UNIVERSIDAD NACIONAL DE COLOMBIA

ASSESSMENT OF NON-CONVENTIONAL PRETREATMENTS FOR AGRICULTURE WASTES UTILIZATION

Laura Vanessa Daza Serna

Universidad Nacional de Colombia
Facultad e Ingeniería y Arquitectura, Departamento de Ingeniería Química
Manizales, Colombia
2015

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Laura Vanessa Daza Serna

Thesis submitted in partial fulfillment of the requirements for the degree of:
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Advisor:
Ph.D, M.Sc, Chemical Engineer Carlos Ariel Cardona
Full professor of Chemical Engineering

Research line:
Lignocellulosic biomass pretreatment
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EVALUACIÓN DE PRETRATAMIENTOS NO CONVENCIONALES PARA EL APROVECHAMIENTO DE RESIDUOS AGRÍCOLAS

Laura Vanessa Daza Serna

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Universidad Nacional de Colombia
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“Me enseñaste a encender la bondad como el fuego”

To my grandmother and brother

For everything, for being here

To my mom and grandfather

For being in my heart keeping it warm.

“…Quedó una luz como una estela que el cuarto antes no conocía”

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Abstract

The current pretreatments of lignocellulosic biomass conventional involve the intensive use of high temperatures, pressures, long times of residence, added to high chemical inputs requirements and elevated energy consumption which sometimes producing inhibitory compounds and sugars degradation. As an alternative to their use two non-conventional pretreatments such as ultrasound assisted pretreatment and supercritical carbon dioxide pretreatments were used to evaluate pretreatment yields and enzymatic digestibility of rice husk, cassava stem and plantain pseudostem. Some operational conditions such as temperature, amplitude, pretreatment time were evaluated during the ultrasound assisted pretreatment whereas the moisture content, pressure and temperature of the pretreatment were evaluated for supercritical carbon dioxide pretreatment. The best performance of ultrasound assisted pretreatment was presented by plantain pseudostem improving the reducing sugars production to 2-3 folds compared the dilute pretreatment (0.78 g total reducing sugars/g of biomass recovered from pretreatment stage). This yield was reached at 60°C and 100% of ultrasound amplitude. By the other hand, for supercritical pretreatment the best performance was presented using rice husk as raw material obtaining reductions in crystallinity index and redistribution of the lignocellulosic matrix.

It could be established that different effects according to the raw material specifications can be reached. Finally it was concluded as a first approach that through these pretreatments would be significantly impacted the technological, economic and environmental performance of scales.

Keywords: sugars production, utilization of agricultural wastes, supercritical fluids, ultrasonic waves, lignocellulosic biomass, digestibility, recalcitrance, digestibility.

Resumen

Los pretratamientos convencionales de biomasa lignocelulósica involucran el uso intensivo de altas temperaturas, presiones, largos tiempos de residencia sumado a los requerimientos de productos químicos y los elevados consumos energéticos pueden llevar a promover la producción de compuestos inhibitorios así como la degradación de azúcares. Como una alternativa a su uso, dos pretratamientos no convencionales (Pretratamiento asistido con ultrasonido y pretratamiento con fluidos supercríticos) fueron empleados para evaluar los rendimientos de este etapa y la digestibilidad enzimática de materias primas de origen agrícola como la cascarilla de arroz, el tallo de plátano y el pseudotallo de plátano. Algunas variables operacionales como temperatura, amplitud y tiempos de pretratamiento fueron evaluados durante el Pretratamiento asistido con ultrasonido, mientras que el contenido de humedad, presión y temperatura fueron las variables analizadas en el pretratamiento con dióxido de carbono supercrítico. La materia prima que ofreció un mejor comportamiento al ser pretratada con ultrasonido fue el pseudotallo de plátano incrementando la producción de azúcares reductores en ordenes de 2-3 veces comparado con el pretratamiento con ácido diluido (0.78 g azúcares reductores/ g biomasa pretratada recuperada). Este rendimiento fue alcanzado a una temperature de 60°C y 100% de amplitud. Por otro lado, la materia prima que presentó el mejor comportamiento en reducción de índice de cristalinidad y una adecuada redistribución en la matriz lignocelulósica.

Finalmente, logró establecerse que diferentes efectos pueden ser alcanzados de acuerdo con las características de las materias primas, estos a su vez pueden generar diferentes impactos a nivel técnico, económico y ambiental.

Palabras claves: producción de azúcares, uso de materias primas de origen agrícola, fluidos supercríticos, ondas ultrasónicas, biomasa lignocelulósica, cristalinidad, reclacitrancia, digestibilidad.

Content

Figures

Tables

List of Publications

Research Papers

- i) **Daza Serna L.V**, Orrego Alzate C.E, Cardona Alzate C.A. Supercritical fluids as a green technology for the pretreatment of lignocellulosic biomass. Bioresource Technology. 2016; 199:113-120.

Submitted Papers

- i) **Daza Serna L.V**, Sanchez Rendón J.C, Cardona Alzate C.A. Análisis Tecno-económico de una Biorrefinería a Partir de Residuos del Plátano. Submitted to: Revista de la Facultad de Ciencias Quimicas. Universidad de Cuenca Ecuador.
- ii) **Daza Serna L.V**, Solarte J.C, Cardona C.A. Biorefineries for Energy Production through Agricultural Waste Management: The Colombian Case. Submitted to: Waste and Biomass Valorization.

Book Chapters

- i) González Villa A., **Daza Serna L.V**, Cardona Alzate C.A. The Antioxidants from Amazon: Status and Perspectives. In:Handbook of Anthocyanins: Food Sources, Chemical Applications and Health Benefits. Editorial: Nova Science Publishers, Inc.

Book chapters under review

- i) Hernández **V, Daza L**, Cardona C.A. Citrus residues as raw materials for biomolecules and energy. The orange peel case. In: Citrus Fruits: Production, Consumption and Health Benefits. Editorial: Nova Science Publishers, Inc. Under Submission.

Conference Papers

Congreso Internacional de Ciencia y Tecnología de los Biocombustibles CIBSCOL 2014 Cartagena (Colombia), March 19-21, 2014

- **i)** **Daza Serna L.V**, Idárraga Vélez A.M, Cardona Alzate C.A. Evaluación tecno-económica de una biorefinería a partir de pulpa de café. Oral Presentation

21st International Congress of Chemical and Process Engineering. Prague (Czech Republic), August 23-27 2014

- **ii)** Dávila Rincón J.A, **Daza Serna L.V,** Cardona Alzate C.A. Techno-economic analysis of natural antioxidants extraction from lulo (*Solanum quiroense*) with supercritical carbon dioxide. Oral presentation

13th Mediterranean Congress of Chemical Engineering. Barcelona (Spain) September 30-October 03 3014

- **iii)** Dávila Rincón J.A, **Daza Serna L.V**, Gonzalez Villa, A.A, Cardona Alzate C.A. Reducing sugar production from some agroindustrial Colombian wastes. Poster presentation.

XXVII Congreso Interamericano y Colombiano de Ingeniería Química. Cartagena (Colombia) October 06-08 2014

- **iv)** Hernandez Piedrahita V, **Daza Serna L.V.** Análisis Tecno-económico de una biorefinería a partir de residuos de Plátano. Poster presentation.
- **v)** Dávila Rincón, J.A, **Daza Serna L.V**, Cardona Alzate J.A. Evaluación de la producción de butanol a partir del bagazo de caña panelera. Poster presentation.
- **vi)** **Daza Serna L.V**, Idárraga Vélez A.M, Cardona Alzate C.A. Análisis Tecno-Económico De Una Biorefinería De Borra De Café (Coffea Spp). Oral Presentation.

2014 Annual Meeting AIChE. Atlanta (USA), November 16-21 2014

- **vii)** Hernandez Piedrahita V, Carvajal Ocampo J.C, **Daza Serna L.V**, Cardona Alzate C.A. Green House Gas Emissions from Lignocellulosics used as Raw Material Biorefineries. Oral presentation.

- **viii)** Carvajal Ocampo J.C, **Daza Serna L.V**, Aristizábal Marulanda V, Cardona Alzate C.A. Pretreatment, Saccharification, Fermentation and Extraction Processes to Obtain Ethanol. Oral presentation.
- **ix)** **Daza Serna L.V**, Rojas Restrepo M.A, Cardona Alzate C.A. A New Policy for the Use of Agricultural Residues in Tropical Countries Based on Biorefineries. Poster presentation.
- **x)** Hernandez Piedrahita V., **Daza Serna L.V**, Rojas Restrepo M.A, Cardona Alzate C.A. Analysis of the Production of Biofertilizers from Colombian Lignocellulosics Feedstocks. Oral presentation.
- **xi)** Gonzalez Villa A.A, **Daza Serna L.V**, Cardona Alzate C.A. Improving the Sustainability of Antioxidants Production through Biorefinery Concept. the Blackberry Case. Poster presentation.
- **xii)** Dávila Rincón J.A, **Daza Serna L.V**, Cardona Alzate C.A. Techno-economic analysis of natural antioxidants extraction from lulo (*Solanum quiroense)* with supercritical carbon dioxide. Oral presentation.

3rd International Conference on Sustainable Solid Waste Management. Tinos Island (Greece), July 2nd-4th 2015

- **xiii)** **Daza Serna L.V**, Cardona Alzate C.A. Energy Biorefineries for Agricultural Waste Management: The Colombian Case. Oral Presentation

10th European Congress of Chemical Engineering. Niza (France) September 27th – October 1st 2015

- **xiv)** **Daza Serna L.V**, Betancourt Grajales R, Cardona Alzate C.A. Modeling and Simulation of Supercritical Assisted Pretreatment in Biomass Uses. Oral presentation

VI Simposio Internacional Biofabricas. Medellín (Colombia) August 12th-14th 2015

- **xv)** **Daza Serna L.V**, Gómez Peña A. Cardona Alzate C.A. Evaluación de la prefactibilidad del aprovechamiento de residuos de plátano. Oral presentation.

XXVIII Congreso Colombiano de Ingeniería Química. Bogotá (Colombia) October 28th-30th 2015

- **xvi)** Majé Cardona M, **Daza Serna L.V**, Cardona Alzate C.A. Evaluación de la Producción de Inhibidores en Pretratamientos de Biomasa no Convencionales. Poster Presentation.

xvii) Solarte Toro J.C, **Daza Serna L.V,** Cardona Alzate C.A. Efecto de la reciruclación celular en la producción de bioetanol a partir de miel de caña. Poster presentation.

2015 Annual Meeting AIChE. Salt lake city (USA)

xviii) **Daza Serna L.V**, Betacourt Grajales R, Cardona Alzate C.A. Supercritical Assisted Pretreatment of Cassava and Plantain Residues. Oral presentation.

xix) Caballero Galván A, **Daza Serna L.V**, García Velasquez C.A, Cardona Alzate. Techno-Economic Assessment of Biodiesel Production from Palm Oil By Supercritical Transesterification. Oral presentation.

xx) **Daza Serna L.V**, Pisarenko Y.A, Duarte L.C, Carvalheiro F, Cardona Alzate C.A. Ultrasound Assisted Pretreatment of Rice Husk and Plantain. Poster presentation

xxi) Chacón Pérez Y, **Daza Serna L.V**, Cardona Alzate C.A. Techno-Economic and Environmental Assessment of Citric Acid Production By Aspergillus Niger. Poster presentation

xxii) **Daza Serna L.V**, Higuita Vasquez J.C, Serna Loaiza S, Cardona Alzate C.A. Analysis for Chlorella vulgaris Growth: A Techno-Economical Assessment of Heterotrophic and Autotrophic Cultures. Oral presentation

Participation of this Thesis in Research Projects

- Jóvenes Investigadores. Colciencias “Call 617 of 2013”
- Apoyo proyectos con Ámerica Latina. Colciencias “Call 653 of 2014”
- Evaluación experimental de la gasificación de residuos. DIMA
- “Evaluar la extracción de aceite de café a partir de la borra usando diferentes tecnologías y/o una combinación de ellas, identificando el contenido de ácidos grasos obtenido por cada una”. DIMA

Introduction

According to the US Department of Energy (DOE) at least twelve important building block chemicals can be produced from sugars. In this sense, lignocellulosic biomass can be used substituting typical non-renewable raw materials in production of different families of chemicals such as food additives, pharmaceuticals, surfactants, organic solvents, biomaterials, energy, biofuels among others.

Lignocellulosic biomass is mainly composed by cellulose, hemicellulose and lignin distributed in different shares depending the nature, type and source of the biomass. These compounds are compactly organized in a structure in which lignin and hemicellulose form a complex structure through covalent cross-linked bounds encapsulating and giving support to the cellulose. Cellulose is a highly crystalline compound that confers the structural support to cells. It is mainly composed by cellodextrines polysaccharides linked by β-(1-4) bonds. Hemicellulose is a heteropolysaccharide composed by 5-carbon (xylose, arabinose) and 6-carbon (mannose, glucose, galactose) sugars and the lignin is the responsible compound of rigidness, impermeability and resistance of cells. It is a type of aromatics macromolecules that can be composed by hydroxycinnamyl monomers with various degrees of methoxlytation [1].

Agricultural wastes can be classified as lignocellulosic biomass representing a significant portion of the total agricultural production. The inadequate disposal of these residues can generate consequences to the environment, ecosystems and humans. The principal constraint to fully utilize the biomass is related to the recalcitrant property of biomass that has been defined by *Himmel* et al as “The resistance of plant cell walls to microbial and enzymatic deconstruction” [2].

Pretreatment represents a key process in lignocellulosic utilization. An efficient pretreatment should cover the preservation of hemicellulose and cellulose fractions, to avoid or reduce the need of size particle reduction, to prevent the formation of inhibitors by further sugars degradation, to reduce the energy requirements and be cost-effective. Additionally, an adequate pretreatment process must allow the improving of sugars formation or the capability to form sugars through enzymatic hydrolysis. There exists a wide variety of pretreatments developed for different purposes. The selection of them will depend of the raw material composition and pretreatment purposes.

Hypothesis

“It is possible enhance the yield and performance of conventional process for agriculture waste biomass through the use of non-conventional assisted pretreatments as ultrasonic and supercritical carbon dioxide assisted pretreatment“

General Objective

To carry out the technical, economical and environmental evaluation to two non conventional assisted pretreatment schemes involving ultrasound pretreatment and supercritical carbon dioxide assisted pretreatment.

Specifics Objectives

1. To perform the chemical characterization of the selected raw materials (pseudostem plantain, rice husk and cassava stem).
2. To determine the effect of ultrasound assisted and supercritical carbon dioxide assisted pretreatment in the lignin content of selected raw materials.
3. To determine the effect of ultrasound pretreatment and supercritical carbon dioxide pretreatment in the digestibility of the selected raw materials through enzymatic hydrolysis.
4. To determine the effect of ultrasound assisted and supercritical carbon dioxide assisted pretreatments in the yield of fermentation.
5. To perform the technical, economical and environmental evaluation of schemes that involves the use of ultrasound assisted and supercritical carbon dioxide assisted pretreatments.

Chapter 1: Lignocellulosic Biomass

Overview

According to the information reported by the World Energy Council it is presumed that biomass can provide about 16% of the expected energy consumption of 17208 Mtoe in 2020 (World Energy Council 2013). Many studies have demonstrated the potential use of lignocellulosic biomass as a substitute of typical non-renewable raw materials in production of different families of chemicals. However, some characteristics such as lignin content, recalcitrance and cellulose crystallinity are drawbacks associated to the raw material nature. In this sense some alternatives and strategies are used for biomass transformation to different platforms. This chapter presents a brief summary about biomass and biomass characteristics, as well as some alternatives for its use.

1.1 Biomass

The term biomass was defined by ASTM as “any material, excluding fossil fuel which was a living organism that can be used as a fuel either directly or after conversion process” [3]. The international Energy Agency (IEA) agree with the concept presented defining it as “any organic, i.e. decomposable, matter derived from plants or animals available on a renewable basis. Biomass includes wood and agricultural crops, municipal organic wastes as well as manure” [4]. Other definitions presented by the Food and Agriculture Organization of United Nations (FAO) and the National Renewable Energy Laboratory (NREL) defined biomass as “The mass of live or dead organic matter” [5] and “The organic matter available on a renewable basis” [6] respectively. Finally, in the same way the Colombian Residual Biomass Atlas defined The live matter present as a thin layer located in the terrestrial surface denominated biosphere also representing a small fraction of the earth mass” [7].

It is considered that changes in biomass density represent a measure of carbon releasing or sequestration between terrestrial layer and the atmosphere [5]. Carbon dioxide sequestration by plants is known as terrestrial sequestration. The carbon dioxide caught from the atmosphere is stored by stems and roots plants to be used during photosynthesis process delivering oxygen as waste gas. In this sense, the biomass plays an important role in the earth equilibrium through green house gases (GHG) balance. Additionally, biomass can be considered as the major and most effective solar energy warehouse that can be used to meet a variety of energy needs such as electrical or heat energy, fuel for transport sector or feedstock for chemicals production.

Biomass can be classified by different categories or descriptions including: economic activities subdivided in wastes, standing forests and energy crops [1]; woody characteristics subdivided in hardwood, softwoods and grasses, and finally according to generations subdivided in first, second and third generation [2].

1.2 Lignocellulosic Biomass

Lignocellulosic biomass refers to the biomass mainly composed by cellulose, hemicellulose and lignin, the three major biopolymers linked in a complex matrix. Due to the composition described. The lignocellulosic biomass can be considered as a renewable raw material able to produce different product families as will be described. In fact, some studies reflects an increasing interest in the utilization of the residual biomass for obtaining of value-added products [8], [9]. Nevertheless, the use of lignocellulosic biomass presents disadvantages at two different scales (macro and microscale). Porosity and particle size have been identified as the major macroscale disadvantages whereas cellulose crystallinity, hemicellulose branching levels and total lignin content are the main microscale disadvantages. Different kind of residues such as stalks, leaves, trunks and woods can be classified as lignocellulosic biomass. Nevertheless, the chemical composition can vary according to the raw material nature. This fact is corroborated by the broad range of chemical composition presented in the Table 1.1

Table 1.1. Lignocellulosic chemical composition

Raw Material	**Moisture (%)**	**Extractives (%)**	**Cellulose (%)**	**Hemicellulose (%)**	**Lignin (%)**	**Ash (%)**	**Others (%)**
Whole banana pseudostem[a]	96.7	n.r	46.3	12.5	15.8	10.7	3.9
Oil palm Empty Fruit Bunches[b]	n.r	10.1	33	23	23.8	4.8	5.3
Oil palm Empty Fruit Bunches[c]	63.0	n.r	13.7	14.0	7.8	0.6	n.r
Rice Straw[b]	n.r	4.2	32.5	20.4	11.3	17.8	13.8
Palm fresh fiber[c]	38.9	n.r	24.0	14.0	12.6	3.0	n.r
Sugarcane bagasse[d]	75	8	34.80	28.96	22.62	4.17	1.45
Rice Husk[d]	10.2	1.34	26.45	27.29	28.03	14.89	2.0
Coffee cut-stem[d]	4.12	8.38	37.35	27.79	19.81	2.27	n.r
Potato peel[e]	10.0	n.r	55.25	11.71	14.24	8.8	n.r
Hardwood[e]	n.r	2-8	43-47	25-35	16-24	n.r	n.r
Softwood[f]	n.r	1-5	40-44	25-29	25-31	n.r	n.r
Cotton stalks[g]	n.r	n.r	36.6	18.1	26.4	n.r	n.r
Banana peel[h]	n.r	29.45	11.97	9.76	2.18	9,21	19.03

Sugarcane bagasse[i]	n.r	n.r	46.7	23.62	19.71	1.13	8.79
Coffee cut-stem[i]	n.r	n.r	40.39	34.01	10.13	1.27	14.18
Olive stone[j]	n.r	10.54	20.10	29.92	13.87	0.57	10.54
Rice husk[k]	n.r	7	40	16	26	11	n.r
Rice hulls[l]	10.20	2.25	29.01	26.94	15.0	16.61	n.r
Norway spruce[m]	n.r	n.r	38.8	22.2	26.4	0.3	11.9
Aspen[n]	n.r	n.r	51	29	16	n.r	n.r
Pine[n]	n.r	n.r	44-46.4	8.8-26	29.4	n.r	n.r
Oat Straw[n]	n.r	n.r	41.0	16.0	11.0	n.r	n.r
switchgrass[n]	n.r	n.r	31-37	20.4-29	17.6-19	n.r	n.r

[a]M*anilal* and *Sony*.[10], [b]*Schmidt* and *Thomsen*[11], [c]*Rincón* et al.[12], [d] *Quintero* et al[13], [e] *Leninhan* et al[14], [f] *Zugenmaier*[15], [g] *Haykir* and *Bakir*[16], [h] *Obreoi* et al[17], [l] *Aristizábal* et al [18], [j]*Hernández* et al[19], [k] *Daza* et al[20], [l]*Quintero* and *Cardona*[21], [m]*Agnihotri* et al[22], [n]*Gupta* et al[23]

Cellulose

Cellulose is a nonbranched polysaccharide of variable length composed by 1-4 linked β-D-glucopyranose (glucose) units. The degree of polymerization (DP) as well as the crystallinity index (CrI) are the main cellulose attributes that allow to establish differences between different type and nature of raw materials. DP is a measure of the chain units forming cellulose and can vary according to the raw material nature. Whereas CrI is a measure of the crystalline region within the total structure. Figure 1.1 presents the cellulose structure, the monomers can rotate 180° around the main axis [24]. Cellulose is composed by crystalline and amorphous regions. The second one has a major trend to react and be converted to sugars by enzymatic hydrolysis. The crystalline region is characterized by the lateral linkages that exhibit a kind of parallelism while the less ordered region is denominated the paracrystalline or amorphous region [24].

In addition, all the chemical properties are determined by hydroxyl group linkages. Hydrogen bonds provide to cellulose the hydrophobic property, because of that cellulosic fraction is not easily dissolved or degraded in common solvents [25]. Strong dipolar attractions are characteristics between cellulose molecules are responsible of its thermosetting behavior. Because of the mentioned behavior cellulose structure cannot be modified without chemical decomposition [15].

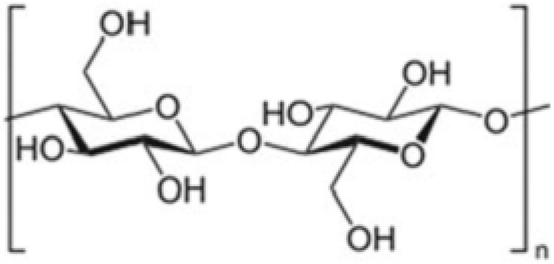

Figure 1.1. Cellulose Structure. Taken from: [1]

It has been reported that approximately 77 kcal of radiational energy is stored in each gram of pure cellulose [24]. The energy required to break down the chemical and hydrogen bonds present in cellulose is approximately 400-1000 kJ/mol and 5-30 kJ-7mol respectively [26]. Knowing these values can be estimated the power, operational conditions and requirements that should be to pretreat and take advantage of cellulose.

Hemicellulose

Hemicellulose is a heterogeneous polysaccharide bonded to other cell wall components such as cellulose, lignin, phenolic compounds by different type of bonds [27]. It is composed by pentoses (β-D-xylose and α-L-arabinose), hexoses (β-D-mannose, β-D-glucose and α-galactose) and/or uronic acids (α-D-glucuronic, α-D-4-O-methylgalacturonic and α-D-galacturonic acids).

Hemicellulose and cellulose have three main reactive sites identified as reducing end groups, glycosidic linkages and hydroxyl groups. Due to its low degree of polymerization, hemicellulose is a easily hydrolizable compound [28]. It has been demonstrated that hemicelluloses hydrolysis promotes C5 or C6 sugars formation according to the raw material nature. This is more prolific in hardwoods than softwoods [29]. For hardwoods, xylan is the dominant fraction of hemicellulose, whereas glucomannan is the corresponding dominant fraction for softwoods [28].,[30]. Recalcitrance of lignocellulosic materials is strongly affected by the content of acetyl groups in hemicellulose chains. These acetyl groups are released according to the severity of reaction during pretreatment stage. Removal of the hemicellulose helps to increase the pore size increasing the digestibility [26].

Lignin

Lignin has been defined as an amorphous polyphenolic material produced by dehydrogenative polymerization of three phenylpropanoid monomers: p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. Those molecules are known as phenylpropanoid monomers, they are conjugated forming a three-dimensional complex lignin structure. Moreover, the lignin composites includes polisaccharides (arabinose, xylose and galactose) often linked by carbon-carbon and ether bonds [31]. Additionally, other types of functional groups are composed by phenolic hydroxyl, benzylic hydrozyl and carbonyl groups. According to the different ratios of these groups the material reactivity can vary.

Rather than be considered as a compound, lignin is considered a composite because of its molecular arrangements [31]. Figure 1.2 presents the molecular structure of lignin monomers.

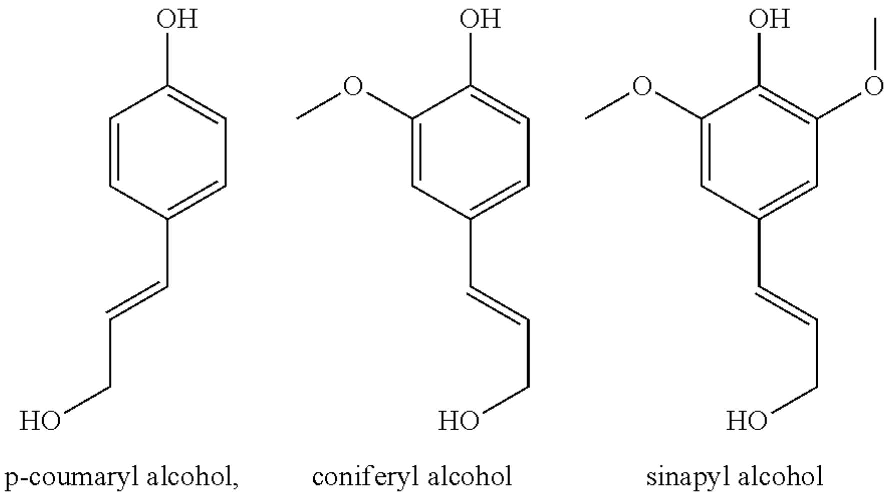

Figure 1.2. Lignin Monomers**.** Taken from:[32]

Chemical and thermal lignin decomposition have been studied to amplify the comprehension level of lignin structure. The chemical decomposition methods includes acidolysis, nitrobenzene oxidation, permanganate oxidation, hydrogenolysis, ozonitation and thermolysis [33]. Thermal decomposition methods are used to determine the structure of lignin samples and the reaction times of producing carbon from lignin sources [33].

There exist different ranges of lignin content according to the raw material configuration. For instance, lignin content is higher for softwood followed by hardwood, grasses and straws as well as, other types of herbaceous plants presents lower lignin contents. Table 1.2 presents a summary of the different lignin models proposed based on the raw material classification.

Table 1.2. Lignin Model Summary

Characteristic Materials	**Model**	**Description**
Softwoods	Guaiacyl lignin	Approximately composed (more than 95%) by coniferyl alcohol followed by p-coumaryl alcohol and trace of sinapyl alcohol

		units.
Hardwoods	Guaiacyl-Syringil lignin	This type of lignin is composed by coniferyl alcohol and sinapyl alcohol units.
Grass	Guaiacyl-Syringil lignin	This type of lignin is composed by coniferyl alcohol and sinapyl alcohol units. Additionally, it contains structural elements derived from p-coumaryl alcohol such as p-coumaric acid and ferulic acid

Lignin from lignocellulosic sources is more complex than other lignin from woody sources due to other structural elements derived from p-coumaryl alcohol as well as other phenolic acids such asferulic acid esters linked to the ɣ-position. In some materials such as rice straw, wheat straw and bagasse p-coumaryl or derived compounds can be found in molar ratios of 15, 22 and 27 respectively, the remaining molar ratio is composed by syringil and guaiacyl fractions [34].

Lignin content is deeply related to cellulose accessibility and digestibility, in this sense a reduction in lignin content should conduce to an increase in cellulose accessibility reflected in higher cellulose hydrolysis yields [35].

1.3 Strategies for Lignocellulosic Utilization

Lignocellulosic biomass has a huge potential to be used as one of the major global primary energy source in the short to medium term. In this sense, different types of assessments have been developed to determine the demand based on the competitiveness and available resource to determine the demand and supply to be potentially covered by lignocellulosic biomass [3]. The different bioenergy options should be evaluated considering the available transformation technology as well as the total biomass availability.

Lignocellulosic biomass has been used to produce electricity and heat, biofuels and biochemical compounds used as feedstocks involving the use of different production technologies. Electricity and heat can be obtained through biomass combustion, gasification and pyrolysis. The use of this raw material compared to the conventional coal combustion produces at least 90% of sulfur dioxide and ashes able to be used as soil additive on farms. By the other hand, other energy carries such as sugars and biofuels involve the use of different processes stage to produce alcohols by fermentation or sugars by hydrolysis stages.

To take advantage of lignocellulosic biomass it is necessary to allow the following steps: i) disrupt the matrix removing the hemicellulose and lignin fraction. ii) Reduce the cellulose crystallinity and iii) cellulose hydrolysis for sugars obtaining [37].

Pretreatment technologies and strategies have been designed to overcome recalcitrance making emphasis in different aspects such as lignin removal, hemicellulose hydrolysis and cellulose solubilization among others. Nevertheless, according to the selected pretreatment technology could be produced different compounds that would represent interferences in fermentative processes. These technologies are described in the second chapter.

1.4 Challenges for Lignocellulosic Utilization

Biomass Recalcitrance

Biomass recalcitrance caused by a formed matrix of lignin and hemicellulose which provides physical defense and structural support to plant tissues also acting as a protection mechanism to preserve the structural sugars from different type of enzymatic or microorganisms attacks [25]. Hemicellulose structure has potential sites for covalent bonds between carbohydrates and lignin; precursors of lignin-carbohydrate complexes [1]. Despite lignin content is strongly related to recalcitrance characteristic [25], [35], other ways as cellulose DP and crystallinity reduction [36] have been recognized as features to treat with the aim to reduce recalcitrance [1]. Other natural factors associated to recalcitrance include the tissue characteristics (epidermal, epiticular waxes, density of vascular bundles, thick wall among others) [25].

The extension of recalcitrance reduction should be determined by increases in sugar yields after pretreatment and/or during enzymatic hydrolysis. Other alternatives to determine biomass recalcitrance include Fourier transform infrared spectroscopy (FTIR), Near infrared spectroscopy (NIR), Nuclear magnetic resonance (NMR) and Mass spectrometry (MS) technologies [1].

Inhibitory Compounds

Currently, some of the pretreatment technologies used to reduce recalcitrance involve the use of harsh pH levels, high temperatures and pressures. As a consequence, due to the severity of reactions there are generated degradation products. Reaction severity or severity factor is a measure of pretreatment intensity represented by $\mathbf{R_0}$, this factor is dependent of the time (t) and temperature (T) of the pretreatment used [38]. On the other hand, pH factor has been included to determine the combined severity factor $\mathbf{Cs}$ [38]. Those products named inhibitory compounds are capable to inhibit or reduce enzymatic saccharification and fermentation yields [39]. Inhibitory compounds can be classified according to their origin in: weak acids, furan derivatives and phenolic compounds. The first group is composed by formic, levulinic and acetic acid derived hemicellulose. The second group is composed by furfural, 5-hydroxy-2-methylfurfural (HMF) derived from cellulose and hemicellulose, and finally, the last group composed by vanillin and syringaldehyde produced as lignin degradation products [40],[41].

$$\mathbf{R_0 = t * exp[(T - 100)]} \qquad \text{Eq 1}$$

$$\mathbf{CS = log\,R_0 - pH} \qquad \text{Eq 2}$$

Acetic acid is generated by the hydrolysis of acetyl groups present in the hemicellulose without sugars degradation[28], [38]. Formic and levulinic acid are formed during furfural or HMF degradation. Acetic acid at concentrations between 4 to 10 g/L can inhibit microbial growth due to its capability to going through the cellular membranes and decrease the intracellular pH affecting the microorganisms metabolism [42]. In agreement some reports indicate that 0.95 g/L of acetic acid is the maximum concentration permitted without toxic or inhibitory effects [43]. Approximately 3 g/L is limit concentration of HMF and furfural for glycolysis inhibition [38].

Furan compounds are capable to inhibit relevant enzymes such as the alcohol dehydrogenase, pyruvate dehydrogenase and aldehyde dehydrogenase [39]. HMF is a stable compound produced from glucose degradation [44]. It is a growth inhibitor of microorganisms more lethal than furfural [42]. Some process requires hot water washes because of formation of degradation products as inhibitory compound, this washes processes could contribute to the decreasing of sugars yields[45]

1.5 Enzymatic Hydrolysis

Despite cellulose is the most abundant sugar source on earth, it cannot be digested by humans and microorganisms because of the reasons beforehand developed. Hydrolysis is the process to reduce or saccharificate cellulose to fermentable sugars such as hexoses (glucose) and pentoses (xyloses, mannans). Sugar production through enzymatic hydrolysis produces high yields minimizing by-products formation, also has low energy requirements with low chemical disposal needing at mild saccharification conditions (50-55°C and atmospheric pressure) [30].

Despite the mechanism of enzymatic hydrolysis is not fully understood three type of enzymes are required to hydrolyze lignocellulosic materials: cellulases, hemicellulases, and other enzymes such as hemicellulose debranching enzymes, phenolic acid esterase enzymes and lignin degrading enzymes. The first group of enzymes, endoglucanases (endo-1,4-β-glucanases- EG, EC 3.2.1.4) attacks the low crystallinity cellulose regions cutting the chains at internal positions. The second group of enzymes, cellobiohydrolases (CBH, EC 3.2.1.91) degrades the molecules by removing cellobiose units from the free chain-ends and the last ones, β-glucosidases (BG, EC 3.2.1.21) hydrolyzes cellobiose to produce glucose [46]. Cellulases deactivation and/or inhibition could be caused by soluble sugars, cellobiose unit, organic acids, furans and phenolic compounds.[47]. Nevertheless, the addition of β-glucosidase in high amounts could contribute to reduce the inhibitory effect caused by cellobiose and glucose [47].

Sasaki et al described the pathway to produce glucose and oligomers from cellulose summarized by the Figure 1.3. In this scheme the glucose epimerizes to fructose or decomposes to erythrose and glycoaldehyde or glyceraldehyde and dihydroxyacetone. Then, the produced fructose also decomposes to the same products. Glyceraldehyde can be converted to dihydroxyacetone, and both glyceraldehydes and dihydroxyacetone dehydrated into pyruvaldehyde. Later, pyrovaldehyde, erytrhose and glycoaldehydefurther decompose to smaller species (acids, aldehydes and 1-3 carbons alcohols) [44].

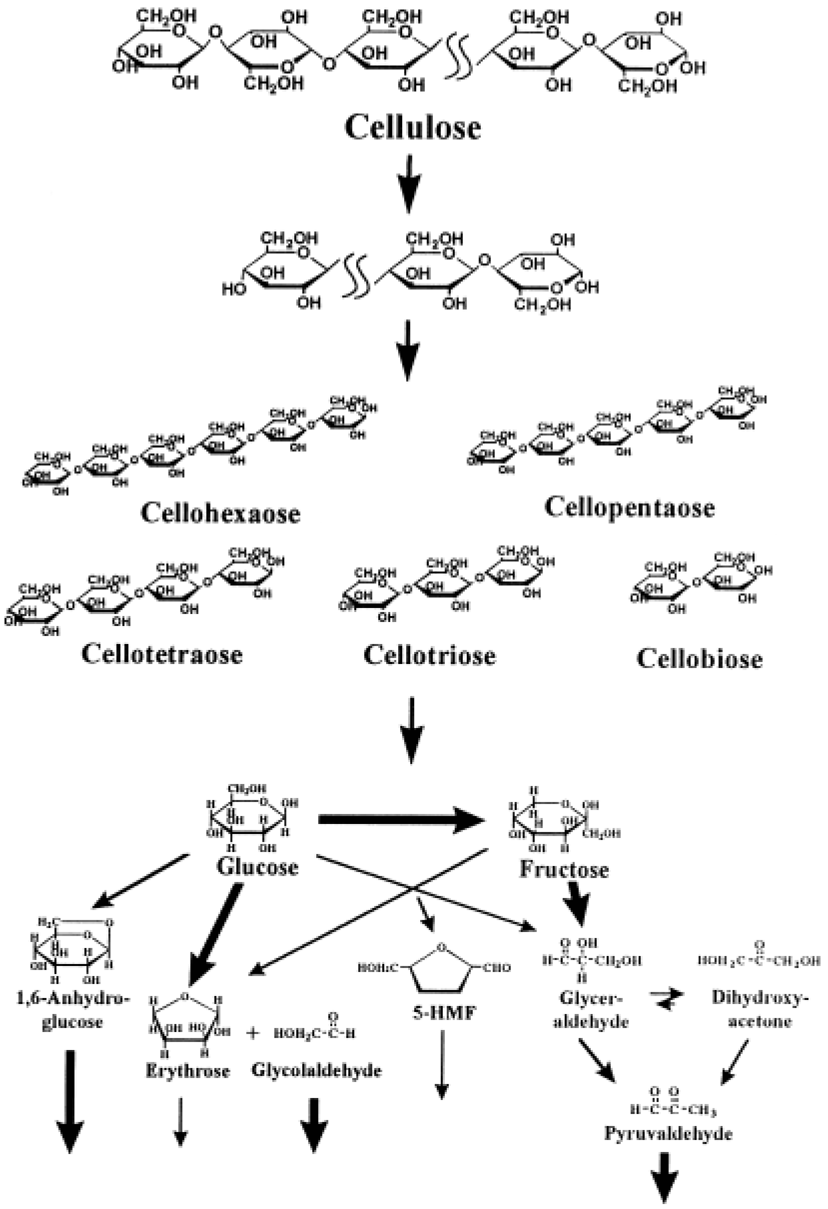

Figure 1.3. Cellulose hydrolysis and secondary reactions. Taken from: [44]

Chapter 2: Pretreatments for Lignocellulosic Biomass

Overview

Recalcitrance represents the main bottleneck for lignocellulosic utilization. To overcome this drawback it is necessary to implement pretreatment stages. There exists a wide variety of pretreatments aimed to break and modify the complex lignocellulosic structure making it accessible to subsequent processes. The main objectives of pretreatment stages include the production of high sugars concentration, the increasing of enzymes and fermentative microorganisms compatibility, minimizing costs and designing scalable processes. This chapter presents a summary of conventional and non-conventional pretreatments including operational conditions as well as the main advantages and disadvantages related to use of each one of them.

2.1 Conventional Pretreatments

Conventional pretreatments refer to a broad range of technologies commonly used to transform lignocellulosic biomass into digestible forms. These technologies can be classified in physical, chemical, hydrothermal and biological pretreatments. The requirements in temperature, pressure, additional chemical inputs, pretreatment times as well as the caused consequences compose the characteristics features of these pretreatments. Due to the higher temperatures used in conventional pretreatments are produced furans compounds. 2-furfuraldehyde (Furfural) and 5-(hydroxymethyl)-2-furfuraldehyde (HMF) are formed from pentoses and hexoses respectively [48].

2.1.1. Physical pretreatments

Cellulose crystallinity can be modified through particle size reduction. Chipping, grinding and milling, as well as different arrangement of them are used for this purpose [45]. These pretreatments do not require the use of chemicals which represents the saving related to cost of chemical inputs and biomass recovering processes.

The ‘Bond Work Index’ is one typical characteristic of raw materials. It is defined as the needed energy to reduce 1 ton of a determinate material until 80% of the material particle size be smaller or equal than 100 mm. The Table 2.1 presents the Work Bond Index summary of some raw materials.

Table 2.1. Bond Work Index Summary

Material	Bond Work Index (kW/ton)	Final particle size	Type of grinder
Agroindustrial wastes[a]	7.0-15.8	3-50 mm	Szego mill
Aspen[b]	70	350-800 µm	Knife mill
Hardwood[c]	130	1.60 mm	Knife mill
Straw[c]	7.5	1.60 mm	Knife mill
Corn stover[c]	14	1.60 mm	Hammer mill
Corn Stover[c]	15	6.35 mm	Knife mill

[a][49], [b][50], [c][45]

Kim et al reported crystallinity index reduction up to 46% by attrition milling and planetary milling. In this pretreatment the biomass was dissolved in a 50 mM acetate buffer solution (pH 5.0) with sodium azide (0.02% w/v) at 300 rpm (Kim, Lee, Kim, Mitchell, & Lee, 2013).

2.1.2 Chemical Pretreatments

Organosolv

Organosolv is a type of pretreatment that requires the use of an aqueous-organic solvent mixture with or without addition of an acid or alkaline catalyst. Delignification (lignin removal in its soluble form) is the main effect caused by organosolv pretreatment. There, water plays the role of nucleophilic agent reacting with the lignin activated centers.

Moreover, organic solvents are responsible for the lignin fragment dissolution [51]. The solvents preferred are these with lows boiling point because that gets easy recovering. Methanol, ethanol, ethyleneglycol, triethyleneglycol, acetone and phenol are some of the most used solvents [52].

Araque et al reported pretreatment and fermentation yields of *Pinus radiate* using dilute acetone 50:50% (v/v) and sulfuric acid as catalyst (0.9% w/w). The average pretreated material yield expressed as the mass of pretreated material obtained from 100 g of dry biomass were 40-54.6% mainly corresponded to the glucan fraction, it means that almost all the hemicellulose fraction was solubilized [40]

Lignol is a technology developed since 197 using ethanol as solvent. In this pretreatment the solubilized lignin fraction can be recovered through precipitation [52].The use of sodium hydroxide as catalyst enhances the selectivity of delignification reactions.[53]. *Hallac* et al reported pretreatment of *Buddleja davidii* using ethanol as solvent. The reported results at 196°C during 60 min with an ethanol concentration 65 (%v/v) gave a glucose recovering up to 85%, nevertheless, the xylose recovering allowed 49%. This fact demonstrates the xylose susceptibility to be degraded [52].

Dilute Acid

The glycosidic linkages of polysaccharides are hydrolyzed to form sugar monomers under acidic conditions. Dilute acid pretreatment is developed at high temperatures between 120°C and 210°C using acid at low concentration between 0.2-2.5% w/w and residence times from 1 min to 1h. Acid pretreatment promotes structural changes to lignocellulosic biomass increasing the specific surface area of fibers and increasing the plant cell wall pore size. As consequences the recalcitrance can be reduced, the amorphous hemicellulose is hydrolysed to different oligomers and sugars. The lignin fraction is degraded and cellulose experiments changes in its crystallinity because of degradation of its amorphous region [54]. Nevertheless, according to *Li* et al in dilute acid pretreatment the remains lignin deposited on the crystalline cellulose surface use to create digestibility problems reducing the yields of enzymatic hydrolysis [55].

The main features affecting this pretreatment are the particle size, temperature, reaction time, the acid concentration and liquid to solid ratio, most of these issues are considered to determine the severity factor of the pretreatment [54]. Authors as *Torres* et al reported that higher severity factors improves the produced sugars yields but producing undesired co-products as furfural and HMF [56].

Among the broad range of used acids can be found: sulfuric, phosphoric, hydrochloric, maleic, fumaric, nitric among others. Sulfuric (H_2SO_4) and phosphoric (H_3PO_4) acids are widely used due to the low costs, high efficiency and moderate environmental impacts caused [54]. Phosphoric acid is less aggressive producing less inhibitory compounds than sulfuric and hydrochloric acids. During this pretreatment, hydronium ions are released by the acid or water promoting the depolymerization of hemicellulose. The hydrolysis is also catalyzed by acetyl and uronic groups released by the lignocellulosic material [35]. This type pretreatment does not requires an additional separation stage after neutralization because the formed salt (sodium phosphate) can be left in fermentation medium as nutrient source [14]

Because of the inhibitory compounds produced, the hydrolysates from dilute acid hydrolysis need to be treated and recovered processed to be used in subsequent operations. In this sense are required stages of concentration, detoxification and neutralization at least. Inhibitory compounds have the capability to reduce the metabolism and enzymatic activity causing damage to the microorganism [14]. The removal of these compounds is often made by filtering off the hydrolyzed liquor followed by an extensive washing of the residues or by use of reverse osmosis with aim to exclude acetic acid, furfural, 5-HMF and other compounds [54]. *Morais* et al reported a significative increase in klason lignin content as a result of dilute acid pretreatment due to the xylan degradation and formation of compounds based on lignin [35].

Sugarcane bagasse, cassava stalks, peanut shell, rice hulls are lignocellulosic materials that have been pretreatred using dilute sulfuric acid reaching hemicelluloses conversion up to 81%, 43.6%, and 45.1% for sugarcane bagasse, peanut shells and cassava stalks respectively. [57]. Moreover, *Ballesteros* et al reported temperatures above 180°C, sulfuric acid concentrations of 0.1-0.2% w/v and 7.5% w/v. At these conditions were recovered 70% and 90% of xylose and glucose. Adittionally, saccharification yields up to 80.2% were reported using cardoon as raw material [58]. *Leninhan* et al reported the optimum temperature and charge of phosphoric acid of 135°C and 2.5% (w/w) obtaining 82.5% of theoretical yield [14].

Alkaline pretreatment

Alkaline pretreatments use non-corrosive compounds such as sodium hydroxide, ammonia, sodium carbonate and calcium hydroxide as pretreatment agents using milder reaction conditions than acid pretreatments [59]. Sodium hydroxide, ammonia and lime are the most important and common pretreatment agents. pH, temperature and alkaline catalyst load are the most important parameters to be considered in this type of pretreatment. Promoting the lignin removal and some acetyl groups as well as various uronic acids linked to hemicellulose structure [35]. Other important effect caused by alkaline pretreatment is the swelling of cellulose increasing the internal surface area followed to a decreasing in polymerization degree. Authors as *Seok* et al agree with lignin and hemicellulose removal increases the crystallinity [59].

Kraft pulping process is one common alkaline pretreatment. It is developed using sodium hydroxide and sodium sulfide, widely known as white liquor [59]. Sodium hydroxide attacks the bounds between lignin and hemicellulose in lignin carbohydrate complexes (ester and carbon-to-carbon in lignin molecules). *Morais* et al reported lignin removal yields on the order of 40% and 80% for eucalyptus and sugarcane bagasse respectively using 15% w/w NaOH [35].

Alkaline hydroxide peroxide can be used in alkaline pretreatment due to lignin and hemicellulose removal capability without furfural and HMF formation [60]. *Diaz* et al reported an enhancement up to 71.15% for pretreated biomass [60].

Lime pretreatment has been used for pretreatment of bagasse, cron stover, poplar wood, news paper among others raw materials using temperatures between 100-150°C and times above 1 to 6 h reporting recovering solid fractions yields up to 96%. Low temperature lime alkaline pretreatment has been probed at temperatures between 25-55°C obtaining 48.4% of delignification during 16 weeks of pretreatments. Lime can be recovered by reaction with CO_2 and then reconverted to lime [61].

Ammonia is an alkaline chemical widely used for pretreatment purposes. Its low volatility that made it easy to recovering [59]. Lignin removal, increase of surface area and pore size are the main effects provided by ammonia pretreatment. Aqueous ammonia pretreatment use a fixed-bed reactor called percolation reactor which minimizes the re-polymerization and re-precipitation of lignin [59]. The ammonia concentration used for this pretreatment is around 10-15% (w/w), and the operational conditions are 150-210°C and 2.3MPa. Approximately, 60-70% of lignin is removed. Other pretreatments involving ammonia are the low liquid ammonia and soaked liquid ammonia. These pretreatment involves milder reaction conditions, lower ammonia and solid charges than aqueous ammonia.

Ammonia fiber explosion/expansion (AFEX) is similar to anhydrous liquid ammonia is developed at 60-120°C. After pretreatment the pressure is rapidly released disrupting the lignocellulosic structure. The ammonia can be recovered in gaseous form and then be reaconditioned, this fact added to the use of high pressures increases significantly the costs and technological drawbacks of this type of pretreatment.

Lime pretreatment has been operated at different operational conditions, among the broad operational conditions are included high temperatures (100-160°C) with short pretreatment times (less than 6 hours), low temperatures (55-65°C) with long pretreatment times (up to 8 weeks) all these with or without oxygen. This pretreatment removes approximately 30% of lignin, despite it does not present higher delignification yields compared to other alkaline pretreatments the main advantage is provided by the low costs associated [59].

Ionic Liquids

Ionic liquids pretreatment uses salts as pretreatment agents. These salts are composed by a small anion and a large organic cation. As characteristics of these pretreatment agents can be highlighted the recyclability and recoverability, the mild operational requirements, low toxicity, the energy efficient product purification, low vapor pressures and high stability at room temperature. These pretreatments have exhibited better performance removing lignin and hemicellulose compared than sulfuric acid [62],[63]. Ionic liquids are capable dissolve cellulose, which can be recovered by precipitation by anti-solvent addition. The most common and effectives anti-solvents are water, methanol, ethanol and acetone [64]. The ionic liquid selection strategy should take into account the desired effect. As a example, solvents as 1-butyl-3-methylimidazolium methylsulfate[Bmim][CF_3SO_3] and 1-hexyl-3methylimidazolium trifluoromethanesulfonate [Hmin][CF_3SO_3] are able to dissolve high lignin concentrations biomass while 1-butyhyl-3-methylpyridinium chloride [Bmpy][Cl], 1-Allyl-3-methylimidazolium chloride [Amim][Cl] and the 1-ethyl-3-methylimidazolium acetate ([Emim][OAc]) are solvents used in cellulose dissolving with low lignin concentrations [64].

Short reaction times (by the order of 10 min) using [Bmpy][Cl] are capable to enhance the biomass recovering of bagasse and *Eucalyptus* up to 93% and 96% respectively [62]. In summary, according to the followed downstream process this pretreatment could allow lignin and cellulose fraction recovering [64].

2.1.3 Hydrothermal Pretreatments

Hydrothermal pretreatments provide a substantial breakdown of lignocellulosic biomass without chemical additions. The most known hydrothermal pretreatment are the steam explosion (SE) and the liquid hot water (LHW).

Steam explosion

Steam explosion takes advantage of pressurization and depressurization processes breaking down the lignocellulosic structure causing hemicellulose hydrolysis and delignification within inhibitory compounds releasing. The operational conditions involved include high temperatures (160-260°C), pressures (0.69-4.83 MPa) and short pretreatment times (2-15 min) [45]. Nevertheless, despite those strong operational conditions it has been proved that energy requirements can be approximately 70% lower than mechanical pretreatments [65]. In addition, acidic impregnation (1% v/v H_2SO_4) represents significant improvements for hemicellulose solubilization up to 74% for sunflower stalks [65]. As advantages, this pretreatment produces lower environmental impact, requires lower capital investment compared with other pretreatments [65].

Liquid Hot Water

The use of water as reaction medium make this pretreatment a feasible economical and environmental process compared to others which involves the use of additional chemicals. Liquid hot water pretreatment removes hemicellulose making cellulose accessible. The operational conditions involve high temperatures 160-200°C, solids loading and pH 4.0-7.0. pH restrictions allows to reduce the monomeric sugars releasing or degradation [66].

2.1.4 Biological Pretreatments

Biological pretreatments take advantage of different microorganisms to degrade and remove lignin and hemicellulose. This pretreatment is low cost effective, safety handle, environmentally friendly, presents low energy requirements and low formation of typical inhibitory compounds such as furfural and hydroxymethylfurfural [30]. This technology uses microorganisms and enzymes as pretreatment agents. The microorganism spectrum includes white-rot fungi such as *Phanerochaete chrysosporium, Pleurotus ostreatus, Ceriporiopsis subvermispora, Postia placenta, Phanerochaete carnosa, Gloephyllum trabeum* and *Trametes versicolor* [67]; brown-rot fungi such as *Serpula lacrymans, Coniophona puteana, Meruliporia incrassate, Laeporeus sulphureus* among others [30]. In addition, lignin peroxidase, manganese peroxidase, phenol oxidase, laccase and versatile peroxidase are the main lignolytic enzymes associated to biological pretreatments[30]

It is presumed that lignin is used by fungi species as carbon and energy source. A loss of dry matter is the main consequence of fungi growth, depending of the degradation selectivity lignocellulosic (lignin, hemicellulose and cellulose) or lignin consumption can be caused. Selectivity of degradation as well as substrate adaptability is strongly related with the selected strain. The main operational parameters related to this pretreatment technology are cultivation time, pH, nutrients and energy levels. Long pretreatment times (by the order of days and weeks) are the main unfeasible factor associated to this pretreatment [67].

2.2 Non-Conventional Pretreatments

Non-conventional pretreatments refer to a variety of pretreatments formulated to overcome the common drawbacks associated to the convetional ones. In this sense, some operational conditions such as reaction severity, residence time as well as equipment configuration among others has been used. The principal expected effects are related to decrease of equipment erosion, maintenance and capital cost reduction, yields increase and inhibitory compounds releasing reduction.

Pulsed electric application [68], biological and chemical mixed configurations [69], ultrasound application [70], [71], [72], supercritical fluids [73] among others have investigated as pretreatment alternatives. There is described the principles and main parameters of two non-conventional pretreatment technologies: Supercritical pretreatment and ultrasound assisted pretreatment.

2.2.1 Supercritical Fluids Theory

Considering a pure phase diagram a substance will present a particular behavior at pressures temperatures above the critical point, these substances are named as supercritical fluids. As shown in Figure 2.1 the phases cannot be clearly distinguished themselves above the critical point. Therefore, a continue transition between from liquid state to supercritical state is presented by temperature increasing at constant pressure. On the other hand, a transition from gaseous state to supercritical is achieved by pressure increasing at constant temperature [74].

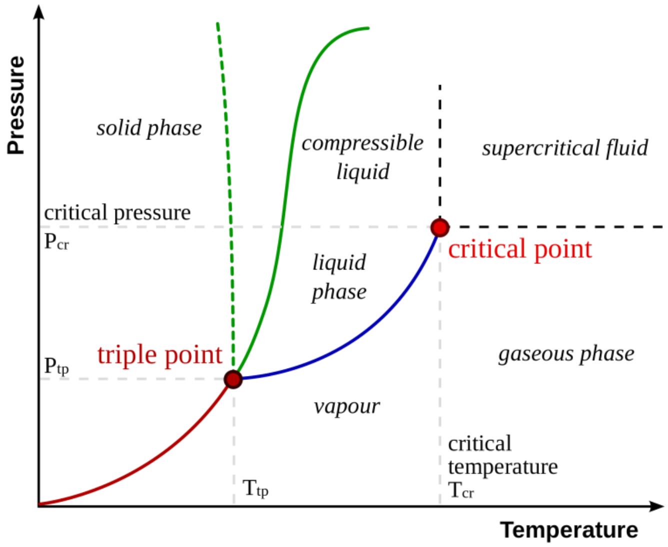

Figure 2.1. Phase Diagram. Taken from:[75]

Fluids over its critical point present properties of liquids and gases. Those fluids have higher diffusivities values (Typical in gases) and higher density values (closer to liquid values and 100-1000 fold than gases). These conditions ensure a proper mass transfer of gases and solvation characteristics similar to liquids [74]. Supercritical fluids can speed up the mass transfer because of its high diffusivity, low viscosity and low surface tension [76]. Higher diffusivities values allow penetrating solid matrix while higher densities enhance the solute dissolving capability [74]. The Table 2.2 presents a brief summary of critical properties of some solvents.

Table 2.2. Critical Properties of some solvents.

Substance	Critical temperature [°C]	Critical pressure [atm]	Characteristic
Acetone	235.5	47.0	Organic
Ammonia	132.5	112.5	Inorganic
Argon	423.8	486.0	Inorganic
Benzene	289.0	48.3	Organic
Carbon dioxide	**31.0**	**72.9**	**Inorganic**
Carbonyl sulfide	104.8	65.0	Inorganic
Hydrogen	306.4	1297	Inorganic
Ethane	32.3	48.2	Organic

Ethanol	243.0	63.0	Organic
Isopropanol	235.2	47.0	Organic
Nitric oxide	-93.0	64.0	Inorganic
Propane	96.7	41.9	Organic
Propylene	91.9	45.6	Organic
Oxygen	427.7	504.3	Inorganic
Water	374.1	218.3	Inorganic

Carbon dioxide is an environmentally friendly, inexpensive, non-toxic, non-flamable, chemically inert and highly selective solvent with mild critical conditions(31°C and 72.9 bar). Because of the mentioned issues CO_2 is the most used fluid for supercritical applications. However, CO_2 is a non-polar substance that cannot interact easily with polar substances [77]. For this reason, sometimes it is necessary the addition of substances called co-solvents with the purpose to increase the mass transfer through polarity modification. Ethanol and methanol are commonly used as co-solvents for the aforementioned purposes [77].

Higher selectivity is recognized as the main advantage of using supercritical fluids in extraction operations compared to liquid extraction [78]. Figure 2.2 presents the density behavior of carbon dioxide calculated based on the equation of state reported by *Span* & *Wagner* at different temperatures and a range of pressure from 100 to 300 bar [79]. According to the selected operational conditions, different density can be achieved and therefore the selectivity of the system can be modified.

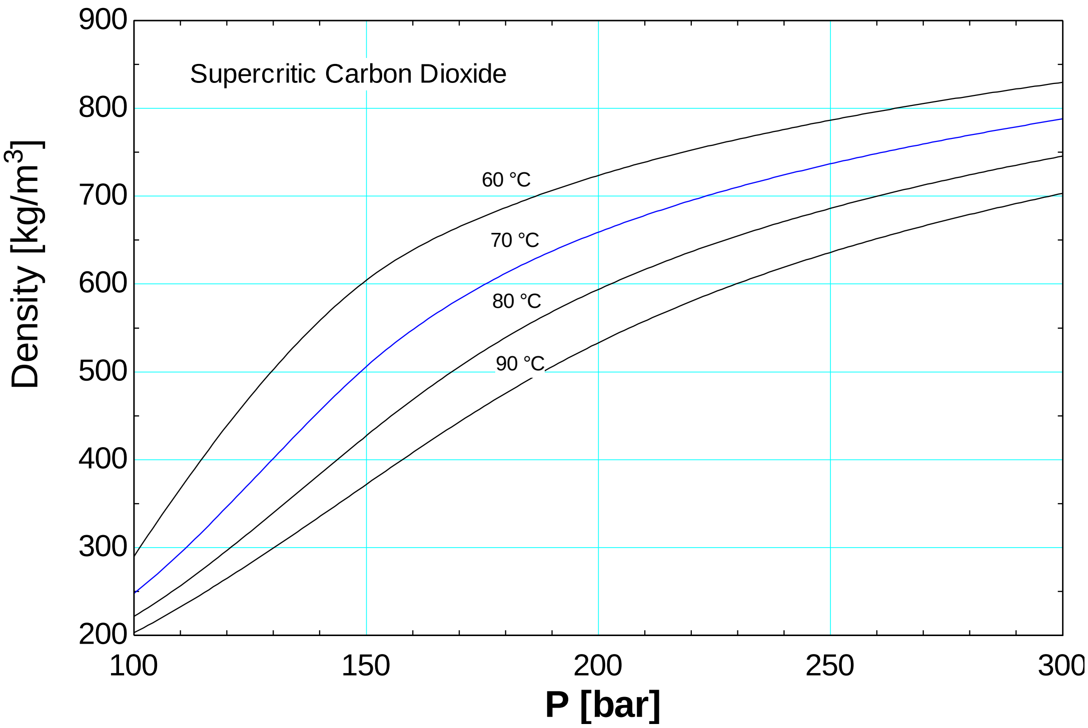

Figure 2.2. SCO_2 density. Based on: [79]

Due to the properties supercritical carbon dioxide (SCO_2) has been used in extraction operations to concentrate different fractions of value-added compounds such as phytosterols [80], Sage [81], antioxidants [82], oil from spent coffee [83], oil from grapeseed [84]. Additionally, SCO_2 has been also used for simultaneous extraction-reaction processes to produce biodiesel from different raw materials [76],[85]. At industrial scale, SCO_2 has been used for coffee decaffeination, tea decaffeination, and fatty acids, spices, flavors and fragrances extraction [86].Other applications include polymerization, polymer fractionation, particle formation for pharmaceutical and military use, textile dyeing and machine cleaning [86].

2.2.1 Supercritical Fluids Assisted Pretreatment

Since 90s supercritical fluids have been used for pretreatment purposes using wide temperature and pressure ranges At high temperatures (190-240°C) SCO_2 pretreatment behaves similar to steam explosion causing changes in lignocellulosic biomass [87]. *Zheng* et al. presented a comparison between SCO_2, nitrogen and helium obtaining glucose yields up to 72.6%, 65% and 67.2%, respectively at 35°C and approximately 206 bar [88]. The phenomena explanation suggested by *Zheng* et al. has been divided in two parts: The first part is related to the carbonic acid formation (because of the reaction between water and SCO_2) that might help the hydrolysis, and the second part is related to the possibility of that CO_2 molecules get into the cavities of crystalline structure causing an explosion that made cellulose more accessible [88],[89]. Nevertheless, *Kim & Hong* and *Yin* et al. affirmed that the effectiveness of the pretreatment depends on the biomass source. For instance, a major effect of the pretreatment was observed for hardwood than for softwood due to the presence of guaiacyl lignin in softwood [73] [90].

On the other hand, *Yin* et al. demonstrated an enhancement in enzymatic hydrolysis when corn residues were pretreated with SCO_2 and ultrasound combining both technologies. First, the residues were pretreated in a ultrasonic device with a power output of 600W, 20 kHz at 80°C, then were submitted to SCO_2 pretreatment at 170°C, 20 Mpa during 0.5h. According to the results significant increases up to 75% in enzymatic hydrolysis yields were observed for corn cob [90].

2.2.1.1 Supercritical Pretreatments Parameters

The pretreatment yields can vary according to the fixed operational conditions. In this sense the pressure, temperature, reaction time and moisture content selection plays important roles to obtain high lignin removal, enzymatic hydrolysis and crystallinity reduction yields. Table 2.3 presents a brief summary of operational conditions as well as raw materials and some observations y effects evidenced. Later on, an explanation of each parameter will be developed.

Table 2.3. SCO_2 pretreatment summary

Raw Material	Type	Temperature [°C]	Pressure [bar]	Pretreatment time	Solvent-Moisture	Observations
Corn stover[a]	Lignocellulosic	150	241	1 h	Moisture content of 75% with water as solvent	Glucose yield increases from 12% to 30%
Switchgrass[a]	Lignocellulosic	150	241	1 h	Moisture content of 75% with water as solvent	Not significantly increases in glucose yields were observed
Pinus taeda wood[b]	Lignocellulosic	190	160	1 h	Ethanol to water ratio 1:1 (v/v)	Delignification extent of 93.1%
Sugarcane bagasse[b]	Lignocellulosic	190	160	1 h	Ethanol to water ratio 1:1 (v/v)	Delignification extent of 88.4%
Red spruce wood[b]	Lignocellulosic	167	325	2 h	Ethanol as cosolvent. Molar fraction= 0.24	Mass loss up to 4.8%
Red spruce wood[b]	Lignocellulosic	190	290	2 h	Water as cosolvent. Molar fraction=0.18	Mass loss up to 35.9%
Red spruce wood[b]	Lignocellulosic	190	290	2 h	Water and ethanol as cosolvent mixture	Mass loss up to 19.3%

Corn stover [c]	Lignocellulosic	180	150	1 h	Water to ethanol ratio 2:1	Sugar yields up to 77.8%
Guayule bagasse [d]	Lignocellulosic	200	275	30 min	Moisture content of 60% with water as cosolvent	Reducing sugar yields of 86%

[a][91], [b][51], [c][92], [d][93]

Pressure

The reported pressure range is 150-350 bar [90]. It is known that carbon dioxide at supercritical conditions has the capability to promote the biomass impregnation. This fact enhances the fluid mobility through the biomass tissues [94]. High pressure help to penetrate the pores of lignocellulosic matrix increasing the interaction between a mixture solvent (or fluids) and the lignin fraction [92],[51].

Temperature

According to the open literature, the temperature range for supercritical pretreatment is 120-240°C [90].Nevertheless, other range of temperature (25-80 °C) has also been used by *Zheng* et al. concluding that below the critical point (31.1°C) the CO_2 molecules are not enough capable to penetrate in lignocellulosic structures [88]. However, temperatures above the critical point are capable to modify the structure of the materials in representative extents.

Yin et al. reported temperature pretreatment in the range of 120-170°C. According to the results higher reducing sugar yields (up to 50% and 29.8% for corn cob and corn stalk, respectively) were obtained at 170°C [90]. *Pasquini* et al demonstrated that higher temperatures and reaction times promote the sugars degradation [94], [95]

Reaction Times

Pretreatment times also represent one of the most important issues to be considered. The common range is between 0.5-4h. Different effects and times requirement are strongly related to the material resilience, property strongly related to raw material hardness. In this sense, different time effects considering the type of material have been reported [90]. Short pretreatment times could be not enough to penetrate and transform the matrix. Nonetheless, long pretreatment times can cause hemicellulose and cellulose decomposition [90].

Moisture Content

It has been considered that moisture content promotes the biomass swelling and the SCO_2 deeper penetration into the fibers [90] [96]. Additionally, it represents a measure of the available water or co-solvent reacting with CO_2 under supercritical state. *Narayanaswamy* et al. probed visually that moisture content does not prevent that CO_2 reaching the supercritical state [91]. These authors also demonstrated that SCO_2 pretreatment at high moisture content increases the enzymatic hydrolysis yields due to the carbonic acid formation [91].

The acidification presented because of biomass moisture content can dissociate the hydrogen-hydrogen bounds linking between hemicellulose polysaccharides and cellulose, therefore cellulose gets more accessible to enzymatic attack [91]. The use of raw materials with high moisture content would represent savings in drying processes. In contrast, moisture content does not causes the same effect for all the pretreatments. For instance, in the steam explosion pretreatment the hemicellulose hydrolysis yields are strongly affected by the samples moisture content difficulting the mass transfer [65].

Carbonic Acid Formation

Carbonic acid is a weak and unstable acid resulting from the aqueous solvation and subsequently hydration of carbon dioxide. This acid also appears in process involving water and steam condensates cycles. Carbonic acid (H_2CO_3) in gaseous state is a mixture of monomers and dimers which ratio is a temperature dependent [97].

Carbonic acid instability makes difficult its determination, due to the acid-base reactions to form carbonate and bicarbonate at environmental conditions, following the stoichiometry presented in equations Eq **3**) and Eq **4**) [98]:

$$\mathbf{CO_{2\,(g)} + 3H_2O \leftrightarrow CO_{2\,(aq)} + 3H_2O \leftrightarrow H_2CO_3 + 2H_2O} \qquad \textbf{Eq 3}$$

$$\mathbf{H_2CO_3 + 2H_2O \leftrightarrow HCO_3^- + H_3O^+ + H_2O \leftrightarrow CO_3^{2-} + 2H_3O^+} \qquad \textbf{Eq 4}$$

Despite it could not be exactly quantified, some authors have affirmed the carbonic acid formation during supercritical pretreatment and its hemicellulose and cellulose hydrolyzing effects [88]. In addition, there exist reports of the formation in-situ of alkylcarbonic acids by the reaction between CO_2 and alcohols. However there is not identified the use or benefit provided by these compounds to supercritical pretreatment of biomass [99].

2.3. Sonochemistry theory

There exist three groups of sound waves according to its vibration frequency :i) Infrasound range (frequencies lower than 20 Hz), ii) Human audible sound range (higher than 20 Hz and lower than 20 kHz) and, iii) Ultrasound range (higher than 20 kHz) [26].

For ultrasonic range has been provided different uses according to the selected frequency. High frequency ultrasound waves (higher than 1 MHz) are used for detection and imaging areas with high accuracy for medical purposes. On the other hand, low frequency ultrasound waves (higher than 20 kHz and lower than 1 MHz) are used to produce physical and chemical changes [26]. Sonochemistry is the term used to describe ultrasound chemical transformations and mechanoacoustic effects corresponds to those physical processes produced by ultrasound application [100].

The diffuse energy sound is concentrated according to the increases in negative pressure application. Cavitation is the main phenomena presented during the ultrasound waves application. As a consequence, it is favored the bubbles formation as function of the energy supplied. This amount of energy must be enough big to initiate the bubble growth. In addition, the medium reactant should have adequate volatility levels to receive and accept the cavitational bubbles [26]. Cavitation production includes: i) Bubble nucleation conformation, ii) Rapid growth together with compression and expansion cycles, and iii) collapse of gaseous microbubbles. Thus, the microbubbles accumulate energy from the oscillations as heat. At continue energy input the bubbles can growth until they reach an unstable structure. Before collapsing microbubbles reach its critical size (inversely to the applied frequency of sounds), the impact will be proportional to the bubble size reached. The microbubbles radii generated by low frequencies (approximately 20 kHz) are between 100-170 µm. on the other hand, higher frequencies (by the order of 1 MHz and higher) produces average microcubble sizes of 3.3 µm. Then, the microbubble will suddenly collapse releasing the stored energy generating different type of chemical, thermal and mechanical effects [100].

The Figure 2.3 presents a schematic representation of acoustic cavitation. During these stages the liquid medium experiments changes of pressure from high pressure (compression) and low pressure (rarefaction). First, the cavitational microbubbles are formed (stage 1). After that, the bubbles formed growth, expanding the sizes (stage 2). Later on, the instability of the bubble cycles increases (stage 3) until collapse is reached (stage 4). Due to the collapse higher local energy densities are reached [29]. The impact generates localized increases in temperature and pressure called local hotspots by the order of 5000K and 1000 bar respectively [26]. As a preliminary conclusion it will be expected that low ultrasound frequencies are most adequate for pretreatment purposes.

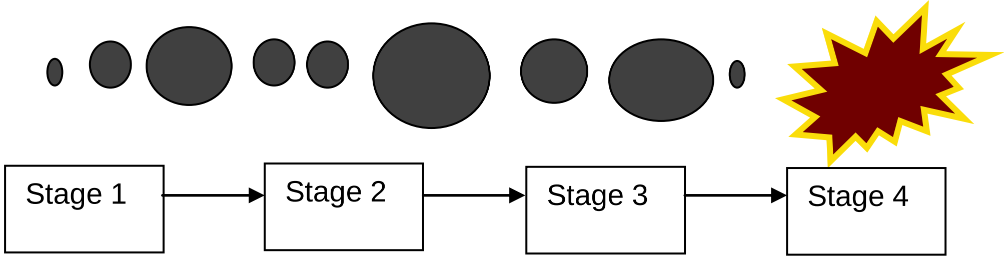

Figure 2.3. Acoustic cavitation phenomen. Adapted from:[47]

2.3.1 Cavitational Effects

Micro-jects, showave impacts and microstreaming are the main effects caused by cavitational microbubble impact. Micro-jets are formed when cavitation takes place near a solid surface of larger size than the microbubble. Its impact is referred to the liquid layer moving through the liquid at high velocities by the order of 100 m/s. Micro-jet impacts are capable to break the cell walls causing an increase in the available surface area [29].

Shockwaves are produced by the instantaneous collapse of microbubbles, generating an impact that might be up to 10^3 MPa [101]. As a consequence of shockwave, micro-streaming is produced referred as the microscopic turbulence within surroundings nearby small solid particles by inducing hydrodynamic shear stress. This last phenomenon enhances the mass transfer and induces changes and damages to the cell walls [29].

In addition, ultrasound can be used enhancing chemical reactions by the following mechanism: firstly, the collapse of cavitational microbubbles produces high energy intermediates such as atomic hydrogen (H*), hydroxyl (OH*), hydrogen peroxide (H_2O_2), superoxide (HO_2) and hydrogen (H_2). These intermediaries have strong oxidants and redactors properties. The equations 5-9 shows the reactions involved in the formation of these compounds[47] :

$$\mathbf{O_2 \rightarrow 2O *} \qquad \text{Eq 5}$$

$$\mathbf{H_2O \rightarrow H * \ + OH *} \qquad \text{Eq 6}$$

$$\mathbf{OH * + OH * \rightarrow H_2O_2} \qquad \text{Eq 7}$$

$$\mathbf{2O * + H * \rightarrow HO_2} \qquad \text{Eq 8}$$

$$\mathbf{H * + H * \rightarrow H_2} \qquad \text{Eq 9}$$

The cavitation behavior is strongly affected by solvent and medium properties such as bulk temperature, viscosity and surface tension. However, ultrasound wave characteristics are also important

2.3.2 Ultrasonic Devices

Systems using ultrasound are generally composed by an ultrasonic frequency generator, a transducer and a reactor. Transducers are the referred to the devices used to convert the supplied energy from a power generator into vibrations (mechanical energy).

There exist two types of transducers: magnetoestrictive and piezoelectric. Magneoestrictive transducers are made by metallic alloys. These devices can allow high levels of acoustic power (greater than 150W/cm2) with lower deterioration possibilities. Piezoelectric transducers are more used than magnetoestrictive, they exhibit higher transfer efficiencies, however, low levels of power intensity as well as short life span are associated to them [47]. Two transducer configurations are the most used: probe and baths. The probe-type ultrasonic systems, the most used in sonochemical reactors, consist in microtips horns or extenders horns (commonly made of high-grade titanium) used to apply directly the ultrasounds waves to liquid mediums.

2.3.4 Ultrasound applications

Sonochemistry application is able to modify the kinetic of chemicals reactions by changing the reaction mechanisms, generating emulsification, promoting the raw materials erosion, crystallization, precipitation among others transference processes [100]. Ultrasound has been applied to enzymatic enhancing the transport of enzyme to the surface of the substrate in pretreatment processes. For starchy materials, short pretreatment times (gelification stage) by the order of 5 min can enhance the liquefaction up to 6.82% compared with the unpretreated ones [102].

Nevertheless, the generation of cavitation bubbles can causes damage to biological molecules. For this reason ultrasound must to be carefully applied to these kind of processes [100]

2.3.5 Ultrasound Pretreatment

It is expected that sonochemical and mechanoacoustic effects produced by ultrasound pretreatment affects the chemical composition as well as the physical configuration of lignocellulosic biomass. The principle behind the ultrasound assisted pretreatment is related to the enhancement of physical effects [100]. In this sense, mass transfer improving is achieved increasing the turbulence and microlevel mixing.

Ultrasound application is a useful tool to reduce the structural rigidity as well as to overcome the mass transfer resistance because of ultrasound pretreatments allows to increase the lignin extraction and hemicellulose solubilization yields by cleavage of lignin-hemicellulose linkages [100]. In addition, this pretreatment can achieve reduction in pretreatment times, reducing the needed amounts of reactants, and mild temperatures reaction [100]. The Table 2.4 presents a summary of ultrasounds application to lignocellulosic biomass including the operational conditions of these processes:

Table 2.4. Ultrasound Applications

Type of Substrate	**Raw Material**	**Process**	**Frequency [kHz]**	**Time**	**Temperature [°C]**	**Irradiation Density [W/mL]**	**Key findings**
Lignocellulosic	Sugarcane bagasse[a]	Pretreatment with 2% NaOH	24	20 min	N.R	N.R	Lignin removal about 75.44%
Lignocellulosic	Sugarcane bagasse[a]	Sono-assisted acid hydrolysis. Pulsing cycle 50%	24	45 min	50	N.R	Glucose and pentose yields were 0.36 g and 0.22 g per gram of sugarcane bagasse respectively. Inhibitory compounds were in admissible levels for fermentative endings (acetic acid 0.95 g/l and furfural 0.1 g/l).
Lignocellulosic	Rice Straw[b]	Acid hydrolysis 10% H_2SO_4, Liquid to solid ratio 7. Power 750 W	20	60 min	80	N.R	Maximum sugar yield 310g/kg biomass
Starchy	Maize[c]	Pretreatment using a probe in	20	5-40 s	Environment	4.8-8.3	Starch conversion improved up to 56.8% from the initial 13.7%

		aqueous medium (3 g/32 mL)					
Starchy	Cassava[c]	Pretreatment using a probe in aqueous medium. 5% total solids	20	10-30 s	Environment	8.5	Reduction in fermentation times up to 50% from 72 h to 36 h.
Cellulosic	Microcrystalline cellulose[c]	Pretreatment using Alkymidazolium dimethylphosphate ionic liquid using an ultrasonic bath	45	30 min	60°C	0.01	Cellulose conversion improved from 75.6% to 95.5%
Starchy	Triticale meal[d]	Pretreatment (liquefaction) and simultaneous saccharification and fermentation (SSF) using an ultrasonic bath	40	5 min	60	N.R	Time of SSF operation was reduced from 72 to 48h achieving the 84.56% of theoretical bioethanol yield.
Lignocellulosic	Rice straw[e]	Simultaneous pretreatment and	20	50 min	80°C	N.R	The maximum sugar yield reached was 31.78/ 100 g of

		saccharificatio. The pretreatment was developed using 10% H_2SO_4 with an ultrasound probe of 750 W, 20% of amplitude.					biomass.
Starchy	*Chlamydomonas fasciata* Microalgae[f]	Starch extraction using a 30W homogenizer. Biomass: water ratio 1:10.	20	30 min	Environment	N.R	Starch extraction up to 93.8% corresponding to 0.408 g-Starch/g-dry microalgae. Additionally, the starch simultaneous saccarification fermentation yield was up to 79.5% of theoretically.

[a]*Velmurugan* and *Muthukumar*[43], [b]*Saif* et al[103], [c]*Luo* et al [101], [d]*Pejin* et al[102], [e]*Rehman* at al[104], [f]*Asada* et al[105]

2.3.5.1 Ultrasound Pretreatment Parameters

Acoustic power, frequency, pretreatment times, solvent nature, temperature and reactor specifications are the main parameters involved in ultrasound pretreatment. A complementary understanding of these parameters as well as the effects caused to lignocellulosic materials is summarized below.

Acoustic Power

The acoustic power is related to the amplitude of vibration proportioned by the ultrasonic source [47]. Therefore, it is expected that an increase in the amplitude of vibration will strength the ultrasonic effect caused. Higher amplitudes are required to treat high viscosity mediums. Nonetheless, higher amplitudes can deteriorate ultrasonic transducers also causing poor transmission into the liquid media due to the formation of a dense cavitation bubbles cloud. It has been demonstrated that increases in acoustic power gives more effective results than other factor as pretreatment times [26]

Frequency

Cavitational effect is strongly related to the operational frequencies. Low frequencies by the order of kHz promotes voids creation, hence cavitation is produced by the compression and rarefaction cycle [47]. Different effects can be achieved according to frequency levels. Low frequencies between 20-80 kHz lead physical effects such as shockwaves, microjects and micro-streaming. On the other hand, high frequencies between 150-2000 kHz enhance the production of radicals by cavitation which mainly produces sonochemical effects [100]. By comparison of the effect of low and high frequencies concluding that delignification purposes are enhanced at low frequencies meanwhile high frequencies contribute to carbohydrates solubilization. In addition, the use of advanced sonic systems involving dual frequencies can produces highly energized zones ensuring constant processing by dead zones elimination.

Pretreatment Times

Times associated to pretreatment covers relevance due to the required energy amounts and the economical aspects associated. *Subhedar* and *Gogate* reported yields for assisted alkaline pretreatment using shorter times by the order of 15 to 35 min. at these conditions an increase in delignification up to 8.4% compared to the base case without sonication [47]. For lignocellulosic materials, approximately 87% of hemicellulose was hydrolyzed within 60 min sonication using 10% H_2SO_4 with ultrasound (20kHz 750W) [103]. According to the raw material nature are recommended different suitable pretreatment times, for starchy and lignocellulosic materials are 5-10 min and 10-20 min [47].

The allowed effects are proportional to the pretreated time, notwithstanding, it was found that long pretreatment times could increase the concentration of inhibitory compounds as acetic acid, in addition has been reported that increases in pretreatment times can produce high crystallinity index of materials because of long pretreatment times using low-power irradiation does not represent significantly changes to CrI or size pore [26], [103].

Solvent Nature

For heterogeneous systems the solvent nature plays an important role. In this sense, aqueous, ionic and organic solvents have been used as reaction medium [29]. Cavitation is favored by those liquids with high vapor pressure and low density because of these properties govern the severity of the cavitational collapse [47]. The use of alkaline or acid solutions must to consider the appropriate concentrations to avoid undesired reactions [29]. Alkaline solutions (sodium hydroxide and lime solutions) have been tested for delignification processes without increasing in lignin content. On the other hand, for pretreatment using acidic solutions the carbohydrates degradation risk increases using long pretreatment times (>45 min) [29].

Temperature

According to the temperature levels two different effects can be presented. As far as the temperature increases the solvent vapor pressure increases and more solvent vapor fills the cavitation microbubbles reducing the sonication effects (microbubbles collapse will be less intense) [47]. At high temperature levels the hydrogen bonding, van de Waals forces and dipole attraction can be disrupted or broken. Additionally, faster diffusion rates occur.

On the other hand, better cavitation is allowed at constant ultrasonic power and low temperatures [47]. Typical temperatures for aqueous solvents between 30-70°C are use, however high temperatures by the order of 90-160°C are used for nonaqueous solvents (including ionic liquids) [29].

Reactor Specifications

The reactor geometry and specifications can affect the power dissipation causing interferences or different patrons of mass transfer within the sonicated system [29]. According to delignification assays developed using comparable volumes horn transducers was more effective than the bath ensuring better mass transfer rates [29].

Ultrasonic horns gives higher cavitational intensity compared to ultrasonic bath. Nevertheless, due to the ultrasound intensity, erosion problems are usually presented [47]. Some typical horn shapes are the “donut shape”, “telsonic horn” [47]. There are highlighted some relevant design aspects to be considered for ultrasonic reactors specifications: i) the position of ultrasonic transducer (horn or tip) to ensure a good distribution or acoustic energy and ii) transducer surface and shape to provide high ultrasonic energy density. In addition, and array of transducers as well as superposition of ultrasonic fields can be tested to reduce the dead zones and intensify the ultrasonic energy [101].

The probe configuration and immersion level allow emphasize on different effects such as ultrasonic energy amplification or intensity amplification. Notwithstanding ,the scaling up probes and horn type its very complex due to the distribution of the acoustic energy through the systems. To develop scaling up methodologies it will be necessary take into account hydrodynamics aspects, mixing and mass transfer to ensure the efficiency en physical and chemical applications.

Ultrasonic baths have numerous transducers vibrating simultaneously. The transducers are attached underneath the metallic tank. The main disadvantage of this arrangement is related to creation of maximum and minimum acoustic zones. Other types of transducers include tubular reactors, parallel-plate reactors, dual frequency flow cell and triple frequency flow cell reactors. As a disadvantage, low intensity cavitation is produced by those devices [29] [47].

Chapter 3: Agricultural wastes

Overview

It is estimated that 90% of the material resources used o related to productive process end up as waste generating a high impact on the environment. Those generated volume would be used as raw materials to obtain different chemical families, however, it is necessary to establish the sustainable and profitable use of these residues. According to the chemical composition would be defined processing routes including the most accurate pretreatment capable to reduce costs. In this sense, the study and knowledge of agricultural waste generation plays an important role to understand compositional and logistics issues. This chapter presents an exploration of some important issues such as raw materials classification, sources as well as some examples of production, harvested areas and common uses for agricultural wastes.

3.1. Raw Material Classification

According to the exposed in the first chapter, three raw material generations have been widely described by authors as *Moncada* et al [106], *Lennartsson* et al [107] among others. This classification include first, second and third generation raw materials.

First generation raw materials is referred to those edible crops used for agroindustrial purposes, among those crops are included starchy, sugar-rich as well as oleaginous plants [107]. Nowadays, Brazil, EEUU, China, France, Germany, Sweden are bioethanol producers from first generation raw material such as sugarcane [108] [109] and maize [110]. The use of first generation raw materials has been widely discussed due to the potential consequences at environmental and social scales as possible increases in food market prices affecting food safety hitting in major extent poor and unequal countries [111]. Moreover, an uncontrolled increase in first generation raw material utilization should influence in the land use measured by the ILUC (Indirect Land-Use Change). The ILUC express the generated impacts due to a possible expansion of feedstock harvesting areas in order to increase the raw materials availability. This fact might cause several effects on biodiversity, soils and hydric resources [112][113].

Second generation raw materials are mainly composed by lignocellulosic materials generated during seeding, cropping, harvesting and processing stages of cultures. Other sources of second generation raw materials are the non-edible crops (i.e Jatropha) [114].

Finally, microalgae are recognized as the third generation raw materials. They exhibit good growth rates as well as higher annual yields of harvested biomass per area compared to crops associated to first and second generation raw materials [115]. The capability to catch CO_2 as well as the photosynthetic efficiency and the adaptability to different growth conditions are the main advantages of third generation raw materials. Some authors as *Moncada* et al [106],[116] and *Rincón* et al [117] [118] have integrated microalgae as raw material for different biofuels production under biorefinery mixed schemes involving the use of first and second raw materials.

3.2. Sources of Agricultural Wastes

Colombia is a tropical country located between Pacific and Atlantic Ocean in the northern side of South America. Some geographical special features made of Colombia a country assorted of a biodiversity because of its variety of soils, weathers, topographies, hydric resources among others.

According to the Colombian Geographical Institute Agustin Codazzi and the Colombian Environmental Information System [119] Colombia has a total surface of 114.174 Mha distributed for different uses presented in the Figure 3.1. **Colombian Land Distribution (a) Colombian Land Use (b) Agricultural Land Distribution**

(a). On the other hand the Figure 3.1 (b) present the agricultural land use distribution, it has been widely noticed the inadequate and excessive use of lands for catering activities with low bovine density also occupying lands that would be destined for crops.

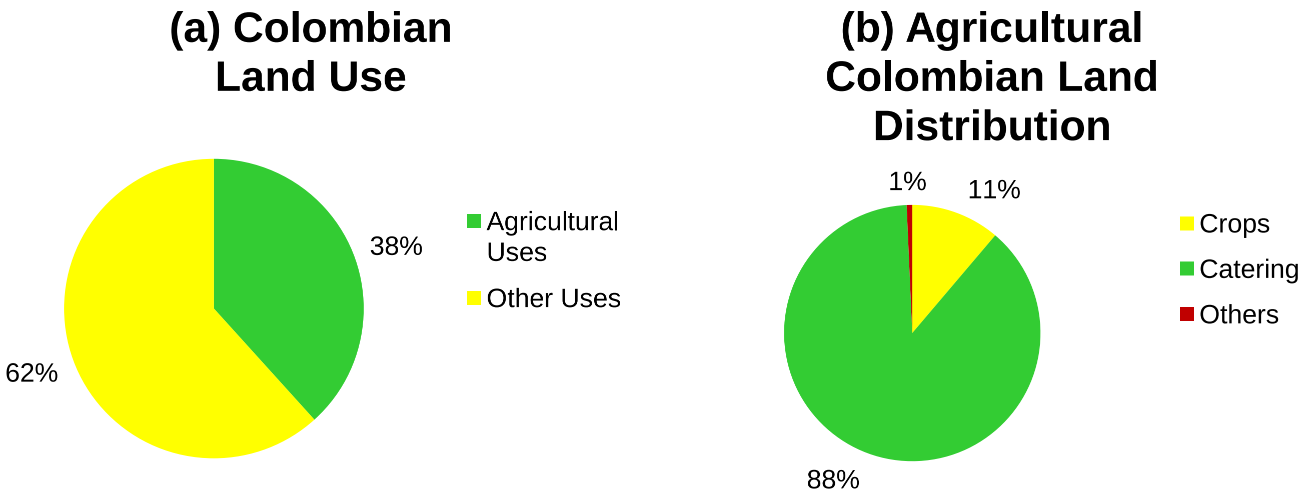

Figure 3.1. Colombian Land Distribution (a) Colombian Land Use (b) Agricultural Land Distribution

Agricultural wastes can be classified as second generation raw materials. These residues are generated in different amounts according to its relationship with the total biomass harvested. Nevertheless, to design processes based on agricultural wastes as raw material it is necessary to take into account the total available biomass is conditioned by the harvesting yields, logistic considerations, storage conditions as well as the constraints associated to other cultural uses (nutrient supplementation, feed, organic matter, compost among others). The Table 3.1 presents a brief summary of some Colombian crops including their harvested areas, productions and some themselves wastes.

Table 3.1. Colombia crops.

Crop	Harvested Area [ha]	Production [ton]	Producers regions	Main wastes
Coffee	707,797	622,282	24	Coffee husk, coffee pulp.
Rice	495,189	2 324,2823	26	Rice bran, rice husk, rice straw.
Wheat	5,915	8,977	6	Wheat straw
Sugarcane	207,462	2 083,6972	7	Sugarcanne bagasse
Maize	527,421	1 206,246	32	Corncob, maize straw
Potato	160,695	3 022,920	17	Potato peel

Plantain	379,771	3 081,915	32	Plantain rachis, plantain pseudostem
Palm oil	344,692	1 150,311	19	Empty fruit bunches, palmoil leaves, palmoil trunk
Cassava	181,513	1 967,444	32	Cassava leaves, cassava trunks.

Following is presented a description of three representative Colombian crops (rice, cassava and plantain) including their farming conditions, statistics about areas and productivities and generation of key wastes with the aim to elucidate the potential associated to the Colombian waste management.

3.2.1. Rice (*Oryza sativa*)

It is a grass from the *Poaceae* family native from the Southeast Asian considered as the third agricultural commodity preceded by sugarcane and maize [120]. This cereal is used by different cultures as a basic food. It represents besides coffee and sugarcane one of the most relevant crops of the Colombian agriculture [124], [123].

This crop is semiannually harvested in 26 Colombian regions, having elevated moisture requirements. For this reason it is common to be found submerged in water at at temperatures (23-35°C) and altitudes (0-2500 mamsl) [121] with annual precipitations by the order of 1200 mm [122]. This culture can be classified according to the culture method used in: irrigated, non-irrigated mechanized and non-irrigated manual. The Colombian Federation of Rice Producers (“FEDEARROZ”, by its Spanish abbreviation) reported that approximately 94% of the Colombian harvest is developed by mechanized methods reducing times and increasing yields. A brief description of these methods is presented in the Table 3.2 :

Table 3.2. Methods for rice harvesting[124]

Technology	Description
Mechanized	It uses tools and mechanized devices during culture development
Manual	It uses manual workers during culture development
Irrigated	The hydric requirement is provided by gravity or pumping

Non-irrigated	The hydric requirement is is only provided by rainfall

During the harvesting stage the rice grains named Green Paddy Rice (GPR) are removed from the plant, the residual non edible part (mainly composed by stem and leaves) is named straw. Approximately 1.5 ton or rice straw is produced per ton of rice processed [125]. After that, the GPR is submitted to cleaning and moisture reduction, at this stage the grain is named Dried Paddy Rice (DPR). Then, the DPR is dehusked and cleaned producing rice bran and the final white rice, rice husk and whole rice grain are generated as co-products of this stage [124]. Rice husk (RH) also denominated rice hull is the innermost layer of rice paddy grain, It has been reported that 0.23 ton of RH are produced from each ton of rice processed [126]. In the Table 3.3 is presented a summary of the generation stage as well as the common uses of the rice residues.

Table 3.3. Common uses of rice residues.

Rice Residue	**Generation stage**	**Common uses**
Straw	Post-harvesting	It is mainly used as feed and as organic matter.
Husk	Rice milling process	It is mainly burned or dumped on landfills as organic matter
Bran	Rice milling process	It is mainly used as feed, food and bakery products manufacturing.

The Figure 3.2Figure 3.6 presents the seeded and harvested area, the Figure 3.3 presents the total and yield of rice production during the period 2007-2012 built according to official statistics provided by the Colombian Agriculture Ministry [127].

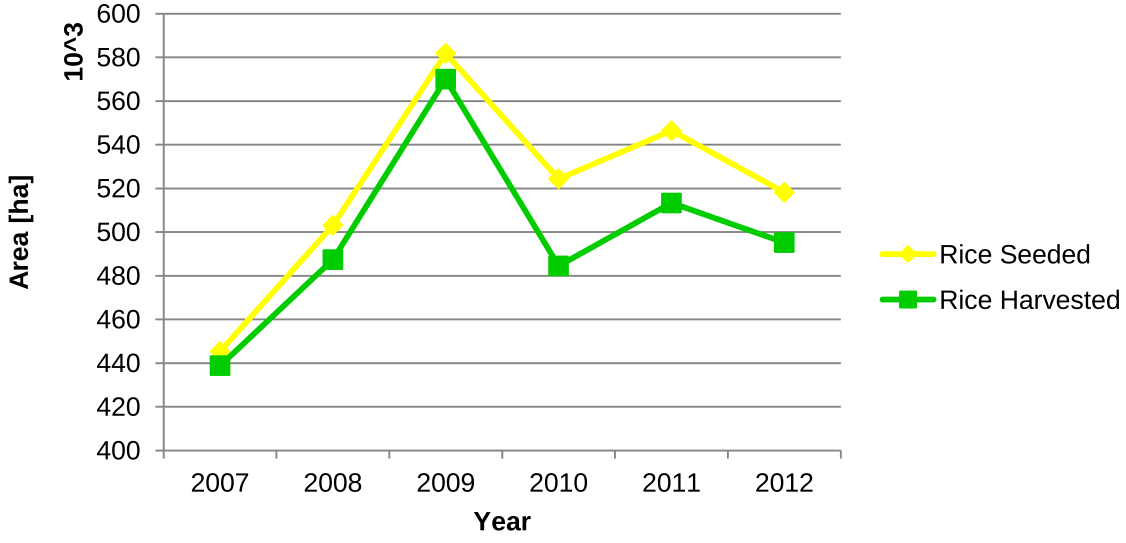

Figure 3.2. Total surface of rice

As illustrate the Figure 3.2. the seeded to harvested ratio held a constant behavior during the period 2007-2009 without significant differences between , but nevertheless, by the 2010-2012 period there is observed a slight gap between the harvested area compared to the seeded ones.

By the other hand the Figure 3.3. presents the total production and yield behavior presented strong variations because of the productivity reduction for the period 2010-2012. Correspondingly, the correspondent yields allowed the lower levels for the overall evaluated period.

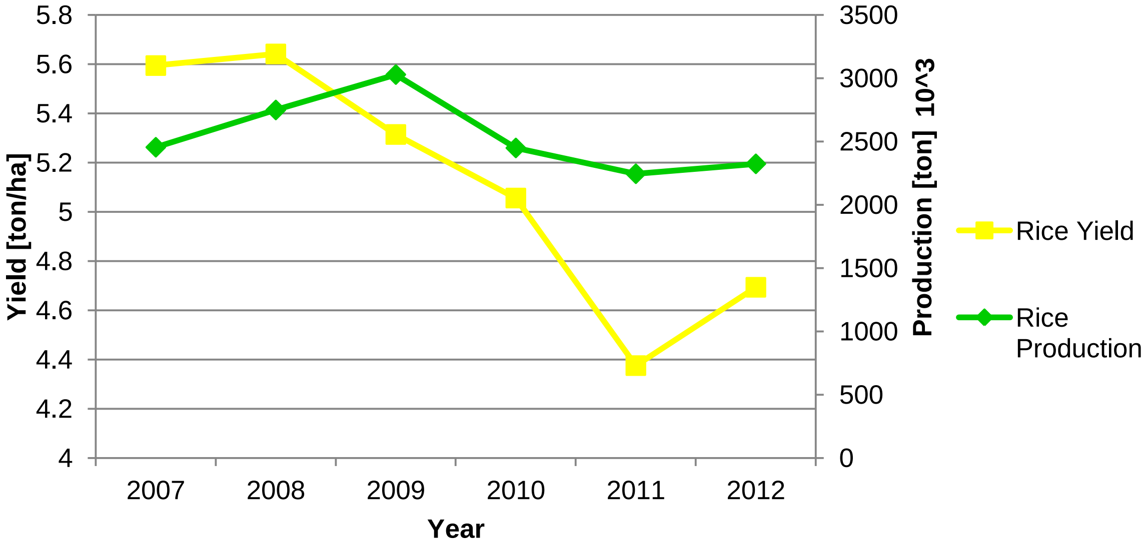

Figure 3.3. Rice production

Considering DPR to RH ratio, approximately 534,000 ton of rice husk were produced in 2012. Due to high amounts of GPR are joined to be submitted to dehusking and milling operations. For this reason all the logistic issues associated to rice husk collecting and storing do not represent a drawback for its use as bioprocess raw material.

3.2.2. Cassava (*Manihot esculenta*)

It is a woody perennial plant from the *Euphorbiaceae* family native from the Amazon basin region [128] considered as the eighth agricultural commodity preceded by sugarcane, maize, rice, wheat and others [120].

Cassava crop is cultivated in 32 Colombian regions at different production scales. This crop assorted by a wide adaptive capability growing in diverse type of weathers and soils [128]. The harvesting conditions include broad ranges of temperature (20-38°C), relative moisture (50-90%) and altitude (0-1800 masl) [128]. In Colombia it is recognized as a subsistance crop harvested in association to other crops (i.e as cocoa, maize, citric fruits among others), also plays an important role for food security due to its high energy content (67 kcal per person-day). The geographical adaptability and the resistance against drought supports the relevance of this culture in the local agriculture [129].

This crop is mainly harvested by small farmers (<3 ha of lands) The Caribbean region contributes with approximately 50% of the total produced. Nevertheless, this region has presented low yields because of the lacking in assistance, technical and technological advances [129]. Few countries as Nigeria, Brazil, Thailand and Indonesia have extensive and technified cassava cultures for biofuel production [23].

The biomass from cassava crop is composed by a woody stem (represents 50% of total produced root [130]) and an edible portion. The edible part of cassava crop consists in leaves and roots. The first ones are provided by proteins, carotenoids, vitamins and minerals whereas the second ones are rich in carbohydrates (starch) widely used in food industry. The pulp is the edible part of the root, it represents approximately 80% whereas the residual 20% correspond to the peel [129].

Cyanogenic compounds have been detected in cassava roots and leaves. According to the concentration of these compounds cassava crops has been classified as sweet and sour (able or not for human and animal consumption) [128]. Sour cassava crops are mainly used for agroindustrial purposes (i.e production of bioethanol, flour, starch, etc). The Table 3.4 presents a summary of residues and common uses of this crop.

Table 3.4. Common uses for Cassava Residues [128],

Residue	**Generation stage**	**Common uses**
Leaves	Post-harvesting	It is used as feed, additionally it would be used to prepare flour for human consumption.
Stem	Post-harvesting	Approximately 10-20% is used for cassava propagation.

Despite the low harvesting yields associated to cassava crop (presented by the Figure 3.4) the seeded surface has kept constant during the period 2007-2012 according to official statistics provided by the Colombian Agriculture Ministry [127], corroborating its importance in food security and other applications.

Figure 3.4. Total Surface of Cassava

The trend presented in the Figure 3.5. allows concluding that low harvesting yields is a common phenomena associated to cassava cultures. Notwithstanding, the seeded surface has kept constant during the period 2007-2012, this fact reaffirm the importance of this crop.

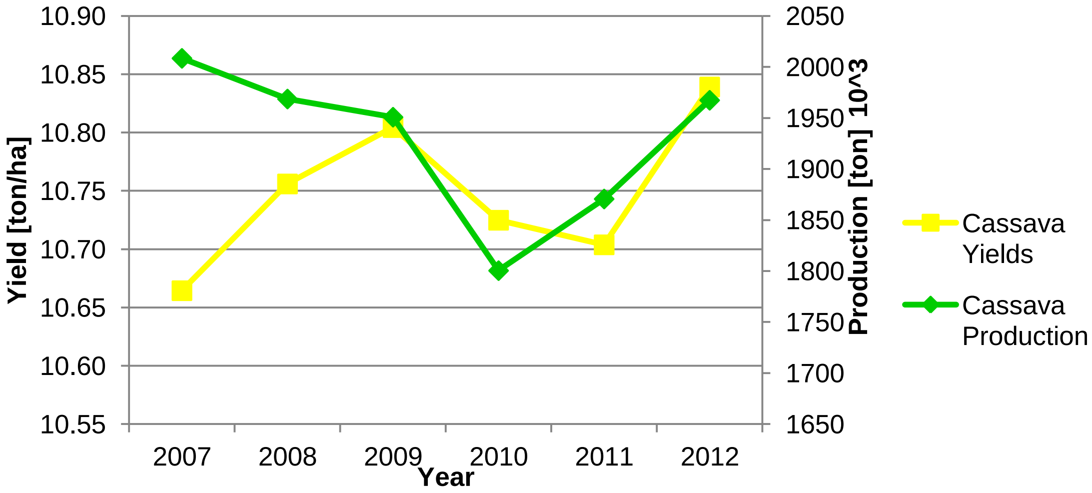

Figure 3.5. Cassava Production

According to the Figure 3.5 by 2012 were produced approximately 2,000 ton of cassava roots producing 1,000 ton of stems.

Colombian yields by the order of 10-11 ton/ha are lower than Asian and Latin America average yields of 16.4 and 12.8 ton/ha respectively [128]. The unstable harvested area as well as the low productivity added to the lack of technical assistance represents the main drawbacks of cassava crop as an alternative use for agroindustrial purposes.

3.2.3. Plantain (*Musa paradisiaca*)

It is a herbaceous perennial plant from the *Musaceae* family native from the Southeast Asian [131]. This crop is characterized by its big size provided by non-woody stems that supports the fruit bunches and leaves[132][133].

By 2013 Colombia was the third world’s exporter preceded by Guatemala and Ecuador [136] representing one of the most traditional sectors in Colombian agriculture cultivated in 31 Colombian regions, widely cultivated in warm climates at wide range of temperatures (18-38°C) and altitudes (0-2000 mamsl) with an average annual precipitation requirement of 1800 mm [134]. This crop has traditionally been cultivated by small farmers (less than 3 hectares) mainly associated to other crops such as coffee, cocoa, cassava and fruit crops.

The total plantain biomass is composed by an edible and a non-edible part. Approximately 20% of total plantain biomass is represented by the edible part called plantain bunches (fruit bunches), whereas the non edible part is composed mainly by plantain pseudostem (representing the 50% of total biomass) [137], and the remaining 30% is composed by rachis and low quality edible parts (discarded by size and weight) [131]. Its fruit is traded for the local (playing a key role in food security) as well as international markets [135]. The Table 3.5 presents the unavailable fraction and common uses of some post-harvesting plantain residues. This is a feature that must be considered for productive processes design.

Table 3.5. Common uses of Plantain Residues

Residue	Unavailable fraction	Generation stage	Common uses
Pseudostem	79%	Harvesting	It is mainly used as nutrient support for new plants. Other uses include clothes, laces and papers manufacturing.
Rachis and leaves	87%	Harvesting	Used in catering feed and flour manufacturing.

Low quality bunches	Not available	Post-harvesting	This fraction is commonly commercialized at low prices for human consumption.

PP is commonly used for nutrient assistance of new plants during the agronomic stage [131]. The Figure 3.6 and Figure **3.7** present the seeded and harvested plantain area and the total production and yields for the period 2007-2012 in Colombia. Both of them were built according to official statistics provided by the Colombian Agriculture Ministry [138].

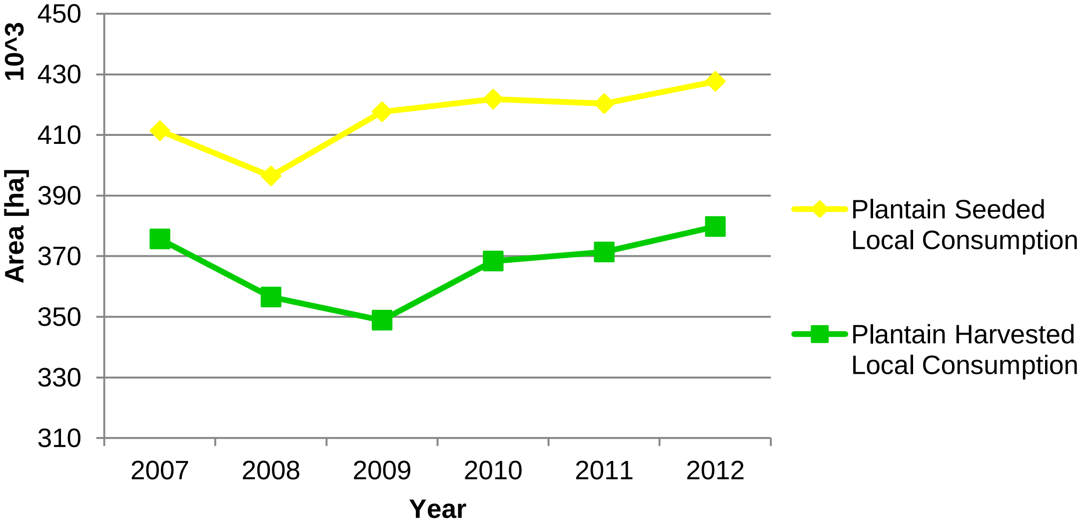

Figure 3.6. Total Surface of Plantain

According to the Figure 3.6 the seeded and harvested surface area has kept a constant behavior during recent years. In the same way the seeded/harvested ratio demonstrate the stability of this culture related to its average used land.

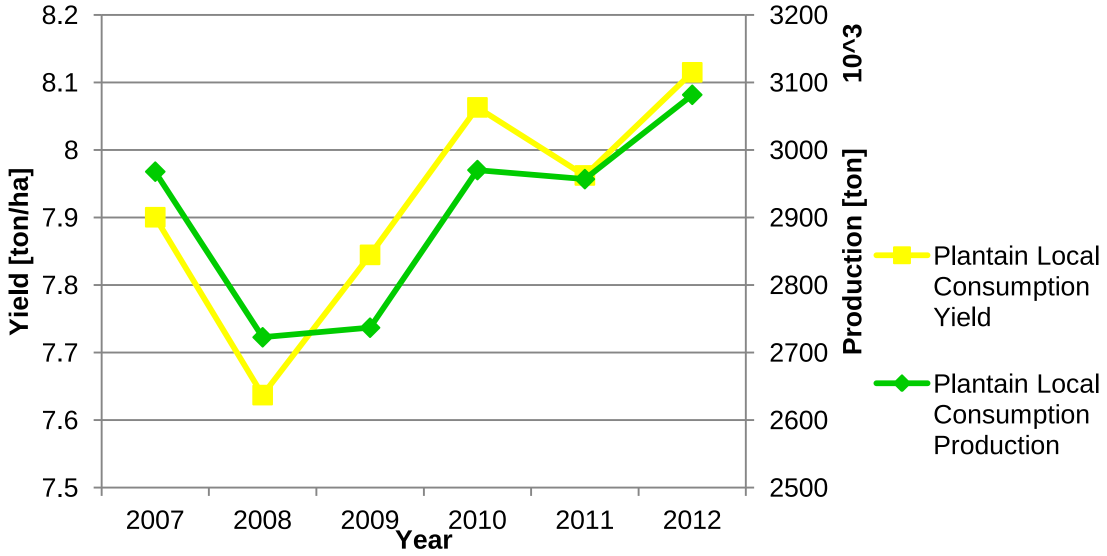

Figure 3.7. Plantain Production

The Figure 3.7 shows that in 2012 approximately 3.08 million tons of plantain bunches were produced with yields of 8.1 ton/ha corresponding to the highest yield registered during the period 2007-2012. Nevertheless, the produced plantain as well as the production yield did not preserved the same trend of the seeded and harvested area. This particular behavior should be consequence of climatic changes related to abnormally lacks or excesses rainfall presented in recent years [138]. Considering the ratio pseudostem to plantain bunches approximately 7.7 million tons of plantain pseudostem were produced in 2012.

Chapter 4: Methodology

Overview

This chapter describes materials and methods used for the development of the present work beginning by the raw materials description and characterization methods. Then, experimental procedures followed by raw materials pretreatments such as conventional dilute sulfuric acid, ultrasound assisted dilute sulfuric acid and supercritical pretreatment are presented. Finally, simulation procedure, including techno-economic and environmental assessment of the mentioned pretreatments are also presented.

4.1. Raw materials

Rice husk (RH)

Rice husk (from *Oryza Sativa* variety) used in this study was provided by a rice mill company located at Tolima region between the Magdalena and Cauca river valleys (central zone of Colombia, 2º 52' 59'' and 5º 19' 59'' N, 74º 24' 18'' and 76º 06' 23'' W) during January 2015. This region is characterized by a warm weather with average values of temperature (28°C), annual rainfall (1988 mm) and moisture (72%). RH was stored at room temperature (17°C) at the Instituto de Biotecnología y Agroindustria (IBA, Universidad Nacional de Colombia-Manizales Campus) before its characterization and processing.

Cassava stem (CS)

Cassava stem (from *Manihot esculenta* variety) used in this study was obtained as by-product from a small farm located at Bugalagrande town in Valle del Cauca region (Southwest zone of Colombia, 4° 12' 27'' N and 76° 09' 27''O) during January 2015. This region is characterized by a mild weather with average values of temperature (24°C), annual rainfall (1927 mm) and moisture (75-87%). CS was milled, dried and stored at room temperature (17°C) at the Instituto de Biotecnología y Agroindustria (IBA, Universidad Nacional de Colombia-Manizales Campus) before its characterization and processing.

Plantain pseudostem (PP)

Plantain pseudostem (from *Musa paradisiaca* variety) used in this study was obtained as by-product from a small farm located at Bugalagrande town in Valle del Cauca region (Southwest zone of Colombia, 4° 12’ 27’’ N and 76° 09’ 27’’O) during January 2015. This region is characterized by a mild weather with average values of temperature (24°C), annual rainfall (1927 mm) and moisture (75-87%). PP was dried and stored at room temperature (17°C) at the Instituto de Biotecnología y Agroindustria (IBA, Universidad Nacional de Colombia-Manizales Campus) before its characterization and processing.

4.2. Raw material characterization methods

The physicochemical characterization was performed by triplicate for each raw material according comprising the experimental determination of extractives [1]; moisture [2]; holocellulose, cellulose, hemicellulose and lignin [5]; and, ash content. These characterization procedures were performed at the Instituto de Biotecnología y Agroindustria and Laboratorio de Equilibrio y Cinéticas Enzimáticas from Universidad Nacional de Colombia (Manizales, Colombia).

Reagents

The chemical reagents used for this study were of analytical grade. Anhydrous glucose and sodium hydroxide were purchased from Merck. Acetic acid, sodium and potassium tartrate, sulfuric acid, and anhydrous ethanol were purchased from Carlo Erba. 3,5-dinitrosalicylic acid (DNS) was bought from Sigma-Aldrich. Sodium chlorite was obtained from Khemra Technologies. Celluclast 1.5L was purchased by Novozymes

The physicochemical characterization for the selected agricultural wastes with three replicates was carried out.

Sample preparation

Moisture contents were measured at 105ºC using Shimadzu moisture balance MOC - 120H. All the materials were dried in an oven (Thermo Precision model 6545) at 45°C during 24 hours (until constant weight was reached). Finally,oven dried materials were milled (Retsch GmbH SR200 Gusseisen) to pass 40 mesh (0.4mm) using a Wiley® Mill.

Extractives content

Extractives determination was performed with aim to remove all the non-structural material from biomass. The non-structural material is referred to inorganic material, non-structural sugars, nitrogenous material, chlorophyll, waxes among others compounds which are soluble in water and ethanol. This procedure was developed according to ASTM Standard Test Method E 1690 “Determination of Ethanol Extractives in Biomass” [139].

Approximately 10 g of sample were weighted and put on a preweighed extraction bags and then were placed in a soxhlet extraction unit. Then 250 ml of ethanol were served in a 500 ml round bottom flask with boiling chips to prevent bumping. The heating rates were adjusted to provide a minimum of 6-10 siphon cycles per hour. The extractions were carried out during 24 h in a ventilated chemical fume hood. Finally, the residual samples were dried and weighted to determine the extractives content. The extractives share was calculated according to the equation 10 :

$$\mathbf{Extractives}\ (\%) = \frac{\mathbf{B_1}}{\mathbf{B_2}} * \mathbf{100}\ \% \qquad \text{Eq 10}$$

Where $\mathbf{B_1}$ is the final biomass dried after extractives extraction (g), and $\mathbf{B_2}$ initial dry biomass before extractives extraction (g)

Ash content

The ash content was determined according to National Renewable Energy Laboratories (NREL/TP-510-42622) [140]. The ash content of fiber is defined as the residue remaining after temperature ramp program indicated below:

Approximately 5 mg of biomass (particle size corresponding to 40-mesh) were weighted into porcelain crucibles. Then, was programmed a ramp from room temperature to 105°C. Hold at 105°C for 12 minutes. Then, an increasing ramp to 250°C at 10°C per minute was programmed. It was held at 250°C for 30 minutes. After that, was programmed a ramp to 575°C at 20°C per minute and then it was held at 575°C for 180 minutes. Finally, the temperature dropped to 105°C and held at 105°C until samples removing. After ignition carefully remove the crucible from the furnace directly into a desiccator and cooled. At last, the samples were weighted on the analytical balance. Finally, the total ash content was calculated according to the equation 11:

$$\mathbf{Ashes}\,(\%) = \frac{\mathbf{RM_2}}{\mathbf{RM_1}} * \mathbf{100}\,\% \qquad \text{Eq 11}$$

Where $\mathbf{RM_2}$ represents the residual mass after ignition (mg) and $\mathbf{RM_1}$is the free moisture mass fed (mg)

Holocellulose content

Holocellulose is defined as the water insoluble carbohydrates fraction composed of cellulose and hemicellulose. The samples were submitted to a chlorination process to remove lignin. The followed method was adapted from *Rabemanolontsoa* and *Saka* [141]. About 2.5 g of moisture and extractive-free sample were placed in a 250 ml Erlenmeyer flask with an inverted 25 ml Erlenmeyer flask in the neck of the reaction flask, then 80 ml of hot distilled water were added and placed on a water bath at 70°C. After one hour were added 1 g of sodium chlorite and 0.5 ml of acetic acid. This procedure was repeated during 6 h until the delignification process was completed [141]. Then, the reaction mixture was left on the water bath 24 h. Later on, the samples were cooled, filtered on cualitative filter paper (Munktell & Filtrak 125 mm id) using a Buchner funnel equipment. The samples were dried and weighted. The holocellulose should not contain any lignin and the lignin content of holocellulose should be determined and subtracted from the weight of the prepared holocellulose.

$$\mathbf{Holocellulose}\,(\%) = \frac{\mathbf{w_2}}{\mathbf{w_1}} * \mathbf{100}\,\% \qquad \text{Eq 12}$$

Where w_2 represents the final weight after chlorination process (g) and $w_1$represents the initial free moisture and extractives weight (g).

Cellulose and hemicellulose content

The preparation of α-cellulose is a continuous procedure after holocellulose determination procedure in pursuit of the ultimately pure form of fiber [142]. Thus, hemicellulose fraction is determined by the difference between holocellulose and α-cellulose content.

About 2 g of vacuum-oven dried free of extractives holocellulose was weighted and placed into a 250 ml glass beaker provided with a glass cover. Then 25 ml of 17.5% NaOH were measured and maintained at 20°C. After, were added 10 ml of 17.5% NaOH solution to the holocellulose in the 250 ml beaker, covered with a watch glass, and maintained at 20°C in a water bath. The holocellulose was manipulated lightly with a glass rod with the flat end so that all of the material becomes soaked with the NaOH solution.

By 2 minutes intervals, the material was manipulated with the glass rod by pressing and stirring until the particles are separated from one another. After the addition of the first portion of 17.5% NaOH solution to the material, at 5 minute intervals, 5 ml of NaOH solution were added and the mixture was thoroughly stirred with the glass rod, until the NaOH was gone. The mixture was stood at 20°C during 30 minutes, making the total time for NaOH treatment 45 minutes. After that, 33 ml of distilled water at 20°C were added to the mixture mixing thoroughly and then was stood at 20°C for 1 h before filtering.

The α-cellulose was vacuum filtered into alkali-resistant fitted-glass medium porosity crucibles. The residual fraction was transferred to the crucible, washing with 100 ml of 8.3% NaOH solution at 20°C. After the NaOH wash solution has passed through the residue in the crucible, continue the washing at 20°C with distilled water, making certain that all particles have been transferred from the 250 ml beaker to the crucible. Then, 15 ml of 10% acetic acid (at room temperature) were poured into the crucible, drawing the acid into the cellulose by suction but, while the cellulose is still covered with acid, the suction was released. Finally, the crucible was dried on the bottom and sides with a cloth, placed during overnight in a vacuum oven at 100-105°Ch. The crucible was cooled and weighted. The α-cellulose and hemicellulose were calculated according to the equations

$$\boldsymbol{\alpha}\mathbf{Cellulose}\ (\%) = \frac{\mathbf{w_2}}{\mathbf{w_1}} * \mathbf{100}\% \qquad \textbf{Eq 13.}$$

$$\mathbf{Hemicellulose}\ (\%) = \mathbf{holocellulose}\ (\%) - \boldsymbol{\alpha}\mathbf{Cellulose}\ (\%) \qquad \text{Eq 14.}$$

Where w_2 represents the final dry weight of the sample and w_1 represents the initial weight of the holocellulose sample.

Lignin content

This procedure is a modified version of TAPPI T222 acid-insoluble lignin in wood and pulp [142]. The sample should be extractive and moisture free.

Approximately 100 mg of dry matter were weighted and placed in 150 mL erlenmeyers. Then 1 mL of 72% H_2SO_4 was added. The flasks were kept at 30°C in a water bath for 1 h, after that were added 28 mL of distillate water. The samples were covered using aluminium foil and placed in autoclave at 121°C for 1 h. The insoluble fraction (Klason Lignin) was filtered using filter paper (Munktell & Filtrak 125 mm id) and washed with hot water until acid free. The filter paper with the solid fraction was oven-dried (Retsch GmbH SR200 Gusseisen) at 60°C during 24 h and weighted to determine the klason lignin content. Lignin content was calculated according to the equation

$$\boldsymbol{Lignin}\ (\%) = \frac{\boldsymbol{Bm_2}}{\boldsymbol{Bm_1}} * \mathbf{100}\% \qquad \text{Eq 15}$$

Where Bm_2 represents the residual dry solid after pretreatment (mg) and Bm_1represents the weight of the initial moisture and extractives free sample.

4.3 Experimental Work

This section describes the methodological procedures related to the experimental pretreatments developed including the conventional dilute acid as well as the ultrasound assisted and the supercritical pretreatment.

Dilute Acid Pretreatment (DAP)

This pretreatment was developed based on the methodology used by *Triana* [143]. Approximately 10 g of sample were weighted and placed in flasks to be pretreated.

The acid pretreatment of raw materials was carried out in an autoclave device using a dilute sulfuric acid (H_2SO_4 2 %v/v) with a solid to dilute-acid ratio of 1:10 (w/w) at 121ºC during 60 min [144], [145] After pretreatment, the solid fractions and liquor were separated by filtration. Solid fraction was washed using distillated warm water. This pretreatment was based in methodology exposed by C. Triana, 2010 [143].

Ultrasound Assisted Pretreatment (UASP)

This pretreatment was developed based on the DAP assisted by ultrasonic waves generated by an ultrasonic processor purchased UP50H (Hielscher Ultrasound Technology).

The acid pretreatment of raw materials was carried out in an experimental assembly using a beaker submerged in a water bath. The pretreatment medium used was dilute sulfuric acid (H_2SO_4 2 %v/v) with a solid to dilute-acid ratio of 1:10 (w/w), different temperatures, amplitudes and exposition times were evaluated to determine the effect [144], [145] After pretreatment, the solid fractions and liquor were separated by filtration. Solid fraction was washed using distillated warm water.

For RH, CS and PP pretreatment were developed different cases of study (according to the raw material) evaluating different temperatures and amplitudes. For each case the approximately 10 g of sample (0.5-0.2 mm particle size) were weighted and placed in a beaker, then diluted as previously described, and submerged in a water bath, the samples were sonicated and finally, the biomass was recoved by vacuum filtration.

4.3.3 Supercritical Carbon Dioxide Pretreatment (SCDP)

All the runs were performed in a laboratory unit, consisting of a line for conditioning CO_2 composed by a cooler and a pump, a reaction chamber and a collector vessel. The Figure 4.1(a) shows a schematic representation of supercritical reactor and the Figure 4.1(b) presents a picture of the lab scale supercritical reactor used.

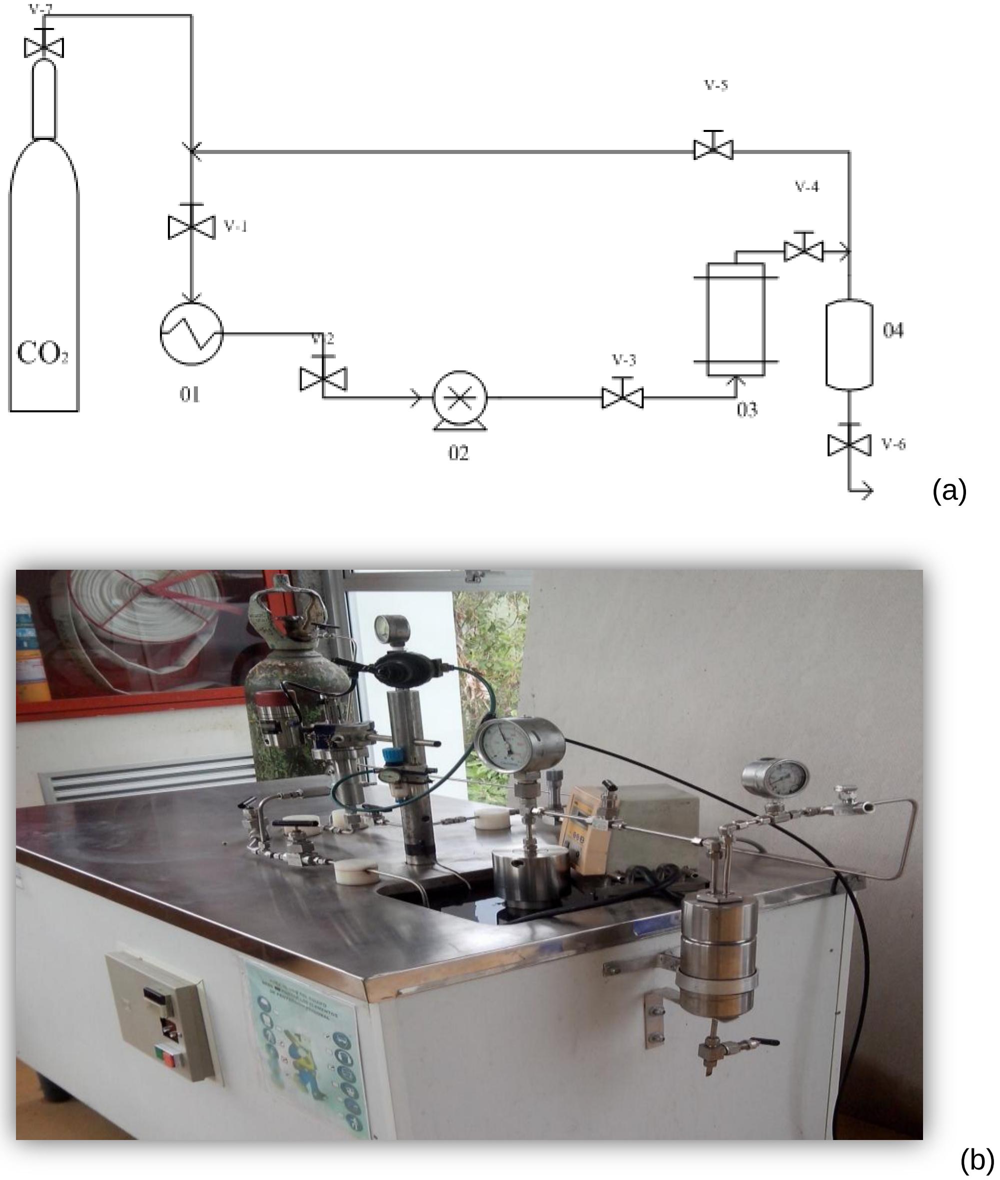

Figure 4.1. Supercritical Reactor (a)Scheme (b) Equipment

According to the Figure 4.1 The gas is passed through a cooler system (equipment 1) using ethylene glycol as cooling fluid. In this stage the CO_2 is carried to liquid state at -20°C. Then, the liquid CO_2 is pressurized by a pneumatic pump until the desired pressure (equipment 2, a Dual Seal pneumatic plunger pump WILLIAMS Chemical Metering Pumps.) The maximum endured pressure is 350 bar and the reactor capacity is 254 mL. Later on, the pressurized CO_2 was put in contact with the sample in the extraction or reaction chamber (equipment 3, made of stainless steel of 10,000 psi). In this equipment approximately 10 g of raw material got in contact with the supercritical carbon dioxide. The reactor was immersed in a water bath at the desired temperature (atmospheric water bath that can reach 93°C). The CO_2 then endured a pressure drop while it is conducted to the collector (equipment 4). In this stage due to the pressure drop the CO_2 returned to gaseous state (all the solid and liquid compounds extracted or dragged by the CO_2 were kept in the collector) and then it would be recycled or purged returning or leaving the system practically clean, with no additional recovering steps [146].

4.4 Assessment of Pretreatment Effects

Crystallinity, delignification yield and enzymatic hydrolysis were the procedures developed to determine the pretreatment effects.

Crystallinity determination

X-ray diffraction (XRD) is a technique widely used for crystallinity characterization of lignocellulosic biomass and composites [147]. This characterization was performed using a RIGAKU MINIFLEX II diffractometer, using Cu-K_a radiation at 30 kV and 15 mA with a scan rate of 5°(2θ) min^{-1}, a sampling width of 0.02°(2θ), and over a range between 5° and 50° (2θ). The crystallinity index defined as the crystalline to amorphous ratio was calculated based on the method proposed by *Segal* et al.[148] according to the equation 16

$$\textbf{Crystallinity Index}\ (\%) = \frac{\mathbf{I_{002} - I_{am}}}{\mathbf{I_{002}}} * \mathbf{100}\% \qquad \text{Eq 16.}$$

I_{002} represents the maximum intensity (measured in arbitrary units) of the diffraction from the plane at 2θ ≈ 22.6° for cellulose I and I_{am} represents the intensity of the background scatter measured at 2θ ≈ 19°.

Delignification yield

The delignification yield refers to the lignin fraction removed as a pretreatment consequence. This yield was calculated according to the equation 17.

$$\textbf{Delignification yield}\ (\%) = \frac{\mathbf{Lignin_1 - Lignin_2}}{\mathbf{Lignin_1}} * \mathbf{100}\% \qquad \text{Eq 17.}$$

Where Lignin_1 represents the initial lignin content and Lignin_2represents the lignin content after pretreatment.

Enzymatic Hydrolysis

After pretreatment stage the samples were washed as was described in the preconditioning stage. The protocol followed was based on the “Laboratory Procedure 009: Enzymatic Saccharification of Lignocellulosic Biomass” developed by the National Renewable Energy Laboratory [149]. The samples were hydrolyzed by a cellulolytic using Celluclast 1.5L. The samples were dissolved in sodium citrate 0.1 M (pH 4.8). The enzymatic hydrolysis was carried out at 50 °C and 100 rpm during 48 hours. After enzymatic hydrolysis the sugar content was determined in each sample by the colorimetric DNS method [150].

Reducing Sugars Concentration

DNS method was used for determining the total reducing sugar content during enzymatic hydrolysis and ethanolic fermentation [150]. The reducing sugars were quantified by the 3,5-Dinitrosalicylic acid (DNS) method with a Jenway 6405 UV/Vis Spectrophotometer. The sample 1:20 in DNS reagent in a test tube were diluted and mixed. Tubes were placed in boiling water bath for 9 minutes, transferred to ice to rapidly cool down for 3 minutes. Then, absorbance was measured at a wavelength of 540 nm, against a blank. A calibration curve was made from an anhydrous glucose standard solution (1, 2, 3, 4 and 5 g l^{-1}) and the blank was prepared with distilled water.

Inhibitory Compounds Determination

Furfural was selected as the reference inhibitory compound. It was determined through High Performance Liquid Cromatography. The method used was developed based on *Davies* et al [39]. and *Farre* et al [151]. All the samples were diluted in methanol (Dilution Factor=5), centrifuged and filtered using Whatman syringe filters (GF/C 25 mm filter diameter 1.2µm pore size) purchased from Whatman International Ltd.

Furfural was analyzed using an HPLC system LC2010 purchased by Shimadzu Corporation. The mobile phase used was a binary mixture of 1% acetic acid (Solvent A) and methanol (Solvent B) with an overall flow rate of 1.0 ml/min. The volume of sample injected was 10 µl.

4.5 Process Assessment

Process simulation

To understand the potential of the proposed pretreatments, it must be defined the structure of the process and to simulate the selected technological pretreatment schemes. With the aim to contrast the techno-economic performance of the pretreatments analyzed in this work with the industry scales, the processing of 10 ton/h of raw material were assessed.

For this purpose the commercial package Aspen Plus v8.2 (Aspen Technologies, Inc., USA) was the main tool used based on models for every step of the flowsheet showing one or more inlet streams entering into the system's first unit operation (i.e., heat exchanger, compressor, reactor etc.) and continues through the process, illustrating all intermediate unit operations and the interconnecting streams. When the calculations are complete, the software lists the results, stream by stream and unit by unit, so it is possible to know not only the change in species and also on properties of the flows. The most complex and detailed technological schemes are obtained through rigorous simulations, involving sensitivity analysis with the aim to looking for the most adequate operation conditions. All thermodynamic properties for conventional molecules were obtained from aspen plus properties database, NIST database and the developed database for biofuels components [152].

Therefore, the biomass was introduced into the simulator database according to the determined chemical composition previously described. The thermodynamic model used for simulation purposes was the Non-Randonm Two-Liquid (NRTL), this method allows to calculate activity coefficients correlating the activity coefficients of a compounds with its mole fraction in the liquid phase. In addition, UNIFAC Dortmund method was used to predict the phase equilibria taking advantage of the method capability to predict behaviors of mixture such as vapor-liquid equilibria (VLE), activity coefficients at infinite dilution, excess enthalpies, liquid -liquid equilibria (LLE), solid -liquid equilibria of eutectic systems

(SLE) and azeotropic data over a wide temperature range, through the simultaneous fitting of the group interaction parameters for a given mixture.

Economic assessment

The capital and operating costs were calculated using the Aspen Process Economic Analyzer v8.2 software (Aspen Technologies, Inc., USA). This assessment corresponded to the Colombian conditions considering the local income tax, annual interest rate, labor salaries among others. This analysis was estimated in US dollars during 10-year period (8000 h/period). Prices and economic data used in this analysis correspond to Colombian conditions such as the costs of the raw materials, income tax, labour salaries, among others, were incorporated in order to calculate the production costs per kilogram of product. The Table 4.1 presents a summary of the economic parameters involves in the techno-economical assessment:

Table 4.1. Prices of feedstocks, utilities and products

Parameter	**Unit**	**Value**
Rice husk[a]	USD/ton	5
Cassava stem[+]	USD/ton	5
Plantain pseudostem[+]	USD/ton	5
Sulfuric acid[b]	USD/kg	0.08
Calcium Hydroxyde[b]	USD/kg	0.07
Nutrients [+]	USD/kg	1
Enzymes[+]	USD/kg	1
Glucose [c]	USD/kg	0.8
Xylose [c]	USD/kg	1.5
Income tax [d]	%	25
Annual interest rate [e]	%	17
Operator labor cost [f]	USD/h	2.14
Supervisor labor cost [f]	USD/h	4.29

Low pressure steam [f]	USD/ton	1.57
Medium pressure steam [f]	USD/ton	8.18
Electricity [f]	USD/kWh	0.14
Potable water [f]	USD/m^3	0.74
Fuel [f]	USD/MWh	24.58

[a] *Quintero* et al [13], [b]Icis Pricing[153], [c] Alibaba [154], [d] KPMG [155], [e] KPMG [156], [f] *Moncada* et al [116], [+]Estimated prices

Environmental Assessment

The environmental assessment was carried out using the Waste Reduction Algorithm (WAR Algorithm) designed by the Environmental Protection Agency of the United States (US EPA) by WARGUI software. This algorithm is based on the determination of the Potential Environmental Impact (PEI) [157]–[159]. It is defined as the effect caused to the environment by some specie.

The PEI are quantified in Human Toxicity Potential by Ingestion (HTPI), Human Toxicity Potential by Exposure (HTPE), Aquatic Toxicity Potential (ATP), Terrestrial Toxicity Potential (TTP), Global Warming Potential (GWP), Ozone Depletion Potential (ODP), Photo-chemical Oxidation Potential (PCOP) and Acidification Potential (AP). Some PEI's of compounds such as lignin, cellulose and hemicelluloses are not in database of WARGUI software, so they were taken from several material safety data sheet, articles, among others. The WAR algorithm is a tool to compare process configurations from the environmental point of view. The bounds of the systems were defined from the inlet to the outlet plant door, in this sense the PEI contributions of the manufacture process for each input were ignored in this evaluation. The selected product stream was the dilute glucose stream while the other streams were considered as non-product streams. In addition, this by the using of this tool was taken into account the energy source and requirements.

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Chapter 5: Experimental characterization results.

Overview

This chapter presents the cellulose, hemicellulose, lignin and ash content as main components of the analyzed raw materials (RH, CS and PP). Correspondingly, the chemical characterization of these types of raw materials allows determining the potential uses in agroindustry. Nevertheless, the yields conversion through biotechnological processes is highly dependent of tissues characteristics and cell wall organization, therefore, recalcitrance and crystallinity index (CrI) dependent.

5.1 Raw materials criteria selection

RH, CS and PP were the agricultural wastes selected as raw materials for the development of this thesis. The main criterion considered was related to the source of these residues as well as the agricultural requirements associated to them. That is supported by the wide capability to growth in different regions assorted of soils variety.

Rice, Cassava and Plantain are crops harvested respectively in 26, 31 and 32 Colombian regions. This fact illustrates the potential of RH, CS and PP to be obtained and possibly used in different regions. However, these possibilities can be constrained by the harvesting yields as well as agricultural management customs.

5.3 Rice Husk (RH)

The Table 5.1 presents the results of the experimental characterization of rice husk (*Oryza sativa* specie) and some characterization data reported by different authors [160]–[162]. Despite the source and raw material origin could produce compositional differences in this case the experimental characterization agree with those reported by *Quintero* et al[160], *Díaz* et al [161] and *Soltani* et al. In addition, the extractive-free material can be interpreted as a measure of the theoretical lignocellulosic biomass able to be transformed, at the same time the high ash content should increase the interest of this raw material to produce silica based compounds as well as C5 and C6 based compounds.

Table 5.1. Rice Husk Characterization

Feature	**Content (wt%)**			
	This work	***Quintero* et al**[160]	***Díaz* et al** [161]	***Soltani* et al** [162]
Moisture	11.5	10.2	n.r	n.r
Extractives	7.69 ± 1.50	1.34	n.r	n.r
Cellulose	39.30 ± 2.90	26.45	29.8	34
Hemicellulose	15.88±3.56	27.29	16.6	24.3
Lignin	25.98±7,74	18.91	22.7	19.2
Ash	11.14±1.14	14.89	n.r	15-20

(D.B): Dry Basis, nr: Not reported

In addition, the low moisture content represents an advantage for rice husk utilization. This fact would imply energy savings for the preconditioning stage related to the material drying for those operations in which low moisture content is required.

5.4 Cassava Stem (CS)

The Table 5.2 presents the results of the experimental characterization of cassava stem (*Manihot esculenta* specie) and some characterization data reported by different authors [11], [163], [164]. These results agree with other authors. There are observed slight compositional differences between other reported compositions and the other one reported by this work. However, in this case the characterizations presented by *Klinpratoom* et al[163]. and *Han* et al [164] were recollected from Thailand and Indonesia respectively, hence, the origin of the samples as well as the soils characteristics and harvesting practices would influence the chemical composition.

Table 5.2. Cassava Stem Characterization

Feature	**Content (wt%)**			
	This work	*Schmidt* & *Thomsen* [11]	*Klinpratoom* et al[163].	*Han* et al [164].
Extractives	12.69 ± 0.60	8.9	n.r	n.r
Cellulose	41.54 ± 4.18	33.1	42.10	35.2
Hemicellulose	16.65 ± 8.93	13.7	14.85	24.3
Lignin	24.69 ± 4.18	28.3	35.63	33.8
Ash	4.42 ± 3.51	4.1	5.97	2.2

(D.B): Dry Basis, nr: Not reported

In addition, otherauthors have been reported a starchy fraction up to 30% in cassava stem [130], [165], nevertheless, authors do not usually report the corresponding lignocellulosic composition and inversely.

5.5 Plantain Pseudostem (PP)

The Table 5.3 presents the results of the experimental characterization of plantain pseudostem (*Musa paradisiaca* specie) and some characterization data reported by different authors [166]–[168]. There is evidenced some differences between the reported compositions. Nevertheless, this fact should be explained by the variety of species from *Musaceae* as well as the different conceptions and naves gave to plantain fruits and crops. In addition, particle size reduction of raw PP produces an exudate fraction (not characterized by this work) that would contain starch of easy mechanical extraction, moreover, has been reported heavy losses (up to 72%) of this fraction stored above shadow as well as being left in the fields [169].

Table 5.3. Plantain Pseudostme Characterization

Feature	**Content (wt%)**			
	This work	*Sosa* et al [166]	*Cordeiro* et al [167]	*Darkwa* [168]
Moisture	83	n.r	n.r	n.r
Extractives$^{(D.B)}$	2.50 ± 1.46	9.56	14.5	4.77
Cellulose$^{(D.B)}$	43.46 ± 7.23	55.65	40.2	42.09

Hemicellulose$^{(D.B)}$	33.77 ± 4.48	n.r	25	19.04
Lignin$^{(D.B)}$	20.14 ± 3.54	11.56	12.7	13.53
Ash$^{(D.B)}$	0.14 ± 0.001	n.r	14.6	6.44

(D.B): Dry Basis, nr: Not reported

According to the presented chemical composition, the moisture content of plantain pseudostem would represent one significant drawback for its agroindustrial utilization because of the energy costs associated to drying stage.

5.6 Raw Materials Crystallinity

The Figure 5.1 presents the XRD plots for raw (a) rice husk, (b) cassava stem and (c) plantain pseudostem. There is observed differences in peaks definition and intensities which would be related to the matrix composition.

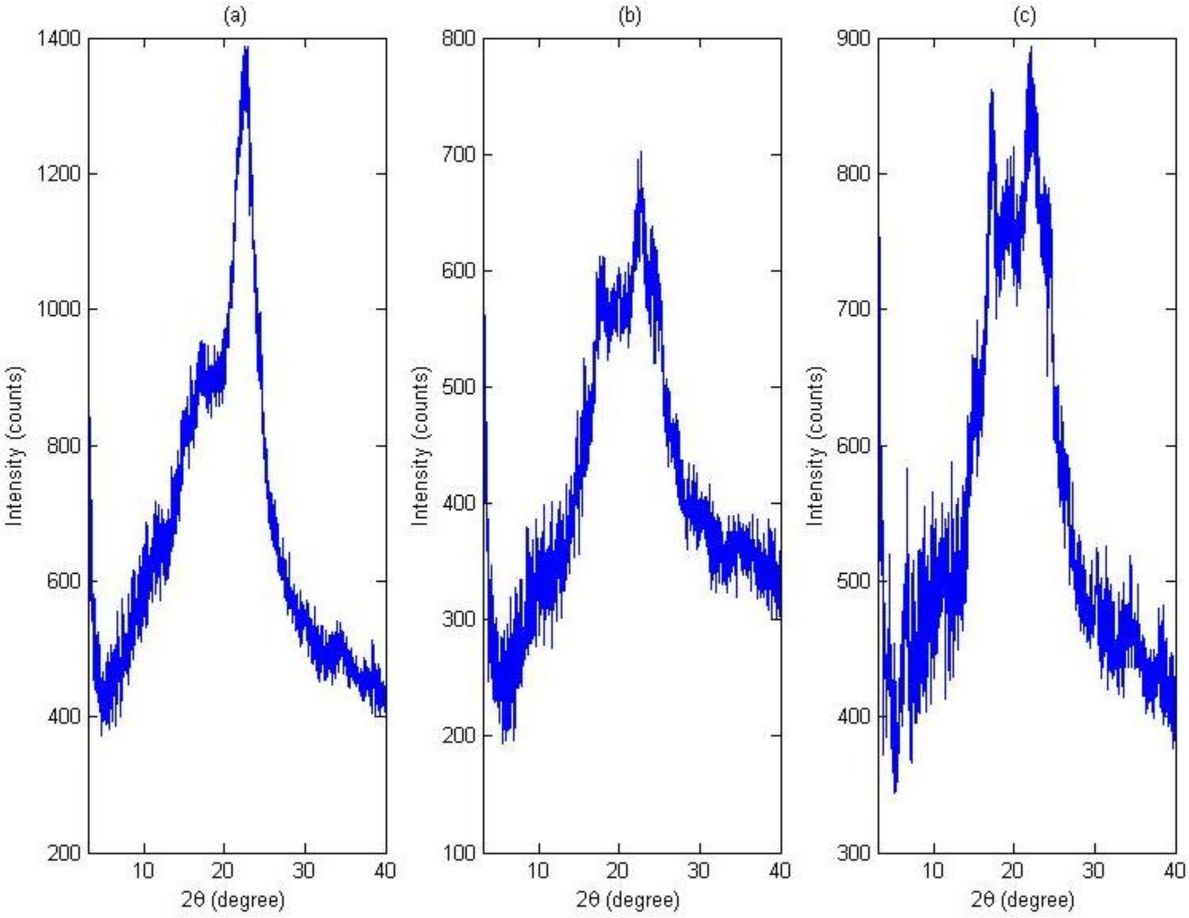


Figure 5.1. XRD plots (a) Rice Husk, (b) Cassava Stem, (c) Plantain Pseudostem

CrI as a characteristic feature of raw materials, for instance have been reported CrI of cassava bagasse and rice straw by the order of 53.3 % [170] and 14.52% [171] respectively. Additionally, it has been demonstrated that higher values of CrI by the order of 74% (cotton nanofibers) and 78.4% (sugarcane bagasse nanofibers) can be allowed through different pretreatments [171]

According to the procedure described in the section 4.5.1 the crystallinity index were estimated for comparative purposes using the method proposed by *Segal* et al.[148]. The CrI were 24%, 36% and 16% for RH, CS and PP respectively. In this sense the most crystalline raw material was is the cassava stem followed by the rice husk, by last, plantain pseudostem as the lower crystalline waste.

5.7 Final Remarks

In this work the selected samples produced by different farms from two different Colombian regions under different handling and storage conditions were characterized. The chemical composition of the selected raw materials would be a useful tool to identify possible advantages and disadvantages related to the use of each one of them as agroindustry raw materials. The high cellulose contents allows to identify the RH, CS and PP potential for hexoses obtaining whereas the hemicellulose content in PP would be also used for pentoses extraction. Nevertheless, the lignin content and crystallinity index are barriers to overcome

.

Chapter 6: Results of Ultrasound Assisted Pretreatment

Overview

The use of ultrasound should improve the mass transfer providing different benefits to processes such as time reduction and yields enhancing among others [1]–[3]. The application of ultrasonic waves is able to intensify the effect of sulfuric acid pretreatment, therefore through glucosidic bonds cleavage it is expected that individual crystallites are released [170]. This chapter presents the results of RH, CS and PP pretreatment considering the experimental assessment of ultrasound irradiation at two different amplitudes and temperatures.

6.1 Experimental stage

The ultrasound device used in this thesis was a sonicator UP50H purchased from Hielscher (Hielscher ultrasonics gmbh, Germany). This device works at constant frequency (30 kHz) with an automatically adjust of frequency and 50W of power, in addition the amplitude can be fixed in the range of 20-100%. This device was provided by a horn-type piezoelectric ultrasonic transducer made of titanium (3 mm id). The internal diameter of the transducer was a determinant factor of the maximum volume to be pretreated, hence the mass load is also constrained by the transducer internal diameter.

According to the literature it would be expected because of increases in mass transfer, the pretreatment times should be reduced. In this sense was developed a pre-experimental stage to determine the effect of different pretreatment times. For this purpose PP samples were submitted to 20 min and 60 min pretreatment. Finally, the results corroborated in agreement with the open literature [47] that no long pretreatment times are needed to redistribute the lignocellulosic content by similar extent. Taking into account this finding, the ultrasound assisted pretreatment developed in this thesis used 20 min as pretreatment time at different temperature levels (80°C and 40°C)

Ultrasound power is also considered as a key factor in ultrasound pretreatment [26]. As was previously mentioned, it is expected that higher ultrasound amplitudes produces strength ultrasonic effect. However, high density of bubbles can cause poor transmission into the liquid media [26]. In this work the samples were pretreated using two amplitude levels.

Another important issue for ultrasound assisted pretreatments is the applied volume-specific acoustic energy (EV) [10]. The EV is a measure of the energy applied to a determinate pretreated volume per unit time establishing a relationship between the pretreatment time and the power supplied [10]. The EV is calculated by the equation Eq **18**:

$$EV = \frac{P * t}{V} * 60 \qquad \text{Eq 18}$$

Where: P is power (W), t is time (min) and V is pretreated volume (l). Some EV values were estimated taking into account the specifications of the used device at three different amplitudes (20%, 60% and 100%), for 0.1 l of total pretreated volume. The results are presented in the Figure 6.1. From this figure can be concluded about short pretreatment times as well as low amplitude values would be useful to reduce the costs associated to the supply of energy.

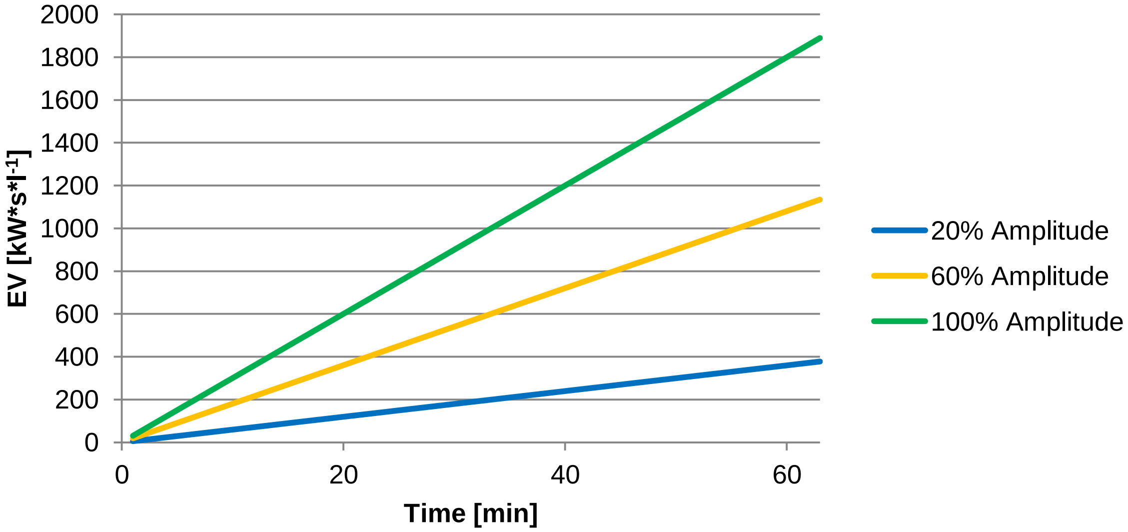


Figure 6.1. Applied volume-specific acoustic energy

Taking into account that hemicellulose and lignin are the main compounds removed during dilute acid pretreatment, it is presented a redistribution of holocellulose, in consequence the lignin in the residual solid. These values added to CrI would provide information about the pretreatment extent.

The empirical method proposed by *Segal* et al. allows to determine the cellulose crystallinity exclusively for comparison purposes due to the approximations made of the amorphous region [148].

Finally, with the aim to determine the impact caused by ultrasound application at least two temperatures (40° and 80°C) and amplitudes (20% and 100%) values were evaluated. The pretreatments were developed using dilute sulfuric acid (2% v/v) with a solid to liquid ratio 1:10. Then, based on crystallinity index, holocellulose content, recovered biomass and reducing sugar results were selected the best operational conditions for ultrasound assisted pretreatment.

6.2.1 Rice Husk (RH)

The conditions of the evaluated cases are presented in the Table 6.1. For this raw material were evaluated two additional operational conditions. In this sense medium temperature and amplitude levels with the aim to determine the effects at 60°C and 60% amplitude.

Table 6.1. Rice husk cases description

Case	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Temperature (°C)	80	80	80	60	60	60	40	40	40
Amplitude (%)	100	60	20	100	60	20	100	60	20

For the first case of study developed at the harshest conditions was visually observed an increase in the medium viscosity (not quantified), as evidence the filtration stage spent more time than other samples. This fact would be associated to modifications in the material porosity causing saturation of the filter paper.

Crystallinity Index

The Figure 6.2 presents the crystallinity index of pretreated RH samples. These results evidence different trends in CrI according to the selected amplitude. However, all of them differs from the initial (CrI 24%) as a pretreatment consequence. At the highest temperature level all the amplitudes were able to reduce CrI up to 13% revealing a kind of synergism between temperature and ultrasonic waves. Whereas, the moderate and low temperature levels presented decreases and increases in CrI compared to the unpretreated sample, the highest CrI for each level of amplitude assessed was presented at 60°C.

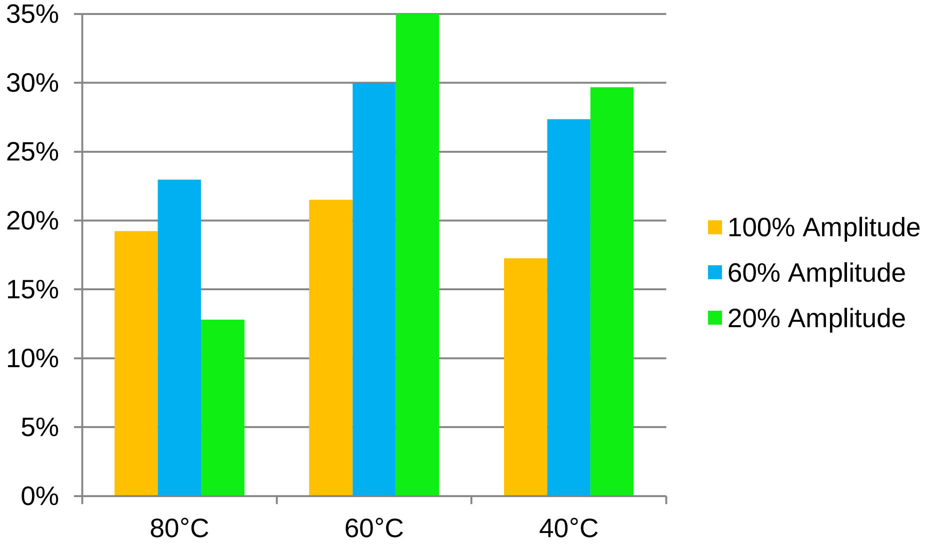


Figure 6.2. Rice husk Cristallinity Index

Finally, it can be concluded that high amplitude values (100%) ensure similar effects in CrI regardless the temperature selected (CrI values of 19.23, 12.80 and 17.25% for 80, 60 and 40°C respectively). As a highlighted feature, it was observed that low temperatures (by the order of 40°C) added to high amplitude are able to reduce the RH crystallinity up to 17.25%.

Holocellulose content

The Figure 6.3 presents the holocellulose fraction in RH samples after pretreatment. The holocellulose content of pretreated samples was between 59-70% representing increases by the order of 7-27% compared to the initial 55.18%. Increases in holocellulose content should be related to delignification of the material and hemicellulose hydrolysis extent.

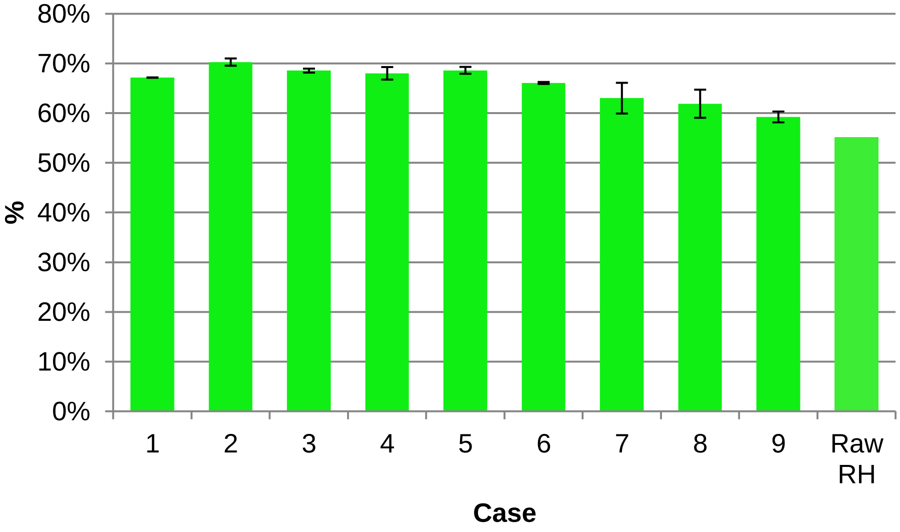


Figure 6.3. Rice husk holocellulose content

The Figure 6.4 presents the CrI and holocellulose content as combined results.

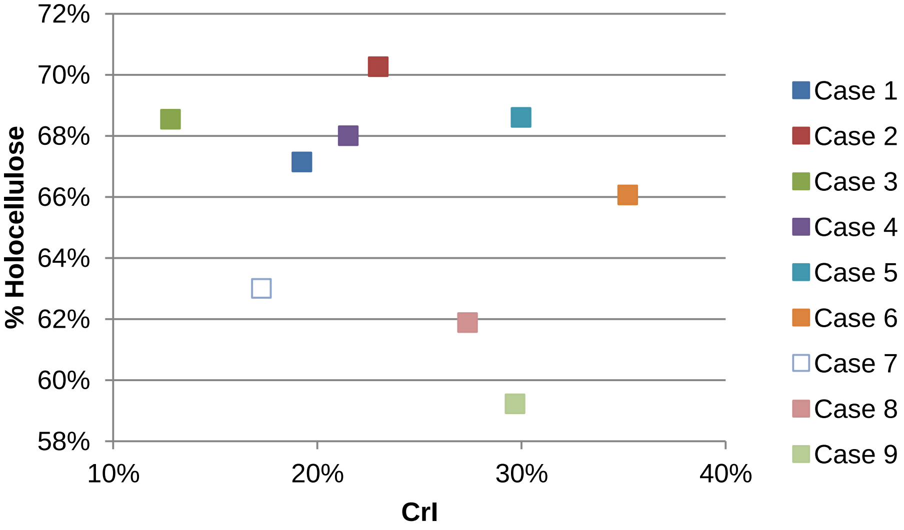


Figure 6.4. Rice husk combined results

According to the Figure 6.4 and based on CrI reduction and holocellulose increase criteria were identified the samples with considerable pretreatment effects corresponding to the cases 1, 3, 4 and 7. All those cases showed the higher holocellulose increases and CrI decreases at the higher amplitude (cases 1, 4 and 7) and temperature (case 3) levels.

Pretreatment yields

Taking into account the results before presented were developed new pretreatment runs considering the higher and lower temperature and amplitude. Additionally, the total content of reducing sugars, the reducing sugars yield, furfural concentration and biomass recovered were determined. Those results are addressed in the Table 6.1:

Table 6.1. RH pretreatment results

Case	**1**	**2**	**3**	**4**
Temperature (°C)	40	40	80	80
Amplitude (%)	100	20	100	20
Reducing sugars (g/l)	0.40	0.41	1.15	1.01
Reducing sugars yield (g/g dried biomass)	0.004	0.004	0.010	0.008
Furfural (g/l)	N.D	N.D	N.D	N.D
Biomass recovered (%)	93	92	89	92

N.D: Not detectable

According to the Table 6.1, the best results were obtained (case 3) at the harshest conditions. However, despite of CrI reduction, low reducing sugars concentration and hemicellulose hydrolysis yields by the order of 1.15 g/l and 6.4% were reached without detectable furfural production. That means that under the evaluated operational conditions the dilute acid pretreatment assisted ultrasound is not able to produce high yields of RH hydrolysis.

6.2.2 Cassava Stem (CS)

The conditions of the evaluated cases are presented in the Table 6.2:

Table 6.2. Cassava stem cases description

Case	**1**	**2**	**3**	**4**
Temperature (°C)	80	80	40	40
Amplitude (%)	100	20	100	20

Crystallinity Index

The Figure 6.5 presents the crystallinity index of pretreated CS samples. These results evidence different CrI trends according to the selected temperature. However, all of them were capable to reduce the initial value (CrI 36%) as a pretreatment consequence. According to the obtained results, low amplitudes added to low temperatures favored the CrI reduction whereas, for high temperature causes the opposite effect.

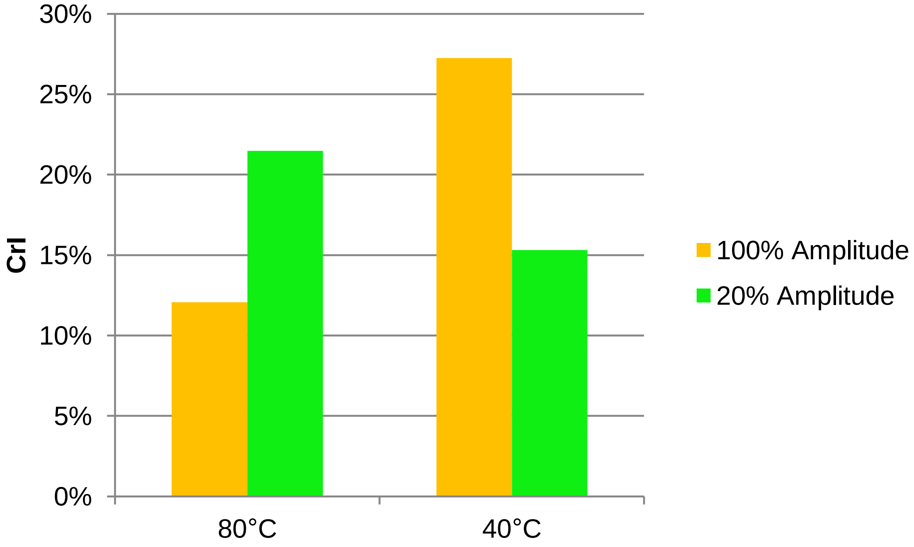


Figure 6.5. Cassava stem Crystallinity Index

In this case, for low amplitude values, the decrease of CrI was favored at low temperature whereas for high temperature values the decrease of CrI was favored at high amplitude values. The lowest CrI value was reached at 80°C and 100% amplitude, this behavior would be presented because of the synergism between temperature and ultrasonic waves, able to overcome barriers caused by bubbles density generated at high amplitude levels.

Holocellulose content

The Figure 6.6 presents the holocellulose fraction in CS samples after pretreatment. The holocellulose content of pretreated samples was between 52.8-60.5% representing reductions by the order of 9-21% compared to the initial 66.6%. From these holocellulose results it would expect higher hydrolysis yields compared to those presented by RH.

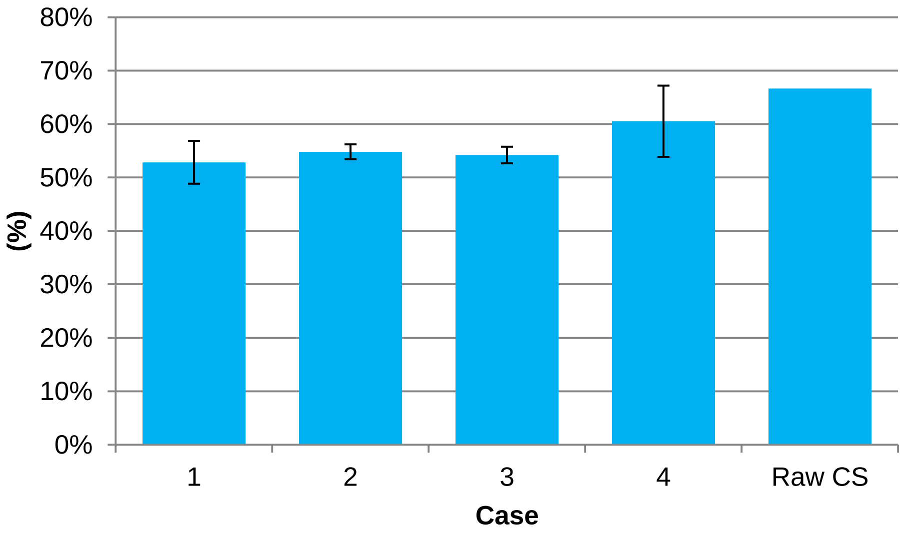


Figure 6.6. Cassava stem holocellulose content

The Figure 6.7 presents CrI and holocellulose content as combined results.

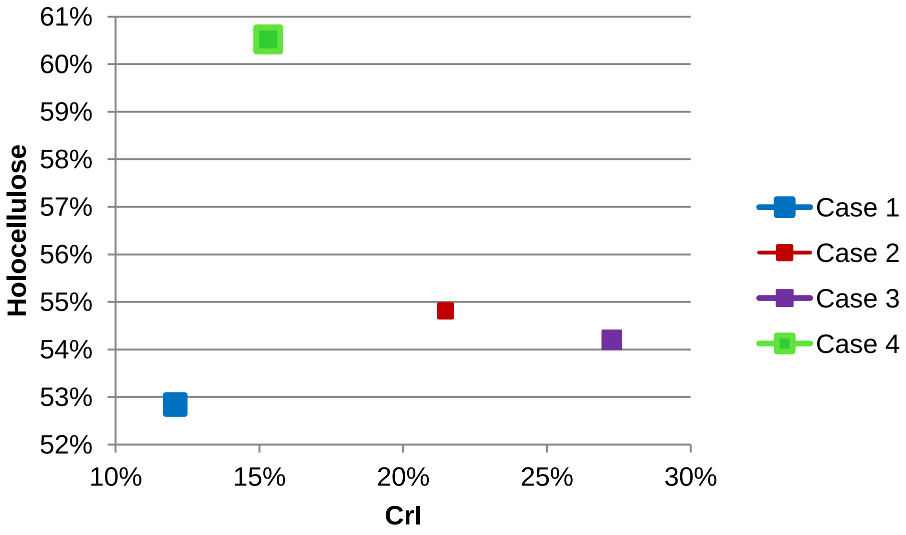


Figure 6.7. Cassava stem combined effects

According to the Figure 6.7 the cases 1 and 4 exhibited the higher CrI reductions whereas the holocellulose content reduction was higher at high temperature and amplitude (case 1), hence, higher yields and extent of hemicellulose hydrolysis would be expected.

Pretreatment yields

Taking into account the reduction in CrI and holocellulose contents before presented were developed new pretreatment runs. For this case were considered the same cases (based on the high and low temperature and amplitude values). Additionally, the total content of reducing sugars, the reducing sugars yield, furfural concentration and biomass recovered were determined. Those results are addressed in the Table 6.3:

Table 6.3. CS pretreatment results

Case	**1**	**2**	**3**	**4**
Temperature (°C)	80	80	40	40
Amplitude (%)	100	20	100	20
Reducing sugars (g/l)	3.30	3.18	2.13	2.58
Reducing sugars yield (g/g dried biomass)	0.030	0.029	0.019	0.023
Furfural (mg/l)	4.32	9.66	0.33	0.82
Biomass recovered (%)	92.4	96.3	98.8	98.0

According to the Table 6.3 the higher pretreatment yields were reached at the harshest operational conditions in agreement with the behavior showed in the Figure 6.7. In this case it was reported furfural production for all the evaluated cases, because of severity of the reaction the higher temperature scale produced more furfural than the other one. However, the furfural produced was kept by the order of ppm which does not represent a threat for microorganisms.

Finally, for CS pretreatment the cases 1 and 2 presented the highest values of reducing sugars concentration and hemicellulose hydrolysis yields by the order of 3.30 g/l and 19% respectively. Despite of the increase in hemicellulose yields compared to RH pretreatment the concentrations produced are still low for biotechnological transformation purposes. However, an increase in the sugars concentration at this stage could involve the use of large amounts of energy, also affecting the production costs.

6.2.3 Plantain Pseudostem (PP)

The conditions of the evaluated cases are presented in the Table 6.4:

Table 6.4. Plantain Pseudostem cases description

Case	**1**	**2**	**3**	**4**	**5**	**6**
Temperature (°C)	40	40	60	60	80	80
Amplitude (%)	100	20	100	20	100	20

At the maximum temperature and amplitude level (case 5) was visually observed an increase in the medium viscosity (not quantified), as evidence the filtration stage spent more time than other samples. This fact would be associated to modifications in the material porosity causing saturation of the filter paper.

Crystallinity Index

The Figure 6.8 presents the crystallinity index of pretreated PP samples. These results evidence close similarities in CrI at mild and low temperature levels and all the amplitudes evaluated. However, all of them differs from the initial (CrI 16%) as a pretreatment consequence.

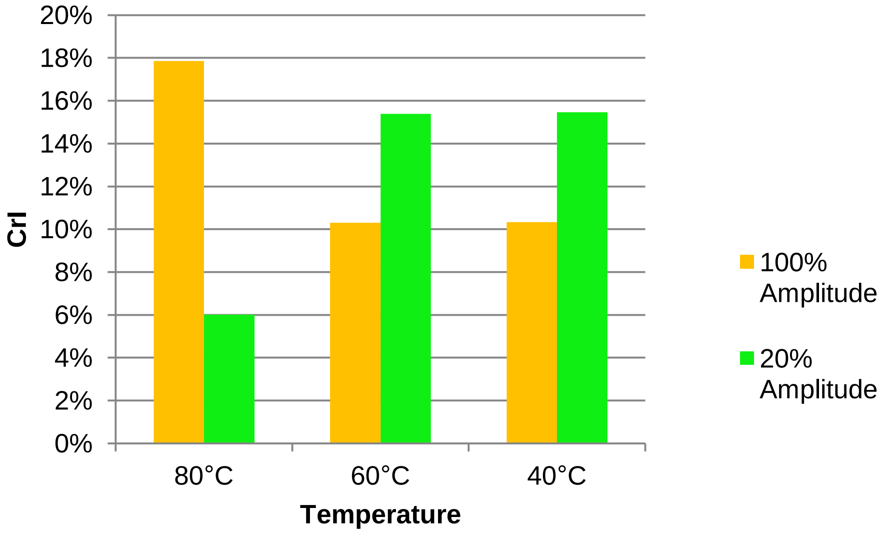


Figure 6.8. Plantain pseudostem Crystallinity Index

According to the results obtained it was identified two interesting operational configurations for CrI reductions for PP pretreatment. The first configuration using low amplitude and high temperatures and the second configuration using high amplitude and low temperature.

Holocellulose content

The Figure 6.9 presents the holocellulose fraction in PP samples after pretreatment. The holocellulose content of pretreated samples was between 19-71% representing reductions by the order of 71-11% compared to the initial 79%. From these holocellulose results it would expected highest hydrolysis yields compared to those presented by RH and CS.

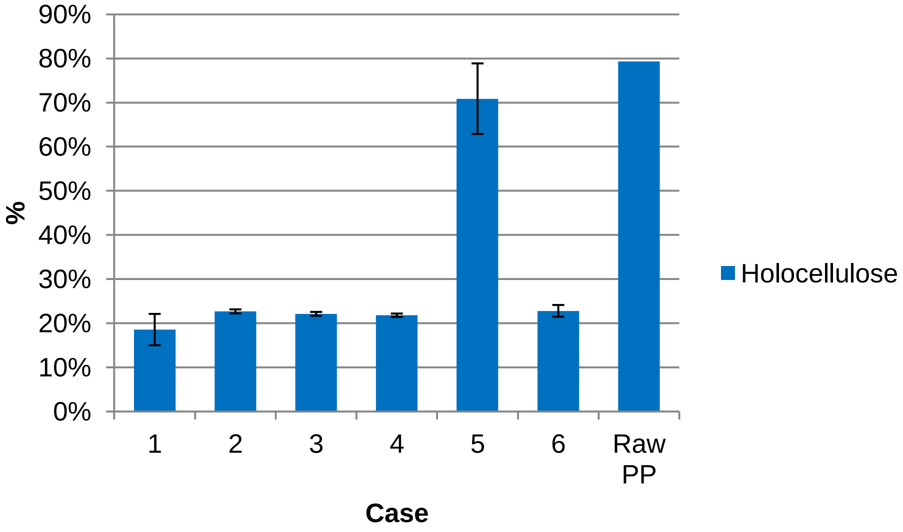


Figure 6.9. Plantain pseudostem holocellulose

The Figure 6.10 presents CrI and holocellulose content as combined results.

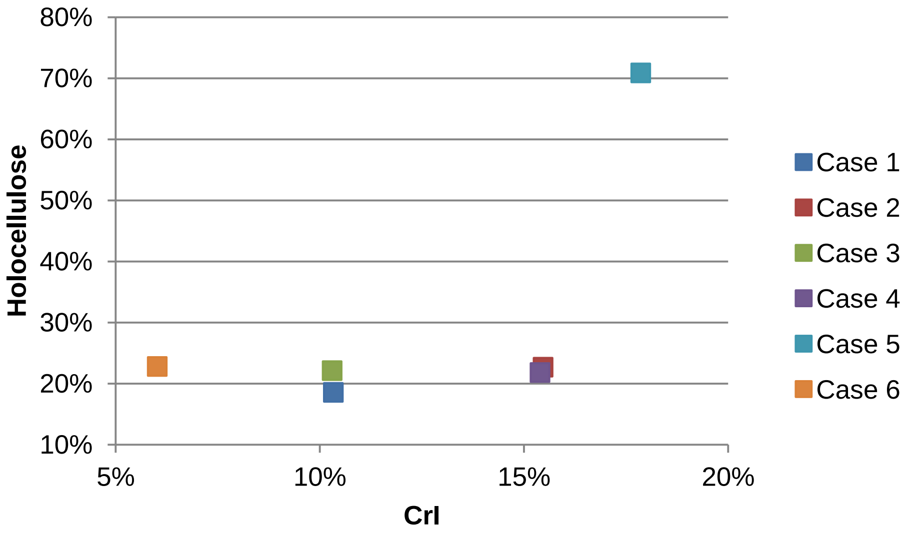


Figure 6.10. Plantain pseudostem combined effects

According to this figure, the cases 1, 3 and 6 present the higher holocellulose and CrI reductions. Hence, as an interesting fact higher yields and extent of hemicellulose hydrolysis would be expected at different temperature and amplitude level

Pretreatment yields

Taking into account the reduction in CrI and holocellulose contents before presented were developed new pretreatment runs for PP. For this case were considered the same cases (based on the high and low temperature and amplitude values). Additionally, the total content of reducing sugars, the reducing sugars yield, furfural concentration and biomass recovered were determined. Those results are addressed in the Table 6.5

Table 6.5. PP Pretreatment results

Case	**1**	**2**	**3**	**4**	**5**	**6**
Temperature (°C)	40	40	60	60	80	80
Amplitude (%)	100	20	100	20	100	20
Reducing sugars (g/l)	14.41	7.96	16.59	15.26	14.85	16.24
Reducing sugars yield (g/g dried biomass)	0.126	0.071	0.148	0.137	0.133	0.144
Furfural (mg/l)	0.4101	0.3075	0.4189	0.4164	0.455	0.5268
Biomass recovered (%)	74	74	73	73	49	55

In agreement with the behavior consigned in the Figure 6.10, the results consigned in the Table 6.5 confirms that highest concentration values of reducing sugars were reached at 60°C and 80°C using 100% and 20% ultrasound amplitudes respectively. On the other hand, furfural production was kept by the order of ppm (low values compared to conventional dilute acid pretreatments). To sum up, the behavior presented at the evaluated cases allow to identify that not significantly increases are presented by temperature varioations. Nevertheless, this raw material behave similar than CS because of the increases in furfural concentrations at high temperature and low amplitudes values.

Finally, under the conditions corresponding to cases 3 and 6 it was achieved hemicellulose hydrolysis yields up to 45% and 44% respectively. These results allow concluding that dilute acid pretreatment assisted with ultrasound is a feasible technology for PP pretreatment.

6.3 Assessment of the Enzymatic Digestibility.

This section presents the results of enzymatic digestibility assessment of the samples with better pretreatment performances (significant CrI reduction, holocellulose increases and hemicellulose hydrolysis yields). In consequence, cassava stem (CS) and plantain pseudostem (PP) were the raw materials selected to be submitted to enzymatic hydrolysis using Celluclast 1.5L and Viscozyme l purchased by novozymes. The followed procedure was based on the Laboratory Analytical Procedure (LAP-009) developed by the National Renewable Energy Laboratory for enzymatic saccharification of lignocellulosic biomass [149]. The biomass and the enzyme load was modified as signed in the **Table 6.6**.

Table 6.6. Enzymatic Hydrolysis Conditions

Pretreated Samples		
Sample	Temperature (°C)	Amplitude (%)
Plantain Pseudostem (PP1)	60	100
Plantain Pseudostem (PP2)	80	20
Cassava Stem (CS1)	80	100
Enzymatic Hydrolysis		
Feature	Units	Value
Biomass load	Wt%	5

Celluclast 1.5L load	FPU/ g cellulose	40
Viscozyme I load	FBG/g cellulose	30

Two additional cases were submitted to enzymatic hydrolysis, PP3 and CS2 samples were pretreated using dilute sulfuric acid (2%v/v) during 60 min in autoclave to compare overall pretreatment yields. All the samples were placed in erlenmeyer flasks and diluted in a 0.05M sodium citrate buffer (pH 4.6-4.9) to adjust the load desired, after that werw placed in a water bath at 50°C, 120 rpm during 41 h. The Table 6.7 presents the average values of the biomass recovered and the experimental results for the enzymatic hydrolysis:

Table 6.7. Enzymatic Hydrolysis Yields

Sample	**PP1**	**PP2**	**PP3**	**CS1**	**CS2**
Recovered biomass from pretreatment (%)	73	55	18	92	43
g total reducing sugars/g recovered biomass	0.78	0.89	0.311	0.26	0.14

Considering the recovered biomass (%) from pretreatment stage, different amount of biomass were used for the enzymatic hydrolysis. CS presented the higher recovering biomass yields (for all the cases), despite of that, low enzymatic hydrolysis yields were obtained due to its lower content of hemicellulose and higher CrI (compared to PP).

For PP pretreatment the highest hydrolysis yield (0.89 g total reducing sugars/g recovered biomass) was presented by the PP2 sample pretreated at 80°C and 20% amplitude. However, taking into account the pretreatment recovering yields (%) it can be concluded that PP1 operational conditions are the most adequate for plantain pseudostem pretreatment assisted by ultrasound. Finally, the obtained hydrolysis yields allow affirming that ultrasound assisted pretreatment representing 2-3-fold yield increases compared to the biomass pretreated at conventional conditions.

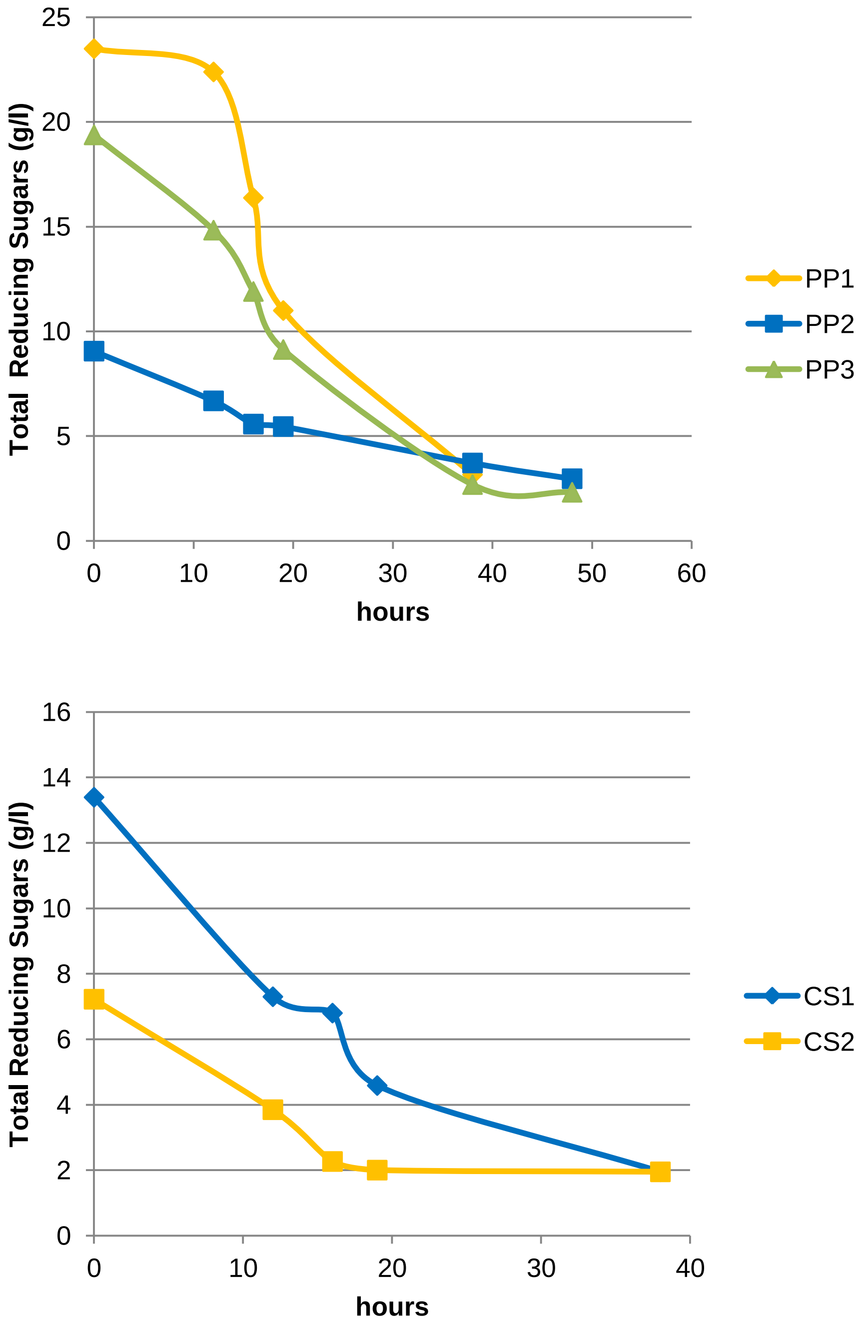
6.4 Assessment of Sugars Uptake.

With the purpose to determine the sugars uptake it was developed an alcoholic fermentation using *Saccharomyces cerevisiae* during 41 h at 30°C and 100 rpm. The total volume: According to the enzymatic hydrolysis yields were prepared the initial concentration described in the Table 6.8.

Table 6.9. Fermentation Samples

Sample	**Raw Material**	**Pretreatment**	**Initial Reducing Sugars (g/l)**
PP1	Plantain Pseudostem	Ultrasound 60°C 100% Amplitude	28.9
PP2	Plantain Pseudostem	Conventional 121°C	11.9
PP3	Plantain Pseudostem	Ultrasound 80°C 20% Amplitude	20.7
CS1	Cassava Stem	Conventional 121°C	15.1
CS2	Cassava Stem	Ultrasound 80°C 100% Amplitude	19.6

The **Figure 6.11** presents the sugars uptake rates for PP and CS cases:



(a)

(b)

Figure 6.11. *Saccharomyces cerevisiae* sugars uptake (a) PP cases, (b) CS cases

6.5 Techno-economical Assessment of Ultrasound Assisted Pretreatment

This section presents the techno-economical results of PP pretreatment and enzymatic hydrolysis. For this purpose, an assumed PP feed (10 ton/h) was pretreated. The pretreatments were evaluated considering the experimental characterizations, pretreatment yields, biomass recovered and enzymatic hydrolysis yields. Taking into account the method used for the reducing sugars determination (DNS Method), it was not feasible determine the specific amounts of pentoses and hexoses in the mixture. However, these results represent an approach used for simulation purposes to specify the type of sugars produced in each stage (i.e TRS Xylose-equivalent and TRS Glucose-equivalent).

The results of the conventional dilute acid pretreatment and ultrasound assisted pretreatment (at the most appropriate operational conditions developed in this chapter: 60°C, 20 min, 50 W, 30kHz) are presented in the **Table 6.10**:

Table 6.10. Products and inputs mass balance

Process Inputs		
Plantain Pseudostem	ton/h	10
Dilute Sulfuric Acid	m3/h	90
Calcium Hydroxyde	ton/h	3.3
Enzymatic Cocktail	kg/h	5-10
Conventional pretreatment		
Temperature	°C	121
Time	min	60
Conventional pretreatment Results		

TRS Xylose-equivalent (46 wt%)	kg/h	9645.0
TRS Glucose-equivalent (75 wt%)	kg/h	863.6
Furfural	kg/h	92.3
Ultrasound assisted pretreatment		
Temperature	°C	60
Time	Min	20
Amplitude	%	100
Ultrasound assisted pretreatment		
TRS from Xylose (46 wt%)	kg/h	3226.946
TRS from Glucose (75 wt%)	kg/h	7514.6

According to the results presented in this table, at harsher conditions (Conventional pretreatment) more structural carbohydrates (hemicellulose and cellulose) are hydrolyzed to sugars. Nevertheless, lower amounts of biomass are recovered from this pretreatment leading low enzymatic hydrolysis yields.

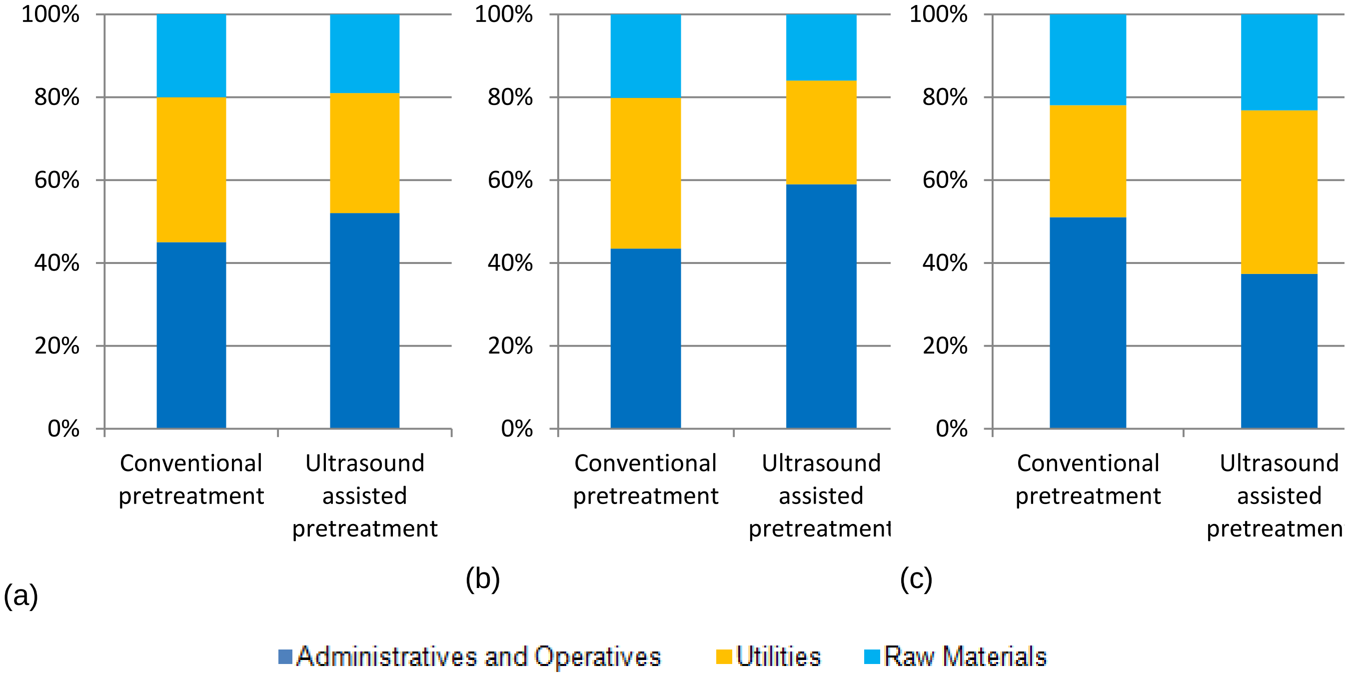
On the other hand, ultrasound assisted pretreatment produced the highest yields of enzymatic hydrolysis with low or undetectable production of inhibitory compounds. The **Table 6.11** presents the production costs associated to conventional pretreatment and ultrasound assisted pretreatment:

Table 6.11. Pretreatment and Enzymatic Hydrolysis Costs

Feature	**Conventional Pretreatment**		**Ultrasound Assisted Pretreatment**	
	Xylose	**Glucose**	**Xylose**	**Glucose**
Raw Materials	$ 2,895,080	$ 792,920	$ 2,895,080	$ 792,920

Utilities	$ 2,416,397	$ 426,423	$ 1,238,655	$ 825,770
Administratives and Operative	$ 8,540,469	$ 1,708,093	$ 5,725,315	$ 2,610,843
Total/period	$ 13,851,946	$ 2,927,436	$ 4,929,525	$ 2,114,766
USD/kg	**$ 0.16**	**$ 0.42**	**$ 0.19**	**$ 0.035**

Considering the xylose and glucose production costs consigned in the Table 6.11 the increases in enzymatic hydrolysis yields represents significantly reductions in the overall costs. The use of conventional pretreatment involving high temperature values affect the costs because of temperature, additional recovering processes and utilities required. The **Figure 6.12** (a) presents the total production costs and its distribution (Raw materials, utilities as well as and administrative and operative costs).



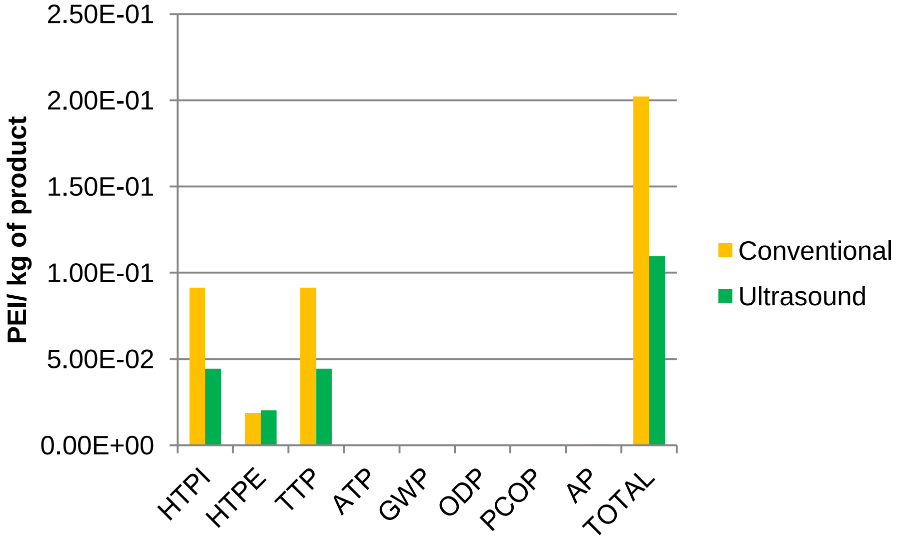
(a) (b) (c)

Figure 6.12. Share of pretreatment costs (a) Technology Comparison, (b) Share of xylose production costs (c) Share of glucose production costs

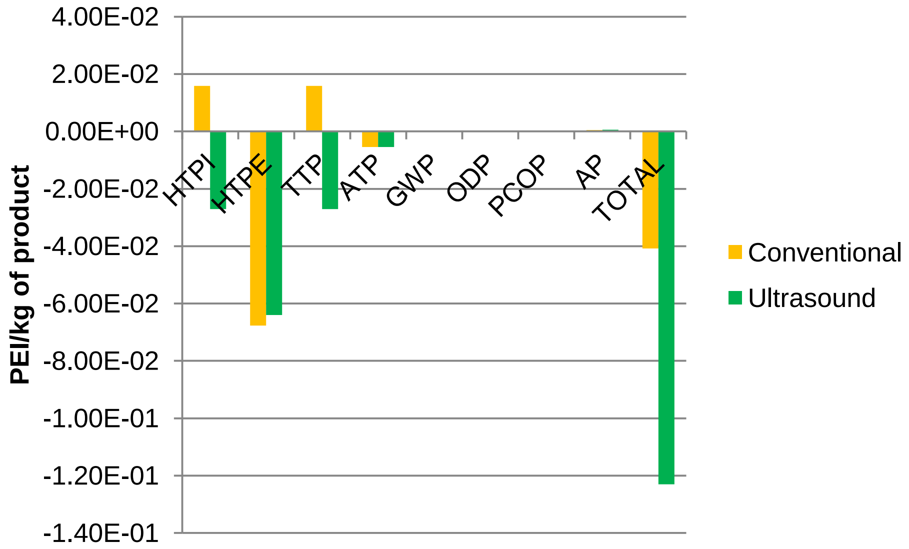
The Figure 6.12 presents a comparative parallel of the distribution costs. For ultrasound assisted pretreatment the raw material costs represent for each plant the higher share of production costs, due to recovering and chemical inputs. Whereas, for conventional pretreatment the requirements associated to equipment as well as utilities increases significantly the transformation costs.

6.6 Environmental Assessment of the Ultrasound Assisted Pretreatment

The environmental assessment was made to determine in a comparative scenario the Potential Environmental Impact (PEI) of each pretreatment. The PEI is related to the effect that a determined material or process would have on the environment, being an interesting tool and approximation to understand the environmental efficiency of the process. The PEI is measured by eight dimensionless impact categories: HTPI (Human Toxicity Potential by Ingestion), HTPE (Human Toxicity Potential by Exposure), ATP (Aquatic Toxicity Potential), TTP (Terrestrial Toxicity Potential), GWP (Global Warming Potential), ODP (Ozone Depletion Potential) and AP (Acidification Potential). The results of the environmental assessment are consigned in the Figure 6.13:



(a) PEI Leaving the system



(b) PEI Generated by the system

Figure 6.13. Potential of Environmental Impact

According to the Figure 6.13 the dilute acid pretreatment as process generates the highest PEI. The major contributions are based on the toxicity potential by ingestion and the terrestrial toxicity potential. The HTPI is related to the lethal dose that produce death in 50% of rats by oral ingestion meaning that are needed lower amounts of products or wastes produced in dilute acid pretreatment to affect a determinate population in comparison with the supercritical pretreatment. Thereby the values for HTPI and TTP (both toxicological potentials) should be explained by the nature of inhibitory compounds released by this conventional pretreatment (represented by furfural) and the residues formed during the detoxification stage.

6.7 Final Remarks

According to the results obtained ultrasound waves are capable to modify crystallinity and holocellulose content through enhancing pretreatment stage at moderate temperatures and short pretreatment times (60°C and 20 min) without production of significant amounts of inhibitory compounds and high biomass recovering rates. As a consequence of this pretreatment it was proved the increasing of the saccharification yields obtaining 2-3-fold higher enzymatic yields compared to the conventional case. Due to the dilute sulfuric acid uses, this pretreatment is more applicable to hemicelluloses-rich raw materials as Pseudostem plantain than lignin-rich raw materials such as rice husk and cassava stem.

In addition, it would exists a relationship between pretreatment efficiency and apparent density of the material, this is the case of RH pretreatment which presented lower pretreatment yields compared to cassava stem which as a higher CrI and similar lignin content.

Finally, the techno-economical and environmental assessment allowed to conclude that ultrasound assisted pretreatment would be a promissory technology for hemicellulose-rich raw materials producing higher overall yields, nevertheless, the lacking of effective scaling up methodologies is the main drawback to overcome.

Chapter 7: Results of Supercritical Pretreatment

Overview

As has been previously introduced, a wide number of different processes exist to reduce the recalcitrance and cellulose crystallinity of lignocellulosic biomass. Supercritical fluids have been described and used for pretreatment purposes. This technology is able to remove lignin increasing the substrate digestibility. This chapter presents the technical, economical and environmental assessment of supercritical fluids pretreatment including a comparison with the most used pretreatment: dilute sulfuric acid pretreatment.

7.1. Experimental stage

The most important parameters to be considered are the moisture, temperature, pressure and pretreatment time. Water, as moisture content in biomass combined with supercritical carbon dioxide generates a carbonic acid mixture which generates a kind of weak acidic environment, intensifying the mass transfer conditions and promoting the hemicellulose hydrolysis [95]. In addition, different works have reported that ethanol-water mixtures are capable to improve the solubility of lignin fragments, hence the delignification capability is enhanced [92].

During the initial stage of this thesis were developed some pre-experiments aimed to determine the most suitable moisture content and solvent characteristics. The temperatures used were selected according to the capacity of the device used for heating. Additionally, short pretreatment times by the order of 20-30 min have been commonly used for pretreatment purposes with the aim to avoid sugars degradation. Holocellulose and lignin content was altered by varying the solvents amounts for each treatment. For supercritical pretreatment the results indicated that delignification can be controlled by varying the solvent characteristics, particularly the water:ethanol content. This effect can be interpreted as a kind of organosolv pretreatment using ethanol-water as solvent mixture catalyzed by some carbonic acid which could be formed due to a reaction between the carbon dioxide and water present. Additionally, the ethanol as polar co-solvent (with polar characteristics) can promote the pretreatment based on the cleavage of the lignocellulosic matrix. The last statements agree with those discussed by other authors [92], [172].

Considering the time and available temperature ranges, rice husk samples were pretreated at two different moisture levels (25% and 75%) and two solvent specifications (water and water:ethanol). In this sense, four samples M1 (moisture content 25% adjusted with water), M2 (moisture content 75% adjusted with water), M3 (moisture content 25% adjusted with water:ethanol), M4 (moisture content 75% adjusted with water:ethanol) were pretreated at 300 bar and 80°C, the water to ethanol ratio (2:1) was selected based on the literature [172]. The results for holocellulose determination of pre-experimental stage are consigned in the Figure 7.1:

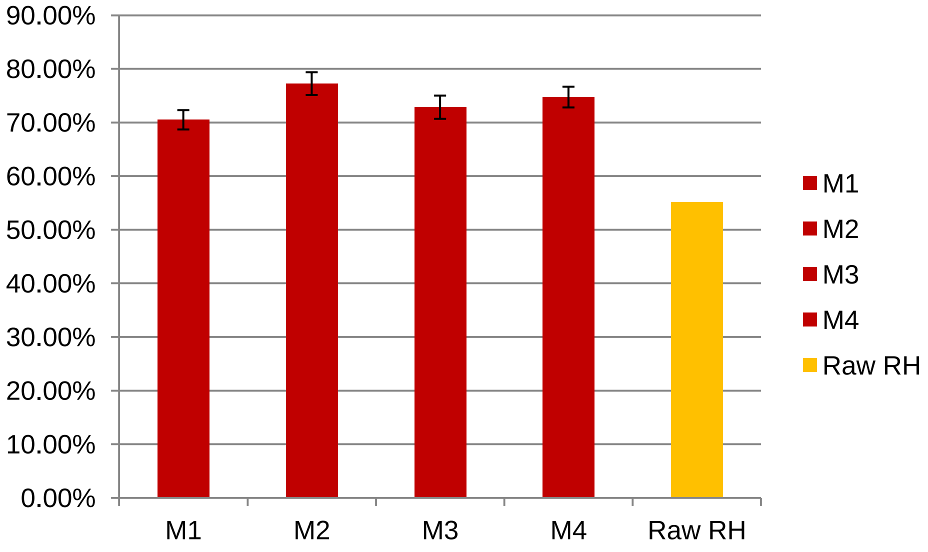


Figure 7.1. Pre-experimental stage results

In this figure it is observed an increase in holocellulose fraction as effect of delignification extent. In addition, high moisture content enhances the delignification process, representing higher amounts of water able to react with carbon dioxide producing carbonic acid. According to the results obtained in this stage it was selected the maximum moisture content for the development of the experiments later presented,

7.2.1 Rice Husk (RH)

Due to its lignin content, water-ethanol was selected as the cosolvent mixture, because of its wide use for delignification purposes. Finally, were evaluated 4 cases considering two levels of temperature and pressure presented in the Table 7.12:

Table 7.12. Rice husk cases description

Case	**1**	**2**	**3**	**4**
Temperature (°C)	60	60	90	90
Pressure (bar)	300	100	300	100

Crystallinity Index

The Figure 7.2 presents the crystallinity index of pretreated RH samples. These results evidence different CrI trends according to the selected temperature and pressure; however, all of them differs from the initial (CrI 24%) as a pretreatment consequence.

At low pressure levels, increases in the temperature can produce reductions in CrI, whereas for high pressure levels there are not significantly differences in CrI by temperature changes.

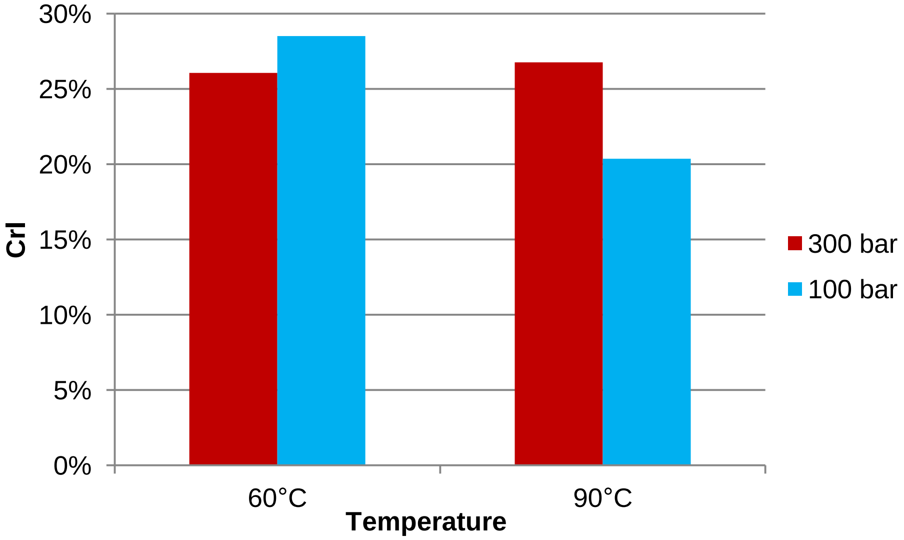


Figure 7.2. Rice husk Cristallinity Index

Holocellulose content

The Figure 7.3 presents the holocellulose fraction in RH samples after pretreatment. The holocellulose content of pretreated samples was between 66-84% representing increases by the order of 20-51% compared to the initial 55.18%. Increase in holocellulose content is related to delignification of the material.

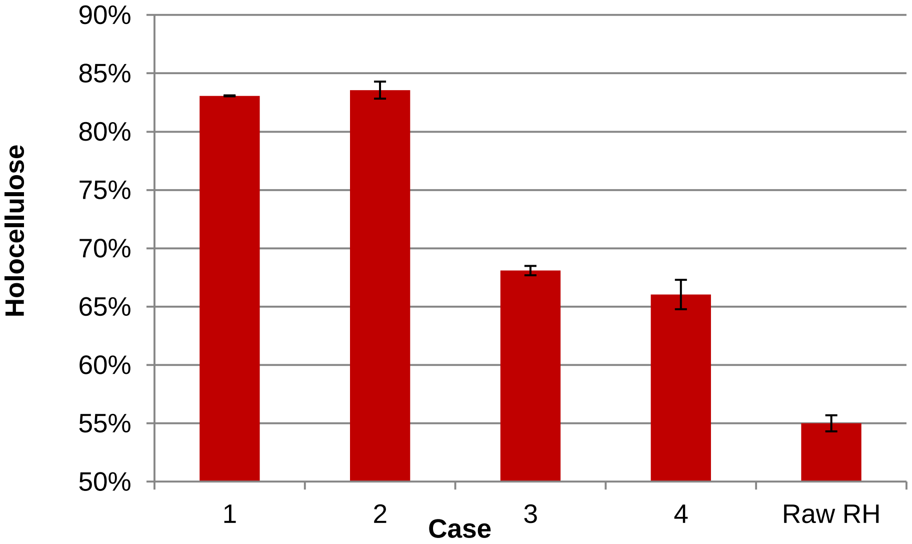


Figure 7.3. Rice husk holocellulose content

The Figure 7.4 presents the CrI and holocellulose content as combined results.

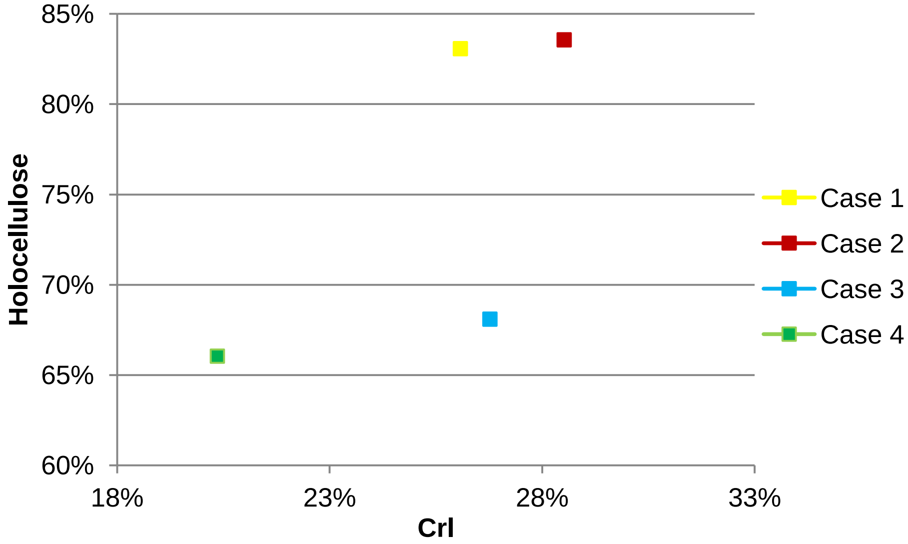


Figure 7.4. Rice husk combined results

According to the Figure 7.4 and based on CrI reduction and holocellulose increase criteria the sample of the case 4 was presented the higher pretreatment yields. The main trend identified in this graph corresponds to the behavior at low temperatures which increases the holocellulose an CrI in different amounts by pressure variation. In addition, increases in holocellulose content added to increases in CrI would imply the removal of compounds (mainly lignin) from the amorphous region producing a kind of purified material with high and pretreated cellulose.

On the other hand, biomass recovering provides an approximate about the mass fraction removed, released or broken during pretreatment. This fraction would be associated to extractives, and disrupted lignin or hemicellulose. Those results are addressed in the Table 6.1:

Table 7.13. RH pretreatment results

Case	**1**	**2**	**3**	**4**
Temperature (°C)	60	60	90	90
Pressure (bar)	300	100	300	100
Biomass recovered (%)	94.62	96.49	95.94	95.27

N.D: Not detectable

According to the Table 6.1, for all the temperature and pressure levels similar amounts of biomass are recovered. However differences in CrI and holocellulose content allow to conclude that different effects (delignification or hemicellulose disruption) are mainly promoted.

7.2.2 Cassava Stem (CS)

Due to its lignin content (similar to RH), water-ethanol was selected as the cosolvent mixture, taking into account its wide use for delignification purposes. The conditions of the evaluated cases are presented in the:

Table 7.14. Cassava stem cases description

Case	**1**	**2**	**3**	**4**
Temperature (°C)	90	90	60	60
Pressure (bar)	300	100	300	100

Crystallinity Index

The Figure 7.5 presents the crystallinity index of pretreated CS samples. These results evidence a general decreasing trend for CrI according to the selected temperature and pressure:

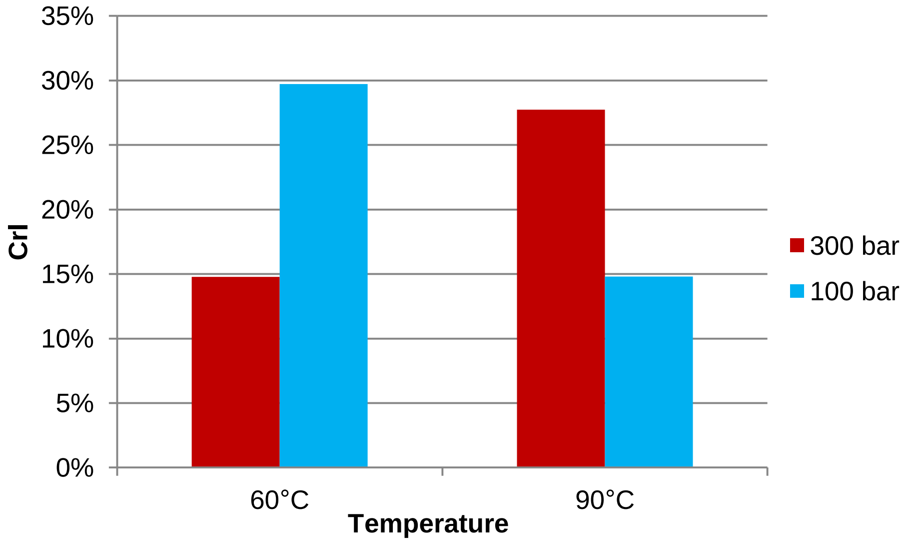


Figure 7.5. Cassava stem Crystallinity Index

In this case, at low pressure some increases in temperature enhance the reduction in CrI whereas at high pressure values a reduction in temperature will affect positively the CrI reduction.

Holocellulose content

The Figure 7.6 presents the holocellulose fraction in CS samples after pretreatment. The holocellulose content of pretreated samples was between 53.8-61.2% representing reductions by the order of 7-18% compared to the initial 66.6%.

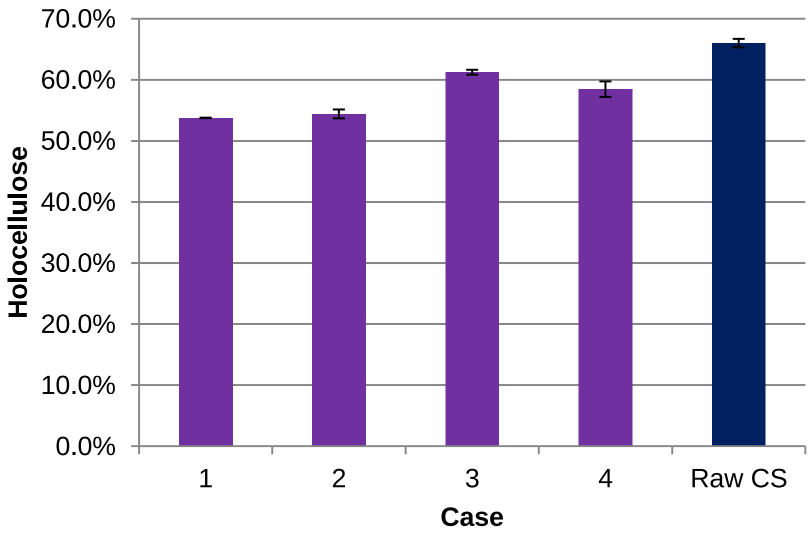


Figure 7.6. Cassava stem holocellulose content

The Figure 7.7 presents CrI and holocellulose content as combined results.

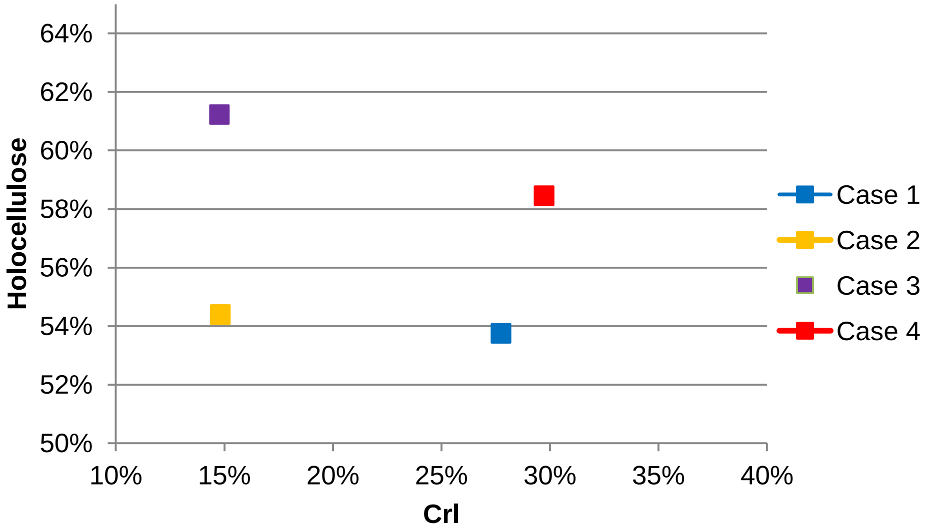


Figure 7.7. Cassava stem combined effects

According to the Figure 7.7 the cases 2 and 3 presented the lower CrI with contents above and below the initial value at low and high pressure level respectively. In this sense it would be expected higher enzymatic yields at these conditions.

Biomass recovering fraction of the evaluated cases is addressed in the Table 6.3:

Table 7.15. CS pretreatment results

Case	**1**	**2**	**3**	**4**
Temperature (°C)	90	90	60	60
Pressure (bar)	300	100	300	100
Biomass recovered (%)	94.24	96.12	94.27	96.26

According to the Table 6.3 the higher delignification yields were reached at 60°C and 300 bar. This affirmation is supported in the CrI reduction added to the removed biomass and final holocellulose content.

7.2.3 Plantain Pseudostem (PP)

Based on the initial hemicellulose-lignin content of PP, it were developed 8 cases of study evaluating different solvent and solvent composition (water and water-ethanol ratio 2:1). The conditions of the evaluated cases are presented in the :

Table 7.16. Plantain Pseudostem cases description

Case	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Moisture content (75%)	Water				Water:Ethanol			
Temperature (°C)	90	90	60	60	90	90	60	60
Pressure (bar)	300	100	300	100	300	100	300	100

Crystallinity Index

The Figure 6.8 presents the crystallinity index of pretreated PP samples. These results evidence wide changes in CrI as answer of temperature, pressure and solvent characteristic variations, all of them differs from the initial (CrI 16%) as a pretreatment consequence.

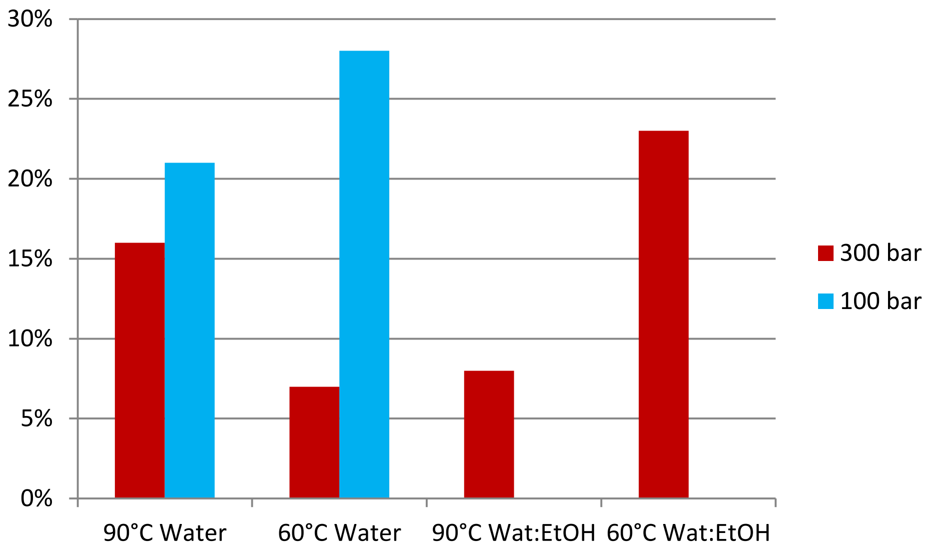


Figure 7.8. Plantain pseudostem Crystallinity Index

According to the results obtained it was identified two interesting operational configurations for CrI reductions in PP pretreatment. The first configuration used low amplitude and high temperatures whereas the second configuration used high amplitude and low temperature.

Holocellulose content

The Figure 6.9 presents the holocellulose fraction in PP samples after pretreatment. The holocellulose content of pretreated samples was between 19-71% representing reductions by the order of 71-11% compared to the initial 79%. From these holocellulose results it would expect highest hydrolysis yields compared to those presented by RH and CS.

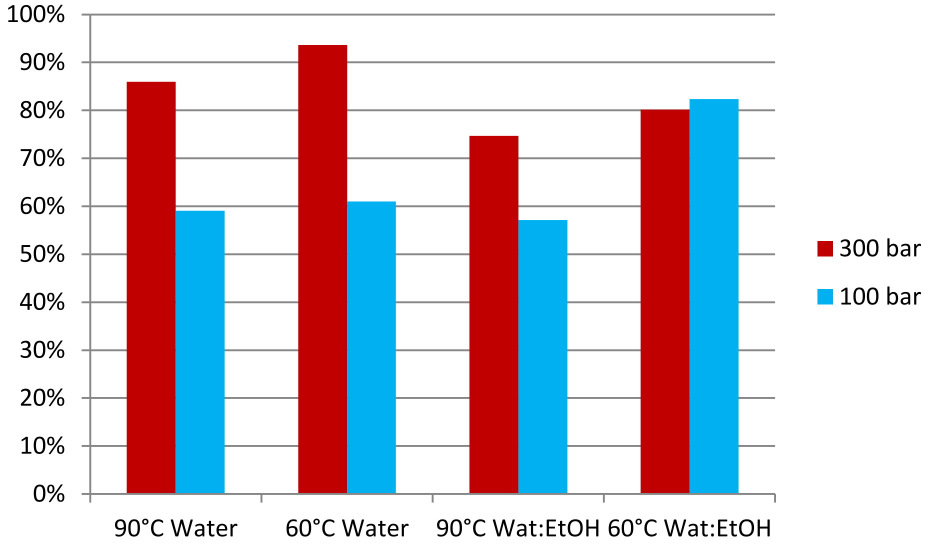


Figure 7.9. Plantain pseudostem holocellulose

The Figure 6.10 presents CrI and holocellulose content as combined results, using water as pretreatment solvent. The solvent selection was based on the chemical composition of PP and its trend to be pretreated in aqueous mediums.

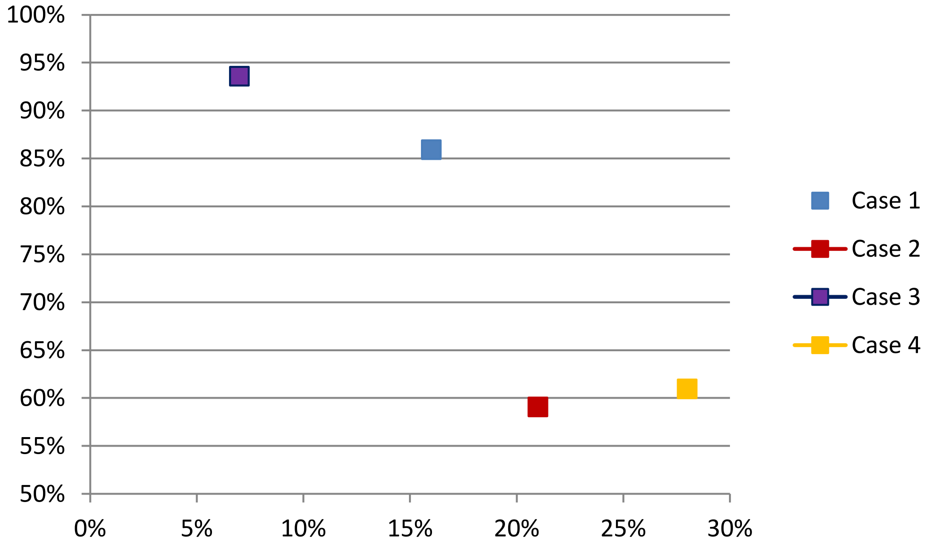


Figure 7.10. Plantain pseudostem combined effects

According to this figure, the case 3 (60°C and 300 bar) presented the higher holocellulose increase and CrI reductions with an average biomass recovering yield of 93%, this fact allow to conclude that PP can be pretreated at this operational conditions with the expectative to obtain high cellulose conversion yields.

7.3 Assessment of the Enzymatic Digestibility

This section presents the results of enzymatic digestibility assessment of the samples with better pretreatment performances (significant CrI reduction and holocellulose increases). For this purpose RH was pretreated. In consequence, RH samples were submitted to enzymatic hydrolysis using Celluclast 1.5L and Viscozyme l purchased by novozymes. The followed procedure was based on the Laboratory Analytical Procedure (LAP-009) developed by the National Renewable Energy Laboratory for enzymatic saccharification of lignocellulosic biomass [149]. The biomass and the enzyme load was modified as signed in the Table 7.17:

Table 7.17. Enzymatic Hydrolysis Conditions

Pretreated Samples		
Sample	Solvent	Moisture (%)
Rice husk (RH1)	Water	25
Rice husk (RH2)	Water	75
Rice husk (RH3)	Water:ethanol 2:1	25
Rice husk (RH4)	Water:ethanol 2:1	75
Rice husk (RH5)*	N.A	N.A
Enzymatic Hydrolysis		
Feature	Units	Value
Biomass load	Wt%	10
Celluclast 1.5L load	FPU/ g cellulose	60
Viscozyme l load	FBG/g cellulose	64

All the samples were placed in erlenmeyer flasks and diluted in a 0.05M sodium citrate buffer (pH 4.6-4.9) to adjust the load desired, after that werw placed in a water bath at 50°C, 120 rpm during 72 h.

These samples were selected considering the holocellulose content and the changes performed to crystallinity index. The obtained results report approximate cellulose conversion yields up to 55% ± 1.99 and 53% ± 0.56 for RH4 and RH5. It is important to note that the addition of β-glucosidase to the samples with best performances improves clearly the digestibility, based on the cellobiose conversion to glucose.

7.4 Techno-economical Assessment of Supercritical Carbon Dioxide Pretreatment

This section presents the techno-economical results of PP pretreatment and enzymatic hydrolysis. For this purpose, an assumed RH feed (10 ton/h) was pretreated. The pretreatments were evaluated considering the experimental characterizations, biomass recovered after pretreatment and enzymatic hydrolysis yields. Taking into account the method used for the reducing sugars determination (DNS Method), it was not feasible determine the specific amounts of pentoses and hexoses in the mixture.

It is necessary to clarify that large amounts of liquid fraction were not generated during supercritical pretreatment stage, because of the solvent or solvent mixture added increases the moisture content of the samples without lixiviate generation.

Table 7.18.Products and inputs mass balance

Process Inputs		
Rice husk	ton/h	10
Dilute Sulfuric acid	m3/h	90
Calcium Hydroxyde	ton/h	3.3
Dilute water:ethanol solvent	m3/h	30
Enzymatic Cocktail	kg/h	5-10
Conventional pretreatment		
Temperature	°C	121
Time	min	60
Supercritical pretreatment		
Temperature	°C	80
Pressure	bar	300
Conventional pretreatment results		

Glucose (80 wt%)	kg/h	2570
Ultrasound assisted pretreatment		
Glucose (80 wt%)	kg/h	2667

The results of techno-economic assessment performed are presented in the It considers the inputs, raw materials, the total utilities costs and other administrative costs associated to the processes considering saccharification yields for RH pretreated with supercritical carbon dioxide and conventional process. Initially, the pretreatment, detoxification and enzymatic hydrolysis stages were evaluated for the conventional pretreatment (dilute sulfuric acid). For the second case (supercritical pretreatment) it was evaluated the pretreatment (including the supercritical carbon dioxide and 2:1 water-ethanol solvent mixture as well as the recovering stages) and lately, the enzymatic hydrolysis stage.

Table 7.19. Pretreatment and Enzymatic Hydrolysis Costs

Feature	**Acid Pretreatment [USD/Period]**	**Supercritical Pretreatment [USD/period]**
Total Raw Materials Cost	$ 34,400,000	$ 3,165,400
Total Utilities Cost	$1,541,000	$ 1,113,865
Administratives and Operative	$ 3,769,785	$ 957,638.00
Total [USD/Period]*	**$ 39,710,785**	**$ 5,236,903**
Production Cost [USD/kg]**	**$ 1.93**	**$0.25**

It has been reported in literature a conventional pilot or near to industry cases [173] costs close to 0.27 USD$/kg of sugar. Last show a possible feasibility, but additional research is needed. Once again the tendency of supercritical pretreatment is to be lower in costs than dilute acid. The scale (fed flow of RH to be transformed) has a huge influence on the sugars production costs. For the case of dilute acid pretreatment the high amounts of inputs represents the main disadvantage. For the supercritical pretreatment, the highest impact was on the utilities costs, due to the energy needed for CO_2 conditioning. As a positive aspect of this technology is the possibility of recycling the solvent and cosolvent for delignification purposes. The Figure 7.11 presents the distribution of the production costs associated to both pretreatments:

(b)

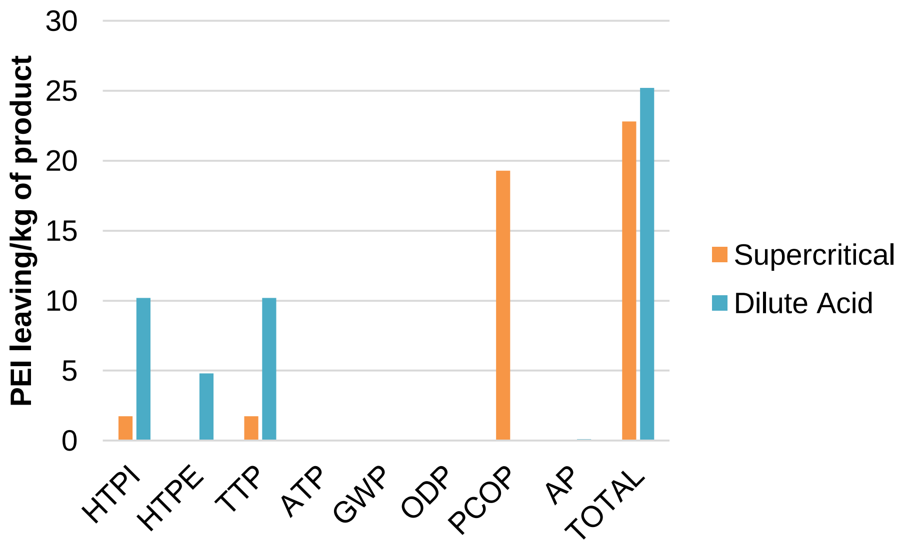
(a)

Figure 7.11. Glucose Cost distribution (a) Conventional pretreatment, (b) Supercritical pretreatment

In the Figure 7.11 the raw material costs, for the dilute acid pretreatment there are involved reagents that cannot be recycled or reprocessed (acid and reagents for detoxification). In the supercritical pretreatment the reuse of solvents is not so difficult (carbon dioxide and water-ethanol recycling). Total utilities costs are larger for supercritical pretreatment due to the operational conditions (high pressures and energy required for carbon dioxide liquefaction) nevertheless the raw materials costs and the general and operating costs are higher for dilute acid pretreatment due to the inputs and the devices used in this pretreatment associated to additional stages (detoxification and biomass recovering). The simulation doesn't consider (due to the complex task to include it into simulation.) the recovering of the residual energy contained in carbon dioxide (still at high pressures that can be used for example in a turbine to generate electricity) leaving the chamber after pretreatment. This would represent perhaps, the main challenge to improve the economic performance of this type of nonconventional pretreatment.

7.5 Environmental Assessment of Supercritical Carbon Dioxide Pretreatment

The environmental assessment was made to determine in a comparative scenario the Potential Environmental Impact (PEI) of each pretreatment. The PEI is related to the effect that a determined material or process would have on the environment, being an interesting tool and approximation to understand the environmental efficiency of the process. The PEI is measured by eight dimensionless impact categories: HTPI (Human Toxicity Potential by Ingestion), HTPE (Human Toxicity Potential by Exposure), ATP (Aquatic Toxicity Potential), TTP (Terrestrial Toxicity Potential), GWP (Global Warming Potential), ODP (Ozone Depletion Potential) and AP (Acidification Potential). The results of the environmental assessment are consigned in the Figure 7.12:



(a) PEI Leaving the system

Figure 7.12. Potential of Environmental Impact

According to the Figure 7.12 dilute acid is the pretreatment that generates the highest PEI. Approximately 88% of the PEI leaving the system is generated because of the raw material transformation process. The major contributions are based on the toxicity potential by ingestion and the terrestrial toxicity potential. The HTPI is related to the lethal dose that produce death in 50% of rats by oral ingestion meaning that are needed lower amounts of products or wastes produced in dilute acid pretreatment to affect a determinate population in comparison with the supercritical pretreatment. Thereby the values for HTPI and TTP (both toxicological potentials) should be explained by the nature of inhibitory compounds released by this conventional pretreatment (represented by furfural) and the residues formed during the detoxification stage.

7.6 Final Remarks

The use of supercritical fluids as a pretreatment technology is mainly focused on lignin removal. It is highly dependent on the operational parameters, raw material composition and CrI. Different behaviors were observed according to the pressure and temperature selected. For instance, those raw materials with high lignin content (i.e RH and CS) CrI was reduced using low temperatures and high pressures with water-ethanol solvent, Some cases reported decreases in holocellulose content, this fact would suggest hemicellulose bonds disruption. The techno-economical assessment developed for dilute acid pretreatment showed the highest cost because of it was assumed only for glucose syrup production. The results for the second pretreatment suggested as a preliminary result that supercritical pretreatment would be used in lignocellulosic pretreatment. Nevertheless, different drawbacks should be overcome such as residual energy use to reduce costs. The perspectives of the supercritical pretreatment using the carbon dioxide as a green solvent for continuous processing of biomass depends on the results for other lignocellulosic residues with different composition characteristics .

Chapter 8: Conclusions and Recommendations

Overview

This chapter presents the most relevant conclusions of this thesis considering the advantages and drawbacks associated to the agricultural waste utilization as well as the technological features to be considered. In addition, some recommendations are consigned with the aim to determine possible challenges of pretreatments using ultrasound and supercritical carbon dioxide.

Conclusions

As result of this thesis was determined that in different extent and considering the source and characteristic of raw materials ultrasound assisted pretreatment and supercritical carbon dioxide pretreatments are technologies able to enhance the overall performance associated to the agricultural waste pretreatment. The following sections will deepen in the results and behavior observed in each pretreatment technology.

Ultrasound Assisted Pretreatment

The results presented in the chapter 6 allowed to identify different effects for each case considering the raw material evaluated. There was evidenced that different physicochemical characteristics proper of each raw material can influence the pretreatment performance. In this case, two raw materials with high lignin content (RH and CS) and one with high hemicellulose content (PP) were selected.

A significant biomass recovering from pretreatment would be desired for subsequent processes as a way to ensure the equilibrated production of pentoses from the hemicellulose fraction and hexoses from the cellulose fraction.

The results presented for the two lignin-rich raw materials (RH and CS) were observed different trends. For these cases the pretreatment yields obtained differed at least in one magnitude order (estimated hemicellulose hydrolysis of 6.4% and 17% respectively) despite the lignin content (approximately 26% and 25% respectively) and contrary to the CrI behavior (24% and 36% respectively). This behavior would be related to the influence of the apparent density in the process performance reflected in the apparent viscosity observed during pretreatment this fact would have reduced the effectiveness of mass transfer or the intensity of physical effects caused by ultrasound.

On the other hand, the ultrasound assisted pretreatment of PP filled the expectations producing high reducing sugars from the hemicellulose as well as the cellulose fraction. The PP results presented high levels of hemicellulose solubilization (by the order of 45%) added to the elevated recovering percentages by the order of 55-73% which are higher than 18% provided by dilute acid pretreatment developed at conventional conditions. In addition, the low production of inhibitory compounds represents other advantage of this pretreatment technology strongly related to the pretreatment severity factor due to the temperature and pretreatment times used. Other benefits demonstrated were the notorious increases in enzymatic hydrolysis 2-3 fold at the same charge of enzymes compared to the results obtained from dilute acid pretreatment, and the sugar uptake as a sign of sugars digestibility.

However, despite the before developed benefits some drawbacks were identified and bellow listed. Albeit the low amounts of furfural released the use of acids in this pretreatment require additional stages for pH conditioning with similar input requirements than the base case. Another additional feature is related to the energy consumption which is function of the volume, power requirements and pretreatment times. Nonetheless, short pretreatment times added to the low operative amplitudes would mitigate the economic impact. In contrast, the use of short pretreatment times would impact the capital costs associated to the volume of reactors as well as the space occupied in plant or increase the total productivity treating higher amounts of raw material in plants with similar capacity.

In summarize, it can be conclude that dilute acid pretreatment assisted is an attractive alternative for hemicellulose-rich waste pretreatment at moderate temperatures and low amplitude values enhancing the enzymatic and microorganism digestibility. In addition, it was also concluded that CrI coupled with holocellulose content represents a good indicator of the pretreatment usefulness for dilute acid pretreatments. These are findings that can represent possibilities for creating novel functionalities from biomass to achieve existing and open new markets.

Supercritical carbon dioxide pretreatment

The results presented in the chapter 7 allowed to identify the main drawbacks and potentialities of supercritical carbon dioxide pretreatment. There were evidenced modifications to CrI and holocellulose content, however, these results did not followed a particular trend as was evidenced in the ultrasound assisted pretreatment.

Because of the pretreatment specifications related to the raw materials moisture content it was not recovered a liquid fraction from the pretreatment stage. In consequence, if hemicellulose fractions were solubilized it were not recovered in high amounts at least to be measured. The lacking of the total reducing sugars from hemicellulose fraction would represent competitive disadvantages for this pretreatment compared to others. Nevertheless, similar concentrations of total reducing sugars were allowed by supercritical carbon dioxide pretreatment using lower pretreatment times and without compared to those exhibited by dilute acid. Considering the without trend behavior presented in this pretreatment would be considered the formation or extractives non-removal, this fact would represents interferences in the determination of holocelllulose and lignin content.

Notwithstanding, the sugar production costs for this non-conventional pretreatment was lower than the offered by the selected case base because of the elevated amounts of inputs required by the conventional pretreatment selected as the case base.

The use of this technology must to overcome some challenges related to the implementation of a cycle for energy recovering capable to use the energy content in pressurized carbon dioxide to produce energy able to be used in the process.

To sum up, supercritical carbon dioxide pretreatment should be tested and used for those rich-cellulose and rich-lignin raw materials (to avoid significantly losses of reducing sugars from hemicellulose) with elevated moisture content at higher temperature conditions, more closer to those reported by the literature (higher than 140°C) with the purpose to evaluate better results. Considering the characteristics of the pretreated materials, these lasts would be tested in simultaneous processes to determine the real accessibility and digestibility by enzymes and microorganisms.

Agricultural waste utilization

In this thesis were evaluated the use of three different agricultural wastes exhibiting different performances according to its physicochemical characterization. RH, CS and PP has different moisture contents, different lignocellulosic composition, different apparent density (visually observed) and different CrI. Those market differences made PP and RH raw materials more feasible for ultrasound assisted pretreatment and supercritical carbon dioxide pretreatment respectively.

Nevertheless, both raw materials have different gathering characteristics. RH is more close to be an agroindustrial waste because of the needed dehusking process making easier the collection of this raw material.

On the other hand PP residues are land-left due to its production as a waste from harvesting stage; this fact added to the dispersion of the crop represents one of the main drawbacks for its utilization. Besides, it has not exists established logistical plans for its utilization and gathering. Other disadvantage of the PP utilization is related to its high moisture content needing adequate storage conditions to ensure an acceptable phytosanitary state of the raw material.

However, the use of these agricultural raw materials would be assumed as a challenge for different sectors including govern and involved farmers. Among the possible challenges would be highlighted the strengthen of the agriculture sector, the promotion of different associative structures with governmental or private assistance impacting at technical, economical and environmental levels.

Recommendations

Some recommendations that would represent challenges for future works in these areas are related to different issues are developed in this section.

To be deeper in the techno-economical assessment would be necessary the characterization of ultrasound fields in large reactors to identify the most suitable configuration to allow better or similar results than presented by this work. In addition, considering the mass transfer rates allowed by the ultrasound pretreatment it would be considered assessment of lower acid concentrations as well as biomass load increases to determine the economic and environmental impacts. These impacts would be measured by the reduction of the input requirements associated to pH adjusting, reduction in probe and equipment erosion among others.

On the other hand, the use of specific determination technologies would contribute to establish more accurately the impact on raw material structure. In this sense, it is recommended the pore size determination as a measure of cellulose digestibility. Another useful tool to deep on the phenomenological comprehension of the processes would be related to the implantation of other chromatographic tests to understand and specify the sugars released by the pretreatments.

For future works in environmental assessment would be considered the use of other environmental assessment tools such as LCA (Life Cycle Assessment) evaluated for different raw materials according to its context (at different organization levels) to determine the environmental impact with the purpose to extend the system boundaries. The boundaries system extension would provide suitable information about the real impact of agricultural waste management for the Colombian context.

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