioplastics containing hemicelluloses presented lower swelling than one from only chitosan, the formulation with the highest hemicelluloses addition showed the lowest swelling (229.2%), and all formulations were statistically different. A negative linear correlation ($r = 0.95$) between mass proportions of added hemicelluloses and swelling values was observed. The formulation with the highest hemicelluloses addition showed to be statistically different and revealed the highest values of tensile strength (18.7 MPa) and Young's modulus (66.1 MPa). And, according to thermogravimetric analysis, the mass loss of the bioplastics occurred in four stages, the hemicelluloses addition decreased the temperature at maximum mass loss, and the formulation with the lowest addition of hemicelluloses showed a degradation rate higher than the other ones.

Keywords: Biomass, natural polymers, biopolymer, bio-based films

4.1. Introduction

As projected by the Organisation for Economic Co-operation and Development (OECD), the world production of plastics in 2023 will be 489.14 million tons, from which only 0.51% will correspond to bioplastics, and it is estimated a whole production annual growth of 2.9% (OECD, 2023). The traditional plastics, also named synthetic ones, have delivered several benefits to humanity, however, problems involving their origin (non-renewable sources), final disposal, huge generation and accumulation of non-biodegradable wastes, soil and water contamination, low recycling and greenhouse emissions from its lifecycle, have aroused much attention (ABE *et al.*, 2022b; CONVERSIO, 2020; OECD, 2020; PLASTICS EUROPE, 2022). To overcome these concerns, bioplastics have arisen as an alternative to substitute the synthetic ones since coming from biomass and could include better biodegradation (ABE *et al.*, 2022b). Biomass, especially one that comes from wastes, has advantages such as renewability, abundance, availability and avoids competence with either crops or animals destined for human food (ABE *et al.*, 2021; GENT *et al.*, 2017; PONNUSAMY; MANI, 2022b). The chemical structure of biomass mainly consists of natural polymers, which can be extracted/isolated for bioplastic production (MACEDO; ABE; *et al.*, 2022; MACEDO; MARTINS; *et al.*, 2022). To obtain bioplastics, cellulose, hemicelluloses (HC), lignin, pectin, starch, and chitin, are the most common natural polymers found in the biomass (JACOB *et al.*, 2022).

The most common, studied, and developed bioplastics are ones based on starch, however, there are other precursors with strong potential such as the HC and chitosan (Ch) that can also be used (ABE *et al.*, 2022b; AMARAWEEERA *et al.*, 2021; PRIYADARSHI; RHIM, 2020). HC are considered the second most plentiful carbohydrate compound on earth and are made by heteropolysaccharides such as xylan, mannan, xyloglucan, and xylogalactan, which have backbones constituted by xylose, mannose, glucose and galactose, respectively (AHMAD; ZAKARIA, 2019; OLORUNSOLA *et al.*, 2018). These monomers/monosaccharides are considered the building blocks of HC, nevertheless, there are molecules located on side chains that belong to HC chemical structure too, among them are acetyl groups, arabinose, galactose, and methylglucuronic acid (ZHANG; TU; PAICE, 2011b). Nowadays, HC have taken great importance for developing value-added materials but its use has been mainly focused on food packaging applications through obtaining of polymeric films (ABE *et al.*, 2022b; MACEDO; MARTINS; *et al.*, 2022; ZHAO *et al.*, 2020b).

On the other hand, Ch is deemed a natural polymer too despite being industrially obtained by modification from chitin, the most classic sources of chitin are the crustacean shells generated from the seafood industry, and the most commonly employed modification process is the chemical deacetylation (HUQ *et al.*, 2022; LI *et al.*, 2021). Ch is a versatile and linear copolymer composed of glucosamine and N-acetyl-glucosamine molecules, and the higher deacetylation degree the lower content of N-acetyl-glucosamine units (PELLIS; GUEBITZ; NYANHONGO, 2022). Ch-based films have aroused great industrial interest due to its ease of film formation, and antimicrobial, biocompatibility and biodegradability properties that can be harnessed for food packaging and coatings (PRIYADARSHI; RHIM, 2020).

Individual properties of both polysaccharides (HC and Ch) have been well discussed in several applications of bioproduct obtaining. Nonetheless, researches about combining them still are necessary, especially when HC and Ch are used for film formation since together could deliver bioplastics with new properties (PRIYADARSHI; RHIM, 2020; ZAMORA *et al.*, 2022; ZHAO *et al.*, 2020b). Thus, the objective of this study was to produce plasticized films based on commercial Ch and HC extracted from spent liquor generated in alkali organosolv pretreatment of banana plant pseudostem. And, the films were characterized through moisture content, solubility in water, opacity, water absorption, tensile assays (tensile strength, breaking elongation, and Young's modulus), and TGA tests.

4.2. Experimental

4.2.1. Materials

Banana plant pseudostem was collected from the Institute for Research in Bioenergy at São Paulo State University in Rio Claro - São Paulo state, Brazil - (-22° 23' 58.94" N -47° 34' 17.6" W). It was dried by sunlight during 24 h, and a mean particle size smaller than 0.841 mm (mesh 20) was achieved by comminution and sieving (FERNANDES *et al.*, 2020a). After that, the biomass was dried again upon 24 h in an oven at 50 °C. C3646 Chitosan from shrimp shells (source: SLCK9065 and PCode: 1003345975) was purchased from Sigma-Aldrich company and its deacetylation degree was determined by potentiometric (81.2±0.65%) and Fourier Transform Infrared (FTIR) spectroscopy (80.6±0.08%) methods (BRUGNEROTTO *et al.*, 2001;

YUAN *et al.*, 2011). Glycerin p.a., ethanol 92.8%, and Sodium hydroxide p.a. and 99.8% glacial acetic acid p.a., were purchased from local companies, Labsynth®, Safra Industria and Neon Comercial, respectively.

4.2.2. Hemicelluloses (HC) extraction

The biomass was pretreated by organosolv process catalyzed by alkali at 170 °C during 1 h, with 45 min of prior soaking. 3 g of banana plant pseudostem, 1.8 g of NaOH (60% m/m with respect to biomass) and 50 mL of aqueous ethanolic solution 30% (m/m) were put inside a stainless-steel reactor. After organosolv process was completed, the pretreated biomass (solid fraction) was separated by filtration (filter paper) and the filtrate (spent liquor) was collected. HC precipitation was fulfilled in plastic bottles adding ethanol 92.8% to the spent liquor and the HC were isolated by decantation after 24 h; the new two fractions (the solid and liquid ones) were separated on the hand using pipette for suction and the solid new fraction (The HC) was dried at 60 °C for 72 h in an oven.

4.2.3. Preparation of bioplastics

Bioplastic formulations were prepared by the casting method after mixing individual solutions of HC and Ch. The Ch solution was prepared dissolving Ch in 2% (m/m) aqueous solution of acetic acid in continuous magnetic stirring for 4 h at room temperature and 450 rpm. HC were solubilized in hot distilled water using magnetic stirring during 15 min at room temperature and 500 rpm, after solubilization, the solution was heated in microwave oven for 20 s (10 s interval for hand agitation) and filtered using 100 mesh sieve. Both solutions rested by at least 18 h to ensure the elimination of bubbles. HC solution was poured into Ch solution and glycerin was added at a proportion of 20% (m/m) with respect to the total mass of polysaccharides. The final mixing was stirred at room temperature during 30 min and 350 rpm, subsequently, it was undergone to ultrasound for 30 min (to eliminate bubbles), rested at room temperature for 30 min and filtered through 100 mesh sieve. The filmogenic solution was poured in acrylic plate maintaining 0.705 g/cm² as condition for all formulations and drying at 37 °C during 2 days. Table 4-1 shows the mass proportions used to formulate the bioplastics.

Table 4-1 - Mass proportions of bioplastic formulations

ID	%Ch	%HC
Ch100HC0	100	0
Ch95HC5	95	5
Ch90HC10	90	10
Ch85HC15	85	15
Ch80HC20	80	20
Ch75HC25	75	25

%Ch: Mass percentage of chitosan;%HC: Mass percentage of hemicelluloses

Source: By author

4.2.4. Characterization of bioplastics

4.2.4.1. Moisture content and solubility in water

From every bioplastic formulation were randomly cut 1.0 x 5.0 cm strips (in triplicate) and immediately weighed, afterwards the samples were undergone to drying at 105 °C for 24 h in an oven and again weighed. The moisture content was reported as the percentage of the mass difference between the reported weights. Every dried strip was put inside a glass beaker containing 50 mL of distilled water, and then was carried out shaking at 110 rpm at 25 °C during 24 h. When this period finished, the water was removed, and the strip was again oven-dried at 105 °C for 24 h and weighed. The solubilization percentage in water was calculated by the mass difference of the strip previous to water immersion (dried strip) and after immersion plus drying (MEDINA-JARAMILLO *et al.*, 2017a).

4.2.4.2. Thickness and opacity

Strips of 0.9 x 3.0 cm (in triplicate) were randomly cut from each bioplastic formulation. The thickness was measured at three different points of every strip (center and ends) by a digital micrometer with 0.001 mm accuracy. Each strip (the same used for thickness determination) was put at an internal wall of a glass cuvette for absorbance reading by spectrophotometer at 600 nm. The empty cuvette was

configured as zero and the opacity (mm^{-1}) was calculated dividing absorbance by thickness (ZHAO; WANG; LIU, 2022a).

4.2.4.3. Water absorption (swelling)

Each bioplastic sample (in triplicate) of 0.9 x 3.0 cm was oven-dried at 50 °C during 24 h and weighed (m_1). The dried sample was immersed in glass beaker containing 25 mL distilled water at 23 °C for 24 h. Following immersion, the water excess at the surface of the samples was removed with filter paper as absorbent and then the samples were again weighed (m_2). After this procedure, the wet samples were oven-dried at 50 °C during 24 h and reweighed (m_3). The mass percentage of water absorption was calculated as the sum of water-soluble matter lost (mass percentage difference between m_1 and m_3) plus the increase in weight during immersion (mass percentage difference between m_1 and m_2) (ASTM, 2022; ISO, 2021).

4.2.4.4. Tensile assays

Before the tensile assay, bioplastics were conditioned as follows, every whole film of every formulation was taken off from acrylic plate and put inside a desiccator at relative humidity 56 - 57%, and this system (desiccator and film) was placed inside an incubator at 23 ± 2 °C during 48 h. After this stage, the tensile tests were performed on conditioned films; strips of 1.0 x 10 cm (10 replicates) were cut from each formulation and the thickness (measured in 10 points of the strip) was determined using a digital micrometer with 0.001 mm accuracy (ASTM, 2018). The equipment model TXT-ENG-5K with a displacement speed of 5 mm/min and 5 kg of load cell was used for tests. The free length of each strip during tensile assays was 8.2 cm (0.9 cm stayed trapped in each grip of the equipment). Tensile strength, breaking elongation, and Young's modulus were calculated according to the standard test method ASTM D882-18.

4.2.4.5. Thermogravimetric analysis (TGA)

The bioplastics Ch100HC0, Ch95HC5, and Ch75HC25 were subjected to analysis of thermal stability using a TGA Q500 model equipment from the TA Instrument brand in the temperature range of 25 to 800 °C, with a heating rate of 10

°C/min in a nitrogen atmosphere at a flow rate of 50 mL/min. This resulted in the obtaining of thermograms for the samples analyzed.

4.2.5. Statistical analysis

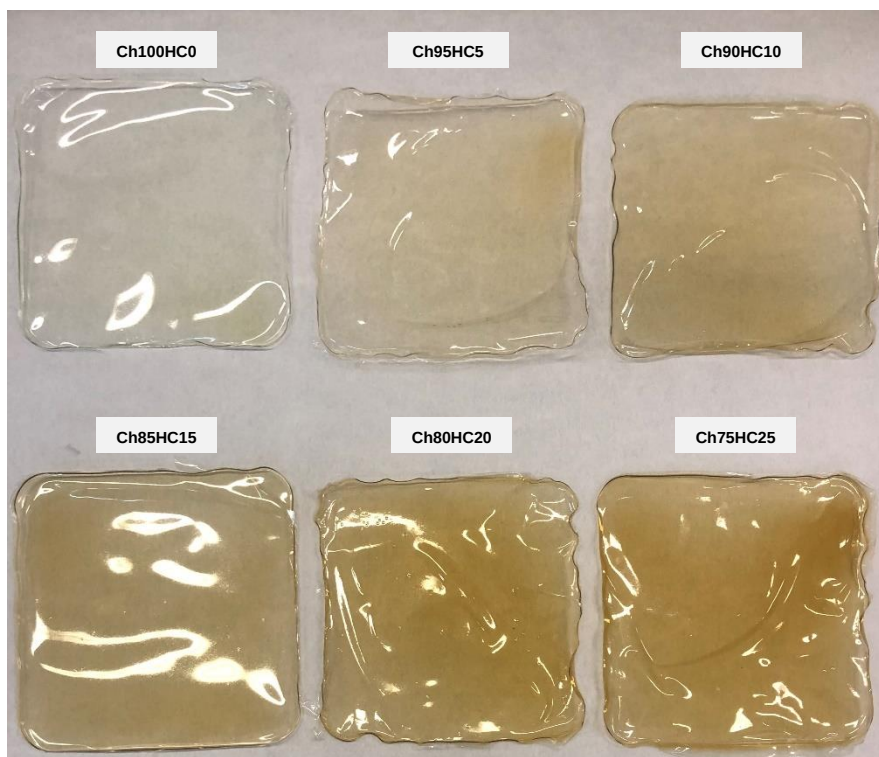
IBM SPSS software v.28.0 was employed for the statistical study, it consisted of the Tukey HSD test to check significant differences among the bioplastic formulations. When p-value was inferior to 0.05 ($p < 0.05$), differences were considered statistically significant.

4.3. Results and discussion

4.3.1. Preparation of bioplastics

The aspect of the six preparations of bioplastic can be observed in Figure 4.1. Bioplastics without cracks were obtained from each formulation described in the Table 4-1, and all of them presented homogeneous structure. However, the increase of HC content led to an increase in the color presence. Besides that, all formulations used for the bioplastic production let to obtain thin and flexible films, and none of them showed fragile and brittle structure, which helped to take off from the acrylic plates. Despite favorable visual characteristics cited earlier, each formulation (excepting Ch100HC0) exhibited at least one well-defined region with non-equal distribution/dispersion probably caused by accumulation of HC. This behavior could be attributed to mutual affinity between Ch and HC was not complete, mixing conditions (room temperature and 300 rpm for 30 min) to obtain the filmogenic solutions were mild, or solvents of the filmogenic solution did not have a uniform evaporation at drying stage. Although the Ch and HC compatibility for bioplastic production has been established as good and suitable, studies of compatibilizers could be included to analyze improving of dispersion in films (KUSUMASTUTI *et al.*, 2020; ZHANG *et al.*, 2020). Finally, the formulations with the highest HC content (Ch80HC20 and Ch75HC25) were the most wrinkled, it could be caused by the presence of large amounts of HC located in specific regions (darker ones) of the bioplastics that originated the formation of clusters, which could be a result of the bonding increasing among HC chains (ABE *et al.*, 2022b).

Figure 4.1 - Bioplastics based on Ch and HC using different mass proportions listed in Table 4-1



Source: By author

4.3.2. Characterization of bioplastics

Table 4-2 summarized the characterization performed on every formulation of bioplastic in terms of moisture content (%), solubility in water (%), thickness (mm), opacity (mm^{-1}), water absorption (%), tensile strength (MPa), elongation at break (%) and Young's modulus (MPa).

Table 4-2 - Moisture, solubility in water, thickness, opacity, water absorption, and tensile properties of bioplastics

ID	Moisture (%)	Solubility in water (%)	Thickness (mm)	Opacity (mm^{-1})	Water absorption (%)	Tensile strength (MPa)	Breaking elongation (%)	Young's modulus (MPa)
Ch100HC0	14.6 $\pm$ 0.78 ^a	18.7 $\pm$ 0.70 ^{ac}	0.084 $\pm$ 0.0086 ^a	0.73 $\pm$ 0.073 ^a	608.8 $\pm$ 9.72 ^a	14.5 $\pm$ 1.57 ^{abcd}	24.9 $\pm$ 1.28 ^{acf}	58.3 $\pm$ 1.71 ^{acf}
Ch95HC5	17.7 $\pm$ 0.97 ^b	22.4 $\pm$ 0.63 ^b	0.082 $\pm$ 0.0056 ^a	0.74 $\pm$ 0.054 ^a	533.3 $\pm$ 5.40 ^b	11.4 $\pm$ 1.17 ^{abcde}	37.1 $\pm$ 2.00 ^{bde}	30.8 $\pm$ 1.79 ^{bde}
Ch90HC10	22.3 $\pm$ 0.87 ^c	21.3 $\pm$ 1.16 ^b	0.110 $\pm$ 0.0300 ^a	0.94 $\pm$ 0.159 ^a	378.2 $\pm$ 8.20 ^c	12.9 $\pm$ 1.87 ^{abcde}	25.4 $\pm$ 1.50 ^{ac}	58.0 $\pm$ 1.87 ^{abc}
Ch85HC15	17.5 $\pm$ 0.72 ^b	21.6 $\pm$ 1.16 ^b	0.094 $\pm$ 0.0044 ^a	1.15 $\pm$ 0.048 ^a	328.3 $\pm$ 4.83 ^d	12.9 $\pm$ 1.10 ^{abcde}	35.5 $\pm$ 1.34 ^{cd}	36.4 $\pm$ 1.42 ^{cde}
Ch80HC20	17.5 $\pm$ 1.19 ^b	20.4 $\pm$ 0.99 ^{abc}	0.106 $\pm$ 0.0252 ^a	1.28 $\pm$ 0.227 ^a	288.9 $\pm$ 8.78 ^e	10.8 $\pm$ 1.58 ^{bce}	36.4 $\pm$ 1.04 ^{ce}	29.6 $\pm$ 2.15 ^{cde}
Ch75HC25	19.0 $\pm$ 0.56 ^b	21.2 $\pm$ 0.97 ^{abc}	0.083 $\pm$ 0.0089 ^a	1.95 $\pm$ 0.254 ^b	229.2 $\pm$ 9.94 ^f	18.7 $\pm$ 1.40 ^f	28.4 $\pm$ 1.85 ^{af}	66.1 $\pm$ 2.19 ^{acf}

*Significative statistical differences (p-value lesser than 0.05) were symbolized by distinct letters in the same column.

Source: By author

4.3.2.1. Moisture content and solubility in water

The formulations Ch95HC5 – ChHC6 (those with HC incorporation) exhibited higher moisture contents than Ch100HC0 (bioplastic made only of Ch), being the Ch90HC10 (Ch 90% and HC 10%) the bioplastic with the highest one (22.3%) and statistically different to other formulations. This finding can be chemically explained; HC are hydrophilic mainly due to the presence in its structure of hydroxyl groups, which for having a distribution of negative charge can attract water molecules (hydrogen bonding). Aside from -OH groups, HC also could contain other functional groups such as uronic acids that contribute to its hydrophilic nature. An additional important factor that contributes to hydrophilicity is that HC molecular structure with pending groups is highly hydrated, which increases the overall surface area that is available for hydrogen bonding with water molecules (PATEL; PARSANIA, 2018). This hydrophilicity behavior is natural since HC help in water regulation and nutrient transport in live plants, as well as maintaining the water proper balance of the cell walls (FRY, 2003). Conversely, Ch is not attracted to water and tries to repel it mainly due to its cationic nature in environments with acetic acid aqueous solutions, which cause protonation of amino (-NH₂) groups (LUO; WANG, 2014). Another factor that could contribute to water repelling is that Ch has a linear (unbranched) and slightly hydrated structure (KUMARI; KISHOR, 2020), which would help to reduce the presence of hydrophilic sites. In addition, the water repelling of Ch could be affected by the degree to which acetyl groups are removed from chitin during the deacetylation process; as more acetyl groups are removed, Ch water repelling probably would increase because of the growth of positive charges attributed to protonation reactions. On the other hand, the moisture content of bioplastics could be programmed or fixed through studies of time, air humidity, and temperature variations at the drying stage (GEANKOPLIS, 1993). All moisture contents found herein were in concordance with the Agusman *et al.* (2022) research, who reported values between 12.5 and 24.0% for bioplastics based on Ch with high deacetylation degree (>89%), however, incorporating agar in different proportions (26 – 65%). And, Merino *et al.* (2023) found moisture values in the range

6 – 10% in bioplastics dried at room temperature based on Ch (deacetylation degree: 75 – 85%) in combination with hydrolyzed orange peel (pectin high content).

Regard to the solubility of the formulations in water, the results had the same trend than the ones for moisture content; those with HC integration (Ch95HC5 – ChHC6) revealed higher values of solubility in water than the bioplastic from only Ch (Ch100HC0), and the Ch95HC5 (Ch 95% and HC 5%) formulation showed the maximum solubility (22.4%) and was statistically different to Ch100HC0. As was previously mentioned, the chemical structure of HC has the presence of hydroxyl groups, which are responsible for causing solubility in water through forming of hydrogen bonding between -OH groups and water molecules (PATEL; PARSANIA, 2018). For this reason, the solubility increased in those bioplastics with HC incorporation. The highest solubility achieved herein was superior to one reported by Carpinelli *et al.* (2022), who found a value of 20.3% for a bioplastic plasticized with an identical amount of glycerin (20%) and made with the same HC (xylan from sugarcane) proportion (5%) but instead of using Ch was employed soluble starch. Manabu *et. al.* (2022) reported a solubility of 17.5% for a bioplastic formulation that was plasticized with an identical proportion of glycerin (20%) and had the lowest HC (xylan from sugarcane) content (10%) but was performed based on soluble starch instead of Ch. This result was inferior respect to the reported one herein. Conversely, high solubility values of bioplastics from natural polymers have been reported. Merino *et al.* (2023) presented in their study solubilities, between 45 and 72%, of different bioplastic formulations made of Ch (deacetylation degree: 75 – 85%) plasticized with polyglycerol-3 (proportion 30%) and hydrolyzed orange peel (pectin high content) using the casting method. And, Rodsamran and Sothornvit (2019) produced bioplastics based on lime peel pectin and using a 20% ratio of glycerin, they found solubilities of 62.4% and 44.0% for the formulations with and without lime peel extract, respectively. To manufacture bioplastics come from natural polymers with high values of solubility in water could limit the performance of those mainly in applications for food packaging (RODSAMRAN; SOTHORNVIT, 2019). When making comparisons among different studies of bioplastics, it is important to take into account where these come from (the sources), extraction processes of natural polymers, production methods and conditions, compositions (proportions), and thickness, as these factors can have an impact on various physicochemical properties such as its solubility.

4.3.2.2. Thickness and opacity

Table 4-2 shows that the average thickness measurements for each bioplastic formulation varied from 0.083 to 0.110 mm, however, there were no statistically significant differences, mainly because all formulations were prepared with the same total mass of polysaccharides (see Section 4.2.3). The thickness of bioplastics produced using the casting method depends on raw materials (such as natural polymers) used in film obtaining, on the concentration of the individual solutions employed to solubilize each natural polymer and on the amount of filmogenic solution poured onto a plate. Generally, the greater the amount of filmogenic solution and the concentrations of individual solutions used, the greater the resulting thickness (BATISTA *et al.*, 2019; GALDEANO *et al.*, 2013; RODRIGUES *et al.*, 2021). Therefore, it was not possible to find previous researches with the same or similar conditions of film processing, so, few works are mentioned for comparison. An alike thickness (0.120 mm) was reported by Lavorgna *et al.* (2010), who from Ch of deacetylation degree >75% (as base) mixed with clay nanocomposites manufactured films plasticized with glycerin in proportion 25% (respect to the Ch mass) through casting method and preparation of individual solutions but including room and vacuum conditions at drying stage. Merino *et al.* (2023) designed films using the individual solutions and casting methods based on Ch (deacetylation degree: 75 – 85%) in combination with hydrolyzed orange peel (pectin high content), with thickness of 0.150 mm. And, Zhang *et al.* (2020) found thickness measurements between 0.063 and 0.069 mm with no significant difference ($p > 0.05$) in films made of Ch (deacetylation degree: > 95%), HC extracted from *Eucalyptus* wood by an alkali route and including TiO₂ nanoparticles. However, this research used plasticizer (glycerin) proportions of 20 – 40%, drying stage at room conditions and HC were added to the end of process through soaking of dried films. To measure the polymer thickness is important since the mass transfer and tensile properties depend on this parameter (CANEVAROLO JR., 2010; GEANKOPLIS, 1993). The final thickness can also be influenced by other factors, such as viscosity, surface tension and drying conditions of the filmogenic solution. Probably, higher viscosity and surface tension can result in thicker films, and if the solvent evaporation rate during the drying stage is too rapid, the resulting film may be thinner than desired (FRIED, 2014; PROVDER; URBAN, 2001).

On the other hand, opacity values showed an increasing trend as HC amounts were gradually incremented, which could be confirmed through the positive linear correlation ($r = 0.86$) showed in Figure S4.1 at supplementary information. Among the produced formulations, the Ch75HC25 (75% of Ch and 25% of HC) had the highest opacity value (1.95 mm^{-1}), which was statistically different from the other ones. As informed by Xu *et al.* (2019a), HC incorporation into Ch films produced a reduction in transparency measured at wavelengths of 500 – 800 nm. This research consisted of film processing through the casting method of mixed individual solutions (Ch and HC in the same proportions, 1:1) but including low amounts of cellulose nanofiber (5%) and xylooligosaccharides (1.79 – 7.18%). And, Moalla *et al.* (2021) reported opacities of 0.47 and 1.63 mm^{-1} for films made of only Ch (deacetylation degree of 75 – 85%) and Ch plus *Artemisia campestris* essential oil, respectively, using the casting method and drying in an oven at 35 °C during 48 days. Shady bio-based packaging can provide protection to a specific product from harmful effects caused by light exposure. However, it could also have some impacts on the product's sales since the opacity of packaging material may appear different to consumers. Furthermore, when it comes to medical applications, an opaque material can make it difficult to observe the healing process (AHMAD *et al.*, 2020).

4.3.2.3. Water absorption (swelling)

The films at swollen state maintaining their body intact and could be manipulated without breaking apart. The water immersion caused the films to become more flexible, which could be a desirable characteristic for certain applications. However, the water absorption could contribute to notable alterations in the tensile properties. Each bioplastic showed significant statistical difference, and the formulations containing HC (Ch95HC5 – ChHC6) exhibited lesser water absorption than the bioplastic made only of Ch (Ch100HC0). The formulation with the highest HC proportion (Ch75HC25: Ch 75% and HC 25%) was the specimen with the lowest swelling (229.2%). Swelling property showed a decreasing trend as HC amounts were gradually incremented, which could be confirmed through the negative linear correlation ($r = 0.95$) showed in Figure S4.2 (supplementary information). Water absorption is a mass transport phenomenon that occurs within the structure of a solid material due to water diffusion through spaces among solid molecules. During this

diffusion, water molecules randomly move (but always from regions of water with high concentration to regions of low concentration) and locate (when equilibrium concentration is achieved) within the solid structure causing separation among solid molecules that led to the solid expansion (swelling) (GEANKOPLIS, 1993). Based on the above, there is no doubt that HC incorporation into Ch films affected the water absorption capacity of all bioplastic formulations. This because HC molecules possibly filled some spaces among Ch molecules and formed bonding, which led to decrease the water diffusion and, consequently, to a reduction in the swelling percentage of bioplastics with HC addition. Besides that, the drop in the water absorption degree of the bioplastics that incorporated HC, compared to the Ch100HC0 formulation, could be caused by the proportional substitution of the Ch amounts by HC. The swelling property of bioplastics produced using the casting process depends on the method used to calculate it and could be affected by the same factors than thickness (mentioned in the preceding section). Thus, it was not possible to find previous studies with the same or similar conditions of film processing, so, few works using chitosan as the base with the incorporation of some additives, and the immersion method for 24 h and 23 °C were considered for comparison.

Moalla *et al.* (2021) and Janik *et al.* (2021) reported that the incorporation of additives to bioplastics based on Ch (as the main component) and plasticized with glycerin resulted in a decrease in the water absorption degree. The first research indicated a reduction of 88.3 to 53.9% in the swelling index when *Artemisia campestris* aqueous extract was added (in unknown proportion) to Ch solution, and the bioplastic was confectioned using the casting method and drying at 35 °C during 24 h. The second study developed bioplastics made of Ch, starch, and PVA, in which lignin was considered as the additive, here it was informed a decreasing from 200 to 90% in the bioplastic with the additive (at low amount) but the processing route was through hot-pressing. The swelling degree in bioplastics is closely associated with the crosslinking degree that has taken place within the polymeric network, and therefore can significantly impact the water resistance of bioplastics. Generally, bioplastics with low swelling degrees exhibit high water resistance due to the decreased ability of water molecules to diffuse and disrupt the polymeric matrix (NGUYEN *et al.*, 2020). Additionally, a low swelling index could be related to low degradation rates due to oxidation processes could be limited, however, it is necessary to include detailed studies to determine the real degradation (ABE; BRANCIFORTI; BRIENZO, 2021b).

4.3.2.4. Tensile strength

There was a variation from 10.8 to 18.7 MPa without trend with the proportional increase of HC in the bioplastics, however, the HC addition of 25% improved the tensile strength value. The formulation with the highest content of added HC (Ch75HC25) revealed significant statistical difference from the other ones. It can be established that the bioplastic Ch75HC25 was stronger (more resistant to break) than one made only of Ch. This outcome could have been caused by the intermolecular interactions, such as hydrogen bonding, that possibly took place between HC and Ch, which contributed to obtain a strong film with better tensile strength than that made only of Ch (KAMDEM *et al.*, 2019; LIU *et al.*, 2016; ZHANG *et al.*, 2020). Similar values of tensile strength were reported by the Solier *et al.* (2022) study, where bioplastics obtained through the casting method and made of commercial Ch and xylan (extracted from sugarcane bagasse) with 30:70 and 20:80 ratios, had alike values (9.5 and 22.0 MPa, respectively) to those found in the present research. Nevertheless, it is important to mention that the Ch:xylan ratio used by Solier *et al.* (2022) was different from the ratios employed herein (Ch was the main component). Likewise, in a previous study published by Zhang *et al.* (2020), it was found that a bioplastic made with commercial Ch and HC (extracted from *Eucalyptus* wood), plasticized with glycerin (20%) and prepared using the casting method, had a tensile strength of 13.7 MPa. Nonetheless, the reported value differed in that it included TiO₂ nanoparticles (20%), used HC low proportion (1%), and the HC were added at the end of the film processing by soaking of the dried Ch films.

Although the tensile strengths found in the present study are similar to those mentioned in the cited studies, there are other works that have reported better results in terms of resistance, albeit with low ductility. Xu *et al.* (2019) produced a bioplastic with a tensile strength of 38.6 MPa and elongation at break of 10.1% from Ch (commercial) and HC (extracted from *Eucalyptus grandis* holocellulose) with mass ratio of 1:1 but including 5% (based on the dry weight of both polysaccharides) of cellulose nanofibers. The bioplastic was developed by casting method (including vacuum drying) and using glycerin as plasticizer at a proportion of 20%. The same authors but in a different publication, manufactured a bioplastic with tensile strength and an elongation at break of 58.5 MPa and 5.5%, respectively, based on the same

proportions of polysaccharides and film processing used in the previous work but without including of any type of plasticizer (XU; XIA; YUAN; *et al.*, 2019). In the Kamdem *et al.* (2019) study, tensile strengths of approximately 88.0, 95.0 and 102.0% were reported for formulations made from Ch and xylan with mass ratios of 90:10, 80:20 and 70:30, respectively. These bioplastics were produced using the casting method without addition of any plasticizer, which led to obtain films with low ductility (the highest elongation break reported was 4.5%). According to the earlier discussion, there was no doubt that the addition of any type of plasticizer, such as glycerin, during the bioplastic processing stage plays a key role in determining the resistance and ductility of the resulting material. Generally, the tensile strength increases when any kind of plasticizer is not used and decreases when it is added. On the contrary, the plasticizer inclusion in the formulation of a specific material can increase its ductility (CALLISTER; RETHWISCH, 2018). There are other factors that could affect the resistance properties of a finished bioplastic based on Ch and HC, among them are the molecular mass of both polysaccharides, the film preparation method, the used mass ratios (or proportions) of the compounds, and the deacetylation degree of the Ch. High values of tensile strength, such as those found by Xu *et al.* (2019) and Kamdem *et al.* (2019), mean that the materials had high resistance before their breaking. However, those strong bioplastics showed to have low withstanding to deformation, which implies little ductility.

4.3.2.5. Elongation at break

Considering the elongation at break results, these varied from 24.9 to 37.1%, with the lowest value corresponding to the formulation Ch100HC0 (that one made only of Ch), and the highest one corresponding to the formulation Ch95HC5 (that with the lowest content of HC: 5%). The formulations with HC (Ch95HC5 - Ch75HC25) demonstrated to have higher elongation values than Ch100HC0. The last one could be associated with the degree to which the structure of films was organized in regular and repeated patterns of molecules (crystallinity degree) (SCHNELL *et al.*, 2017). A highly ordered structure with molecules arranged in regular lattices would mean high crystallinity of the films, and a low crystallinity would be related to a more disordered structure with molecules arranged in less regular patterns (ABDAN *et al.*, 2020). Thereby, the bioplastics containing HC likely contributed to form bioplastics with lower

crystallinity than the formulation made only of Ch. Probably, the HC pending groups/branched structures collaborated to decrease the film crystallinity. As consequence, the resulting bioplastics with HC incorporation exhibited increasing in elongation at break (SCHNELL *et al.*, 2017; ZAMORA ZAMORA *et al.*, 2022). Zhang *et al.* (2020) reported an alike range of elongation at break values (23.9 – 39.6%) to that found herein (25.4 – 37.1%) for bioplastics plasticized with the same glycerin proportion (20%), prepared through casting method (drying at room temperature), and composed of commercial Ch and HC extracted from *Eucalyptus* wood. However, the cited research also included TiO₂ nanoparticles (20%) and the HC addition was performed by soaking of Ch dried films. Similarly, Solier *et al.* (2022) found 21.5 and 25.6% for films produced through slow and fast solvent evaporation, respectively, developed by casting method in silicone molds, and composed of commercial Ch and xylan extracted from sugarcane bagasse. Nevertheless, the mentioned study used a xylan:Ch ratio of 70:30, which was very different from the ones employed herein where Ch was the predominant component.

Conversely, Xu *et al.* (2019) produced a bioplastic from Ch (commercial), HC (extracted from *Eucalyptus grandis* holocellulose) and cellulose nanofibers in a mass ratio of 47.5:47.5:5, respectively, which achieved an elongation at break of 10.1%. The bioplastic was obtained by the casting method (including vacuum drying) and using glycerin as plasticizer at same proportion (20%). Kamdem *et al.* (2019) reported the values of 4.5, 4.3 and 3.7% for preparations based on Ch and xylan with mass ratios of 90:10, 80:20 and 70:30, respectively, which were produced through the casting method involving room conditions during the drying, however, the processing did not use any plasticizer. Plasticizers are commonly added to bioplastics aiming to improve their ductility, thus low values of elongation at break can be obtained when plasticizer are not used, as showed by the Kamdem *et al.* (2019) research (CANEVAROLO JR., 2010; PATEL; PARSANIA, 2018). Low values of elongation at break, such as those mentioned in the last 2 cited studies, mean that a material undergoes little deformation before it breaks. This could indicate that the material is brittle and prone to fracture, rather than being ductile and able to withstand deformation without breaking (CALLISTER; RETHWISCH, 2018). Thus, the elongations at break achieved in the present research can be considered as intermediate values since were higher than the ones found by Xu *et al.* (2019) and Kamdem *et al.* (2019), and lower than one found

by Sun *et al.* (2019), who reported 104.1% as the highest value for a film manufactured from Ch and starch (ratio: 1:0.6).

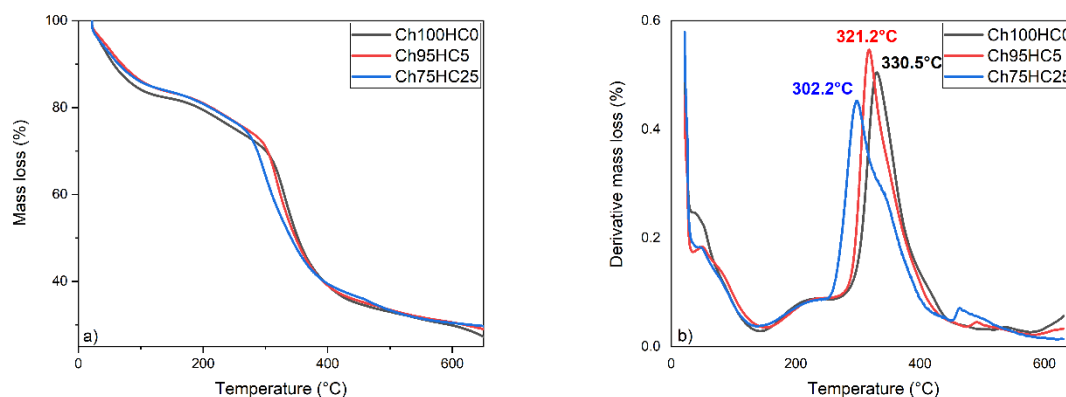
4.3.2.6. Young's modulus

Higher values of tensile strength (or stress at break), elongation at break (or strain at break) and young's modulus (or elastic modulus) indicate greater resistance to lengthwise stress, ductility and stiffness of a material, respectively (CALLISTER; RETHWISCH, 2018). The results of the Young's moduli varied between 30.8 and 66.1 MPa, and the formulation containing the highest amount of added HC (Ch75HC25) showed the maximum value and statistical significant difference compared to the other ones. Thereby, it can be affirmed that the formulation Ch75HC25 was more rigid than one made only of Ch (Ch100HC0). This observed result possibly was produced by the intermolecular interactions, such as hydrogen bonding, that probably happened between HC and Ch, which contributed to obtain a more tightly packed (and therefore more rigid) chemical structure in the film Ch75HC25 compared to Ch100HC0 (KAMDEM *et al.*, 2019). The range of the elastic moduli found herein seemed to be unique for the bioplastics produced under the processing conditions described in the methodology section, therefore, few studies are mentioned to compare. Jakubowska *et al.* (2020) reported a similar value (58.4 MPa) for a film made only of Ch, plasticized with deep eutectic solvent based on choline chloride and malonic acid using 20% (m/m) as proportion, however, the film had a thickness of 0.229 mm (almost tripling the value achieved for Ch75HC25) and its elongation at break value (19.2%) was around 1.5 times lesser than the found one for Ch75HC25. Zaman *et al.* (2018) reported a Young's modulus of 0.4 MPa for a bioplastic consisting of Ch and *Garcinia atroviridis* extract (2%), manufactured by the casting method and plasticized with glycerin (0.5% v/v). The resulting film had a similar thickness (0.081 mm) to Ch75HC25 but with lower values of elongation at break (17.1%) and tensile strength (6.6 MPa). Conversely, Kamdem *et al.* (2019) found high elastic moduli; 3.0, 3.8 and 4.0 GPa for formulations made from Ch and xylan with mass ratios of 90:10, 80:20 and 70:30, respectively. These bioplastics were produced using the casting method but excluding plasticizer use, which led to obtain films with high stiffness and very poor ductility (the highest elongation break reported was 4.5%).

4.3.2.7. Thermogravimetric analysis

The stability of bioplastics during thermal changes is one of the properties that has aroused great interest for packaging applications, and its importance is extended to the bioplastic uses such as storage (at low or room temperatures) or protection/preservation of hot food (JAKUBOWSKA *et al.*, 2020a). Thus, the TGA was performed aiming to assess the effect of HC addition on the thermal behavior of Ch films. Mass loss thermograms of the formulations Ch100HC0 (composed only by Ch), Ch95HC5 (with the lowest content of HC) and Ch75HC25 (with the maximum content of HC) are depicted in the Figure 4.2a. Four intervals of mass loss were observed in the Ch and HC films. The temperature range of 25 to 110 °C represented the first stage, here the water and residual acetic acid underwent evaporation, and the mass average loss was around 16% (COSTA *et al.*, 2021; MOALLA *et al.*, 2021). A mass average loss of approximately 10% occurred between 105 to 270 °C (the second stage), which is attributed to the HC softening, and degradation of glycerol and of low molecular weight Ch (KAMDEM *et al.*, 2019). When HC were incorporated into the bioplastic formulations, the mass loss in the first and second stage decreased as compared to the film Ch100HC0. The third stage occurred between 270 and 410 °C and very probably corresponded to the initial pyrolysis (decomposition and depolymerization) of both polysaccharides (COSTA *et al.*, 2021; KAMDEM *et al.*, 2019). In this zone, the formulations with HC addition showed increasing in the mass loss compared to film manufactured only with Ch, and the mass average loss achieved its highest value (33%). Finally, the temperature range of 410 to 650 °C corresponded to the fourth stage, where Ch of high molecular weight possibly completed its degradation (KAMDEM *et al.*, 2019; YANG *et al.*, 2007). In the final region, the formulations, irrespective of the presence or not of HC, indicated a mass average loss of roughly 10%, and the films containing HC did not exhibit increasing or decreasing mass loss with respect to Ch100HC0. Kamdem *et al.* (2019) found a similar path of mass loss by thermal analysis for films based on Ch and xylan, however, the formulations (processed without plasticizer) included other kinds of additives such as carvacrol and Span 80. The first, second, third, and fourth stages of mass loss corresponded to temperature ranges of 50 – 110 °C, 110 – 240 °C, 220 – 410 °C and 410 – 650 °C, respectively.

Figure 4.2 - Thermogravimetric (TG) a) and derivative thermogravimetric (DTG) b) curves of the bioplastics Ch100HC0, Ch95HC5, and Ch75HC25



Source: By author

On the other hand, based on the information presented in the Figure 4.2b, it was evident that the HC inclusion in the film formulations caused decreasing in the temperature where happened the maximum mass loss (T_{MAX}); 330.5, 321.2 and 302.2°C were the temperatures for the formulations Ch100HC0, Ch95HC5 and Ch75HC25, respectively. However, the degradation rate (%/°C) at T_{MAX} increased in Ch95HC5 and decreased in Ch75HC25 with respect to the Ch100HC0, which indicates that the formulation with the lowest content of HC degraded at a higher rate than the other ones. These same behaviors were revealed by Zhang *et al.* (2020), who reported decreasing of T_{MAX} from 307.5 to 302.2 °C for films made only of Ch and Ch plus HC with a mass ratio of 95:5 (the same proportion used for the formulation Ch95HC5), respectively. Besides that, this same research exposed that the bioplastic 95:5 (Ch:HC) increased the degradation rate (%/°C) at T_{MAX} compared to the formulation produced only from Ch.

4.4. Conclusions

Thin, and flexible bioplastics with homogeneous structures were produced, and their color variations were caused by the proportional incorporation of HC. Formulations with the highest HC contents (Ch80HC20 and Ch75HC25) revealed accumulation regions of HC, non-uniform color/distribution and the most wrinkled aspect. The formulations containing HC exhibited higher moisture content and

solubility in water than that one made only of Ch. Regarding opacity, the values increased as the HC amount was gradually increased, (behavior represented by the positive linear correlation), being the bioplastic with the highest HC content the opaqueness. According to water solubility, all formulations were statistically different, the bioplastics containing HC revealed inferior values to that one from only Ch, and the formulation with the highest HC content exhibited the lowest swelling percentage. The swelling index showed a negative linear correlation with the mass proportion of HC added to bioplastics. About tensile properties, the formulation with the highest HC content showed to be statistically different from the other ones and revealed the highest values of tensile strength and Young's modulus. And, regarding TGA, the mass loss of the bioplastics occurred in four stages, the HC addition decreased the temperature at maximum point of mass loss, and the formulation with the lowest HC content showed a degradation rate higher than one made only of Ch. Based on the opacity and swelling results, the bioplastics produced in this research have great potential since both properties exhibited changes depending on mass proportion of added HC, which following trends represented by the linear models. Thus, the bioplastics could be used in packaging applications, especially in those (food or medicines) where will be necessary to control light exposition and water diffusion.

Statements and Declarations

Competing Interests: The authors declare no competing interests

Acknowledgments

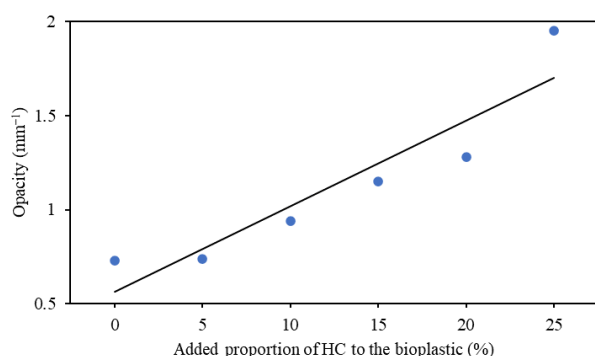
The authors are grateful to the Brazilian Improvement Coordination of Higher Education Personnel (CAPES), Brazilian National Council for Scientific and Technological Development (process number 303039/2021-2), and São Paulo Research Foundation (FAPESP, process number 2017/22401-8; 2021/10839-4) for its financial support.

4.5. Supplementary Information

List of figures

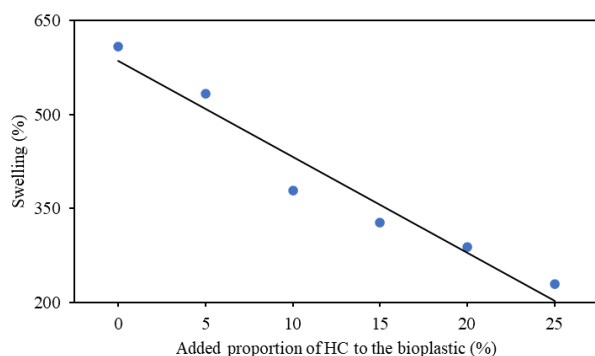
Figure S4.1	Linear correlation between added proportions of HC and opacity of the formulations
Figure S4.2	Linear correlation between added proportions of HC and swelling of the formulations

Figure S4.1 - Linear correlation between added proportions of HC and opacity of the formulations



Source: By author

Figure S4.2 - Linear correlation between added proportions of HC and swelling of the formulations



Source: By author

References

ABDAN, K. B.; YONG, S. C.; CHIANG, E. C. W.; TALIB, R. A.; HUI, T. C.; HAO, L. C. Chapter 6 - Barrier properties, antimicrobial and antifungal activities of chitin and chitosan-based IPNs, gels, blends, composites, and nanocomposites. *In*: GOPI, S.; THOMAS, S.; PIUS, A. B. T.-H. of C. and C. (eds.). **Handbook of Chitin and Chitosan V2**. Amsterdam: Elsevier, 2020. p. 175–227. DOI 10.1016/B978-0-12-817968-0.00006-8. Available at: <https://www.sciencedirect.com/science/article/pii/B9780128179680000068>.
 ABE, Mateus M; BRANCIFORTI, M. C.; BRIENZO, M. Biodegradation of Hemicellulose-Cellulose-Starch-Based Bioplastics and

Microbial Polyesters. **Recycling**, Basel, vol. 6, no. 1, p. 1–21, 2021. DOI 10.3390/recycling6010022. Available at: <https://www.mdpi.com/2313-4321/6/1/22>. Accessed on: 14 Feb. 2023.

ABE, Mateus M.; MARTINS, J. R.; SANVEZZO, P. B.; MACEDO, J. V.; BRANCIFORTI, M. C.; HALLEY, P.; BOTARO, V. R.; BRIENZO, M. Advantages and Disadvantages of Bioplastics Production from Starch and Lignocellulosic Components. **Polymers**, Basel, vol. 13, no. 15, 2021. DOI 10.3390/polym13152484. Available at: <https://www.mdpi.com/2073-4360/13/15/2484>. Accessed on: 27 Dec. 2023.

ABE, Mateus Manabu; BRANCIFORTI, M. C.; NALLIN MONTAGNOLLI, R.; MARIN MORALES, M. A.; JACOBUS, A. P.; BRIENZO, M. Production and assessment of the biodegradation and ecotoxicity of xylan- and starch-based bioplastics. **Chemosphere**, Amsterdam, vol. 287, no. Part 3, p. 1–10, 2022. DOI 10.1016/j.chemosphere.2021.132290. Available at: <https://www.sciencedirect.com/science/article/pii/S0045653521027624>.

AGUSMAN; FRANSISKA, D.; MURDINAH; WAHYUNI, T.; IRIANTO, H. E.; PRIAMBUDI, P.; FATEHA; ABDULLAH, A. H. D.; NISSA, R. D.; FIRDIANA, B.; NURHAYATI. Physical properties of bioplastic agar/chitosan blend. **IOP Conference Series: Earth and Environmental Science**, Bristol, vol. 978, no. 1, p. 1–11, 2022. DOI 10.1088/1755-1315/978/1/012046. Available at: <https://iopscience.iop.org/article/10.1088/1755-1315/978/1/012046>. Accessed on: 11 Feb. 2023.

AHMAD, Naveed; TAYYEB, D.; ALI, I.; K. ALRUWAILI, N.; AHMAD, W.; UR REHMAN, A.; KHAN, A. H.; IQBAL, M. S. Development and Characterization of Hemicellulose-Based Films for Antibacterial Wound-Dressing Application. **Polymers**, Basel, vol. 12, no. 3, p. 1–16, 2020. DOI 10.3390/polym12030548. Available at: <https://www.mdpi.com/2073-4360/12/3/548>. Accessed on: 16 Feb. 2023.

AHMAD, Norlailiza; ZAKARIA, M. R. Chapter 8 - Oligosaccharide From Hemicellulose. In: ARIFFIN, H.; SAPUAN, S. M.; HASSAN, M. A. B. T.-L. for F. B. (eds.). **Lignocellulose for Future Bioeconomy**. 1st ed. Amsterdam: Elsevier, 2019. p. 135–152. DOI 10.1016/B978-0-12-816354-2.00008-6. Available at: <https://www.sciencedirect.com/science/article/pii/B9780128163542000086>.

AMARAWEEERA, S. M.; GUNATHILAKE, C.; GUNAWARDENE, O. H. P.; FERNANDO, N. M. L.; WANNINAYAKA, D. B.; DASSANAYAKE, R. S.; RAJAPAKSHA, S. M.; MANAMPERI, A.; FERNANDO, C. A. N.; KULATUNGA, A. K.; MANIPURA, A. Development of Starch-Based Materials Using Current Modification Techniques and Their Applications: A Review. **Molecules**, Base, vol. 26, no. 22, p. 1–30, 2021. DOI 10.3390/molecules26226880. Available at: <https://www.mdpi.com/1420-3049/26/22/6880>. Accessed on: 1 Feb. 2023.

ASTM. **D570-22: Standard Test Method for Water Absorption of Plastics**. West Conshohocken, United States: [s. n.], 2022. Available at: <https://www.astm.org/d0570-22.html>.

ASTM. **D882-18: Standard Test Method for Tensile Properties of Thin Plastic Sheeting**. West Conshohocken: [s. n.], 2018. Available at: <https://www.astm.org/d0882-18.html>.

BATISTA, J. T. S.; ARAÚJO, C. S.; PEIXOTO JOELE, M. R. S.; SILVA, J. O. C.; LOURENÇO, L. F. H. Study of the effect of the chitosan use on the properties of biodegradable films of myofibrillar proteins of fish residues using response surface methodology. **Food Packaging and Shelf Life**, Amsterdam, vol. 20, p. 1–10, 2019. DOI 10.1016/j.fpsl.2019.100306. Available at: <https://www.sciencedirect.com/science/article/pii/S2214289418303259>. Accessed on: 15 Feb. 2023.

BRUGNEROTTO, J.; LIZARDI, J.; GOYCOOLEA, F. M.; ARGÜELLES-MONAL, W.; DESBRIÈRES, J.; RINAUDO, M. An infrared investigation in relation with chitin and chitosan characterization. **Polymer**, Amsterdam, vol. 42, no. 8, p. 3569–3580, 2001. DOI 10.1016/S0032-3861(00)00713-8. Available at: <https://www.sciencedirect.com/science/article/pii/S0032386100007138>. Accessed on: 1 Feb. 2023.

CALLISTER, W. D.; RETHWISCH, D. G. **Materials Science and Engineering: An Introduction**. 10th ed. Hoboken: Wiley, 2018.

CANEVAROLO JR., S. V. **Ciência dos Polímeros**. 2nd ed. São Paulo: Artlber, 2010.

CONVERSO. **Global Plastics Flow 2018**. Mainaschaff: [s. n.], 2020. Available at: <https://www.bkv-gmbh.de/studies/study-global-plastics-flow-2018-conversio.html>.

COSTA, S. M.; FERREIRA, D. P.; TEIXEIRA, P.; BALLESTEROS, L. F.; TEIXEIRA, J. A.; FANGUEIRO, R. Active natural-based films for food packaging applications: The combined effect of chitosan and nanocellulose. **International Journal of Biological Macromolecules**, Amsterdam, vol. 177, p. 241–251, 2021. DOI 10.1016/j.ijbiomac.2021.02.105. Available at: <https://www.sciencedirect.com/science/article/pii/S0141813021003846>. Accessed on: 14 Feb. 2023.

FERNANDES, É. S.; BUENO, D.; PAGNOCCA, F. C.; BRIENZO, M. Minor Biomass Particle Size for an Efficient Cellulose Accessibility and Enzymatic Hydrolysis. **ChemistrySelect**, Hoboken, vol. 5, no. 25, p. 7627–7631, 7 Jul. 2020. DOI 10.1002/slct.202001008. Available at: <https://chemistry-europe.onlinelibrary.wiley.com/doi/full/10.1002/slct.202001008>. Accessed on: 23 Sep. 2022.

FRIED, J. R. **Physical chemistry of surfaces**. 3rd ed. Hoboken: Prentice-Hall International, 2014.

FRY, S. C. Cell Walls and Fibers | Cell walls. In: THOMAS, B. B. T.-E. of A. P. S. (ed.). **Encyclopedia of Applied Plant Sciences**. Oxford: Elsevier, 2003. p. 75–87. DOI 10.1016/B0-12-227050-9/00014-4. Available at: <https://www.sciencedirect.com/science/article/pii/B0122270509000144>.

GALDEANO, M. C.; WILHELM, A. E.; MALI, S.; GROSSMANN, M. V. E. Influence of thickness on properties of plasticized oat starch films. **Brazilian Archives of Biology and Technology**, Curitiba, vol. 56, no. 4, p. 637–644, Aug. 2013. DOI 10.1590/S1516-89132013000400014. Available at: <https://www.scielo.br/j/babt/a/QYr8c6j4KHT5SDT8q4DzpZK>. Accessed on: 15 Feb. 2023.

GEANKOPLIS, C. **Transport Processes and Unit Operations**. 3rd ed. Englewood Cliffs: Prentice-Hall International, 1993.

GENT, S.; TWEDT, M.; GEROMETTA, C.; ALMBERG, E. Chapter Two - Introduction to Feedstocks. In: GENT, S.; TWEDT, M.; GEROMETTA, C.; ALMBERG, E. B. T.-T. and A. A. of B. T. (eds.). **Theoretical and Applied Aspects of Biomass Torrefaction**. Oxford: Butterworth-Heinemann, 2017. p. 17–39. DOI 10.1016/B978-0-12-809483-9.00002-6. Available at: <https://www.sciencedirect.com/science/article/pii/B9780128094839000026>.

HUQ, T.; KHAN, A.; BROWN, D.; DHAYAGUDE, N.; HE, Z.; NI, Y. Sources, production and commercial applications of fungal chitosan: A review. **Journal of Bioresources and Bioproducts**, Beijing, vol. 7, no. 2, p. 85–98, 2022. DOI 10.1016/j.jobab.2022.01.002. Available at: <https://www.sciencedirect.com/science/article/pii/S2369969822000020>. Accessed on: 1 Feb. 2023.

ISO. **ISO 62:2008: Plastics - Determination of water absorption**. Vernier: [s. n.], 2021. Available at: <https://www.iso.org/standard/41672.html>.

JACOB, J.; GOMES, F.; HAPONIUK, J. T.; KALARIKKAL, N.; THOMAS, S. **Natural Polymers: Perspectives and Applications for a Green Approach**. 1st ed. London: Taylor & Francis Group, 2022. DOI 10.1201/9781003130765. Available at: <https://www.taylorfrancis.com/books/edit/10.1201/9781003130765>.

JAKUBOWSKA, E.; GIERSEWSKA, M.; NOWACZYK, J.; OLEWNIK-KRUSZKOWSKA, E. Physicochemical and storage properties of chitosan-based films plasticized with deep eutectic solvent. **Food Hydrocolloids**, Amsterdam, vol. 108, p. 1–10, 2020. DOI 10.1016/j.foodhyd.2020.106007. Available at: <https://www.sciencedirect.com/science/article/pii/S0268005X2030059X>. Accessed on: 12 Dec. 2022.

JANIK, W.; WOJTAŁA, A.; PIETRUSZKA, A.; DUDEK, G.; SABURA, E. Environmentally Friendly Melt-Processed Chitosan/Starch Composites Modified with PVA and Lignin. **Polymers**, Basel, vol. 13, no. 16, p. 1–14, 2021. DOI 10.3390/polym13162685. Available at: <https://www.mdpi.com/2073-4360/13/16/2685>. Accessed on: 20 Feb. 2023.

KAMDEM, D. P.; SHEN, Z.; NABINEJAD, O.; SHU, Z. Development of biodegradable composite chitosan-based films incorporated with xylan and carvacrol for food packaging application. **Food Packaging and Shelf Life**, Amsterdam, vol. 21, p. 1–9, 2019. DOI 10.1016/j.fpsl.2019.100344. Available at: <https://www.sciencedirect.com/science/article/pii/S2214289418301790>. Accessed on: 3 Mar. 2023.

KUMARI, S.; KISHOR, R. Chapter 1 - Chitin and chitosan: origin, properties, and applications. In: GOPI, S.; THOMAS, S.; PIUS, A. B. T.-H. of C. and C. (eds.). **Handbook of Chitin and Chitosan V1**. Amsterdam: Elsevier, 2020. p. 1–33. DOI 10.1016/B978-0-12-817970-3.00001-8. Available at: <https://www.sciencedirect.com/science/article/pii/B9780128179703000018>.

KUSUMASTUTI, Y.; PUTRI, N. R. E.; TIMOTIUS, D.; SYABANI, M. W.; ROCHMADI. Effect of chitosan addition on the properties of low-density polyethylene blend as potential bioplastic. **Heliyon**, Cambridge, vol. 6, no. 11, p. 1–8, 2020. DOI 10.1016/j.heliyon.2020.e05280. Available at: <https://www.sciencedirect.com/science/article/pii/S240584402032123X>. Accessed on: 8 Feb. 2023.

LAVORGNA, M.; PISCITELLI, F.; MANGIACAPRA, P.; BUONOCORE, G. G. Study of the combined effect of both clay and glycerol plasticizer on the properties of chitosan films. **Carbohydrate Polymers**, Amsterdam, vol. 82, no. 2, p. 291–298, 2010. DOI 10.1016/j.carbpol.2010.04.054. Available at: <https://www.sciencedirect.com/science/article/pii/S0144861710003358>. Accessed on: 20 May 2022.

LI, J.; TIAN, X.; HUA, T.; FU, J.; KOO, M.; CHAN, W.; POON, T. Chitosan Natural Polymer Material for Improving Antibacterial Properties of Textiles. **ACS Applied Bio Materials**, Washington, vol. 4, no. 5, p. 4014–4038, 17 May 2021. DOI 10.1021/acsabm.1c00078. Available at: <https://pubs.acs.org/doi/10.1021/acsabm.1c00078>. Accessed on: 1 Feb. 2023.

LIU, Y.; SUN, B.; WANG, Z.; NI, Y. Mechanical and water vapor barrier properties of bagasse hemicellulose-based films. **BioResources**, Raleigh, vol. 11, no. 2, p. 4226–4236, 2016. DOI 10.15376/biores.11.2.4226-4236. Available at: <https://bioresources.cnr.ncsu.edu/issues/vol11-issue2/page/10/>. Accessed on: 7 Mar. 2023.

LUO, Y.; WANG, Q. Recent development of chitosan-based polyelectrolyte complexes with natural polysaccharides for drug delivery. **International Journal of Biological Macromolecules**, Amsterdam, vol. 64, p. 353–367, 2014. DOI 10.1016/j.ijbiomac.2013.12.017. Available at: <https://www.sciencedirect.com/science/article/pii/S0141813013006673>. Accessed on: 11 Feb. 2023.

MACEDO, J. V. C.; ABE, M. M.; SANVEZZO, P. B.; GRILLO, R.; BRANCIFORTI, M. C.; BRIENZO, M. Xylan-starch-based bioplastic formulation and xylan influence on the physicochemical and biodegradability properties. **Polymer Bulletin**, Berlin, , p. 1–26, 2022. DOI 10.1007/s00289-022-04385-x. Available at: <https://link.springer.com/article/10.1007/s00289-022-04385-x>. Accessed on: 13 Feb. 2023.

MACEDO, J. V. C.; MARTINS, J. R.; ABE, M. M.; BRANCIFORTI, M. C.; BRIENZO, M. Hemicellulose Application for the Production of Bioplastics and Biomaterials. In: BRIENZO, M. (ed.). **Hemicellulose Biorefinery: A Sustainable Solution for Value Addition to Bio-Based Products and Bioenergy**. Singapore: Springer Nature Singapore, 2022. p. 231–273. DOI 10.1007/978-981-16-3682-0_8. Available at: https://link.springer.com/chapter/10.1007/978-981-16-3682-0_8.

MEDINA-JARAMILLO, C.; OCHOA-YEPES, O.; BERNAL, C.; FAMÁ, L. Active and smart biodegradable packaging based on starch and natural extracts. **Carbohydrate Polymers**, Amsterdam, vol. 176, p. 187–194, 2017. DOI 10.1016/j.carbpol.2017.08.079. Available at: <https://www.sciencedirect.com/science/article/pii/S0144861717309554>. Accessed on: 1 Aug. 2022.

MERINO, D.; BELLASSI, P.; PAUL, U. C.; MORELLI, L.; ATHANASSIOU, A. Assessment of chitosan/pectin-rich vegetable waste composites for the active packaging of dry foods. **Food Hydrocolloids**, Amsterdam, , p. 108580, 2023. DOI 10.1016/j.foodhyd.2023.108580. Available at: <https://www.sciencedirect.com/science/article/pii/S0268005X23001261>. Accessed on: 11 Feb. 2023.

MOALLA, S.; AMMAR, I.; FAUCONNIER, M.-L.; DANTHINE, S.; BLECKER, C.; BESBES, S.; ATTIA, H. Development and characterization of chitosan films carrying Artemisia campestris antioxidants for potential use as active food packaging materials. **International Journal of Biological Macromolecules**, Amsterdam, vol. 183, p. 254–266, 2021. DOI 10.1016/j.ijbiomac.2021.04.113. Available at: <https://www.sciencedirect.com/science/article/pii/S0141813021008795>. Accessed on: 16 Feb. 2023.

NGUYEN, T. T.; THI DAO, U. T.; THI BUI, Q. P.; BACH, G. L.; HA THUC, C. N.; HA THUC, H. Enhanced antimicrobial activities and physicochemical properties of edible film based on chitosan incorporated with Sonneratia caseolaris (L.) Engl. leaf extract. **Progress in Organic Coatings**, Amsterdam, vol. 140, p. 1–9, 2020. DOI 10.1016/j.porgcoat.2019.105487. Available at: <https://www.sciencedirect.com/science/article/pii/S030094401931402X>. Accessed on: 20 Feb. 2023.

OECD. Global Plastics Outlook: Greenhouse gas emissions from primary plastics. 2020. DOI doi.org/10.1787/1ac580ff-en. Available at: https://www.oecd-ilibrary.org/environment/data/global-plastic-outlook_c0821f81-en. Accessed on: 30 Jan. 2023.

OECD. Global Plastics Outlook: Plastics use by polymer - projections. 2023. DOI 10.1787/b9bae4d1-en. Available at: https://www.oecd-ilibrary.org/environment/data/global-plastic-outlook_c0821f81-en. Accessed on: 30 Jan. 2023.

OLORUNSOLA, E. O.; AKPABIO, E. I.; ADEDOKUN, M. O.; AJIBOLA, D. O. Emulsifying Properties of Hemicelluloses. In: KARAKUŞ, S. (ed.). **Science and Technology Behind Nanoemulsions**. 1st ed. London: IntechOpen, 2018. p. 29–42. DOI 10.5772/intechopen.74473. Available at: <https://www.intechopen.com/chapters/59915>. Accessed on: 10 Feb. 2021.

PATEL, J. P.; PARSANIA, P. H. 3 - Characterization, testing, and reinforcing materials of biodegradable composites. In: SHIMPI, N. G. B. T.-B. and B. P. C. (ed.). **Woodhead Publishing Series in Composites Science and Engineering**. Sawston: Woodhead Publishing, 2018. p. 55–79. DOI 10.1016/B978-0-08-100970-3.00003-1. Available at: <https://www.sciencedirect.com/science/article/pii/B9780081009703000031>.

PELLIS, A.; GUEBITZ, G. M.; NYANHONGO, G. S. Chitosan: Sources, Processing and Modification Techniques. **Gels**, Basel, vol. 8, no. 7, p. 1–27, 2022. DOI 10.3390/gels8070393. Available at: <https://www.mdpi.com/2310-2861/8/7/393>. Accessed on: 1 Feb. 2023.

PLASTICS EUROPE. **Plastics-The facts 2021**. Brussels: [s. n.], 2022. Available at: <https://plasticseurope.org/knowledge-hub/plastics-the-facts-2021/>.

PONNUSAMY, P. G.; MANI, S. Material and Environmental Properties of Natural Polymers and Their Composites for Packaging Applications—A Review. **Polymers**, Basel, vol. 14, no. 19, p. 1–22, 2022. DOI 10.3390/polym14194033. Available at: <https://www.mdpi.com/2073-4360/14/19/4033>. Accessed on: 31 Jan. 2023.

PRIYADARSHI, R.; RHIM, J.-W. Chitosan-based biodegradable functional films for food packaging applications. **Innovative Food Science & Emerging Technologies**, Amsterdam, vol. 62, p. 1–20, 2020. DOI 10.1016/j.ifset.2020.102346. Available at: <https://www.sciencedirect.com/science/article/pii/S1466856420302927>. Accessed on: 1 Feb. 2023.

PROVDER, T.; URBAN, M. W. **Film Formation in Coatings: Mechanisms, Properties, and Morphology**. 1st ed. Washington:

American Chemical Society, 2001. DOI 10.1021/bk-2001-0790. Available at: <https://pubs.acs.org/doi/book/10.1021/bk-2001-0790>.

RODRIGUES, A. A. M.; COSTA, R. R. da; SANTOS, L. F. dos; SILVA, S. de M.; BRITTO, D. de; LIMA, M. A. C. de. Properties and characterization of biodegradable films obtained from different starch sources. **Food Science and Technology**, Campinas, vol. 41, no. suppl 2, p. 476–482, 1 Feb. 2021. DOI 10.1590/fst.28520. Available at: <https://www.scielo.br/j/cta/a/GtX8N67GRGSNmTqp6ncKnwJ/>. Accessed on: 15 Feb. 2023.

RODSAMRAN, P.; SOTHORNVIT, R. Lime peel pectin integrated with coconut water and lime peel extract as a new bioactive film sachet to retard soybean oil oxidation. **Food Hydrocolloids**, Amsterdam, vol. 97, p. 1–9, 2019. DOI 10.1016/j.foodhyd.2019.105173. Available at: <https://www.sciencedirect.com/science/article/pii/S0268005X1930757X>. Accessed on: 13 Feb. 2023.

SCHNELL, C. N.; GALVÁN, M. V.; PERESIN, M. S.; INALBON, M. C.; VARTIAINEN, J.; ZANUTTINI, M. A.; MOCCHIUTTI, P. Films from xylan/chitosan complexes: preparation and characterization. **Cellulose**, Berlin, vol. 24, no. 10, p. 4393–4403, 2017. DOI 10.1007/s10570-017-1411-x. Available at: <https://link.springer.com/article/10.1007/s10570-017-1411-x>. Accessed on: 2 Mar. 2023.

SOLIER, Y. N.; SCHNELL, C. N.; GALVÁN, M. V.; MOCCHIUTTI, P.; ZANUTTINI, M. Á.; INALBON, M. C. Fast Preparation of Flexible Wet-Resistant and Biodegradable Films From a Stable Suspension of Xylan/Chitosan Polyelectrolyte Complexes. **Journal of Polymers and the Environment**, Berlin, vol. 30, no. 1, p. 114–124, 2022. DOI 10.1007/s10924-021-02181-8. Available at: <https://link.springer.com/article/10.1007/s10924-021-02181-8>. Accessed on: 2 Mar. 2023.

SUN, K.; LI, F.; LI, J.; LI, J.; ZHANG, C.; CHEN, S.; SUN, X.; CUI, J. Optimisation of compatibility for improving elongation at break of chitosan/starch films. **RSC Advances**, Cambridge, vol. 9, no. 42, p. 24451–24459, 2019. DOI 10.1039/C9RA04053F. Available at: <https://pubs.rsc.org/en/content/articlelanding/2019/ra/c9ra04053f>. Accessed on: 6 Mar. 2023.

XU, J.; XIA, R.; YUAN, T.; SUN, R. Use of xylooligosaccharides (XOS) in hemicelluloses/chitosan-based films reinforced by cellulose nanofiber: Effect on physicochemical properties. **Food Chemistry**, Amsterdam, vol. 298, p. 125041, 2019. DOI 10.1016/j.foodchem.2019.125041. Available at: <https://www.sciencedirect.com/science/article/pii/S0308814619311434>. Accessed on: 16 Dec. 2023.

XU, J.; XIA, R.; ZHENG, L.; YUAN, T.; SUN, R. Plasticized hemicelluloses/chitosan-based edible films reinforced by cellulose nanofiber with enhanced mechanical properties. **Carbohydrate Polymers**, Amsterdam, vol. 224, p. 1–9, 2019. DOI 10.1016/j.carbpol.2019.115164. Available at: <https://www.sciencedirect.com/science/article/pii/S0144861719308318>. Accessed on: 3 Mar. 2023.

YANG, H.; YAN, R.; CHEN, H.; LEE, D. H.; ZHENG, C. Characteristics of hemicellulose, cellulose and lignin pyrolysis. **Fuel**, Amsterdam, vol. 86, no. 12, p. 1781–1788, 2007. DOI 10.1016/j.fuel.2006.12.013. Available at: <https://www.sciencedirect.com/science/article/pii/S001623610600490X>. Accessed on: 14 Dec. 2023.

YUAN, Y.; CHESNUTT, B. M.; HAGGARD, W. O.; BUMGARDNER, J. D. Deacetylation of Chitosan: Material Characterization and in vitro Evaluation via Albumin Adsorption and Pre-Osteoblastic Cell Cultures. **Materials**, Basel, vol. 4, no. 8, p. 1399–1416, 12 Aug. 2011. DOI 10.3390/ma4081399. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5448679/>. Accessed on: 20 May 2022.

ZAMAN, N. B. K.; LIN, N. K.; PHING, P. L. Chitosan film incorporated with Garcinia atroviridis for the packaging of Indian mackerel (*Rastrelliger kanagurta*). **Ciência e Agrotecnologia**, Lavras, vol. 42, no. 6, p. 666–675, Dec. 2018. DOI 10.1590/1413-70542018426019918. Available at: <https://www.scielo.br/j/cagro/a/4HKNNzXqRCSXxRCvQ8DfPPj>. Accessed on: 13 Mar. 2023.

ZAMORA ZAMORA, H. D.; OLAYIWOLA, H. O.; JACOBUS, A. P.; GROSS, J.; TYHODA, L.; BRIENZO, M. Hemicelluloses Role in Biorefinery Systems of Cellulosic Bioethanol, Particleboard, and Pulp and Paper Industries. In: BRIENZO, M. (ed.). **Hemicellulose Biorefinery: A Sustainable Solution for Value Addition to Bio-Based Products and Bioenergy**. Singapore: Springer Nature Singapore, 2022. p. 1–37. DOI 10.1007/978-981-16-3682-0_1. Available at: https://link.springer.com/chapter/10.1007/978-981-16-3682-0_1. Accessed on: 5 Jul. 2022.

ZHANG, Xiao; TU, M.; PAICE, M. G. Routes to Potential Bioproducts from Lignocellulosic Biomass Lignin and Hemicelluloses. **BioEnergy Research**, Berlin, vol. 4, no. 4, p. 246–257, 2011. DOI 10.1007/s12155-011-9147-1. Available at: <https://link.springer.com/article/10.1007/s12155-011-9147-1>. Accessed on: 10 Feb. 2021.

ZHANG, Xueqin; WEI, Y.; CHEN, M.; XIAO, N.; ZHANG, J.; LIU, C. Development of functional chitosan-based composite films incorporated with hemicelluloses: Effect on physicochemical properties. **Carbohydrate Polymers**, Amsterdam, vol. 246, p. 1–8, 2020. DOI 10.1016/j.carbpol.2020.116489. Available at: <https://www.sciencedirect.com/science/article/pii/S0144861720306639>. Accessed on: 8 Feb. 2023.

ZHAO, J.; WANG, Y.; LIU, C. Film Transparency and Opacity Measurements. **Food Analytical Methods**, Amsterdam, vol. 15, no. 10, p. 2840–2846, 2022. DOI 10.1007/s12161-022-02343-x. Available at: <https://link.springer.com/article/10.1007/s12161-022-02343-x>. Accessed on: 6 Feb. 2022.

ZHAO, Y.; SUN, H.; YANG, B.; WENG, Y. Hemicellulose-Based Film: Potential Green Films for Food Packaging. **Polymers**, Basel, vol. 12, no. 8, p. 1–14, 2020. DOI 10.3390/polym12081775. Available at: <https://www.mdpi.com/2073-4360/12/8/1775/htm>. Accessed on: 15 Feb. 2022.

CHAPTER 5 - Fractionation of guava seed cake through organosolv pretreatment catalyzed by alkali

Abstract

The purposes of this study were to assess the alkali organosolv pretreatment as a fractionation strategy of the industrial waste generated after oil extraction process on guava seed (waste hereafter referred to as guava seed cake), and to concurrently extract hemicelluloses and lignin. After pretreatment, stages of ethanol addition, solvent evaporation, and acid precipitation were completed to extract hemicelluloses and lignin, respectively. Digestibility of pretreated biomass through enzymatic hydrolysis using Cellic® Ctec2 was performed aiming to evaluate the effectiveness of the organosolv pretreatment. A central composite design 2^3 was developed to analyze the behavior of the extraction mass yields of hemicelluloses and lignin, and cellulose conversion using different configurations of alkali concentration, temperature, and ethanol aqueous solution concentration at pretreatment stage. The factors that exhibited the most significant statistical effects were the alkali concentration and temperature for the extraction mass yield of hemicelluloses, and the temperature for the extraction mass yield of lignin and cellulose conversion. At 170 °C, ethanol aqueous solution concentration of 30% and alkali concentration of 60% (w/w, respect to raw biomass), the highest mass yield of extracted hemicelluloses was 97.3%. The highest mass yield of extracted lignin (45.0%) was reached at 170 °C, ethanol aqueous solution concentration of 70% and alkali concentration of 60%. And, the maximum cellulose conversion (50.3%) was obtained under conditions of 170 °C, ethanol aqueous solution concentration of 30%, and alkali concentration of 60%. The experimental conditions allowed to obtain hemicelluloses high recovery.

Keywords: Fruit waste, hemicelluloses, lignin, enzymatic hydrolysis, biorefinery.

Statement of Novelty

At present, the use of biorefineries for obtaining value-added bioproducts examining new lignocellulosic (LC) industrial wastes is deemed a topic of great importance. Thus, this research is the first one showing the physicochemical

characterization of guava seed cake, which is a local (Brazilian) industrial waste obtained after oil extraction from guava seed. In addition, this is the first study carried out in Brazil involving the evaluation of changes in chemical compositions of untreated and pretreated guava seed cake, as well as the impact of alkali ethanolic organosolv pretreatment (OHEOP) to fraction the waste to extract hemicelluloses (HC) and lignin (LG). After pretreatment, ethanol was added to the liquid fraction to precipitate hemicelluloses (HC), which could then be extracted. Likewise, to guarantee lignin (LG) precipitation, the liquid fraction obtained after HC extraction underwent solvent evaporation. HC and LG extraction are keys from the industrial point of view since are regarded as platform (intermediate) chemicals for biofuel and biopolymer obtaining.

5.1. Introduction

Currently, common practices related to fossil sources, such as exploration processes, refining techniques, and derivative uses are closely linked to greenhouse gas emissions, which are responsible for climate change causing negative consequences on ecosystems. Thereby, to decrease or eradicate the use of fossil sources, appropriate alternatives based on renewable sources have been explored to obtain bioproducts by analogous routes to the petroleum refinery (biorefinery). Vegetal biomass is a renewable precursor that has been identified as the best substitute for oil, natural gas, and coal. However, recent studies are focus on producing bioproducts from LC wastes (OREJUELA-ESCOBAR; LANDÁZURI; GOODELL, 2021).

Worldwide, numerous studies have been completed involving LC fruit waste, such as peel, seed, pomace, and other byproducts from the plant itself to obtain bioproducts through biorefinery schemes (MANHONGO *et al.*, 2022b). This type of waste is commonly generated in huge quantities from agroindustrial production and industrial processing of fruits, and improper disposal can result in negative consequences for both the environment and the agroindustry (GÜZEL; AKPINAR, 2019). The top 3 countries leading the fruit world production are China, India, and Brazil with 253.9, 107.9, and 39.8 millions of metric tons (STATISTA, 2023).

Among the fruits, the guava is highlighted because is economically important in several regions around the world due to its excellent agricultural yield and the consistent demand for diverse products that can be industrially obtained from its fruit (ANGULO-LÓPEZ *et al.*, 2021). The industrial processing of guava for pulp extraction

results in a byproduct composed of seeds (mostly) and peel, commonly referred to as guava seed (BIBWE *et al.*, 2022). Which is a waste with LC composition that can be used as feedstock to produce, biofuels, biopolymers, and other bioproducts using a biorefinery scheme (PEREIRA *et al.*, 2022a). Although guava seed is often discarded, its oil content (~ 13%) has led to implement methods to add value to this waste through the oil extraction (ANGULO-LÓPEZ *et al.*, 2021).

Considering guava seed with LC structure, then the cake will likely have a similar constitution of cellulose, HC, and LG, which are deemed as platform chemicals for the development of diverse industrial applications (PEREIRA *et al.*, 2022a). Nevertheless, extracting HC and LG is a technical challenge since LC structures present natural resistance (recalcitrance) caused by physicochemical bonding forces among the macromolecules (ZOGHLAMI; PAËS, 2019b). In order to beat recalcitrance, a pretreatment stage must be completed before extraction process of HC and LG (SHIMIZU; ZAMORA; *et al.*, 2020b). Based on the desirable products (final or intermediate), pretreatments are used in biorefineries to obtain biomass with its macromolecules (cellulose, HC, and LG) more accessible, better digestibility (it means, high production of monosaccharides after enzymatic hydrolysis), increased porosity and surface area, reduced cellulose crystallinity, limited inhibitor formation (vital for following enzymatic stages) and reduced degradation of the carbohydrates (cellulose and HC) (SHIMIZU *et al.*, 2020b).

The organosolv process is deemed as one of the most effective pretreatments used in biorefineries since contributes to the valorization of the LC biomass through selective separation into isolated fractions of cellulose, HC, and LG (THORESEN *et al.*, 2020). The use of organic solvent such as ethanol is characteristic of the organosolv pretreatment. Generally, ethanol is used due to its affordability, low toxicity, comes from renewable raw materials, has ability to be recovered and recycled, and exhibits higher water miscibility compared to other solvents (ZHANG; PEI; WANG, 2016b). Organosolv using ethanol can be completed either with or without catalyst addition, where the first option operates at intermediate temperatures (130 to 200 °C) while the second one works at temperatures upper than 200 °C (FERREIRA; TAHERZADEH, 2020). From both options, the most suitable is the first one since operating below 200 °C, cellulose would avoid to be chemically degraded (KLAMRASSAMEE *et al.*, 2013b). Thereby, the most usually organosolv process established at industrial, pilot, and laboratory levels is the ethanolic one catalyzed by

acid (ZHANG *et al.*, 2020b). However, acids can cause issues such as breaking down of carbohydrates and generation of inhibitors (by dehydrating reactions of hexoses and pentoses) that no doubt going to interfere with subsequent biological processes (SHIMIZU; ZAMORA; *et al.*, 2020b; ZHANG *et al.*, 2020b). Therefore, recent studies have analyzed other catalysts like the alkalis, principally for biorefinery systems where is desired to preserve the carbohydrates and where biological steps take place after the pretreatment stage (WEI *et al.*, 2021b).

In the present study was presented the evaluation of an organosolv pretreatment using ethanol and catalyzed by NaOH (OHEOP) that allowed to fraction the waste guava seed cake for the subsequent obtaining of HC and LG. Also, enzymatic hydrolysis (Novozymes Cellic® CTec2) was carried out to study the digestibility of pretreated biomass (PB). Adding ethanol to the liquid fraction after pretreatment (spent liquor) and adding sulfuric acid to the liquid remnant after HC extraction allowed to extract HC and LG, respectively. A central composite design 2^3 was executed with the factors: alkali concentration (OHC), temperature (T), and ethanol aqueous solution concentration (EC). The response variables studied were extraction mass yield of HC (HCy) and extraction mass yield of LG (LGy), and conversion of cellulose (CLX).

5.2. Material and methods

5.2.1. Biomass preparation

The guava seed cake, corresponding to the press cake generated after the oil extraction from guava seed, was kindly supplied (in dry state) by the AGGTEC company located at Dois Córregos - São Paulo state - Brazil (-22° 22' 6.9" N -48° 23' 51.01"W). During the oil extraction process, both oil and cake are obtained, where the cake contains around 4 - 5% of residual oil (information provided by the AGGTEC company). All of the research was developed using the waste with an average particle size smaller than 0.841 mm (mesh 20). For storage, the waste (after milling and sieving processes) was dried at 50 °C during 24 h. And the chemical composition (ethanol and water extractives, ash, cellulose, HC, and LG), was determined according to the methods established by the National Renewable Energy Laboratory (SLUITER *et al.*, 2012b; SLUITER; HAMES; *et al.*, 2008b; SLUITER; RUIZ; *et al.*, 2008b).

5.2.2. NaOH-catalyzed organosolv pretreatment using ethanol (OHEOP), for hemicelluloses (HC) and lignin (LG) extraction

The pretreatment configuration was carried out using stainless steel reactors that operated (each one) with 10 g of biomass and 50 mL of ethanolic aqueous solution (biomass loading: 20% m/v = solid/liquid ratio: 1:5). A 45 min soaking stage was implemented before pretreatment, which had a reaction time of 1 h. A central composite design 2³ (Table 5-1) was performed by duplicate in order to assess the pretreatment performance. The factors of the statistical design were OHC, T, and EC, and the response variables were HCy, LGy and CLX. The statistical analysis was executed using the StatSoft Statistica Enterprise v.12 software at a significance level of 95%.

Table 5-1 - Central composite design 2³

Factor	Levels				
	-1	+1	-1.682	0	+1.682
T (°C)	120	170	103.0	145	187.0
EC (% m/m)	30	70	16.4	50	83.6
OHC (%)	10	60	0.0	35	77.0

T: Temperature, EC: Ethanol aqueous solution concentration, OHC: alkali concentration (respect to raw biomass)

Source: By author

After pretreatment, the biphasic mixture was separated into a solid fraction and a liquid one using filter paper. The solid fraction (frequently called pretreated biomass) was washed until to achieve the pH of distilled water (pH~7.0), and the liquid fraction (commonly denominated spent liquor) was collected in a plastic bottle. Ethanol (92.8% of purity) addition to the spent liquor and decantation for at least 18 h were the successive stages used to extract HC. Once HC were extracted, the added ethanol was evaporated maintaining a temperature of 80 °C. For LG extraction were necessary three stages, firstly was added concentrated sulfuric acid to the liquid remnant (after ethanol boiling) until to reach pH below 2, then a resting period of at least 18 h was completed, and finally the LG was recovered by separation using filter paper (the LG was washed up to reach the pH of distilled water). The extracted HC and LG were

dried at 60 °C during 72 h. The mass yields of HC and LG extraction were calculated considering the mass of each extracted compound and the respective content of HC and LG present in the raw biomass.

5.2.3. Characterization of pretreated biomass (PB), and extracted hemicelluloses (HC) and lignin (LG)

The determination of cellulose, HC, and LG content (using the National Renewable Energy Laboratory method) was performed to chemically characterize the PB (SLUITER *et al.*, 2012b). And, in the case of the extracted HC and LG, Fourier Transform Infrared (FTIR) spectroscopy was completed using 32 scans, resolution of 4 cm⁻¹, spectrum region of 4000 – 400 cm⁻¹, and a mass ratio of 1:125 (KBr:sample) (JAVIER-ASTETE; JIMENEZ-DAVALOS; ZOLLA, 2021b). Birchwood xylan (XYN - Sigma Aldrich, Lot 067K0701) and kraft lignin (LGK - Sigma Aldrich, Lot MKCP5496) were included in the FTIR analysis for comparison. Both spectra were used as comparison references for the HC and LG extracted from guava seed cake. Normalization and baseline correction were applied in all spectra by maximum intensity and 3 points methods, respectively. Besides FTIR spectroscopy, molecular weight of the extracted HC was determined by Ultra Performance Liquid Chromatography (UPLC) following the methodology described by De Freitas and Brienzo (2022).

5.2.4. Digestibility of the pretreated biomass (PB)

The PB digestibility was studied through enzymatic hydrolysis using Cellic® Ctec2 (Novozymes company). Briefly, 15 FPU/g (enzyme dosage respect to PB mass), 5 mL of sodium citrate buffer 50 mmol/L (pH 4.8), and 0.1 g of PB (dry basis) were placed in falcon tube of 50 mL. The hydrolysis took place with the tilted tubes (~45°) at 150 rpm during 24 h and at 50 °C. Finally, the supernatant obtained after centrifugation at 6000 rpm by 20 min was used for glucose quantification by high performance liquid chromatography (HPLC) employing the methodology described by Schmatz *et al.* (2021). The CLX (cellulose conversion in glyucose) was the parameter used to analyze the hydrolysis performance.

5.3. Results and discussion

5.3.1. Chemical characterization of guava seed cake

The guava seed cake biomass has not been characterized by any previous study, the results were compared with the compositions of guava seed, which can be considered as the most similar waste found in previous works (Table 5-2). Although the difference between both biomasses is the oil content (4 – 5% and ~ 13%, respectively), it was found great similarity in their compositions, excepting the CL value (19.3%). The difference in CL composition could be caused firstly, by using distinct procedures to characterize the same (or very similar) LC biomasses, secondly, by unknowing of internal processes applied by industries that originated the wastes, and finally, by variation of the oil contents (YAN *et al.*, 2020b). In addition, it is worth mentioning that the chemical composition of a particular LC biomass, and therefore its byproducts, is contingent upon the cultivation characteristics and environmental conditions (KOUTSOUKIS *et al.*, 2019b). Regarding the ash content, it was observed that its value (0.6%) was low, this result due to the fact that the waste was originated by the guava industry, which likely involved the washing of fruits with water during processing, a stage that largely contributed to remove non-structural ash (HUANG *et al.*, 2017).

Table 5-2 - Chemical composition (dry basis) of guava seed cake and guava seed from the food industry

CL (%)	HC (%)	LG (%)	Ex (%)	Ash (%)	Reference
19.3±0.14	14.1±0.14	40.3±0.4	13.6±0.01	0.6±0.02	This work
31.4	14.3	40.2	--	0.1	Rahman; Saad, 2003*
28.0	15.5	41.7	16.8 ¹	0.4	Largitte <i>et al.</i> , 2016*

CL: Cellulose, HC: Hemicelluloses, LG: Lignin; Ex: Extractives. *guava seed, ¹ ethanol-benzene mixture

Source: By author

LC contents of some wastes from fruit industries that are similar to guava seed cake are presented in Table 5-3. Based on this data, it would be possible to affirm that

guava seed cake could be a potential raw material to produce value-added bioproducts and bioenergy.

Table 5-3 - LC composition (dry basis) of some wastes from fruit industries

Waste	Objective	CL (%)	HC (%)	LG (%)	Reference
Guava seed cake	HC & LG extraction	19.3 $\pm$ 0.14	14.1 $\pm$ 0.14	40.3 $\pm$ 0.40	This work
Tamarind seed	Bioenergy	20.9	15.8	28.8	Alves <i>et al.</i> , 2020b
Apple pomace	Pectin & CL extraction	17.7	10.9	15.4	Ma; Luo; Xu, 2019
Grape pomace	Various value-added applications	10.5	6.1	31.9	Gowman <i>et al.</i> , 2019
Pear pomace	HC extraction	32.4	20.2	40.3	Rabetafika <i>et al.</i> , 2014b

CL: Cellulose, HC: Hemicelluloses, LG: Lignin; Ex: Extractives

Source: By author

5.3.2. Extraction, and characterization of hemicelluloses (HC) and lignin (LG)

The mass yields of HC and LG extracted from guava seed cake (abbreviated HCy and LGy, respectively), and the LC characterizations of the PB obtained after each pretreatment configuration (based on the established statistical design) are summarized and shown in Table 5-4. Later on, will be presented and analyzed the characterizations performed on the extracted HC and LG compared with commercial one.

Table 5-4 - Extraction mass yields of HC and LG, and LC composition and CLX of pretreated guava seed cake

Exp	Factors			Yield (%)			LC composition (% dry basis)			CLX* (%)
	T (°C)	EC (%)	OHC (%)	PB	HCy	LGy	CL	HC	LG	
G1	120	30	10	75.8±0.22	35.6±0.24	11.7±0.66	29.9±0.48	20.5±0.74	44.7±1.50	11.0±0.27
G2	120	30	60	58.3±0.21	64.5±0.43	5.0±0.09	41.6±0.59	19.1±0.24	44.5±0.01	22.0±0.11
G3	120	70	10	77.3±0.28	20.1±0.57	13.3±0.23	28.7±0.23	20.7±0.81	51.2±1.40	11.4±0.11
G4	120	70	60	60.6±0.21	65.8±0.38	11.5±0.95	40.4±0.46	21.0±0.31	42.0±0.40	22.1±0.59
G5	170	30	10	64.5±0.43	47.4±0.76	19.3±0.80	33.8±0.32	19.6±0.38	48.0±1.50	42.0±0.95
G6	170	30	60	32.1±0.21	97.3±0.82	33.0±0.28	31.7±0.07	10.5±0.09	36.2±1.36	50.3±0.19
G7	170	70	10	64.1±0.21	18.2±0.36	25.7±0.17	34.7±1.44	22.0±0.13	46.1±0.22	36.8±0.32
G8	170	70	60	24.6±0.57	92.3±0.55	45.0±0.19	63.5±1.20	25.5±0.46	15.6±0.30	28.9±1.02
G9	103	50	35	75.3±1.34	40.6±1.01	3.4±0.50	33.2±0.87	20.7±0.84	48.7±1.08	10.8±1.23
G10	187	50	35	39.0±0.42	69.7±0.95	37.7±0.38	42.9±0.69	19.1±1.07	33.4±1.14	47.3±0.59
G11	145	16.4	35	58.0±0.35	65.3±0.48	15.0±0.87	31.7±0.96	15.8±1.02	44.9±0.53	33.6±1.13
G12	145	83.6	35	56.3±0.59	45.0±1.19	26.1±0.66	44.4±0.53	23.9±0.88	37.2±1.03	22.6±0.96
G13	145	50	0	75.7±0.79	20.4±0.74	16.3±0.68	29.3±0.34	21.1±0.57	50.1±0.64	24.2±1.00
G14	145	50	77	34.9±0.94	96.8±1.00	25.7±0.39	48.5±0.72	18.5±0.47	30.2±0.85	32.7±0.27
G15(c)	145	50	35	57.2	55.2	20.6	38.0	19.9	41.0	28.1
G16(c)	145	50	35	56.8	56.0	21.4	39.5	20.0	39.8	29.4
G17(c)	145	50	35	58.0	55.9	19.8	38.2	20.3	40.5	28.0

T: Temperature, EC: Ethanol aqueous solution concentration, OHC: Alkali concentration, PB: Yield of recovered pretreated biomass HCy: Extraction yield of hemicelluloses, LGy: Extraction yield of lignin, CL: Cellulose, HC: Hemicelluloses, LG: Lignin, CLX: Cellulose conversion. * For untreated biomass = 10.7±0.13%

Source: By author

Table 5-5 shows the variance analysis (ANOVA) at 95% of significance completed for determining the effect of the factors (T, EC, and OHC) on the response variables (HCy, LGy, and CLX). The resulting ANOVAs from full experimental design 2^3 with central point did not show statistical significance for curvatures (except HCy, however, its value was so low that was neglected). To verify the insignificance, it was run the same full design but with the inclusion of axial points, once again results indicated curvatures were not significant, thus, it was selected the linear model for the influence of the factors on the response variables (Eq 1).

$$y_{ijkl} = \mu + T_i + EC_j + OHC_k + (T \cdot EC)_{ij} + (T \cdot OHC)_{ik} + (EC \cdot OHC)_{jk} + (T \cdot EC \cdot OHC)_{ijk} + \varepsilon_{ijkl} \quad (1)$$

Here y_{ijkl} is the response variable (HCy, LGy, or CLX), μ is the global average, T_i corresponds to the effect of i-th level of the factor temperature, EC_j is the effect of j-th level of the factor ethanolic aqueous solution concentration, OHC_k is the effect of k-th level of the factor alkali concentration, the interactions of the factors are represented by $(T \cdot EC)_{ij}$, $(T \cdot OHC)_{ik}$, $(EC \cdot OHC)_{jk}$ and $(T \cdot EC \cdot OHC)_{ijk}$, and ε_{ijkl} is the random error (MONTGOMERY, 2001).

Table 5-5 - ANOVA of the influence of factors and its interactions on response variables

<u>Response variable: HCy</u>			$R^2 = 0.999$		
Factor	SS	df	MS	F-value	p-value
Curvature	1.29	1	1.290	5.42	0.036706*
T (°C)	1198.89	1	1198.891	5035.73	0.000000
EC (%)	586.85	1	586.851	2464.96	0.000000
OHC (%)	9845.60	1	9845.601	41354.70	0.000000
T-EC	98.51	1	98.506	413.76	0.000000
T-OHC	611.33	1	611.326	2567.77	0.000000
T-EC-OHC	419.23	1	419.226	1760.88	0.000000
Error	13.51	1	13.506	56.73	0.000004
Total	3.09	13	0.238	--	--
<u>Response variable: LGy</u>			$R^2 = 0.997$		
Factor	SS	df	MS	F-value	p-value
Curvature	0.011	1	0.011	0.031	0.863472
T (°C)	1672.810	1	1672.810	4717.252	0.000000

EC (%)	175.563	1	175.563	495.079	0.000000
OHC (%)	150.063	1	150.063	423.170	0.000000
T-EC	26.523	1	26.523	74.792	0.000001
T-OHC	430.563	1	430.563	1214.168	0.000000
T-EC-OHC	28.090	1	28.090	79.213	0.000001
Error	0.160	1	0.160	0.451	0.513529
Total	4.610	13	0.355	--	--

Response variable: CLX

$R^2 = 0.997$

Factor	SS	df	MS	F-value	p-value
Curvature	0.812	1	0.812	2.067	0.174191
T (°C)	2099.931	1	2099.931	5347.522	0.000000
EC (%)	172.266	1	172.266	438.678	0.000000
OHC (%)	122.656	1	122.656	312.345	0.000000
T-EC	184.281	1	184.281	469.275	0.000000
T-OHC	111.831	1	111.831	284.779	0.000000
T-EC-OHC	67.651	1	67.651	172.274	0.000000
Error	62.016	1	62.016	157.924	0.000000
Total	5.105	13	0.393	--	--

T: Temperature, EC: Ethanol concentration, OHC: Alkali concentration, HCy: Extraction yield of hemicelluloses, LGy: Extraction yield of lignin, CLX: Cellulose conversion, SS: Sum of squares, df: Degrees of freedom, MS: Mean squares. * Significance so small that was neglected

Source: By author

According to correlation coefficients (R^2), all linear models showed superior values than 0.99, demonstrating strong relations between the factors (T, EC, and OHC) and the response variables (HCy, LGy, and CLX) (MONTGOMERY, 2001). In the supplementary information section can be accessed the respective response surfaces.

5.3.3. Hemicelluloses (HC) extraction and characterization by FTIR

After each OHEOP configuration, it was possible to extract HC from guava seed cake. Under the conditions of 170 °C, EC 30%, and OHC 60% (= 3.0 mol/L = 12.0% m/v) at the OHEOP, it was achieved the highest HCy (97.3% regarding the HC content in the raw waste = 13.7% regarding the total mass of the waste). Also, the maximum HC extraction occurred when the pretreated material showed the lowest content of HC (10.5%). The linkage between both results could be related to the fact that the low HC content in PB suggests that HC during pretreatment was solubilized, which contributed to reach the maximum extraction yield (DE FREITAS *et al.*, 2021b). Because this study

is the first one in researching HC extraction from spent liquor after OHEOP, the HCy results were compared to those achieved after hot water pretreatments typically carried out with alike settings of time (1 h) and T (170 °C). The highest HCy obtained herein was superior to the reported one by Wolf *et al.* (2022), who from depectinized apple pomace (biomass loading at pretreatment: 5% m/v) reached 4.3% (regarding the total mass of biomass) using similar temperature (155 °C) and same reaction time (1 h). The maximum HCy, also informed in the Wolf *et al.* (2022) study, was 8.4% (regarding the total mass of initial biomass) after 20 min at 125 °C of pretreatment (in pilot-scale). And, Gallina *et al.* (2018) informed that from *Eucalyptus globulus* pruning (liquid/solid ratio = 150:1) pretreated during 40 - 80 min (reaction time) at 160 °C and 9 bar using a system of cascade reactor operated by batch, the maximum HCy (40.3%, regarding the HC content in raw biomass) was obtained.

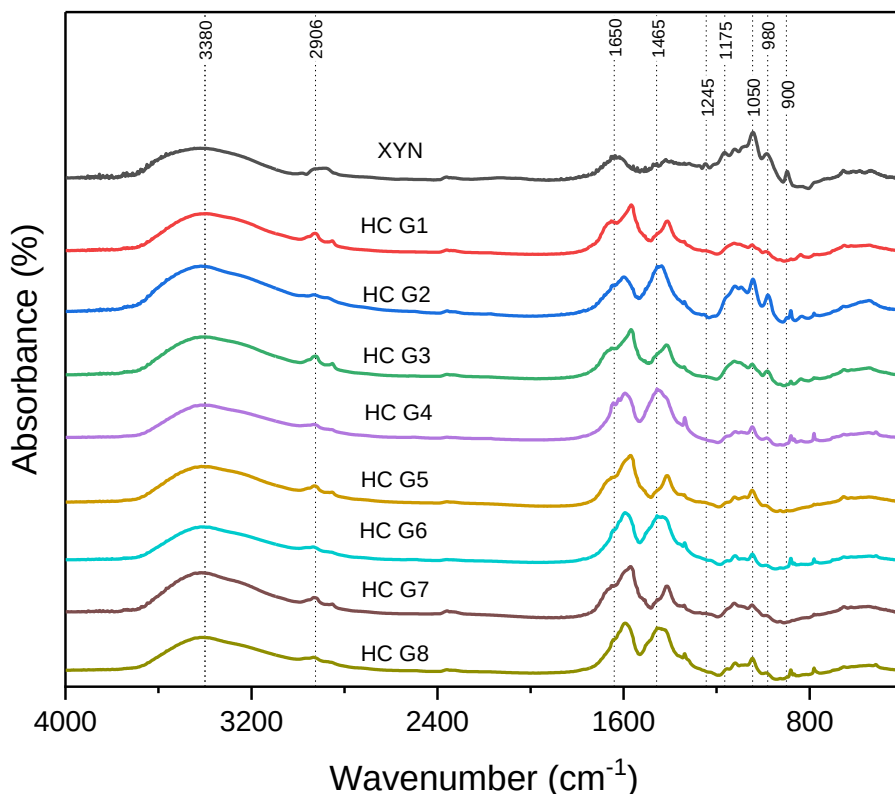
Alkalies have generally been included in hydrothermal pretreatments to enhance the effectiveness of HC extraction (YAO *et al.*, 2015). For example, Rabetafika *et al.* (2014) obtained, from dried pear pomace (biomass loading at pretreatment: 5% m/v), 61.8% (based on the HC content in biomass) as the best HCy after pretreatment with NaOH 4 mol/L at 60 °C during 18 h and including sodium borohydride (NaBH₄) as reducing agent at concentration of 3 g/L. In a further case of alkali inclusion, Puițel *et al.* (2022) reported 62.0% (regarding the HC content in biomass) as the maximum HCy (xylan) from wheat straw pretreated with a liquid/solid ratio of 30:1 and NaOH 6% (m/m) at 100 °C for 90 min.

All previously cited studies showed HCy below one found herein, nevertheless, some studies proved that it is technically feasible to obtain yields of about 95% (regarding HC content in biomass) using the alkaline method. This is the case of Xu *et al.* (2018), who employed a pretreatment during 3 h with a liquid/solid ratio of 30:1, solution of 5% KOH, temperature of 50 °C and 30 min of ultrasound obtained the best HCy (95.2%) from *Eucalyptus grandis* holocellulose. Another example is the Singh *et al.* (2018) study, in which two sequential steps with 10% (m/v) NaOH were implemented (incubation at 65°C for 8 h, followed by a hydrothermal process at 121°C for 1 h) to obtain HCy greater than 94% from areca nut shells (liquid/solid ratio of 10:1). From pear pomace holocellulose (biomass loading: 5% m/v) pretreated using NaOH 2 mol/L at 60 °C during 18 h, Rabetafika *et al.* (2014) found 94.5% as the best value for HCy.

According to the ANOVA indicated in the Table 5-5, OHC was the most significant factor affecting HCy, showing a positive influence when the pretreatment was completed at 60%, and the second in importance was the T, favoring the HCy at 170 °C in the pretreatment stage. Although EC was also significant, its effect on HCy was smaller than OHC and T. Keeping the EC at 30% allowed favoring on HCy. The finding about OHC was consistent with recent studies that have shown that HCy was improved when alkali loads (during the pretreatment stage) were increased (PUIȚEL *et al.*, 2022; YAO *et al.*, 2015). There is no doubt that the mass yield of HC extraction depends on the HC solubilization achieved during alkali pretreatment, where OH⁻ groups act on hydrogen bonds (between cellulose and HC) and ester linkages (between HC and LG), and promote cellulose swelling (LU *et al.*, 2021). Concerning the T effect, this was in congruence with the Hanim *et al.* (2012) work, in which the T was the main factor influencing the HC solubilization during the thermal pretreatment. Likewise, Reyes *et al.* (2013) identified that the T effect had statistical significance on HC extraction after alkaline pretreatment, where 170 °C was the temperature that provoked the best HCy. In terms of using EC around 30%, Santos *et al.* (2022) found 1.41 g/L of solubilized HC (one of the best results) from *Pinus radiata* after organosolv pretreatment with EC of 33% at 185 °C during 75 min. Similarly, Li *et al.* (2020) reported the use of EC 40% during OHEOP of sorghum stem rind, from which was possible to obtain a HCy (xylan) of 80.5% (regarding the HC content in biomass).

The characterization of functional groups for the extracted HC was completed through FTIR spectroscopy (Figure 5.1). The observed and analyzed regions were 800 - 1700 cm⁻¹ and 2800 - 3800 cm⁻¹.

Figure 5.1 - FTIR spectra of hemicelluloses (HC) extracted from guava seed cake



XYN: Birchwood xylan; HC G1: Recovered hemicelluloses from guava seed cake in experiment 1

Source: By author

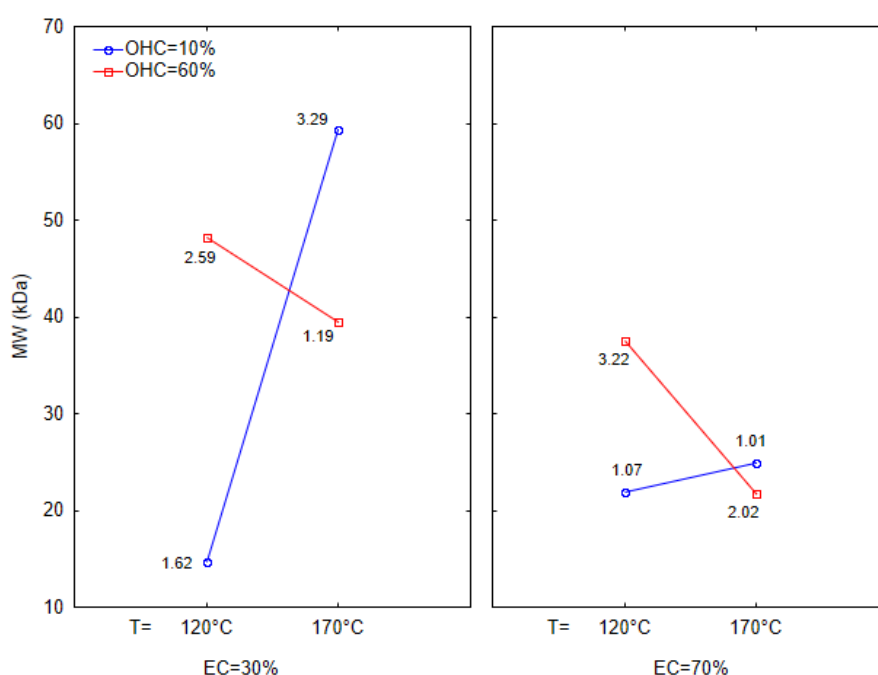
According to Sun *et al.* (2002), the band at 900 cm^{-1} corresponds to C-O-C stretching in β -glycosidic bonds of sugar units in HC. The samples that exhibited this type of stretching were the G2, G4, G6 and G8, from which the G2 exposed the highest absorbance. The arabinan existence in HC structures is indicated by the 980 cm^{-1} band (LIU *et al.*, 2021), which was detected in the samples G2, G3, G4, G6 and G8, with G2 revealing maximum intensity. The C-O-C stretching in xylan is represented by the 1050 cm^{-1} band (JU *et al.*, 2011), all samples exhibited this band with the 2 showing the maximum intensity and sharpness. Arabinosyl groups in HC side chains (1175 cm^{-1}) and C-O stretching corresponding to acetyl groups (1245 cm^{-1}) (BRIENZO, M; SIQUEIRA; MILAGRES, 2009; LI, Feng-he *et al.*, 2012) were not noticed in any spectra. The 1465 and 1650 cm^{-1} bands are attributed to C-H deformations and C=O (conjugated carbonyl) vibrations, respectively (SAMMONS *et al.*, 2013b). In all samples were detected these bands with slight shifting, suggesting contamination by aromatics probably come from LG. Every sample and the commercial one (XYN)

exhibited the 2906 and 3380 cm^{-1} bands, which are associated to O-H and C-H stretchings in HC, respectively (JU *et al.*, 2011).

5.3.4. Molecular weight of extracted hemicelluloses (HC)

The HC extracted from guava seed cake showed a variation of molecular weights between 14.7 and 59.3 kDa (Figure 5.2). The lowest one was similar to the one informed by Jacobs and Dahlman (2001), who extracted HC from softwood kraft pulp by sequential treatment using dimethyl sulfoxide (DMSO) and potassium hydroxide. Similarly, Borovkova *et al.* (2022) reported a molecular weight of 14.2 kDa for HC obtained from aspen wood by oxidative process (acetic acid and hydrogen peroxide medium) at 90 °C during 4h. Regarding the maximum value informed herein, this is in concordance with Farhat *et al.* (2017), who informed a similar molecular weight (64.3 kDa) for HC extracted from partially delignified switchgrass by alkaline treatment (10% NaOH solution). The variability of molecular weights is related to the solubilization and extraction different configurations of HC, and the type of waste used as source (DE FREITAS; BRIENZO, 2022b). Finally, it is important to mention that the samples G4 and G6 presented the most similar molecular weights (37.6 and 39.5 kDa, respectively) to XYN (36.0 kDa).

Figure 5.2 - Average molecular weight (MW) and polydispersity of extracted HC



MW: Molecular weight; T: Temperature; EC: Ethanol aqueous solution concentration; OHC: alkali concentration

Source: By author

5.3.5. Lignin (LG) extraction and characterization by FTIR

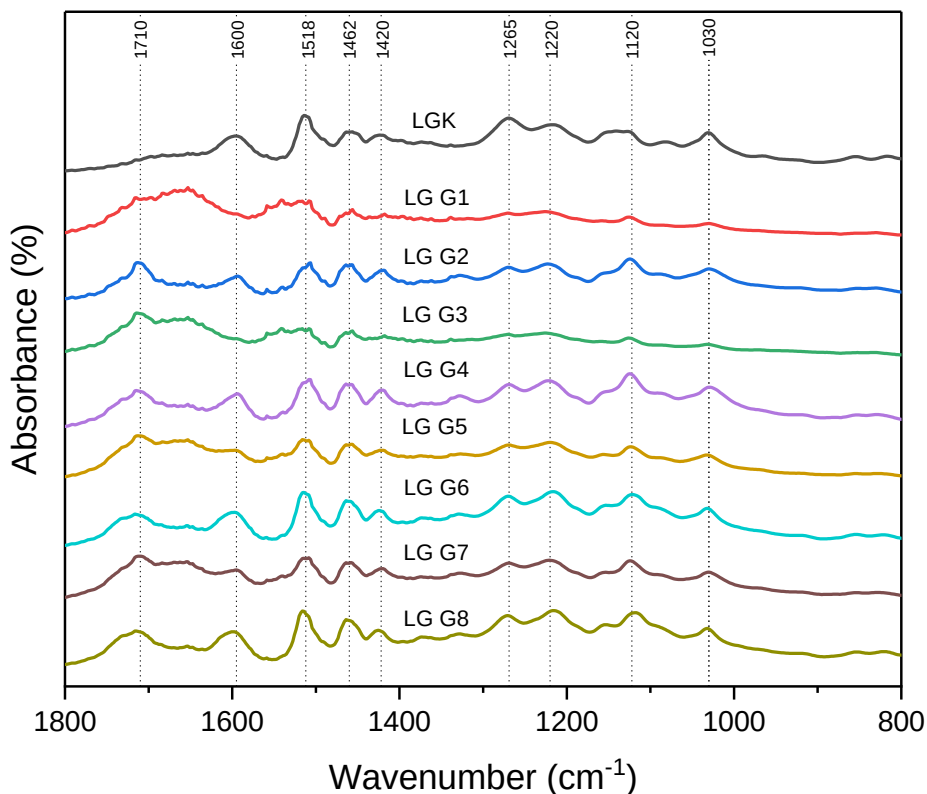
After each OHEOP configuration (Table 5-4), it was possible to extract LG from guava seed cake. Under the conditions of 170 °C, EC 70%, and OHC 60% (= 3.0 mol/L = 12.0% m/v) at the OHEOP, it was achieved the highest LGy (45% regarding the LG content in the raw waste = 18.1% regarding the total mass of the waste) and the lowest content of LG in the PB (15.6%). The corroboration between both findings could be related to the fact that the low LG content in PB suggested that LG was solubilized during pretreatment, which contributed to reach the maximum extraction yield (DE FREITAS *et al.*, 2021b). The maximum value of LGy was slightly higher than the one (42% regarding the LG content in biomass) reported by Başakçılardan and Tanış (2021), who found it from wood sawdust (biomass loading: 10% m/v) after organosolv pretreatment with glycerol and alkali-catalyzed (NaOH loading of 0.4% m/v) at identical T (170 °C) for 2 h. Similarly, Owhe *et al.* (2021) obtained a LGy of 19.0% (regarding the total mass of biomass) from rice husk but using deep eutectic solvent (acetic acid-chlorine chloride) at 155 °C for 2 h and employing a biomass loading of 10% m/m. Conversely, Mondylaksita *et al.* (2020) found that 64.9% (regarding the LG content in biomass) was the maximum LGy obtained from oil palm empty fruit bunch through organosolv pretreatment with ethanolic solution of 50%, a liquid:solid ratio of 10:1, at 210 °C, for 90 min but including 0.07% H₂SO₄ as catalyst. In this case, the high yield was achieved due to the acid route used in pretreatments is advantageous to preserve LG. Likewise, Zhong *et al.* (2021) reported 66% (regarding the LG content in biomass) as the highest LGy obtained from xylose production waste (liquid/solid ratio of 20:1) by NaOH-catalyzed (OHC of 2%) organosolv pretreatment with methanolic solution of 65%, 300 rpm of shaking, and at 100 °C during 1 h. The waste from xylose production had a content of HC around 5%, which could be one of the reasons to achieve the mentioned high yield.

According to the ANOVA indicated in the Table 5-5, T was the most significant factor affecting LGy, showing a positive influence when the pretreatment was completed at 170°C. The second factor in importance was the EC, which favored the

extraction yield when used at 70%, and the third one was the OHC that favored the LGy when kept at 60%. The result of T effect was aligned with the Vaidya *et al.* (2022) review, in which the T is considered a variable with great influence on the fractionation efficiency of LC biomass by alkali organosolv pretreatment, where temperatures between 150 and 200 °C were the most generally used in recent works. Likewise, Suriyachai *et al.* (2018) found that the T effect had the highest statistical significance on LG extraction after organosolv pretreatment, being 170 °C the temperature that provoked the best yields (71.1 - 80.2%). With respect to the EC effect, many studies have pointed out that one of the main reasons to solubilize LG in organosolv pretreatments is the solvent action. The solubilization of LG, in ethanol or methanol presence, occurs by the fracture of the connections between 4-O-methylglucuronic acid esters in HC and α -carbons in LG, and by the partition of α - and β -O-aryl ether bonds between LG units (SANNIGRAHI; RAGAUSKAS, 2013b). Maybe could suppose that a high LG content in LC wastes, such as guava seed cake (40.3%), could need high EC (70%) to solubilize and extract LG. For its part, the OHC finding was in agreement with the reported by Zhong *et al.* (2021), who indicated improvement in LGy when NaOH amounts were increased, and by Başakçılardan and Taniş (2021), who found the best LGy when NaOH was included in organosolv pretreatment. Alkaline extraction of LG happens mainly because ester connections between LG and polysaccharides (CL and HC) are fractured, which leads to obtain part of LG released, which can be solubilized by the ethanol or methanol employed in organosolv pretreatments and subsequently precipitated (SANNIGRAHI; RAGAUSKAS, 2013b; SHAH *et al.*, 2022).

The characterization of functional groups for the extracted LG was completed through FTIR spectroscopy (Figure 5.3). The region showed (800 - 1800 cm^{-1}) has been commonly used to analyze the LG structure.

Figure 5.3 - FTIR spectra of lignin (LG) extracted from guava seed cake



LGK: Kraft lignin; LG B1: Recovered lignin from banana pseudostem in experiment 1

Source: By author

According to ABD *et al.* (2022), the band at 1030 cm^{-1} corresponds to C-O(H) and C-O(C) stretchings in bonds between carbon and hydroxyl functional group (1st order aliphatic OH) present in primary alcohols and between carbon and ether bonds, respectively. All samples (including LGK and excepting G1 and G3) exhibited this band, which means probable contamination by carbohydrates. The aromatic C-H deformation in syringyl units is represented by the band 1120 cm^{-1} (SAMMONS *et al.*, 2013b), which was detected in every spectrum, however, the samples G1 and G3 revealed the lowest intensity. The C-C, C-O and C=O stretches in guaiacyl rings corresponds to the band 1220 cm^{-1} (SAMMONS *et al.*, 2013b), which exhibited low absorbances in the G1 and G3 samples. The 1265 cm^{-1} signal denotes guaiacyl ring stretching vibration (INKROD *et al.*, 2018); the samples G1 and G3 indicated the lowest intensities. C-C stretching in the aromatic skeletal of LG is represented by the band at 1420 cm^{-1} (ABD *et al.*, 2022); G1 and G3 did not showed this type of absorbance. Every sample (excluding G1 and G3) shown C-H asymmetric deformation (in $-\text{CH}_3$ and $-\text{CH}_2-$) in LG and guaiacyl ring stretching, which are denoted by 1462 and 1518 cm^{-1}

bands, respectively (INKROD *et al.*, 2018; SAMMONS *et al.*, 2013b). Finally, the C=O stretching vibration in aromatic rings and unconjugated C=O stretching in phenyl ester correspond to the bands 1600 and 1710 cm^{-1} , respectively (ABD *et al.*, 2022; INKROD *et al.*, 2018; SAMMONS *et al.*, 2013b). G1, G3, and G5 did not showed the C=O stretching vibration, and for LGK and G1 the unconjugated C=O stretching was not detected.

5.3.6. Biomass digestibility through enzymatic hydrolysis

Table 5-4 shows that all CLX achieved after enzymatic hydrolysis of the waste pretreated in each organosolv set up were greater than the value obtained for the raw waste. Thereby, it was possible to confirm that the use of pretreatment on guava seed cake was necessary to overcome recalcitrance, which allowed to extract HC and LG in the following processes. After hydrolysis of the biomass pretreated under the conditions of 170 °C, EC 30%, and OHC 60%, it was achieved the maximum CLX (50.3% = 150 mg/g) and one of the lowest LG contents (36.2%) in the PB. This finding was in accordance to the Singh *et al.* (2022) work, in which an increase in cellulose conversion values, observed after enzymatic hydrolysis of brown midrib sorghums pretreated by an alkaline process, occurred when the PB had reductions in LG contents caused by the pretreatment. On the other hand, the highest CLX reported in the present work was higher than the value informed (~ 130 mg/g) by Weerasai *et al.* (2021), who employed pretreated sugarcane bagasse, identical commercial enzyme (Cellic® CTec2 dosage = 10 FPU/g), a temperature of 50 °C, a reaction time of 72 h, and a vertical agitation of 30 rpm. The pretreatment used was organosolv (biomass loading of 10% m/v) for 30 min in methanol medium (unknown concentration) at 120 °C but employing triethylamine as catalyst (5% m/v) and stirring of 100 rpm. Conversions of cellulose inferior to ~ 70 mg/g indicate enzymatic hydrolysis of low performance, while values superior to 75 - 85% indicate hydrolysis of high performance (ANTCZAK *et al.*, 2018; BATTISTA; BOLZONELLA, 2018). Thus, CLX values of 130 and 150 mg/g could be considered as indicators of enzymatic hydrolysis of intermediate performance. The digestibility of pretreated LC biomasses does not exclusively depend on their LG contents, but also on other factors such as crystallinity of cellulose, porosity and surface area of pretreated fibers, swelling and polymerization

degrees of cellulose and HC, LG remaining quantities in fractions after pretreatment, and inhibition caused by molecules released (cellobiose and glucose) by the cellulases themselves (BATTISTA; BOLZONELLA, 2018; SHARMA; SAINI, 2020b). Additionally, it is worth remarking that the NaOH exhibits higher pretreatment efficiencies on low-content biomasses of LG, which potentially could affect the performance of the enzymatic hydrolysis (KANDHOLA *et al.*, 2017b).

Based on the ANOVA (Table 5-5), all factors and their interactions were significant on CLX after hydrolysis was completed; T, EC and OHC favored the conversions of PB when the pretreatment was performed at 170°C, 30% and 60%, respectively. The T result had coherence with the Vaidya *et al.* (2022) review, in which 170°C was marked as a favorable temperature at pretreatment stage for a production of monosaccharides after enzymatic hydrolysis of biomass pretreated by distinct routes. Regarding to employ EC of 30%, this had correspondence with the Lesar *et al.* (2016) work, in which a similar EC (35%) during ethanolic organosolv pretreatment (non-catalyzed, 220 °C and 3 h) allowed the maximum conversion (71.4%) after enzymatic hydrolysis (Celluclast 1.5FP) using pretreated recycled wood.

5.4. Conclusions

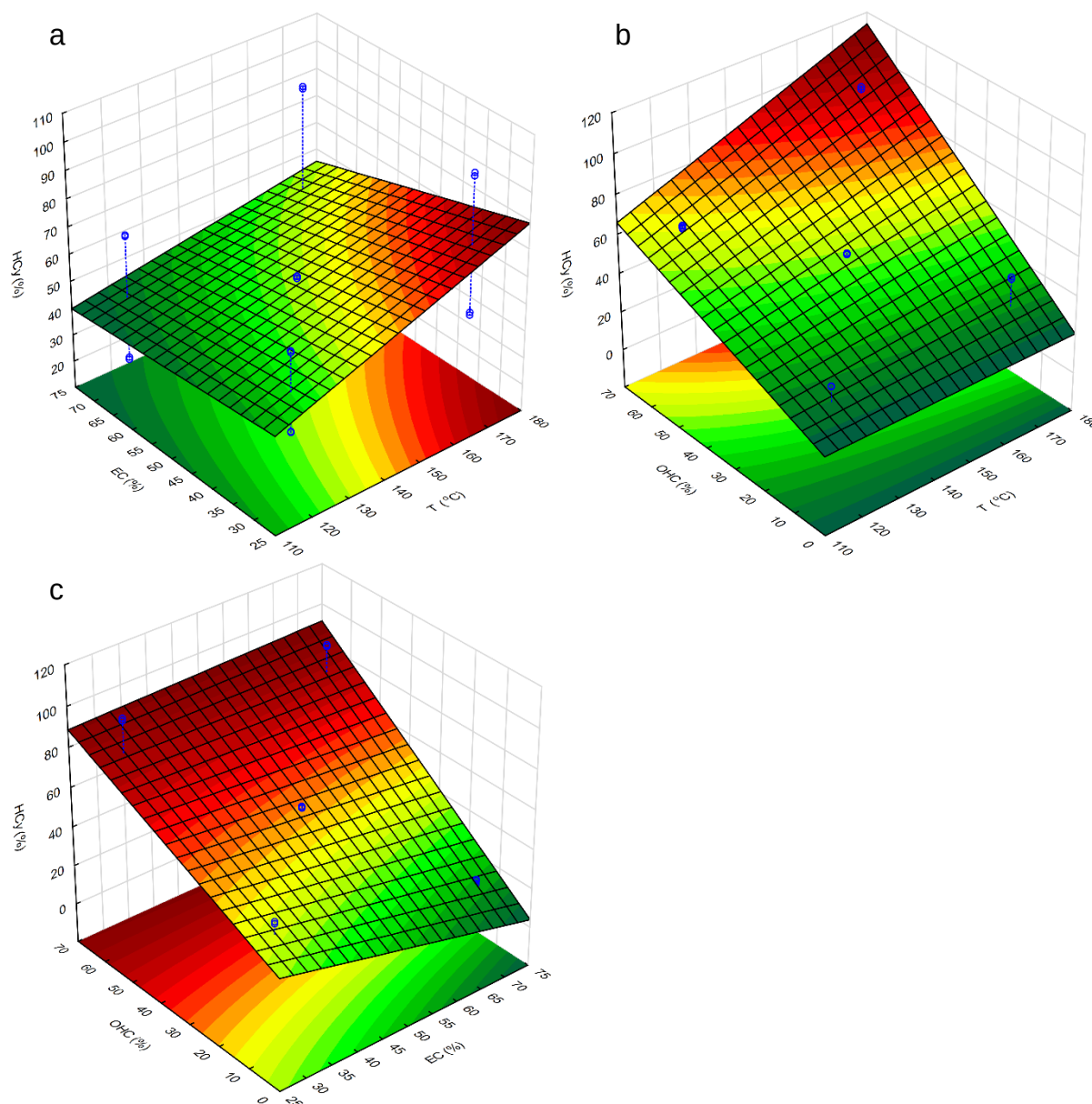
From the points of view of LC composition and LGy, guava seed cake could be considered a suitable source to obtain LG since the raw waste showed high LG content and the extraction yield achieved could be considered intermediate. OHC exhibited the highest significance on HCy, and in the case of LGy and CLX, T had the greatest significance. The consecutive stages of ethanol addition (to spent liquor) and ethanol evaporation (after HC extraction) guaranteed the successive recovery of HC and LG. Although the HC content in the raw waste was low, the HCy was high, and this yield was achieved using the PB with the lowest content of HC among all LC characterizations. Based on the FTIR spectroscopy analysis, each extracted HC and LG revealed an excellent similarity to the commercial ones, respectively. In the HC case, the existence of arabinan and xylan was detected in almost all the samples, however, arabinosyl groups were not noted in any of them (commercial and extracted). And, for the LG case, all bands representing some types of aromatic structure were detected in almost all samples.

5.5. Supplementary Information

List of figures

Figure S5.1	Response surfaces for HCy at EC and T (a), OHC and T (b), and OHC and EC (c)
Figure S5.2	Response surfaces for LGy at EC and T (a), OHC and T (b), and OHC and EC (c)
Figure S5.3	Response surfaces for CLX at EC and T (a), OHC and T (b), and OHC and EC (c)

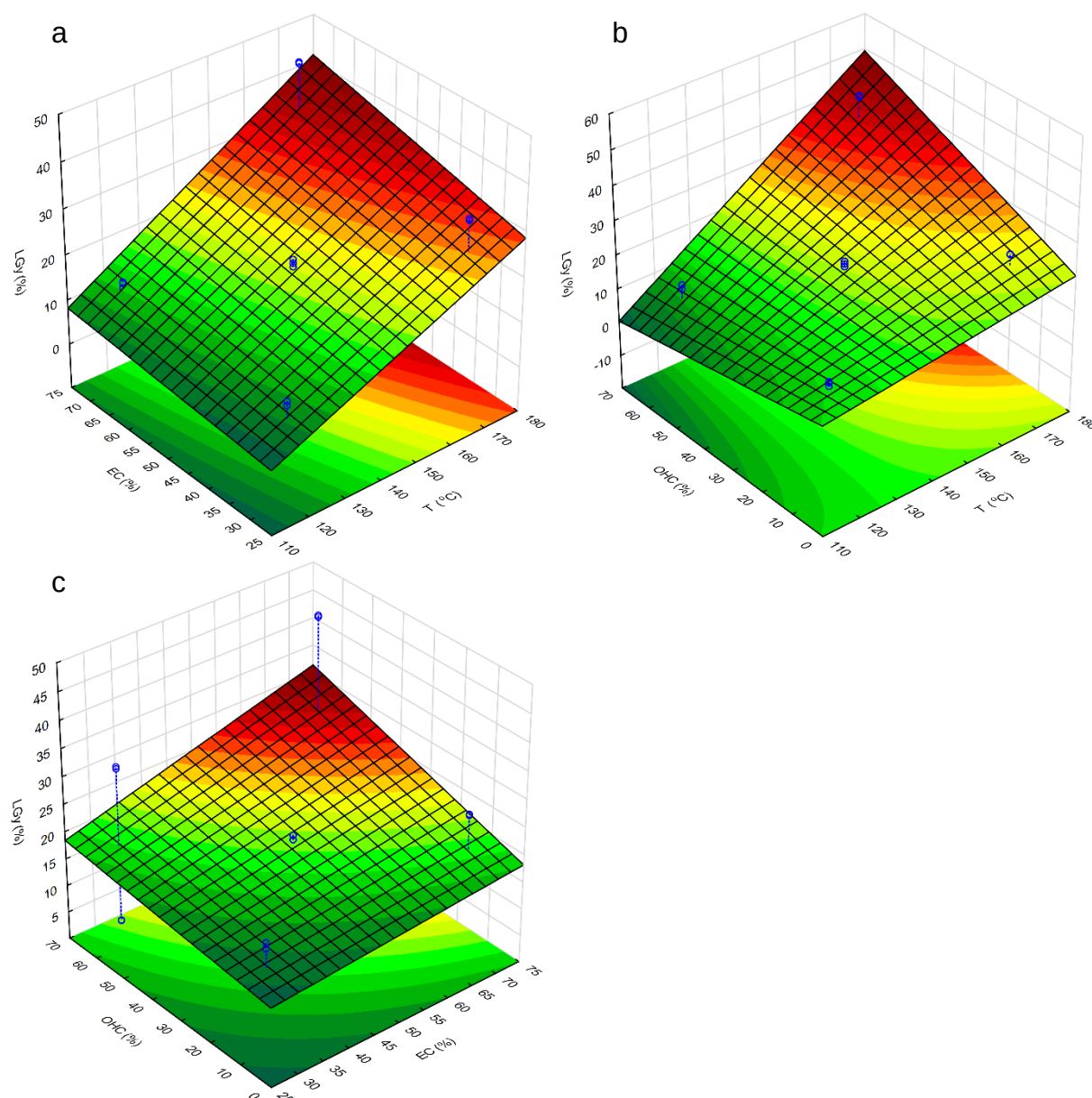
Figure S5.1 - Response surfaces for HCy at EC and T (a), OHC and T (b), and OHC and EC (c)



T: Temperature; EC: Ethanol aqueous solution concentration; OHC: Alkali concentration; HCy: Extraction yield of hemicelluloses

Source: By author

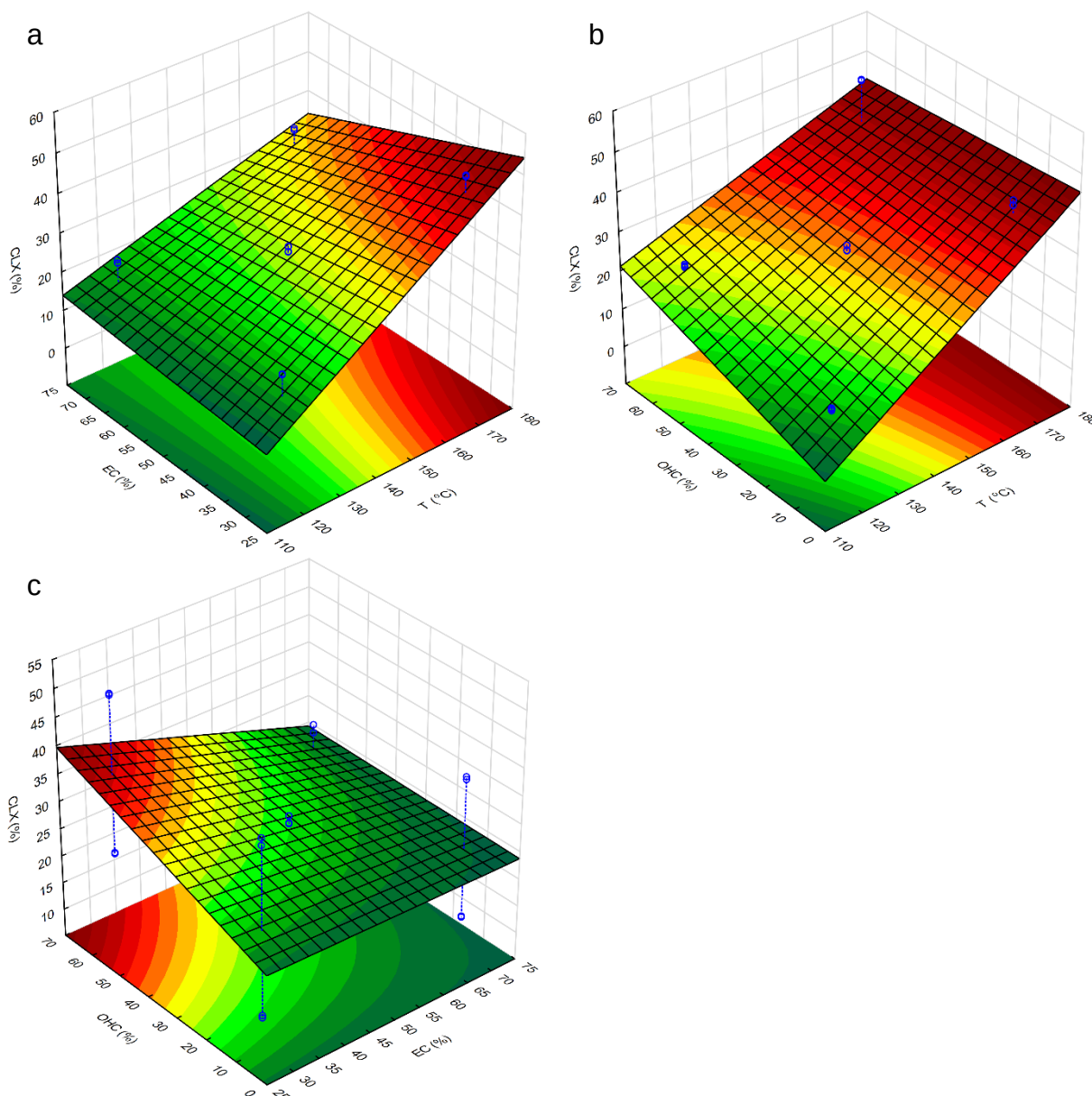
Figure S5.2 - Response surfaces for LGy at EC and T (a), OHC and T (b), and OHC and EC (c)



T: Temperature; EC: Ethanol aqueous solution concentration; OHC: Alkali concentration; LGy: Extraction yield of lignin

Source: By author

Figure 5S.3 - Response surfaces for CLX at EC and T (a), OHC and T (b), and OHC and EC (c)



T: Temperature; EC: Ethanol aqueous solution concentration; OHC: Alkali concentration; CLX: Cellulose conversion.

Source: By author

References

- ABD, N. H.; BROSSE, N.; ZIEGLER-DEVIN, I.; CHRUSIEL, L.; HASHIM, R.; HUSSIN, M. H. A Comparison of Alkaline and Organosolv Lignin Extraction Methods from Coconut Husks as an Alternative Material for Green Applications. **BioResources**, Raleigh, vol. 17, no. 1, p. 469–491, 2022. DOI 10.15376/biores.17.1.469-491. Available at: <https://bioresources.cnr.ncsu.edu/issues/vol17-issue1/page/4/>. Accessed on: 17 Aug. 2022.
- ALVES, J. L. F.; DA SILVA, J. C. G.; MUMBACH, G. D.; DOMENICO, M. Di; DE SENA, R. F.; MACHADO, R. A. F.; MARANGONI, C. Demonstrating the Suitability of Tamarind Residues to Bioenergy Exploitation Via Combustion Through Physicochemical Properties, Performance Indexes, and Emission Characteristics. **BioEnergy Research**, Berlin, vol. 13, no. 4, p. 1308–1320, 2020. DOI 10.1007/s12155-020-10158-z. Available at: <https://link.springer.com/article/10.1007/s12155-020-10158-z>. Accessed on: 23 Mar. 2023.
- ANGULO-LÓPEZ, J. E.; FLORES-GALLEGOS, A. C.; TORRES-LEÓN, C.; RAMÍREZ-GUZMÁN, K. N.; MARTÍNEZ, G. A.;

AGUILAR, C. N. Guava (*Psidium guajava* L.) Fruit and Valorization of Industrialization By-Products. **Processes**, Basel, vol. 9, no. 6, p. 1–17, 2021. DOI 10.3390/pr9061075. Available at: <https://www.mdpi.com/2227-9717/9/6/1075>. Accessed on: 19 Jul. 2022.

ANTCZAK, A.; MARCHWICKA, M.; SZADKOWSKI, J.; DROZDZEK, M.; GAWRON, J.; RADOMSKI, A.; ZAWADZKI, J. Sugars yield obtained after acid and enzymatic hydrolysis of fast-growing poplar wood species. **BioResources**, Raleigh, vol. 13, no. 4, p. 8629–8645, 2018. DOI 10.15376/biores.13.4.8629-8645. Available at: <https://bioresources.cnr.ncsu.edu/issues/vol13-issue4/page/11/>. Accessed on: 23 Dec. 2022.

BAŞAKÇILARDAN KABAKCI, S.; TANIŞ, M. H. Pretreatment of lignocellulosic biomass at atmospheric conditions by using different organosolv liquors: a comparison of lignins. **Biomass Conversion and Biorefinery**, Berlin, vol. 11, no. 6, p. 2869–2880, 2021. DOI 10.1007/s13399-020-00677-2. Available at: <https://link.springer.com/article/10.1007/s13399-020-00677-2>. Accessed on: 3 Mar. 2023.

BATTISTA, F.; BOLZONELLA, D. Some critical aspects of the enzymatic hydrolysis at high dry-matter content: a review. **Biofuels, Bioproducts and Biorefining**, Hoboken, vol. 13, no. 4, p. 711–723, 1 Jul. 2018. DOI 10.1002/bbb.1883. Available at: <https://onlinelibrary.wiley.com/doi/10.1002/bbb.1883>. Accessed on: 23 Dec. 2022.

BIBWE, B.; MAHAWAR, M. K.; JALGAONKAR, K.; MEENA, V. S.; KADAM, D. M. Mass modeling of guava (cv. Allahabad safeda) fruit with selected dimensional attributes: Regression analysis approach. **Journal of Food Process Engineering**, Hoboken, vol. 45, no. 3, p. 1–11, 1 Mar. 2022. DOI 10.1111/jfpe.13978. Available at: <https://onlinelibrary.wiley.com/doi/full/10.1111/jfpe.13978>. Accessed on: 21 Feb. 2023.

BOROVKOVA, V. S.; MALYAR, Y. N.; SUDAKOVA, I. G.; CHUDINA, A. I.; ZIMONIN, D. V.; SKRIPNIKOV, A. M.; MIROSHNIKOVA, A. V.; IONIN, V. A.; KAZACHENKO, A. S.; SYCHEV, V. V.; PONOMAREV, I. S.; ISSAOUI, N. Composition and Structure of Aspen (*Picea pulchra* Mill.) Hemicelluloses Obtained by Oxidative Delignification. **Polymers**, Basel, vol. 14, no. 21, p. 1–18, 2022. DOI 10.3390/polym14214521. Available at: <https://www.mdpi.com/2073-4360/14/21/4521>. Accessed on: 30 Dec. 2023.

BRIENZO, M.; SIQUEIRA, A. F.; MILAGRES, A. M. F. Search for optimum conditions of sugarcane bagasse hemicellulose extraction. **Biochemical Engineering Journal**, Amsterdam, vol. 46, no. 2, p. 199–204, 2009. DOI 10.1016/j.bej.2009.05.012. Available at: <https://www.sciencedirect.com/science/article/pii/S1369703X09001569>. Accessed on: 29 Sep. 2021.

DE FREITAS, C.; BRIENZO, M. Enzymatic Hydrolysis Applied to Banana Pseudostem Biomass Compared to Solubilized Xylan for Xylooligosaccharides Production with High Substrate Concentration. **BioEnergy Research**, Amsterdam, , p. 1–11, 2022. DOI 10.1007/s12155-022-10508-z. Available at: <https://link.springer.com/article/10.1007/s12155-022-10508-z>. Accessed on: 22 Mar. 2023.

DE FREITAS, E.; KHATRI, V.; WU, J.; TAKADA, M.; SCARCELLA, A. S. de A.; MARTINEZ, C. A.; SADDLER, J. N.; POLIZELI, M. de L. T. M. Structural and compositional changes induced by hydrothermal and organosolv pretreatments impacts enzymatic hydrolysis of a tropical forage grass grown under future climate conditions. **Industrial Crops and Products**, Amsterdam, vol. 171, p. 1–13, 2021. DOI 10.1016/j.indcrop.2021.113937. Available at: <https://www.sciencedirect.com/science/article/pii/S0926669021007019>. Accessed on: 24 Mar. 2023.

FARHAT, W.; VENDITTI, R.; QUICK, A.; TAHA, M.; MIGNARD, N.; BECQUART, F.; AYOUB, A. Hemicellulose extraction and characterization for applications in paper coatings and adhesives. **Industrial Crops and Products**, Amsterdam, vol. 107, p. 370–377, 2017. DOI 10.1016/j.indcrop.2017.05.055. Available at: <https://www.sciencedirect.com/science/article/pii/S0926669017303758>. Accessed on: 30 Dec. 2023.

FERREIRA, J. A.; TAHERZADEH, M. J. Improving the economy of lignocellulose-based biorefineries with organosolv pretreatment. **Bioresource Technology**, Amsterdam, vol. 299, p. 1–13, 2020. DOI 10.1016/j.biortech.2019.122695. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852419319248>. Accessed on: 2 Jul. 2022.

GALLINA, G.; CABEZA, Á.; GRÉNMAN, H.; BIASI, P.; GARCÍA-SERNA, J.; SALMI, T. Hemicellulose extraction by hot pressurized water pretreatment at 160°C for 10 different woods: Yield and molecular weight. **The Journal of Supercritical Fluids**, Amsterdam, vol. 133, no. Part 2, p. 716–725, 2018. DOI 10.1016/j.supflu.2017.10.001. Available at: <https://www.sciencedirect.com/science/article/pii/S0896844617303819>. Accessed on: 3 Aug. 2022.

GOWMAN, A. C.; PICARD, M.; RODRIGUEZ-URIBE, A.; MISRA, M.; KHALIL, H.; THIMMANAGARI, M.; MOHANTY, A. K. Physicochemical Analysis of Apple and Grape Pomaces. **BioResources**, Raleigh, vol. 14, no. 2, p. 3210–3230, 2019. DOI 10.15376/biores.14.2.3210-3230. Available at: <https://bioresources.cnr.ncsu.edu/issues/vol14-issue2/page/6/>. Accessed on: 22 Jul. 2022.

GÜZEL, M.; AKPINAR, Ö. Valorisation of fruit by-products: Production characterization of pectins from fruit peels. **Food and Bioproducts Processing**, Amsterdam, vol. 115, p. 126–133, 2019. DOI 10.1016/j.fbp.2019.03.009. Available at: <https://www.sciencedirect.com/science/article/pii/S0960308518308927>. Accessed on: 20 Dec. 2023.

HANIM, S. S.; NORSYABILAH, R.; SUHAILA, M. H. N.; NORAISHAH, A.; KARTINA, A. K. S. Effects of Temperature, Time and Pressure on the Hemicelluloses Yield Extracted Using Subcritical Water Extraction. **Procedia Engineering**, Amsterdam, vol. 42, p. 562–565, 2012. DOI 10.1016/j.proeng.2012.07.448. Available at: <https://www.sciencedirect.com/science/article/pii/S1877705812028482>. Accessed on: 5 Aug. 2022.

HUANG, C.; HUANG, C.; LAI, C.; WU, X.; HUANG, Y.; HE, J.; LI, X.; YONG, Q. Strategy to utilize the high ash content biomass feedstock for fermentable sugars. **BioResources**, Raleigh, vol. 12, no. 4, p. 8306–8319, 2017. DOI 10.15376/biores.12.4.8306-8319. Available at: <https://bioresources.cnr.ncsu.edu/issues/volume-12-issue-4/page/11/>. Accessed on: 25 Jul. 2022.

INKROD, C.; RAITA, M.; CHAMPREDA, V.; LAOSIROPOJANA, N. Characteristics of Lignin Extracted from Different Lignocellulosic Materials via Organosolv Fractionation. **BioEnergy Research**, Berlin, vol. 11, no. 2, p. 277–290, 2018. DOI 10.1007/s12155-018-9895-2. Available at: <https://link.springer.com/article/10.1007/s12155-018-9895-2>. Accessed on: 17 Aug. 2022.

JACOBS, A.; DAHLMAN, O. Characterization of the Molar Masses of Hemicelluloses from Wood and Pulps Employing Size Exclusion Chromatography and Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry. **Biomacromolecules**, Washington, vol. 2, no. 3, p. 894–905, 1 Sep. 2001. DOI 10.1021/bm010050b. Available at: <https://pubs.acs.org/doi/10.1021/bm010050b>. Accessed on: 30 Dec. 2023.

JAVIER-ASTETE, R.; JIMENEZ-DAVALOS, J.; ZOLLA, G. Determination of hemicellulose, cellulose, holocellulose and lignin content using FTIR in *Calycophyllum spruceanum* (Benth.) K. Schum. and *Guazuma crinita* Lam. **PLOS ONE**, San Francisco, vol. 16, no. 10, p. 1–12, 27 Oct. 2021. DOI 10.1371/journal.pone.0256559. Available at: <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0256559>. Accessed on: 20 Jul. 2022.

JU, Y.-H.; HUYNH, L.-H.; KASIM, N. S.; GUO, T.-J.; WANG, J.-H.; FAZARY, A. E. Analysis of soluble and insoluble fractions of alkali and subcritical water treated sugarcane bagasse. **Carbohydrate Polymers**, Amsterdam, vol. 83, no. 2, p. 591–599, 2011. DOI 10.1016/j.carbpol.2010.08.022. Available at: <https://www.sciencedirect.com/science/article/pii/S0144861710006429>. Accessed on: 11 Aug. 2022.

KANDHOLA, G.; DJIOLEU, A.; CARRIER, D. J.; KIM, J.-W. Pretreatments for Enhanced Enzymatic Hydrolysis of Pinewood: a Review. **BioEnergy Research**, Berlin, vol. 10, no. 4, p. 1138–1154, 2017. DOI 10.1007/s12155-017-9862-3. Available at: <https://link.springer.com/article/10.1007/s12155-017-9862-3>. Accessed on: 22 Dec. 2022.

KLAMRASSAMEE, T.; CHAMPREDA, V.; REUNGLEK, V.; LAOSIRIPOJANA, N. Comparison of homogeneous and heterogeneous acid promoters in single-step aqueous-organosolv fractionation of eucalyptus wood chips. **Bioresource Technology**, Amsterdam, vol. 147, p. 276–284, 2013. DOI 10.1016/j.biortech.2013.08.015. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852413012443>. Accessed on: 28 Sep. 2022.

KOUTSOUKIS, C.; ROUKOS, C.; DEMERTZIS, P. G.; KANDRELIS, S.; AKRIDA-DEMERTZI, K. The variation of the chemical composition of the main plant species in a subalpine grassland in northwestern Greece. **Legume Science**, Hoboken, vol. 1, no. 1, p. 1–11, 2019. DOI 10.1002/leg3.23. Available at: <https://onlinelibrary.wiley.com/doi/full/10.1002/leg3.23>. Accessed on: 21 Dec. 2022.

LARGITTE, L.; BRUDEY, T.; TANT, T.; DUMESNIL, P. C.; LODEWYCKX, P. Comparison of the adsorption of lead by activated carbons from three lignocellulosic precursors. **Microporous and Mesoporous Materials**, Amsterdam, vol. 219, p. 265–275, 2016. DOI 10.1016/j.micromeso.2015.07.005. Available at: <https://www.sciencedirect.com/science/article/pii/S1387181115003844>. Accessed on: 23 Mar. 2023.

LESAR, B.; HUMAR, M.; HORA, G.; HACHMEISTER, P.; SCHMIEDL, D.; PINDEL, E.; SIKA-AHO, M.; LIITIÄ, T. Utilization of recycled wood in biorefineries: preliminary results of steam explosion and ethanol/water organosolv pulping without a catalyst. **European Journal of Wood and Wood Products**, Berlin, vol. 74, no. 5, p. 711–723, 2016. DOI 10.1007/s00107-016-1064-8. Available at: <https://link.springer.com/article/10.1007/s00107-016-1064-8>. Accessed on: 23 Dec. 2022.

LI, D.; LONG, L.; DING, S. Alkaline organosolv pretreatment of different sorghum stem parts for enhancing the total reducing sugar yields and p-coumaric acid release. **Biotechnology for Biofuels**, Berlin, vol. 13, no. 1, p. 1–14, 2020. DOI 10.1186/s13068-020-01746-4. Available at: <https://link.springer.com/article/10.1186/s13068-020-01746-4>. Accessed on: 14 Aug. 2022.

LI, F.; HU, H.; YAO, R.; WANG, H.; LI, M. Structure and Saccharification of Rice Straw Pretreated with Microwave-Assisted Dilute Lye. **Industrial & Engineering Chemistry Research**, Washington, vol. 51, no. 17, p. 6270–6274, 2 May 2012. DOI 10.1021/ie202547w. Available at: <https://pubs.acs.org/doi/10.1021/ie202547w>. Accessed on: 11 Aug. 2022.

LIU, X.; RENARD, C. M. G. C.; BUREAU, S.; LE BOURVELLEC, C. Revisiting the contribution of ATR-FTIR spectroscopy to characterize plant cell wall polysaccharides. **Carbohydrate Polymers**, Amsterdam, vol. 262, p. 1–10, 2021. DOI 10.1016/j.carbpol.2021.117935. Available at: <https://www.sciencedirect.com/science/article/pii/S0144861721003222>. Accessed on: 11 Aug. 2022.

LU, Y.; HE, Q.; FAN, G.; CHENG, Q.; SONG, G. Extraction and modification of hemicellulose from lignocellulosic biomass: A review. **Green Processing and Synthesis**, Berlin, vol. 10, no. 1, p. 779–804, 2021. DOI 10.1515/gps-2021-0065. Available at: <https://www.degruyter.com/document/doi/10.1515/gps-2021-0065/html>. Accessed on: 5 Aug. 2022.

MA, Y.; LUO, J.; XU, Y. Co-preparation of pectin and cellulose from apple pomace by a sequential process. **Journal of Food Science and Technology**, Amsterdam, vol. 56, no. 9, p. 4091–4100, 2019. DOI 10.1007/s13197-019-03877-5. Available at: <https://link.springer.com/article/10.1007/s13197-019-03877-5>. Accessed on: 23 Mar. 2023.

MANHONGO, T. T.; CHIMPHANGO, A. F. A.; THORNLEY, P.; RÖDER, M. Current status and opportunities for fruit processing waste biorefineries. **Renewable and Sustainable Energy Reviews**, Amsterdam, vol. 155, p. 1–19, 2022. DOI 10.1016/j.rser.2021.111823. Available at: <https://www.sciencedirect.com/science/article/pii/S1364032121010911>. Accessed on: 7 Jul. 2022.

MONDYLAKSITA, K.; FERREIRA, J. A.; MILLATI, R.; BUDHIJANTO, W.; NIKLASSON, C.; TAHERZADEH, M. J. Recovery of High Purity Lignin and Digestible Cellulose from Oil Palm Empty Fruit Bunch Using Low Acid-Catalyzed Organosolv Pretreatment. **Agronomy**, Basel, vol. 10, no. 5, p. 1–15, 2020. DOI 10.3390/agronomy10050674. Available at: <https://www.mdpi.com/2073-4395/10/5/674>. Accessed on: 31 Dec. 2023.

MONTGOMERY, D. **Design and Analysis of Experiments**. 5th ed. New York: John Wiley & Sons, Ltd, 2001.

OREJUELA-ESCOBAR, L. M.; LANDÁZURI, A. C.; GOODELL, B. Second generation biorefining in Ecuador: Circular bioeconomy, zero waste technology, environment and sustainable development: The nexus. **Journal of Bioresources and Bioproducts**, Amsterdam, vol. 6, no. 2, p. 83–107, 2021. DOI 10.1016/j.jobab.2021.01.004. Available at: <https://www.sciencedirect.com/science/article/pii/S2369969821000050>. Accessed on: 19 Jul. 2022.

OWHE, E. O.; KUMAR, N.; LYNAM, J. G. Lignin extraction from waste biomass with deep eutectic solvents: Molecular weight and heating value. **Biocatalysis and Agricultural Biotechnology**, Amsterdam, vol. 32, p. 1–7, 2021. DOI 10.1016/j.bcab.2021.101949. Available at: <https://www.sciencedirect.com/science/article/pii/S1878818121000451>. Accessed on: 31 Mar. 2023.

PEREIRA, B. S.; DE FREITAS, C.; VIEIRA, R. M.; BRIENZO, M. Brazilian banana, guava, and orange fruit and waste production as a potential biorefinery feedstock. **Journal of Material Cycles and Waste Management**, Berlin, vol. 24, no. 6, p. 2126–2140, 2022. DOI 10.1007/s10163-022-01495-6. Available at: <https://link.springer.com/article/10.1007/s10163-022-01495-6>. Accessed on: 21 Dec. 2023.

PUIŢEL, A. C.; SUDITU, G. D.; DANU, M.; AILIESEI, G.-L.; NECHITA, M. T. An Experimental Study on the Hot Alkali Extraction of Xylan-Based Hemicelluloses from Wheat Straw and Corn Stalks and Optimization Methods. **Polymers**, Basel, vol. 14, no. 9, p. 1–17, 2022. DOI 10.3390/polym14091662. Available at: <https://www.mdpi.com/2073-4360/14/9/1662>. Accessed on: 22 Jul. 2022.

RABETAFIKA, H. N.; BCHIR, B.; BLECKER, C.; PAQUOT, M.; WATHELET, B. Comparative study of alkaline extraction process of hemicelluloses from pear pomace. **Biomass and Bioenergy**, Amsterdam, vol. 61, p. 254–264, 2014. DOI 10.1016/j.biombioe.2013.12.022. Available at: <https://www.sciencedirect.com/science/article/pii/S0961953413005357>. Accessed on: 23 Mar. 2023.

RAHMAN, I.; SAAD, B. Utilization of Guava Seeds as a Source of Activated Carbon for Removal of Methylene Blue from Aqueous Solution. **Malaysian Journal of Chemistry**, Kuala Lumpur, vol. 5, no. 1, p. 8–14, 2003. .

REYES, P.; TEIXEIRA MENDONÇA, R.; RODRÍGUEZ, J.; FARDIM, P.; VEGA, B. Characterization of the hemicellulosic fraction obtained after pre-hydrolysis of pinus radiata wood chips with hot-water at different initial pH. **Journal of the Chilean Chemical Society**, Concepción, vol. 58, no. 1, p. 1614–1618, 2013. DOI 10.4067/S0717-97072013000100024. Available at: <https://www.scielo.cl/pdf/jcchems/v58n1/art24.pdf>. Accessed on: 5 Aug. 2022.

SAMMONS, R. J.; HARPER, D. P.; LABBÉ, N.; BOZELL, J. J.; ELDER, T.; RIALS, T. G. Characterization of organosolv lignins using thermal and FT-IR spectroscopic analysis. **BioResources**, Raleigh, vol. 8, no. 2, p. 2752–2767, 2013. Available at: <https://bioresources.cnr.ncsu.edu/issues/vol8-issue2/page/11/>. Accessed on: 12 Nov. 2022.

SANNIGRAHI, P.; RAGAUSKAS, A. J. Fundamentals of Biomass Pretreatment by Fractionation. In: WYMAN, C. E. (ed.). **Aqueous Pretreatment of Plant Biomass for Biological and Chemical Conversion to Fuels and Chemicals**. Wiley Online

Books. Hoboken: John Wiley & Sons, 2013. p. 201–222. DOI 10.1002/9780470975831.ch10. Available at: <https://onlinelibrary.wiley.com/doi/10.1002/9780470975831.ch10>. Accessed on: 16 Dec. 2022.

SANTOS, T. M.; RIGUAL, V.; DOMÍNGUEZ, J. C.; ALONSO, M. V.; OLLET, M.; RODRIGUEZ, F. Fractionation of *Pinus radiata* by ethanol-based organosolv process. **Biomass Conversion and Biorefinery**, Berlin, , p. 1–14, 2022. DOI 10.1007/s13399-022-02329-z. Available at: <https://link.springer.com/article/10.1007/s13399-022-02329-z>. Accessed on: 8 Aug. 2022.

SCHMATZ, A. A.; SALAZAR-BRYAM, A. M.; CONTIERO, J.; SANT'ANNA, C.; BRIENZO, M. Pseudo-Lignin Content Decreased with Hemicellulose and Lignin Removal, Improving Cellulose Accessibility, and Enzymatic Digestibility. **BioEnergy Research**, Berlin, vol. 14, no. 1, p. 106–121, 2021. DOI 10.1007/s12155-020-10187-8. Available at: <https://link.springer.com/article/10.1007/s12155-020-10187-8>. Accessed on: 23 Feb. 2023.

SHAH, A. A.; SEEHAR, T. H.; SHARMA, K.; TOOR, S. S. Chapter 7 - Biomass pretreatment technologies. In: MAITY, S. K.; GAYEN, K.; BHOWMICK, T. K. B. T.-H. B. (eds.). **Hydrocarbon Biorefinery: Sustainable Processing of Biomass for Hydrocarbon Biofuels**. Amsterdam: Elsevier, 2022. p. 203–228. DOI 10.1016/B978-0-12-823306-1.00014-5. Available at: <https://www.sciencedirect.com/science/article/pii/B9780128233061000145>. Accessed on: 17 Aug. 2022.

SHARMA, D.; SAINI, A. Pretreatment Technologies for Biomass Deconstruction. In: SHARMA, D.; SAINI, A. (eds.). **Lignocellulosic Ethanol Production from a Biorefinery Perspective: Sustainable Valorization of Waste**. Singapore: Springer Singapore, 2020. p. 65–109. DOI 10.1007/978-981-15-4573-3_3. Available at: https://link.springer.com/chapter/10.1007/978-981-15-4573-3_3. Accessed on: 23 Dec. 2022.

SHIMIZU, F. L.; DE AZEVEDO, G. O.; COELHO, L. F.; PAGNOCCA, F. C.; BRIENZO, M. Minimum Lignin and Xylan Removal to Improve Cellulose Accessibility. **BioEnergy Research**, Berlin, vol. 13, no. 3, p. 775–785, 2020. DOI 10.1007/s12155-020-10120-z. Available at: <https://link.springer.com/article/10.1007/s12155-020-10120-z>. Accessed on: 1 May 2022.

SHIMIZU, F. L.; ZAMORA, H. D. Z.; SCHMATZ, A. A.; MELATI, R. B.; BUENO, D.; BRIENZO, M. Biofuels Generation Based on Technical Process and Biomass Quality. In: SRIVASTAVA, N.; SRIVASTAVA, M.; MISHRA, P. K.; GUPTA, V. K. (eds.). **Biofuel Production Technologies: Critical Analysis for Sustainability**. Singapore: Springer Singapore, 2020. p. 37–64. DOI 10.1007/978-981-13-8637-4_2. Available at: https://link.springer.com/chapter/10.1007/978-981-13-8637-4_2. Accessed on: 21 Dec. 2023.

SINGH, R. D.; BANERJEE, J.; SASMAL, S.; MUIR, J.; ARORA, A. High xylan recovery using two stage alkali pre-treatment process from high lignin biomass and its valorisation to xylooligosaccharides of low degree of polymerisation. **Bioresource Technology**, Amsterdam, vol. 256, p. 110–117, 2018. DOI 10.1016/j.biortech.2018.02.009. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852418301834>. Accessed on: 5 Aug. 2022.

SINGH, S. K.; SAULNIER, B. K.; HODGE, D. B. Lignin properties and cell wall response to deconstruction by alkaline pretreatment and enzymatic hydrolysis in brown midrib sorghums. **Industrial Crops and Products**, Amsterdam, vol. 178, p. 1–8, 2022. DOI 10.1016/j.indcrop.2022.114566. Available at: <https://www.sciencedirect.com/science/article/pii/S0926669022000498>. Accessed on: 22 Dec. 2022.

SLUITER, A.; HAMES, B.; RUIZ, R.; SCARLATA, C.; SLUITER, J.; TEMPLETON, D. **Determination of Ash in Biomass**, n. NREL/TP-510-42622. Golden: U.S. Department of Energy, 2008. Available at: <https://www.nrel.gov/docs/gen/fy08/42622.pdf>. Accessed on: 20 Jul. 2022.

SLUITER, A.; HAMES, B.; RUIZ, R.; SCARLATA, C.; SLUITER, J.; TEMPLETON, D.; CROCKER, D. **Determination of Structural Carbohydrates and Lignin in Biomass**, n. NREL/TP-510-42618. Golden: U.S. Department of Energy, 2012. Available at: <https://www.nrel.gov/docs/gen/fy13/42618.pdf>. Accessed on: 28 Sep. 2022.

SLUITER, A.; RUIZ, R.; SCARLATA, C.; SLUITER, J.; TEMPLETON, D. **Determination of Extractives in Biomass**, n. NREL/TP-510-42619. Golden: U.S. Department of Energy, 2008. Available at: <https://www.nrel.gov/docs/gen/fy08/42619.pdf>. Accessed on: 20 Jul. 2022.

STATISTA. Leading producers of fresh fruit worldwide in 2021. 2023. Available at: <https://www.statista.com/statistics/279164/global-top-producers-of-selected-fresh-fruit-worldwide/>. Accessed on: 21 Dec. 2023.

SUN, R. C.; TOMKINSON, J. Characterization of hemicelluloses obtained by classical and ultrasonically assisted extractions from wheat straw. **Carbohydrate Polymers**, Amsterdam, vol. 50, no. 3, p. 263–271, 2002. DOI 10.1016/S0144-8617(02)00037-1. Available at: <https://www.sciencedirect.com/science/article/pii/S0144861702000371>. Accessed on: 8 Aug. 2022.

SURIYACHAI, N.; CHAMPREDA, V.; KRAIKUL, N.; TECHANAN, W.; LAOSIRIPOJANA, N. Fractionation of lignocellulosic biopolymers from sugarcane bagasse using formic acid-catalyzed organosolv process. **3 Biotech**, Berlin, vol. 8, no. 5, p. 1–14, 2018. DOI 10.1007/s13205-018-1244-9. Available at: <https://link.springer.com/article/10.1007/s13205-018-1244-9>. Accessed on: 12 Dec. 2022.

THORESEN, P. P.; MATSAKAS, L.; ROVA, U.; CHRISTAKOPOULOS, P. Recent advances in organosolv fractionation: Towards biomass fractionation technology of the future. **Bioresource Technology**, Amsterdam, vol. 306, p. 1–10, 2020. DOI 10.1016/j.biortech.2020.123189. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852420304600>. Accessed on: 2 Jul. 2022.

VAIDYA, A. A.; MURTON, K. D.; SMITH, D. A.; DEDUAL, G. A review on organosolv pretreatment of softwood with a focus on enzymatic hydrolysis of cellulose. **Biomass Conversion and Biorefinery**, Berlin, , p. 1–16, 2022. DOI 10.1007/s13399-022-02373-9. Available at: <https://link.springer.com/article/10.1007/s13399-022-02373-9>. Accessed on: 12 Dec. 2022.

WEERASAI, K.; LAOSIRIPOJANA, N.; IMMAN, S.; KREETACHAT, T.; SURIYACHAI, N. Reusable alkaline catalyzed organosolv pretreatment and delignification of bagasse for sugar platform biorefinery. **Biomass Conversion and Biorefinery**, Berlin, , p. 1–11, 2021. DOI 10.1007/s13399-020-01269-w. Available at: <https://link.springer.com/article/10.1007/s13399-020-01269-w>. Accessed on: 23 Aug. 2022.

WEI, W.; WANG, B.; WANG, X.; LING, R.; JIN, Y. Comparison of acid and alkali catalyzed ethylene glycol organosolv pretreatment for sugar production from bagasse. **Bioresource Technology**, Amsterdam, vol. 320, no. A, p. 1–9, 2021. DOI 10.1016/j.biortech.2020.124293. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852420315674>. Accessed on: 20 Dec. 2022.

WOLF, M.; BERGER, F.; HANSTEIN, S.; WEIDENKAFF, A.; ENDRESS, H.-U.; OESTREICH, A. M.; EBRAHIMI, M.; CZERMAK, P. Hot-Water Hemicellulose Extraction from Fruit Processing Residues. **ACS Omega**, Washington, vol. 7, no. 16, p. 13436–13447, 26 Apr. 2022. DOI 10.1021/acsomega.1c06055. Available at: <https://pubs.acs.org/doi/10.1021/acsomega.1c06055>. Accessed on: 24 Mar. 2023.

XU, J.-Y.; YUAN, T.-Q.; XIAO, L.; SUN, R.-C. Effect of ultrasonic time on the structural and physico-chemical properties of hemicelluloses from *Eucalyptus grandis*. **Carbohydrate Polymers**, Amsterdam, vol. 195, p. 114–119, 2018. DOI 10.1016/j.carbpol.2018.04.067. Available at: <https://www.sciencedirect.com/science/article/pii/S0144861718304533>. Accessed on: 5 Aug. 2022.

YAN, J.; OYEDEJI, O.; LEAL, J. H.; DONOHOE, B. S.; SEMELSBERGER, T. A.; LI, C.; HOOVER, A. N.; WEBB, E.; BOSE, E.

A.; ZENG, Y.; WILLIAMS, C. L.; SCHALLER, K. D.; SUN, N.; RAY, A. E.; TANJORE, D. Characterizing Variability in Lignocellulosic Biomass: A Review. **ACS Sustainable Chemistry & Engineering**, Washington, vol. 8, no. 22, p. 8059–8085, 8 Jun. 2020. DOI 10.1021/acssuschemeng.9b06263. Available at: <https://pubs.acs.org/doi/full/10.1021/acssuschemeng.9b06263>. Accessed on: 21 Dec. 2022.

YAO, S.; NIE, S.; YUAN, Y.; WANG, S.; QIN, C. Efficient extraction of bagasse hemicelluloses and characterization of solid remainder. **Bioresource Technology**, Amsterdam, vol. 185, p. 21–27, 2015. DOI 10.1016/j.biortech.2015.02.052. Available at: <https://www.sciencedirect.com/science/article/pii/S096085241500231X>. Accessed on: 3 Aug. 2022.

ZHANG, J.; ZHOU, H.; LIU, D.; ZHAO, X. Chapter 2 - Pretreatment of lignocellulosic biomass for efficient enzymatic saccharification of cellulose. In: YOUSUF, A.; PIROZZI, D.; SANNINO, F. B. T.-L. B. to L. B. (eds.). **Lignocellulosic Biomass to Liquid Biofuels**. Cambridge: Academic Press, 2020. p. 17–65. DOI 10.1016/B978-0-12-815936-1.00002-2. Available at: <https://www.sciencedirect.com/science/article/pii/B9780128159361000022>. Accessed on: 27 Sep. 2022.

ZHANG, K.; PEI, Z.; WANG, D. Organic solvent pretreatment of lignocellulosic biomass for biofuels and biochemicals: A review. **Bioresource Technology**, Amsterdam, vol. 199, p. 21–33, 2016. DOI 10.1016/j.biortech.2015.08.102. Available at: <https://www.sciencedirect.com/science/article/pii/S0960852415012080>. Accessed on: 27 Sep. 2022.

ZHONG, L.; WANG, C.; XU, M.; JI, X.; YANG, G.; CHEN, J.; JANASWAMY, S.; LYU, G. Alkali-Catalyzed Organosolv Pretreatment of Lignocellulose Enhances Enzymatic Hydrolysis and Results in Highly Antioxidative Lignin. **Energy & Fuels**, Washington, vol. 35, no. 6, p. 5039–5048, 18 Mar. 2021. DOI 10.1021/acs.energyfuels.1c00320. Available at: <https://pubs.acs.org/doi/10.1021/acs.energyfuels.1c00320>. Accessed on: 9 Aug. 2022.

ZOGLAMI, A.; PAËS, G. Lignocellulosic Biomass: Understanding Recalcitrance and Predicting Hydrolysis. **Frontiers in Chemistry**, Lausanne, vol. 7, p. 1–11, 2019. DOI 10.3389/fchem.2019.00874/full. Available at: <https://www.frontiersin.org/article/10.3389/fchem.2019.00874>. Accessed on: 6 Feb. 2022.

CHAPTER 6 - Incorporating lignin to chitosan-based bioplastics enhances the tensile properties and provides control of swelling and opacity

Abstract

Bioplastics were prepared with different mass proportions of chitosan and lignin from guava seed cake. The casting process was used, and the bioplastics were characterized by thermogravimetric analysis, tensile tests, swelling (water absorption), water solubility, and moisture. All bioplastics that incorporated lignin showed higher moisture percentages than that made only of chitosan (15.7%), with the highest content (19.4%) being reached by the formulation with the lignin maximum addition (10%). No significant statistical differences were found for water solubility and thickness; the average values were 17.4% and 0.0997 mm, respectively. Regarding opacity, the bioplastic with the maximum amount of added lignin showed the highest value (4.75 mm^{-1}), furthermore, a strong linear correlation ($r=0.96$) was revealed for the added lignin proportions and opacity. Likewise, between the property swelling and the lignin proportions was found an inverse linear correlation ($r=0.83$), reducing the swelling from 276.6% (pure chitosan) to 133.5% corresponding to 10% of lignin in the bioplastic. The tensile strength of formulations containing lignin was higher than that made of chitosan (12.5 MPa), and the Young's modulus of the bioplastics with the three highest contents of lignin (6, 8, and 10%) was statistically different from the others and also higher than that made only of chitosan (124.0, 73.1 and 97.0, respectively). According to thermogravimetric analysis, samples experienced their mass losses in four distinct steps, the lignin incorporation raised the temperature at which the bioplastics experienced their maximum mass losses, and the lowest mass loss rate was observed in the preparation with the maximum amount of added lignin.

Keywords: Biomass, natural polymers, biopolymer, bio-based films, agroindustrial waste.

6.1. Introduction

Of the worldwide production of plastics projected for 2030, only 0.5% (almost 3 million tons) will correspond to bioplastics, which are expected to have a production growth of 29.3% for the period 2020 – 2030 (OECD, 2023b). Although the plastics were originally developed to bring benefits to society, they have been responsible for several problems such as greenhouse emissions (from exploration and refining processes, and use of derivatives), their non-renewable source (oil), low recycling and reuse rates, contamination of water and soil (by direct or landfill disposal), air pollution (from open burning), very slow biodegradation, and vast production and buildup of wastes (ABE *et al.*, 2022c; CONVERSIO, 2020; OECD, 2020b; PLASTICS EUROPE, 2022). In order to beat these issues, replacing plastics with bioplastics is highly desirable for future applications, such as food packaging (LUO *et al.*, 2023). This substitution trend since bioplastics are made from natural polymers that are present in biomass, which is considered an accessible, renewable, and plentiful raw material, and if its origin is from wastes, then it would not compete with the biomass intended for food (ABE *et al.*, 2022c; GENT *et al.*, 2017; PONNUSAMY; MANI, 2022a). Starch, lignin (LG), hemicelluloses, cellulose, pectin, and chitin are natural polymers that can be extracted from biomass wastes, and could be used for bioplastic production (JACOB *et al.*, 2022).

Although starch is the most frequently employed natural polymer to produce bioplastics, other raw materials such as LG and chitosan (Ch) have the potential to be considered promising precursors (AGRAWAL *et al.*, 2022; AMARAWEEERA *et al.*, 2021; PRIYADARSHI; RHIM, 2020). LG is considered the second most abundant natural polymer on earth, has a three-dimensional, heterogeneous, and highly irregular (cross-linked and branched) structure, and its composition is based on three basic precursor units (coniferyl, sinapyl, and p-coumaryl alcohol) (SCHMATZ; TYHODA; BRIENZO, 2020b). In plants, LG acts as a shield from environmental threats and provides structural strength, which is considered an important property to be explored in the development of biopolymers (GANEWATTA; LOKUPITIYA; TANG, 2019; LIU, Qingquan; LUO; ZHENG, 2018). However, the LG structure is variable since depends on the type of biomass source, and on the process used for its extraction (SCHMATZ; TYHODA; BRIENZO, 2020b).

Although Ch is derived from chitin, it is also considered a natural polymer that typically is produced in industries through the chemical deacetylation of chitin, which

in turn is extracted from industrial seafood wastes (mainly crustacean shells) (LI, Jianhui *et al.*, 2021). When Ch is of high purity (largely glucosamine chains), this is molecularly similar to cellulose due to the presence of the same β -glycoside bonds, however, Ch has primary amino groups located where cellulose has hydroxyl groups (DUAN *et al.*, 2015). Ch purity usually is determined by the deacetylation degree test, which measures the presence of glucosamine monomers; a high deacetylation degree (high Ch purity) indicates a high content of glucosamine units, conversely, a low deacetylation degree indicates a high content of N-acetyl-glucosamine units (PELLIS; GUEBITZ; NYANHONGO, 2022). Owing to its characteristics of biocompatibility, biodegradation, antimicrobial and processing simplicity, Ch, shows to be promising as raw material to replace plastics (PRIYADARSHI; RHIM, 2020). In recent researches, bioproducts based on Ch, such as gels (tissue engineering), microcapsules (medicine release), and films (packaging) have been developed (AHSAN *et al.*, 2018; PRIYADARSHI; RHIM, 2020). Nevertheless, when gels and films are made of only Ch, these are translucent and fragile (low strength and toughness), which hinder their use as light-protective load-bearing materials (FERNANDEZ; INGBER, 2014; LUO, Jing *et al.*, 2023).

Use LG as an additive in bioplastics based on Ch could result in materials with improved properties. The aims of the present research were to manufacture and evaluate plasticized films using Ch (commercial) and LG extracted from the spent liquor generated after alkali ethanolic organosolv pretreatment of guava seed cake. This industrial waste corresponds to the press cake generated after the oil extraction process from guava seed. This research is the first presenting the manufacturing of bioplastics with LG extracted from fruit industrial waste. Thermogravimetric analysis (TGA), tensile test (tensile strength, breaking elongation, and Young's modulus), moisture, opacity, swelling, and water solubility were used to assess the films produced herein.

6.2. Experimental

6.2.1. Materials

The AGTTEC industry settled in Dois Córregos, São Paulo state, Brazil (-22° 22' 6.9" N -48° 23' 51.01"W), nicely provided the guava seed cake in a dry state. After the size reduction step, the waste reached an average dimension below 0.841 mm (mesh 20) (FERNANDES *et al.*, 2020b). The guava waste was dried for 24 h at 50 °C and stored in plastic bottle. The Ch used in the present study was of commercial type (C3646 of Sigma-Aldrich, source: shrimp shells - SLCK9065, and PCode: 1003345975), and its deacetylation degree was 81.2±0.65% (determined by potentiometric method) and 80.6±0.08% (by Fourier Transform Infrared spectroscopy). Sodium hydroxide p.a., calcium nitrate tetrahydrate p.a., and 99.8% glacial acetic acid p.a. were acquired from Neon Comercial, 92.8% ethanol from Safra Industria, and glycerin p.a. from Labsynth®.

6.2.2. Extraction of lignin (LG)

In a stainless-steel reactor (with a nominal capacity of 75 mL,) the guava seed cake, NaOH, and a 70% (m/m) aqueous ethanolic solution were added, maintaining a waste:alkali:liquid ratio of 10:6:50. The system was mixed with a glass rod and left to soak for 45 min, and the pretreatment was then performed in an oil bath at 170 °C for 1 h. After the reaction, the spent liquor was recovered using filter paper, and hemicelluloses were removed from it. The removal process involved two successive stages: the addition of 92.8% ethanol to the spent liquor, followed by decantation for 18-20 h. After hemicelluloses were removed, the remaining liquid fraction was boiled at 80 °C to evaporate the added ethanol, and then sulfuric acid was poured into the resulting concentrated solution until pH was below 2. Afterward, a resting period of 18-20 h was completed, and LG was recovered by filtration. LG was washed with distilled water and dried at 60 °C for 72 h.

6.2.3. Preparation and formulation of bioplastics

Separated solutions of LG and Ch were prepared at room temperature and then mixed to produce bioplastics through the casting method. LG was dissolved in 80% (m/m) aqueous solution of acetic acid using magnetic stirring (700 rpm) for 20 min, and Ch was dissolved in 2% (m/m) aqueous solution of acetic acid using magnetic stirring

(450 rpm) for 4 h. In order to remove bubbles, the solutions reposed by 20 h, and after that, LG and Ch solutions were mixed along with the plasticizer (20% m/m of glycerin regarding the total mass of LG plus Ch) in an Erlenmeyer flask during 30 min at room temperature and 350 rpm. Later, the mixture was sonicated during 30 min, reposed at room temperature for another 30 min, and then filtered using a sieve of size 100 mesh. All the bioplastic formulations used 0.705 g of filmogenic solution per cm² of the acrylic plate, and the drying stage was performed at 37 °C for 2 days. Table 6-1 exhibits the mass proportions used to prepare the bioplastics.

Table 6-1 - Formulations of the bioplastics (mass proportions)

ID	Ch (%)	LG (%)
Ch100LG0	100	0
Ch98LG2	98	2
Ch96LG4	96	4
Ch94LG6	94	6
Ch92LG8	92	8
Ch90LG10	90	10

%Ch: Mass percentage of chitosan; %LG: Mass percentage of lignin

Source: By author

6.2.4. Characterization of bioplastics

6.2.4.1. Moisture and water solubility

Three rectangular samples of 1.0 x 5.0 cm were cut from each formulation and weighed. Then, the same samples were re-weighed after drying at 105 °C during 24 h. Based on the difference of both weights the moisture mass percentage was calculated. Subsequently, 50 mL of distilled water was used to immerse each dried sample, and the system was shaken for 24 h at 110 rpm and 25 °C. After immersion, the samples were pulled out of the water, dried again under the same conditions mentioned above, and weighed. Considering the difference in weights after the 1st and the 2nd drying steps, the water solubilization percentage was calculated (MEDINA-JARAMILLO *et al.*, 2017b).

6.2.4.2. Thickness and opacity

Three rectangular samples of 0.9 x 3.0 cm were cut from each formulation. Then, with a digital micrometer (0.001 mm accuracy) was measured the thickness at three different points (ends and center). After measuring the thickness, absorbance at 600 nm was read for each sample using a spectrophotometer, and the opacity (mm^{-1}) was calculated by dividing the absorbance by the thickness (ZHAO; WANG; LIU, 2022b).

6.2.4.3. Swelling (water absorption)

Three rectangular samples of 1.0 x 4.0 cm were cut from each formulation, dried at 50 °C during 24 h, and weighed (m_1 : mass 1). Subsequently, 25 mL of distilled water was used to immerse each dried sample, and the immersion lasted 24 h at 23°C (without shaking). After immersion, the samples were pulled out of the water, wiped off with filter paper (to remove the surface water), and weighed (m_2 : mass 2). The samples were dried again under the same conditions mentioned above and once more weighed (m_3 : mass 3). The percentage of increase in weight during immersion was calculated based on the mass difference between m_1 and m_2 , and the percentage of water-soluble matter lost was calculated based on the mass difference between m_1 and m_3 . The mass percentage of water absorption corresponded to the sum of the percentages of increase in weight during immersion and of water-soluble matter lost (ASTM, 2022; ISO, 2021).

6.2.4.4. Tensile test

Prior to testing, all formulations were conditioned for 48 h in an environment where the temperature and relative humidity were maintained at 23 °C and 56 – 57%, respectively. The humidity was achieved using $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (calcium nitrate tetrahydrate), and an incubator was employed to control the temperature. After conditioning, the tensile assay was completed as follows: ten strips of 1.0 cm width and 10 cm length were cut from each formulation, and every strip thickness was

measured at 10 different places using a digital micrometer (0.001 mm accuracy) (ASTM, 2018). All tests were carried out with a load cell of 5 kg, a movement speed of 5 mm/min, and using a testing machine model TXT-ENG-5K. Based on the standard test method ASTM D882-18, were calculated the tensile properties, including tensile strength, elongation at break, and Young's modulus.

6.2.4.5. Thermogravimetric analysis (TGA)

The thermal stability of the Ch100LG0, Ch98LG2, and Ch90HC10 formulations was analyzed through an equipment TGA Q500 (TA Instrument) operating in a nitrogen atmosphere (flow rate of 50 mL/min), and at a range of temperatures between 25 and 800 °C (heating of 10 °C/min). The obtained thermograms represented the thermal behavior of the analyzed formulations.

6.2.5. Statistical analysis

The statistical analysis was carried out using the software IBM SPSS version 28.0. The Tukey HSD test was used to verify significant differences among the formulations at 95% of confidence level ($p < 0.05$).

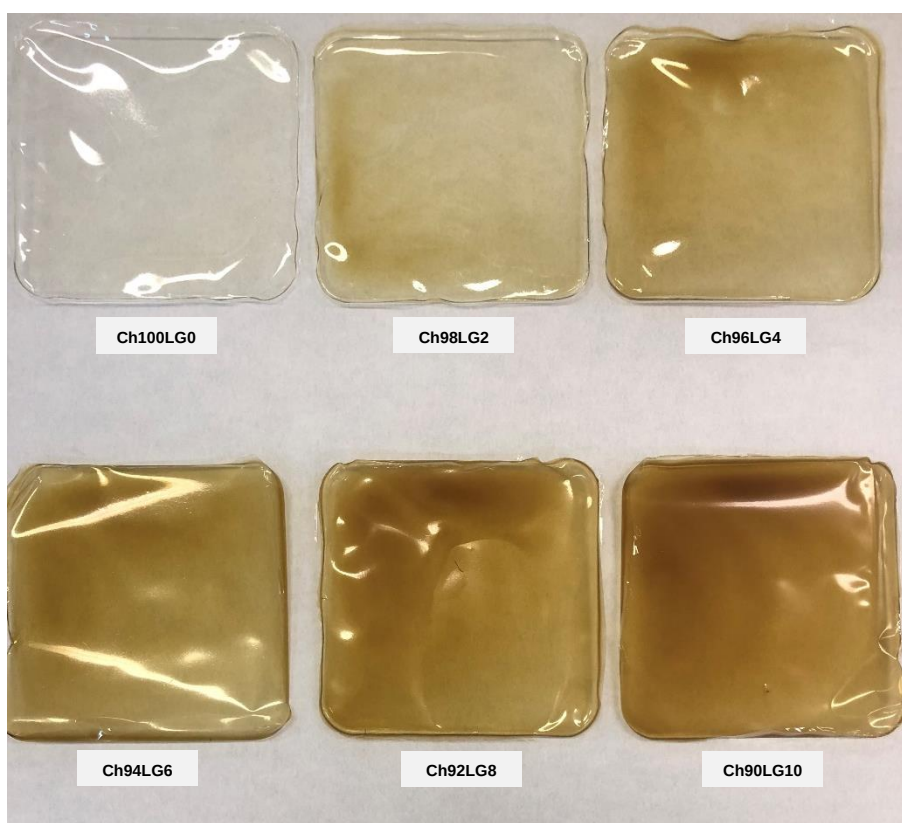
6.3. Results and discussion

6.3.1. Appearance of the bioplastics

All the bioplastic preparations exhibited a single phase, flexibility, and consistent structure without any visible cracks, tears or wrinkles (Figure 6.1 based on proportions of Ch and LG listed in Table 6-1). However, the color intensity proportionally increased according to the mass of added LG. All bioplastics with LG addition exhibited a heterogeneous color distribution, possibly due to the accumulation of LG in certain regions but without the formation of aggregates. The observed behavior could have been caused by several factors, such as incomplete compatibility between Ch and LG, mild mixing conditions (30 min at room temperature and 350 rpm), non-uniform evaporation of solvents during the drying period, or the possibility that the chemical

interactions (bonds) among LG molecules were stronger than the linkages between LG and Ch. Chen *et al.* (2009) suggested a good compatibility of both components probably caused by a strong intermolecular interaction in blend films containing 10 – 30% of LG. However, Rosova *et al.* (2021) reported thermodynamic incompatibility between Ch and LG in composite films containing 10 – 30% of LG but using water as solvent to dissolve LG. In order to overcome the issue of poor mutual affinity of Ch and LG, new studies including the use of compatibilizers could be performed to observe if the LG dispersion in Ch-based films could be improved.

Figure 6.1 - Chitosan and lignin bioplastics using the different mass proportions listed in Table 6-1



Source: By author

6.3.2. Characterization of bioplastics

Moisture (%), water solubility (%), thickness (mm), opacity (mm^{-1}), swelling (%), tensile strength (MPa), elongation at break (%) and Young's modulus (MPa) of all the bioplastic preparations are shown in Table 6-2.

Table 6-2 - Moisture, water solubility, thickness, opacity, water absorption, and tensile properties of the six preparations

ID*	Moisture (%)	Water solubility (%)	Thickness (mm)	Opacity (mm ⁻¹)	Swelling (%)	Tensile strength (MPa)	Breaking elongation (%)	Young's modulus (MPa)
Ch100LG0	15.7±0.97 ^a	17.4±0.75 ^a	0.100±0.0023 ^a	0.62±0.026 ^a	276.6±9.43 ^a	12.5±1.39 ^a	34.6±0.99 ^{ac}	36.1±3.86 ^a
Ch98LG2	17.2±0.55 ^{ab}	17.8±1.00 ^a	0.103±0.0098 ^a	1.00±0.110 ^b	217.3±10.30 ^b	12.6±0.57 ^a	46.2±3.27 ^b	26.1±1.31 ^a
Ch96LG4	16.1±0.81 ^a	16.8±0.83 ^a	0.100±0.0034 ^a	1.74±0.035 ^c	162.4±11.10 ^c	16.6±0.87 ^b	30.5±1.27 ^{ace}	27.5±2.10 ^a
Ch94LG6	17.7±1.05 ^{ab}	18.6±1.10 ^a	0.099±0.0025 ^a	2.32±0.331 ^d	155.1±7.14 ^{cd}	22.5±2.10 ^c	23.3±1.36 ^{def}	124.0±2.96 ^b
Ch92LG8	16.6±0.62 ^a	16.6±0.70 ^a	0.096±0.0023 ^a	3.84±0.087 ^e	154.0±3.57 ^{cd}	18.9±0.50 ^{bc}	26.2±0.87 ^{de}	73.1±3.80 ^c
Ch90LG10	19.4±1.06 ^b	17.4±0.92 ^a	0.100±0.0075 ^a	4.75±0.216 ^f	133.5±4.27 ^d	18.3±1.85 ^b	20.7±1.57 ^{fd}	97.0±2.55 ^d

*ID according to Table 6-1. Different letters in a same column means significant statistical difference (95% of confidence level).

Source: By author

6.3.2.1. Moisture and water solubility

Although there was a small variation in the moisture values among all formulations, those with LG addition showed slightly higher moisture contents than that made only of Ch (15.7%), and the bioplastic with the highest amount of added lignin (Ch90LG10) exhibited the highest moisture value (19.4%) and was statistically different to Ch100HC0 (bioplastic of only Ch). This behavior was in concordance with the Li *et al.* (2023) study, which reported an increase in moisture contents when LG was added (mass proportions of 1 – 3%) to Ch-based films, however, the LG was extracted from bagasse through deep eutectic solvents. Although LG is known to be hydrophobic in nature, its structure contains hydroxyl groups in aliphatic (R-OH), phenolic (Ph-OH) and carboxylic (R-COOH) sites, which have chemical affinity with water (to form hydrogen bonds) due to the distribution of uneven charge in -OH groups (GUAN *et al.*, 2022; LISÝ *et al.*, 2022). Besides these groups, the three-dimensional, heterogeneous, highly cross-linked, and branched structure of LG could provide a large surface area to possibly form hydrogen bonding with water molecules. On the other hand, when Ch is dissolved in an aqueous solution of acetic acid, the amino groups (-NH₂) present in the glucosamine molecules undergo protonation reactions. These results in a partial distribution of positive charges, which contributes to the repulsion of hydroxyl groups decreasing the formation of hydrogen bonds with water molecules (LUO; WANG, 2014). Likewise, the higher the deacetylation degree of

chitosan, the higher the presence of glucosamine units and the lower the presence of N-acetyl-glucosamine units, which would lead to more protonation reactions of amino groups, resulting in an increase of positive charges that would repel -OH groups of the water reducing the number of hydrogen bonds. The moisture (15.7%) for the bioplastic preparation without LG addition (Ch100LG0) was slightly lower than those found by Leceta *et al.* (2013), who reported values of 17.0 and 20.8% for films plasticized with glycerin (15% of mass proportion), manufactured through the casting method, and made of high and low molecular weight Ch (both with a degree of deacetylation higher than 75%), respectively. Conversely, Janik *et al.* (2021) reported a moisture content of around 10% for a bioplastic that was plasticized with glycerin (21.2% of mass proportion), manufactured through mechanical blending and hot-pressing, and made of commercial dealkaline LG (mass percentage of 6.5%), commercial Ch (deacetylation degree higher than 90%) and starch (mass percentage of 18.8%).

In relation to the water solubility results, there were no statistically significant differences, and the range of values was 16.6 - 18.6%. This range was in agreement with the findings informed by Zou *et al.* (2023), who reported water solubility values of 15.2 and 18.3% for Ch-based films with 1% and 5% of added LG, respectively, although the used LG was in nanoparticle form. Both components were the commercial type, and the films were processed using the casting method. Similarly, the highest value found in the present work (18.6%) was similar to the water solubility reported in the Aradmehr and Javanbakht (2020) research, in which, 21.6% was obtained for a film (produced by the casting method), and made of commercial Ch and LG (with a mass proportion of 25%) extracted from bagasse wastes through the acidic-dioxane method. On the contrary, higher solubility values have been reported for bioplastics that used LG as an additive and a base other than Ch. Michelin *et al.* (2020) found solubilities ranging from 23.6 to 34.0% for different bioplastics produced through the casting method, and using a commercial type of sodium carboxymethylcellulose and LG with added amounts of 12.5 – 50%. And, Shankar *et al.* (2015) produced composite films plasticized with glycerin (mass proportion of 40%), based on food grade agar, and using LG with addition of 1 - 10% (based on agar mass), which exhibited solubilities among 26.9 and 34.0%. Solubility in water is an important property that would be determined in bioplastics based on natural polymers because it could limit

their performance, particularly in food packaging applications (RODSAMRAN; SOTHORNVIT, 2019).

6.3.2.2. Thickness and opacity

The thickness values of all formulations did not exhibit statistically significant differences, which were reflected by the low variability achieved in the measurements (0.096 – 0.103 mm). The fact that every bioplastic had almost the same thickness was possibly because all preparations used identical total mass of both components. The thickness of films obtained through the casting technique can be influenced by different factors, such as the solvent/natural polymer ratio employed to dissolve the solid, as well as the surface tension, viscosity, drying rate and used mass or volume of filmogenic solution. Low values of thickness can commonly be obtained using low solvent/natural polymer ratios, and low viscosity, surface tension and amounts of filmogenic solution. Additionally, a fast solvent evaporation (high rate) at drying stage would result in films thinner than expected (BATISTA *et al.*, 2019; FRIED, 2014; PROVDER; URBAN, 2001; RODRIGUES *et al.*, 2021). Luo *et al.* (2023) reported a thickness range of 0.097 – 0.103 mm and without statistically significant differences for composite films produced using the casting method and made of commercial Ch (deacetylation degree of 90%) and LG (extracted from softwood kraft black liquor and modified by sulfomethylation and demethylation). The mass proportions of added LG were from 0.5 to 7.0%, and the film processing was performed without plasticizer addition. A similar thickness (about 0.09 mm) was reported by Chen *et al.* (2009), who from commercial Ch (deacetylation degree 90%) and LG (contents ranged from 5 to 35%) produced composite films, which were processed without plasticizer use, through the casting method, preparing individual solutions, and including a drying stage in an oven at 50 °C. Likewise, Aradmehr and Javanbakht (2020) reported thickness of about 0.09 mm for biofilms produced with commercial Ch and acidic-dioxane LG extracted from bagasse wastes (with contents ranging from 25 to 100%), and through the casting method but including 0.03% silver particles and glutaraldehyde as cross-linking agent. The film processing also included preparation of individual solutions (Ch and LG) and oven-drying at 50 °C. Conversely, Rai *et al.* (2017) found a thickness range of 0.219 - 0.247 mm in films made of Ch (79% deacetylated) and LG from sugar cane bagasse,

extracted by an alkali route. In the research, the process involved LG contents among 5 and 20% without use of any plasticizer, drying stage at 30°C, and solid LG was added to the Ch solution. Thickness measurement is relevant because it affects both the tensile and barrier properties (AMJADI; FATEMI, 2020; BRODNJAK; TODOROVA, 2018), which are important factors that could determine the bioplastic applications. In addition, the thickness measurement could be used in the environmental impact assessment (i.e., carbon footprint) caused during the product lifecycle.

Regarding opacity, all formulations were statistically different, a positive strong linear correlation ($r = 0.96$) was observed between opacity and added LG amount (refer to Figure S6.1 at supplementary information), and the one containing the maximum amount of LG (10%) showed the highest opacity value (4.75 mm^{-1}). According to Li *et al.* (2023), films containing LG exhibited significantly lower transmittance (200 – 800 nm) than the film made only of Ch, where the LG was extracted from bagasse powder using different deep eutectic solvents, the range of added LG was 1 - 3%, and the films were produced in coating machine without plasticizer addition. And, Luo *et al.* (2023) reported a gradual decrease in transmittance as the amounts of added LG increased (0.5 – 7%) in composite films based on Ch (deacetylation degree of 90%) and LG extracted from softwood black liquor, however, the LG used to form the films was chemically modified. Using bioplastics with reduced properties of transmittance would be potentially beneficial, as it would provide protection against photochemical reactions that occur during interactions with light (UV rays) for both the bioplastic itself and the product covered by it. In order to offset modifications in the properties (physical, chemical, and biological) of products such as food, medicines and beverages, bioplastics offering the mentioned protection are being developed (EZATI *et al.*, 2023). However, using opaque (or shady) bioplastics could impact the market since some consumers can prefer to directly see the product inside its packaging before they open it (GIL-PÉREZ; REBOLLAR; LIDÓN, 2020).

6.3.2.3. Swelling (water absorption)

After immersed in water, all preparations were carefully handled to avoid damage. Visually, the swollen bioplastics exhibited increased flexibility, which could be beneficial for specific applications, or disadvantageous due to modifications in their

mechanical properties. The formulations that incorporated lignin (Ch98LG2 – Ch90LG10) showed lower swelling percentage than that made only of chitosan (Ch100LG0), and the bioplastic with the highest amount of added lignin (Ch90LG10) exhibited the minimum swelling (133.5%) and was statistically different to Ch100HC0. Additionally, a negative linear correlation ($r = 0.83$) was observed between swelling and added LG amount (refer to Figure S6.2 at supplementary information). The swelling of solid materials can be explained by the movement of water molecules from one point to another through space that a solid can have. The internal transport of water molecules results from the phenomenon of mass diffusion, which is a mechanism where water molecules move from a zone of high concentration to another of low concentration until they (the water molecules) achieve an equilibrium state of concentration (BIRD; STEWART; LIGHTFOOT, 2002). Thus, the mass diffusion of water within the structure caused the bioplastic molecules to separate, generating expansion in the material. The swelling decrease of the formulations with LG incorporation probably was a consequence of the reduction of water diffusion, which in turn could be caused by LG molecules that occupied or blocked spaces within the material structure to form hydrogen bonds with Ch. Moreover, the gradual replacement of the Ch amounts with LG and the hydrophobic nature of LG could also be responsible for the swelling reduction.

The lowest swelling (133.5%), corresponding to the preparation with the maximum addition (10%) of LG, was slightly higher than that reported by Chen *et al.* (2009), who found a value of 124% for a film manufactured from commercial Ch (deacetylation degree 90%) and LG produced without plasticizer use, through the casting method, preparing individual solutions (Ch and LG), 10% of added LG, and including a drying stage in an oven at 50°C. Similarly, Rosova *et al.* (2021) reported 100% as the lowest swelling for a film containing 10% of commercial LG and 90% of Ch (degree of deacetylation of 86.5%) prepared through the casting method without the use of any plasticizer. Likewise, according to Janik *et al.* (2020), the incorporation (5.2%) of dealkaline commercial LG into a bioplastic based on Ch (high molecular weight), starch, and PVA (Elvanol 71–30), and processed through hot-pressing with glycerin plasticizer, resulted in a decrease in the swelling percentage from 200 to 90%. Conversely, the research of Aradmehr and Javanbakht (2020) reported a higher swelling value (around 175%) for a film made of commercial Ch and acidic-dioxane LG

extracted from bagasse wastes (with content into film of 25%). The films were obtained through the casting method, including 0.03% silver particles and glutaraldehyde as cross-linking agent, and the swelling assay was conducted at 37°C during 24h and pH=7. The reduction of swelling values in bioplastics probably indicates hydrophobicity presence (caused by the diminishing of water diffusion) and decline in degradation rates (caused by the limitation of oxidation processes) (ABE; BRANCIFORTI; BRIENZO, 2021b; NGUYEN *et al.*, 2020).

6.3.2.4. Tensile strength

Those formulations with LG addition showed higher tensile strength values than one made of 100% of Ch (12.5 MPa). The formulation Ch94LG6 (94% of Ch and 6% of LG) revealed the highest tensile strength (22.5 MPa) and was statistically different from the formulation Ch100HC0 (100% of Ch). The difference in resistance to lengthwise stress between the formulations Ch94LG6 and Ch100HC0 could be explained by the formation of hydrogen bonds between Ch and LG, which possibly contributed to enhancing the tensile strength (LUO *et al.*, 2023). Chen *et al.* (2009) also observed an improvement in tensile strength when LG contents of 5 – 20% were incorporated in Ch-based films. Likewise, Jaganathan *et al.* (2018) found the same behavior when LG was added at contents of 1 - 5%, however, the formulation with the highest content of LG (5%) showed a maximum of 0.69 MPa, which can be considered a very low value compared to the highest one achieved in the present work (22.5 MPa) with a similar LG content (6%). Rai *et al.* (2017) revealed a tensile strength of 22 MPa for a film made of Ch (79% deacetylated) and 10% of LG (extracted by an alkali route from sugar cane bagasse). The film was produced by the casting method but without the use of any plasticizer, and the solid LG was added to the Ch solution. Similarly, Luo *et al.* (2023) found a tensile strength of 25.1 MPa for a composite film produced through the casting method without plasticizer addition, and constituted by 97% of commercial Ch (deacetylation degree of 90%) and 3% of LG. However, the LG used in the study was extracted from softwood kraft black liquor and modified by sulfomethylation and demethylation processes. Li *et al.* (2023) reported a slightly lower tensile strength than the maximum one found in the present research. The research revealed a value of 21.3 MPa for two bioplastics obtained through the casting method

and made of commercial Ch and LG (extracted from sugarcane bagasse by chlorine chloride-urea solvent) with 2 and 3% of added mass.

Although the maximum tensile strength observed in the present study was similar to those reported in the cited literature, some studies have revealed superior values. Rosova *et al.* (2021) produced a film with a tensile strength of 54.6 MPa from 80% of commercial Ch (86.5% of deacetylation degree) and 20% of industrial LG (extracted from softwood and modified by a hydrothermal alkaline process). The film was developed by the casting method (including degassing) without any plasticizer, the filmogenic solution was obtained from individual solutions of Ch and LG, and the drying stage occurred at room temperature. And, Aradmehr and Javanbakht (2020) reported a tensile strength of around 47 MPa for a biofilm produced with commercial Ch and 25% of acidic-dioxane LG extracted from bagasse wastes, using the casting method, but including 0.03% silver particles and glutaraldehyde as cross-linking agent. The film processing also included the preparation of the filmogenic solution from individual solutions of Ch and LG, and oven-drying at 50°C.

6.3.2.5. Elongation at break

Those formulations with LG addition (excepting the Ch98LG2) showed lower values of breaking elongation than one made of only Ch (34.6%). The formulation with the maximum content of LG (Ch90LG10 = 90% of Ch and 10% of LG) revealed the lowest elongation (20.7 MPa) and was statistically different to the formulation Ch100HC0 (100% of Ch and 0% of LG). In recent research, LG have been used as an additive in several bioplastics to improve their tensile strength, however, this same addition can affect the elongation of films, since LG can provide rigidity to the polymeric network (DUVAL; LAWOKO, 2014; MARIANA *et al.*, 2021). Thereby, the bioplastics containing LG (except the Ch98LG2) were more rigid than the formulation made of only Ch. The formulations exception, composed of 98% of Ch and 2% of LG (Ch98LG2), exhibited the highest elongation at break (46.2%), which was in great accordance with the findings of Li *et al.* (2023). The research reported that the maximum value (approximately 62%) was achieved when 2% of LG was added to Ch-based films. The film was obtained using a coating machine without plasticizer, the range of LG contents was 1 - 3%, and LG was extracted from bagasse powder through

different deep eutectic solvents. The same research also informed that a film produced from 99% of Ch and 1% of LG (extracted using choline chloride mixed with lactic acid) had the minimum value (around 19%) of elongation at break. Regarding the maximum breaking at break value found in the present research (46.2%), Chen *et al.* (2009) reported a slightly higher number (around 48%), which was attributed to a bioplastic obtained through the casting method and made of commercial Ch and 5% of LG. In contrast, Rosova *et al.* (2021) found values below 9.5% for Ch-based films containing 10 – 30% of LG. Ch with an 86.5% of deacetylation degree was the commercial type and LG came from industry. The film was obtained without plasticizer addition through the casting method, and the filmogenic solution was obtained from individual solutions of Ch and LG.

Although the highest elongation at break achieved in the present study was either superior or similar to those found in the studies cited above, there are other studies that have showed higher values. Aradmehr and Javanbakht (2020) reported the maximum breaking elongation (around 65%) for a biofilm produced with commercial Ch and 25% of acidic-dioxane LG extracted from bagasse wastes using the casting method. The film processing also included 0.03% silver particles, glutaraldehyde as cross-linking agent, preparation of filmogenic solution from individual solutions of Ch and LG, and oven-drying at 50 °C. And, Luo *et al.* (2023) developed a film with a maximum elongation at break of 73.6% from 95% of commercial Ch (90% of deacetylation degree) and 5% of highly sulfomethylated LG. LG was extracted from softwood kraft black liquor and subsequently modified, and the film was processed by the casting method without plasticizer incorporation.

Values of elongation at break such as those informed by Luo *et al.* (2023) and Aradmehr and Javanbakht (2020), mean that the bioplastics made of Ch and LG had the property to withstand high longitudinal deformations before they broke. This could indicate that the bioplastics were ductile, and were not prone to fractures (CALLISTER; RETHWISCH, 2018), characteristics that Rosova *et al.* (2021) probably did not achieve in their bioplastics due to the low values of elongation at break reported. Hence, the maximum elongation at break reported herein could be regarded as an intermediate value because it was higher than those informed by Rosova *et al.* (2021) but lower than those revealed by Luo *et al.* (2023) and Aradmehr and Javanbakht (2020).

6.3.2.6. Young's modulus

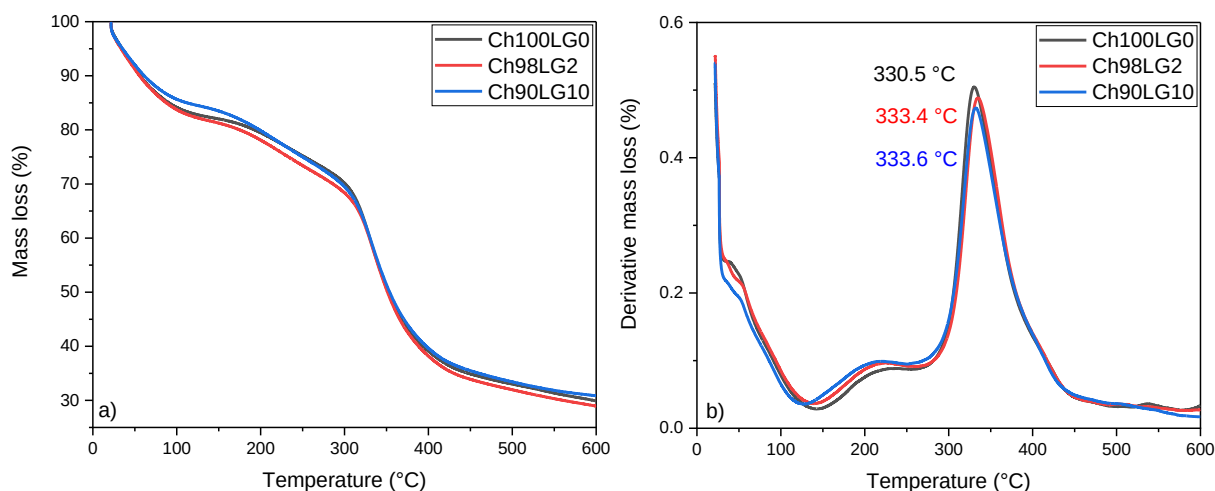
The formulations containing 2 and 4% of LG exhibited smaller Young's moduli than the one made of 100% of Ch, however, the preparations containing 6, 8, and 10% of LG had a higher value. Among them, the formulation containing 6% of added LG (Ch94LG6) showed the highest Young's modulus (124.0 MPa) and was statistically different from the other ones. The most rigid formulation was the Ch94LG6, which possibly developed more hydrogen bonds between Ch and LG, interactions that probably contributed to obtaining a tight chemical structure (LUO, Jing *et al.*, 2023). Crouvisier-Urien *et al.* (2016) reported a similar Young's modulus value (130 MPa) for a Ch-based film with 20% of added LG produced through the casting method (without plasticizer). LG was extracted from sugarcane using an alkali process, and Ch was of commercial type with 90% of deacetylation degree. LG and Ch were solubilized in aqueous solutions of ethanol and acetic acid, respectively. The film had a thickness of 0.102 mm, an elongation at break of 18.7%, and a tensile strength of 7.8%, however, the film was stored in an environment conditioned at 75% relative humidity. This same study reported a Young's modulus of 100 MPa when 10% of LG was used. The film had a thickness, elongation at break, and tensile strength of 0.0755 mm, 56.0%, and 15.6%, respectively. On the contrary, Jaganathan *et al.* (2018) reported values below 0.7 MPa when LG was added at contents of 1 - 5% into Ch-based films. The films showed elongation at break values of 29.8 – 42.6%, and a lower range of tensile strength (0.43 – 0.69 MPa). Another example, Rosova *et al.* (2021) found very high moduli (2.75 – 2.84 GPa) for formulations made from Ch and LG with added contents of 10 – 30%. The films exhibited low values of elongation at break (8.8 – 9.4%), and a tensile strength range of 53.4 – 54.5 MPa. High values of Young's modulus mean that a specific material has high stiffness, however, when the moduli of Ch-based bioplastics are enhanced, the films can result in a reduction of their elongations (CALLISTER; RETHWISCH, 2018; LUO *et al.*, 2023).

6.3.2.7. Thermogravimetric analysis (TGA)

In food packaging applications the thermal behavior analysis of bioplastics is important because they could be used as materials for storage or preservation at

different temperatures (JAKUBOWSKA *et al.*, 2020b). Hence, the influence of LG incorporation into the bioplastics was studied using TGA (Figure 6.2). The curves in Figure 6.2a correspond to the mass loss thermograms of the preparations Ch100LG0, Ch98LG2, and Ch90LG10. All of them exhibited four degradation stages, with the first one corresponding to the evaporation of acetic acid and water between 25 and 120 °C, range in which the three formulations had a mass average loss of approximately 16.3% (CHEN *et al.*, 2009; MOALLA *et al.*, 2021). The second stage represented the degradation of glycerol and Ch fractions (low molecular weight) in the temperature range of 125 - 310 °C, during which a mass average loss of around 16.7% occurred (KAMDEM *et al.*, 2019). The third stage occurred between 310 and 400 °C, a range that corresponded to the Ch decomposition and LG depolymerization, and the mass average loss reached its maximum value (28.2%) (BREBU; VASILE, 2010; GE; WANG, 2014). Finally, the temperature range > 400 °C corresponded to the fourth stage, during which the film carbonization occurred, and an average mass loss of roughly 8.8% was observed (LI *et al.*, 2023). At the end of the first stage, the formulation Ch90LG10 showed a slight decrease in the mass loss compared to the other ones. During the second and final stages, the formulation Ch98LG2 exhibited a small increase in mass loss, however, in the third stage, the bioplastics including LG did not show any increase or decrease in mass loss compared to the formulation Ch100HC0. Li *et al.* (2023) also reported four stages of mass loss for films produced with Ch and LG, however, the LG used was extracted from sugarcane bagasse using chlorine chloride-urea solvent, and the formulations incorporated 0.5 - 3% of LG. The observed temperature ranges were < 100 °C, 100 – 210 °C, 210 – 420 °C and > 420 °C.

Figure 6.2 Thermogravimetric (TG) a) and derivative thermogravimetric (DTG) b) curves of the formulations Ch100LG0, Ch98LG2 and Ch90LG10



Source: By author

On the other hand, Figure 6.2b shows that the LG addition caused an increase (Δ) of 3°C in the temperature at which the highest mass loss occurred (T_{MAX}). T_{MAX} of 330.5 °C was for the formulation Ch100LG0, 333.4 °C for Ch98LG2, and 333.6 °C for Ch90LG10. Nevertheless, the degradation rate (%/°C) at T_{MAX} slightly decreased in the formulations with added LG, which indicated that the formulation composed only by Ch thermally degraded at a higher rate than the other ones. Aradmehr and Javanbakht (2020) also reported an increase in T_{MAX} when LG was added to Ch-based bioplastics, which could indicate that LG had a stabilizing effect on the thermal degradation of such bioplastics. Similarly, Luo *et al.* (2023) reported an increase in T_{MAX} for a Ch-based film incorporating 7% of sulfomethylated LG, however, the ΔT_{MAX} was of 10.3 °C. And, Chen *et al.* (2009) also informed a $\Delta T_{MAX} = 3$ °C for a bioplastic obtained through the casting method and made of 80% of Ch and 20% of LG.

6.4. Conclusions

All proposed formulations resulted in thin bioplastics with one-phase, flexible, and consistent structure. The LG addition to Ch-based films led to changes in their physical and mechanical properties. The gradual inclusion of LG caused a color variation, and a heterogeneous dispersion/distribution of color. The results of water solubility and thickness showed no statistically significant variation. Regarding opacity,

the results were statistically different, and a strong positive linear correlation revealed that the gradual increase of LG content led to increase the opacity too. In terms of swelling, an inverse linear correlation revealed that the same gradual increase of LG content led to a decrease in the swelling values. The LG addition to the bioplastics improved the tensile strength values but decreased the elongation at break. The formulation with the highest LG addition showed the lowest value of elongation at break. And, according to TGA, samples underwent mass loss in four distinct steps, the LG incorporation raised the temperature at which the bioplastics had their maximum mass losses, and the lowest mass loss rate was observed in the preparation with the maximum amount of added LG. The opacity and swelling can be considered promising properties since they showed mathematical dependence with the added LG amounts, which could be observed by the linear models. As a result, the possible applications of this kind of bioplastics could be in the food or medicine fields, since in these the control of light exposure and water diffusion are considered key factors.

Statements and Declarations

Competing Interests: The authors declare no competing interests.

Acknowledgments

The authors are grateful to the Brazilian Improvement Coordination of Higher Education Personnel (CAPES), Brazilian National Council for Scientific and Technological Development (process number 303039/2021-2), and São Paulo Research Foundation (FAPESP, process number 2017/22401-8; 2021/10839-4) for its financial support.

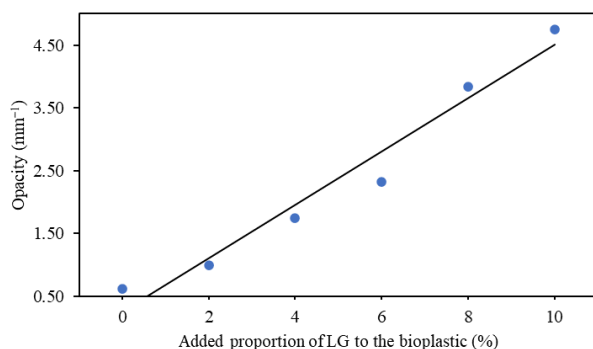
6.5. Supplementary Information

List of figures

Figure S6.1	Linear correlation between added proportions of LG and opacity of the formulations
-------------	--

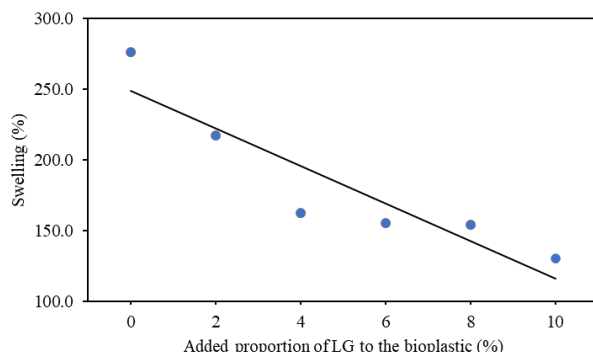
Figure S6.2	Linear correlation between added proportions of LG and swelling of the formulations
-------------	---

Figure S6.1 - Linear correlation between added proportions of LG and opacity of the formulations



Source: By author

Figure S6.2 - Linear correlation between added proportions of LG and swelling of the formulations



Source: By author

References

- ABE, Mateus M.; BRANCIFORTI, M. C.; BRIENZO, M. Biodegradation of Hemicellulose-Cellulose-Starch-Based Bioplastics and Microbial Polyesters. **Recycling**, Basel, vol. 6, no. 1, p. 1–21, 2021. DOI 10.3390/recycling6010022. Available at: <https://www.mdpi.com/2313-4321/6/1/22>. Accessed on: 14 Feb. 2023.
- ABE, Mateus Manabu; BRANCIFORTI, M. C.; NALLIN MONTAGNOLLI, R.; MARIN MORALES, M. A.; JACOBUS, A. P.; BRIENZO, M. Production and assessment of the biodegradation and ecotoxicity of xylan- and starch-based bioplastics. **Chemosphere**, Amsterdam, vol. 287, no. Part 3, p. 1–10, 2022. DOI 10.1016/j.chemosphere.2021.132290. Available at: <https://www.sciencedirect.com/science/article/pii/S0045653521027624>. Accessed on: 6 Apr. 2023.
- AGRAWAL, R.; KUMAR, A.; SINGH, S.; SHARMA, K. Recent advances and future perspectives of lignin biopolymers. **Journal of Polymer Research**, Berlin, vol. 29, no. 6, p. 222, 2022. DOI 10.1007/s10965-022-03068-5. Available at: <https://link.springer.com/article/10.1007/s10965-022-03068-5>. Accessed on: 7 Apr. 2023.
- AHSAN, S. M.; THOMAS, M.; REDDY, K. K.; SOORAPARAJU, S. G.; ASTHANA, A.; BHATNAGAR, I. Chitosan as biomaterial in drug delivery and tissue engineering. **International Journal of Biological Macromolecules**, Amsterdam, vol. 110, p. 97–109, 2018. DOI 10.1016/j.ijbiomac.2017.08.140. Available at: <https://www.sciencedirect.com/science/article/pii/S0141813017318846>. Accessed on: 11 Apr. 2023.
- AMARAWEEERA, S. M.; GUNATHILAKE, C.; GUNAWARDENE, O. H. P.; FERNANDO, N. M. L.; WANNINAYAKA, D. B.;

DASSANAYAKE, R. S.; RAJAPAKSHA, S. M.; MANAMPERI, A.; FERNANDO, C. A. N.; KULATUNGA, A. K.; MANIPURA, A. Development of Starch-Based Materials Using Current Modification Techniques and Their Applications: A Review. **Molecules**, Basel, vol. 26, no. 22, p. 1–30, 2021. DOI 10.3390/molecules26226880. Available at: <https://www.mdpi.com/1420-3049/26/22/6880>. Accessed on: 1 Feb. 2023.

AMJADI, M.; FATEMI, A. Tensile Behavior of High-Density Polyethylene Including the Effects of Processing Technique, Thickness, Temperature, and Strain Rate. **Polymers**, Basel, vol. 12, no. 9, p. 1–14, 2020. DOI 10.3390/polym12091857. Available at: <https://www.mdpi.com/2073-4360/12/9/1857>. Accessed on: 20 Apr. 2023.

ARADMEHR, A.; JAVANBAKHT, V. A novel biofilm based on lignocellulosic compounds and chitosan modified with silver nanoparticles with multifunctional properties: Synthesis and characterization. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, Amsterdam, vol. 600, p. 1–8, 2020. DOI 10.1016/j.colsurfa.2020.124952. Available at: <https://www.sciencedirect.com/science/article/pii/S0927775720305458>. Accessed on: 19 Apr. 2023.

ASTM. **D570-22: Standard Test Method for Water Absorption of Plastics**. West Conshohocken, United States: [s. n.], 2022. Available at: <https://www.astm.org/d0570-22.html>.

ASTM. **D882-18: Standard Test Method for Tensile Properties of Thin Plastic Sheeting**. West Conshohocken: [s. n.], 2018. Available at: <https://www.astm.org/d0882-18.html>.

BATISTA, J. T. S.; ARAÚJO, C. S.; PEIXOTO JOELE, M. R. S.; SILVA, J. O. C.; LOURENÇO, L. F. H. Study of the effect of the chitosan use on the properties of biodegradable films of myofibrillar proteins of fish residues using response surface methodology. **Food Packaging and Shelf Life**, Amsterdam, vol. 20, p. 1–10, 2019. DOI 10.1016/j.fpsl.2019.100306. Available at: <https://www.sciencedirect.com/science/article/pii/S2214289418303259>. Accessed on: 15 Feb. 2023.

BIRD, R. B.; STEWART, W. E.; LIGHTFOOT, E. N. **Transport Phenomena**. 2nd ed. New York: John Wiley & Sons, 2002.

BREBU, M.; VASILE, C. Thermal Degradation of Lignin – A Review. **Cellulose Chemistry and Technology**, Iași, vol. 44, no. 9, p. 353–363, 2010. Available at: [https://www.cellulosechemtechnol.ro/pdf/CCT9\(2010\)/P.353-363.pdf](https://www.cellulosechemtechnol.ro/pdf/CCT9(2010)/P.353-363.pdf). Accessed on: 26 Apr. 2023.

BRODNJAK, U. V.; TODOROVA, D. Chitosan and Rice Starch Films as Packaging Materials. 2018. **International symposium on graphic engineering and design [...]**. Novi Sad: University of Novi Sad, 2018. p. 275–280. DOI 10.24867/GRID-2018-p34. Available at: <https://www.grid.uns.ac.rs/symposium/enzbornik2018.html>.

CALLISTER, W. D.; RETHWISCH, D. G. **Materials Science and Engineering: An Introduction**. 10th ed. Hoboken: Wiley, 2018.

CHEN, L.; TANG, C.; NING, N.; WANG, C.; FU, Q.; ZHANG, Q. Preparation and properties of chitosan/lignin composite films. **Chinese Journal of Polymer Science**, Hackensack, vol. 27, no. 05, p. 739–746, 2009. DOI 10.1142/S0256767909004448. Available at: <https://www.worldscientific.com/doi/10.1142/S0256767909004448>. Accessed on: 20 Dec. 2022.

CONVERSO. **Global Plastics Flow 2018**. Mainaschaff: [s. n.], 2020. Available at: <https://www.bkv-gmbh.de/studies/study-global-plastics-flow-2018-conversio.html>.

CROUVISIER-URION, K.; BODART, P. R.; WINCKLER, P.; RAYA, J.; GOUGEON, R. D.; CAYOT, P.; DOMENEK, S.; DEBEAUFORT, F.; KARBOWIAK, T. Biobased Composite Films from Chitosan and Lignin: Antioxidant Activity Related to Structure and Moisture. **ACS Sustainable Chemistry & Engineering**, Washington, vol. 4, no. 12, p. 6371–6381, 5 Dec. 2016. DOI 10.1021/acssuschemeng.6b00956. Available at: <https://pubs.acs.org/doi/10.1021/acssuschemeng.6b00956>. Accessed on: 25 Apr. 2023.

DUAN, J.; HAN, C.; LIU, L.; JIANG, J.; LI, J.; LI, Y.; GUAN, C. Binding Cellulose and Chitosan via Intermolecular Inclusion Interaction: Synthesis and Characterisation of Gel. **Journal of Spectroscopy**, London, vol. 2015, p. 1–6, 2015. DOI 10.1155/2015/179258. Available at: <https://www.hindawi.com/journals/jspec/2015/179258/>. Accessed on: 11 Apr. 2023.

DUVAL, A.; LAWOKO, M. A review on lignin-based polymeric, micro- and nano-structured materials. **Reactive and Functional Polymers**, Amsterdam, vol. 85, p. 78–96, 2014. <https://doi.org/10.1016/j.reactfunctpolym.2014.09.017>.

EZATI, P.; KHAN, A.; PRIYADARSHI, R.; BHATTACHARYA, T.; TAMMINA, S. K.; RHIM, J.-W. Biopolymer-based UV protection functional films for food packaging. **Food Hydrocolloids**, Amsterdam, vol. 142, p. 1–17, 2023. DOI 10.1016/j.foodhyd.2023.108771. Available at: <https://www.sciencedirect.com/science/article/pii/S0268005X2300317X>. Accessed on: 21 Apr. 2023.

FERNANDES, É. S.; BUENO, D.; PAGNOCCA, F. C.; BRIENZO, M. Minor Biomass Particle Size for an Efficient Cellulose Accessibility and Enzymatic Hydrolysis. **ChemistrySelect**, Hoboken, vol. 5, no. 25, p. 7627–7631, 7 Jul. 2020. DOI 10.1002/slct.202001008. Available at: <https://chemistry-europe.onlinelibrary.wiley.com/doi/full/10.1002/slct.202001008>. Accessed on: 23 Mar. 2023.

FERNANDEZ, J. G.; INGBER, D. E. Manufacturing of Large-Scale Functional Objects Using Biodegradable Chitosan Bioplastic. **Macromolecular Materials and Engineering**, Hoboken, vol. 299, no. 8, p. 932–938, 1 Aug. 2014. DOI 10.1002/mame.201300426. Available at: <https://onlinelibrary.wiley.com/doi/10.1002/mame.201300426>. Accessed on: 11 Apr. 2023.

FRIED, J. R. **Physical chemistry of surfaces**. 3rd ed. Hoboken: Prentice-Hall International, 2014.

GANEWATTA, M. S.; LOKUPITIYA, H. N.; TANG, C. Lignin Biopolymers in the Age of Controlled Polymerization. **Polymers**, Basel, vol. 11, no. 7, p. 1–44, 2019. DOI 10.3390/polym11071176. Available at: <https://www.mdpi.com/2073-4360/11/7/1176>. Accessed on: 7 Apr. 2023.

GE, H.; WANG, S. Thermal preparation of chitosan–acrylic acid superabsorbent: Optimization, characteristic and water absorbency. **Carbohydrate Polymers**, Amsterdam, vol. 113, p. 296–303, 2014. DOI 10.1016/j.carbpol.2014.06.078. Available at: <https://www.sciencedirect.com/science/article/pii/S0144861714006560>. Accessed on: 26 Apr. 2023.

GENT, S.; TWEDT, M.; GEROMETTA, C.; ALMBERG, E. Chapter Two - Introduction to Feedstocks. In: GENT, S.; TWEDT, M.; GEROMETTA, C.; ALMBERG, E. B. T.-T. and A. A. of B. T. (eds.). **Theoretical and Applied Aspects of Biomass Torrefaction**. Oxford: Butterworth-Heinemann, 2017. p. 17–39. DOI 10.1016/B978-0-12-809483-9.00002-6. Available at: <https://www.sciencedirect.com/science/article/pii/B9780128094839000026>.

GIL-PÉREZ, I.; REBOLLAR, R.; LIDÓN, I. Without words: the effects of packaging imagery on consumer perception and response. **Current Opinion in Food Science**, Amsterdam, vol. 33, p. 69–77, 2020. DOI 10.1016/j.cofs.2019.12.006. Available at: <https://www.sciencedirect.com/science/article/pii/S2214799319301353>. Accessed on: 21 Apr. 2023.

GUAN, M.; AN, X.; ZHANG, H.; YANG, G.; TIAN, Z.; NIE, S.; LIU, L.; CHENG, Z.; CAO, H.; LIU, H. Lignin emulsifying rosin for improved sizing performance and mechanical properties of liquid packaging board. **Industrial Crops and Products**, Amsterdam, vol. 187, p. 1–11, 2022. DOI 10.1016/j.indcrop.2022.115276. Available at: <https://www.sciencedirect.com/science/article/pii/S0926669022007592>. Accessed on: 17 Apr. 2023.

ISO. **ISO 62:2008: Plastics - Determination of water absorption**. Vernier: [s. n.], 2021. Available at: <https://www.iso.org/standard/41672.html>.

JACOB, J.; GOMES, F.; HAPONIUK, J. T.; KALARIKKAL, N.; THOMAS, S. **Natural Polymers: Perspectives and Applications for a Green Approach**. 1st ed. London: Taylor & Francis Group, 2022. DOI 10.1201/9781003130765. Available at:

<https://www.taylorfrancis.com/books/edit/10.1201/9781003130765>.

- JAGANATHAN, G.; MANIVANNAN, K.; LAKSHMANAN, S.; SITHIQUE, M. A. Fabrication and characterization of Artocarpus heterophyllum waste derived lignin added chitosan biocomposites for wound dressing application. **Sustainable Chemistry and Pharmacy**, Amsterdam, vol. 10, p. 27–32, 2018. DOI 10.1016/j.scp.2018.08.002. Available at: <https://www.sciencedirect.com/science/article/pii/S2352554118300433>. Accessed on: 24 Apr. 2023.
- JAKUBOWSKA, E.; GIERSEWSKA, M.; NOWACZYK, J.; OLEWNIK-KRUSZKOWSKA, E. Physicochemical and storage properties of chitosan-based films plasticized with deep eutectic solvent. **Food Hydrocolloids**, Amsterdam, vol. 108, p. 1–10, 2020. DOI 10.1016/j.foodhyd.2020.106007. Available at: <https://www.sciencedirect.com/science/article/pii/S0268005X2030059X>. Accessed on: 26 Apr. 2023.
- JANIK, W.; WOJTLA, A.; PIETRUSZKA, A.; DUDEK, G.; SABURA, E. Environmentally Friendly Melt-Processed Chitosan/Starch Composites Modified with PVA and Lignin. **Polymers**, Basel, vol. 13, no. 16, p. 1–14, 2021. DOI 10.3390/polym13162685. Available at: <https://www.mdpi.com/2073-4360/13/16/2685>. Accessed on: 20 Feb. 2023.
- KAMDEM, D. P.; SHEN, Z.; NABINEJAD, O.; SHU, Z. Development of biodegradable composite chitosan-based films incorporated with xylan and carvacrol for food packaging application. **Food Packaging and Shelf Life**, Amsterdam, vol. 21, p. 1–9, 2019. DOI 10.1016/j.fpsl.2019.100344. Available at: <https://www.sciencedirect.com/science/article/pii/S2214289418301790>. Accessed on: 3 Mar. 2023.
- LECETA, I.; GUERRERO, P.; DE LA CABA, K. Functional properties of chitosan-based films. **Carbohydrate Polymers**, Amsterdam, vol. 93, no. 1, p. 339–346, 2013. DOI 10.1016/j.carbpol.2012.04.031. Available at: <https://www.sciencedirect.com/science/article/pii/S0144861712003712>. Accessed on: 18 Apr. 2023.
- LI, C.; ZHENG, C.; HUANG, H.; SU, H.; HUANG, C. Preparation and plasticizing mechanism of deep eutectic solvent/lignin plasticized chitosan films. **International Journal of Biological Macromolecules**, Amsterdam, , p. 1–45, 2023. DOI 10.1016/j.ijbiomac.2023.124473. Available at: <https://www.sciencedirect.com/science/article/pii/S0141813023013673>. Accessed on: 18 Apr. 2023.
- LI, J.; TIAN, X.; HUA, T.; FU, J.; KOO, M.; CHAN, W.; POON, T. Chitosan Natural Polymer Material for Improving Antibacterial Properties of Textiles. **ACS Applied Bio Materials**, Washington, vol. 4, no. 5, p. 4014–4038, 17 May 2021. DOI 10.1021/acsabm.1c00078. Available at: <https://pubs.acs.org/doi/10.1021/acsabm.1c00078>. Accessed on: 1 Feb. 2023.
- LISY, A.; HÁZ, A.; NADÁNYI, R.; JABLONSKY, M.; ŠURINA, I. About Hydrophobicity of Lignin: A Review of Selected Chemical Methods for Lignin Valorisation in Biopolymer Production. **Energies**, Basel, vol. 15, no. 17, p. 1–27, 2022. DOI 10.3390/en15176213. Available at: <https://www.mdpi.com/1996-1073/15/17/6213>. Accessed on: 17 Apr. 2023.
- LIU, Q.; LUO, L.; ZHENG, L. Lignins: Biosynthesis and Biological Functions in Plants. **International Journal of Molecular Sciences**, Basel, vol. 19, no. 2, p. 1–16, 2018. DOI 10.3390/ijms19020335. Available at: <https://www.mdpi.com/1422-0067/19/2/335>. Accessed on: 7 Apr. 2023.
- LUO, J.; GU, Y.; YUAN, Y.; WU, W.; JIN, Y.; JIANG, B. Lignin-induced sacrificial conjoined-network enabled strong and tough chitosan membrane for food preservation. **Carbohydrate Polymers**, Amsterdam, , p. 1–35, 2023. DOI 10.1016/j.carbpol.2023.120876. Available at: <https://www.sciencedirect.com/science/article/pii/S0144861723003417>. Accessed on: 7 Apr. 2023.
- LUO, Y.; WANG, Q. Recent development of chitosan-based polyelectrolyte complexes with natural polysaccharides for drug delivery. **International Journal of Biological Macromolecules**, Amsterdam, vol. 64, p. 353–367, 2014. DOI 10.1016/j.ijbiomac.2013.12.017. Available at: <https://www.sciencedirect.com/science/article/pii/S0141813013006673>. Accessed on: 11 Feb. 2023.
- MARIANA, M.; ALFATAH, T.; H.P.S., A. K.; YAHYA, E. B.; OLAIYA, N. G.; NURYAWAN, A.; MISTAR, E. M.; ABDULLAH, C. K.; ABDULMADJID, S. N.; ISMAIL, H. A current advancement on the role of lignin as sustainable reinforcement material in biopolymeric blends. **Journal of Materials Research and Technology**, Amsterdam, vol. 15, p. 2287–2316, 2021. DOI 10.1016/j.jmrt.2021.08.139. Available at: <https://www.sciencedirect.com/science/article/pii/S2238785421009649>. Accessed on: 24 Apr. 2023.
- MEDINA-JARAMILLO, C.; OCHOA-YEPES, O.; BERNAL, C.; FAMÁ, L. Active and smart biodegradable packaging based on starch and natural extracts. **Carbohydrate Polymers**, Amsterdam, vol. 176, p. 187–194, 2017. DOI 10.1016/j.carbpol.2017.08.079. Available at: <https://www.sciencedirect.com/science/article/pii/S0144861717309554>. Accessed on: 1 Apr. 2023.
- MICHELIN, M.; MARQUES, A. M.; PASTRANA, L. M.; TEIXEIRA, J. A.; CERQUEIRA, M. A. Carboxymethyl cellulose-based films: Effect of organosolv lignin incorporation on physicochemical and antioxidant properties. **Journal of Food Engineering**, Amsterdam, vol. 285, p. 1–9, 2020. DOI 10.1016/j.jfoodeng.2020.110107. Available at: <https://www.sciencedirect.com/science/article/pii/S0260877420302053>. Accessed on: 19 Apr. 2023.
- MOALLA, S.; AMMAR, I.; FAUCONNIER, M.-L.; DANTHINE, S.; BLECKER, C.; BESBES, S.; ATTIA, H. Development and characterization of chitosan films carrying Artemisia campestris antioxidants for potential use as active food packaging materials. **International Journal of Biological Macromolecules**, Amsterdam, vol. 183, p. 254–266, 2021. DOI 10.1016/j.ijbiomac.2021.04.113. Available at: <https://www.sciencedirect.com/science/article/pii/S0141813021008795>. Accessed on: 16 Feb. 2023.
- NGUYEN, T. T.; THI DAO, U. T.; THI BUI, Q. P.; BACH, G. L.; HA THUC, C. N.; HA THUC, H. Enhanced antimicrobial activities and physicochemical properties of edible film based on chitosan incorporated with Sonneratia caseolaris (L.) Engl. leaf extract. **Progress in Organic Coatings**, Amsterdam, vol. 140, p. 1–9, 2020. DOI 10.1016/j.porgcoat.2019.105487. Available at: <https://www.sciencedirect.com/science/article/pii/S030094401931402X>. Accessed on: 20 Feb. 2023.
- OECD. Global Plastics Outlook: Greenhouse gas emissions from primary plastics. 2020. DOI doi.org/10.1787/1ac580ff-en. Available at: https://www.oecd-ilibrary.org/environment/data/global-plastic-outlook_c0821f81-en. Accessed on: 6 Apr. 2023.
- OECD. Global Plastics Outlook: Plastics use by polymer - projections. 2023. DOI 10.1787/b9bae4d1-en. Available at: https://www.oecd-ilibrary.org/environment/data/global-plastic-outlook_c0821f81-en. Accessed on: 6 Apr. 2023.
- PELLIS, A.; GUEBITZ, G. M.; NYANHONGO, G. S. Chitosan: Sources, Processing and Modification Techniques. **Gels**, Basel, vol. 8, no. 7, p. 1–27, 2022. DOI 10.3390/gels8070393. Available at: <https://www.mdpi.com/2310-2861/8/7/393>. Accessed on: 1 Feb. 2023.
- PLASTICS EUROPE. **Plastics-The facts 2021**. Brussels: [s. n.], 2022. Available at: <https://plasticseurope.org/knowledge-hub/plastics-the-facts-2021/>.
- PONNUSAMY, P. G.; MANI, S. Material and Environmental Properties of Natural Polymers and Their Composites for Packaging Applications-A Review. **Polymers**, Basel, vol. 14, no. 19, p. 1–22, 2022. DOI 10.3390/polym14194033. Available at: <https://www.mdpi.com/2073-4360/14/19/4033>. Accessed on: 31 Jan. 2023.
- PRIYADARSHI, R.; RHIM, J.-W. Chitosan-based biodegradable functional films for food packaging applications. **Innovative Food**

Science & Emerging Technologies, Amsterdam, vol. 62, p. 1–20, 2020. DOI 10.1016/j.ifset.2020.102346. Available at: <https://www.sciencedirect.com/science/article/pii/S1466856420302927>. Accessed on: 1 Feb. 2023.

PROVDER, T.; URBAN, M. W. **Film Formation in Coatings: Mechanisms, Properties, and Morphology**. 1st ed. Washington: American Chemical Society, 2001. DOI 10.1021/bk-2001-0790. Available at: <https://pubs.acs.org/doi/book/10.1021/bk-2001-0790>.

RAI, S.; DUTTA, P. K.; MEHROTRA, G. K. Lignin Incorporated Antimicrobial Chitosan Film for Food Packaging Application. **Journal of Polymer Materials**, New Delhi, vol. 34, no. 1, p. 171–183, 2017. Available at: <https://www.printspublications.com/journal/journalofpolymermaterialsaninternationaljournal13712463644>. Accessed on: 20 Apr. 2023.

RODRIGUES, A. A. M.; COSTA, R. R. da; SANTOS, L. F. dos; SILVA, S. de M.; BRITTO, D. de; LIMA, M. A. C. de. Properties and characterization of biodegradable films obtained from different starch sources. **Food Science and Technology**, Campinas, vol. 41, no. suppl 2, p. 476–482, 1 Feb. 2021. DOI 10.1590/fst.28520. Available at: <https://www.scielo.br/fj/cta/a/GtX8N67GRGSNmTqp6ncKnwJ/>. Accessed on: 15 Feb. 2023.

RODSAMRAN, P.; SOTHORNVIT, R. Lime peel pectin integrated with coconut water and lime peel extract as a new bioactive film sachet to retard soybean oil oxidation. **Food Hydrocolloids**, Amsterdam, vol. 97, p. 1–9, 2019. DOI 10.1016/j.foodhyd.2019.105173. Available at: <https://www.sciencedirect.com/science/article/pii/S0268005X1930757X>. Accessed on: 13 Feb. 2023.

ROSOVA, E.; SMIRNOVA, N.; DRESVYANINA, E.; SMIRNOVA, V.; VLASOVA, E.; IVAN'KOVA, E.; SOKOLOVA, M.; MASLENNIKOVA, T.; MALAFEEV, K.; KOLBE, K.; KANERVA, M.; YUD, V. Biocomposite Materials Based on Chitosan and Lignin: Preparation and Characterization. **Cosmetics**, Basel, vol. 8, no. 1, p. 1–17, 2021. DOI 10.3390/cosmetics8010024. Available at: <https://www.mdpi.com/2079-9284/8/1/24>. Accessed on: 14 Apr. 2023.

SCHMATZ, A. A.; TYHODA, L.; BRIENZO, M. Sugarcane biomass conversion influenced by lignin. **Biofuels, Bioproducts and Biorefining**, Hoboken, vol. 14, no. 2, p. 469–480, 2020. DOI 10.1002/bbb.2070. Available at: <https://onlinelibrary.wiley.com/doi/10.1002/bbb.2070>. Accessed on: 1 Dec. 2022.

SHANKAR, S.; REDDY, J. P.; RHIM, J.-W. Effect of lignin on water vapor barrier, mechanical, and structural properties of agar/lignin composite films. **International Journal of Biological Macromolecules**, Amsterdam, vol. 81, p. 267–273, 2015. DOI 10.1016/j.ijbiomac.2015.08.015. Available at: <https://www.sciencedirect.com/science/article/pii/S0141813015005504>. Accessed on: 19 Apr. 2023.

ZHAO, J.; WANG, Y.; LIU, C. Film Transparency and Opacity Measurements. **Food Analytical Methods**, Amsterdam, vol. 15, no. 10, p. 2840–2846, 2022. DOI 10.1007/s12161-022-02343-x. Available at: <https://link.springer.com/article/10.1007/s12161-022-02343-x>. Accessed on: 12 Apr. 2023.

ZOU, Z.; ISMAIL, B. B.; ZHANG, X.; YANG, Z.; LIU, D.; GUO, M. Improving barrier and antibacterial properties of chitosan composite films by incorporating lignin nanoparticles and acylated soy protein isolate nanogel. **Food Hydrocolloids**, Amsterdam, vol. 134, p. 108091, 2023. DOI 10.1016/j.foodhyd.2022.108091. Available at: <https://www.sciencedirect.com/science/article/pii/S0268005X22006117>. Accessed on: 19 Apr. 2023.

GENERAL CONCLUSIONS

Raw banana pseudostem and guava seed cake were chemically characterized, thereby, it was found that their compositions fit to a similar lignocellulosic biomasses that have been considered as potential sources to obtain different types of bioproducts from its main components (cellulose, hemicelluloses and lignin).

With the alkali organosolv pretreatment was possible to fraction each fruit waste (banana pseudostem and guava seed cake) in three solid fractions (cellulose-rich, hemicelluloses, and lignin). The ethanol addition (to the spent liquor) and the solvent evaporation (after hemicelluloses extraction), allowed hemicelluloses and lignin precipitations, respectively, and its subsequent extractions with significative yields. Digestibility (enzymatic hydrolysis) of each pretreated biomass confirmed that the alkali organosolv pretreatment greatly contributed to obtain materials with loose lignocellulosic structure. Thus, the alkali organosolv pretreatment ratified that it can be used as fractionation strategy of lignocellulosic biomass when a biorefinery scheme is applied.

To the pretreated biomass from every configuration of alkali organosolv pretreatment was determined the lignocellulosic composition, which had direct relation with the mass yields of extracted hemicelluloses and lignin. High yields of extracted hemicelluloses and lignin matched with low hemicelluloses and lignin contents, respectively, in each pretreated biomass.

FTIR spectroscopy used to analyze the hemicelluloses and lignin extracted from banana pseudostem and guava seed cake, allowed to conclude that those macromolecules showed great correspondence with the commercial ones.

Two types of bioplastics were successfully produced using the mass proportions established for hemicelluloses and lignin. The hemicelluloses and lignin affected the color, opacity, moisture content, solubility, swelling, tensile and thermal properties of the obtained bioplastics. The bioplastics with the highest contents of hemicelluloses and lignin had a less distribution in color, and showed the maximum opacities. Hemicelluloses and lignin additions improved the tensile strength and Young's modulus properties. Besides that, the addition of hemicelluloses decreased the temperature at which the bioplastics lost mass during the thermal degradation analysis. Conversely, the same analysis showed that the lignin incorporation raised the

temperature at which the bioplastics had their maximum mass losses. Overall, the study suggests that these bioplastics could be used in food or medicine packaging, where light and water control are important factors.