I am CELISE

Sustainable production of Cellulose-based products and additives to be used in SMEs and rural areas

Deliverable D1.2. Pre-treatments of lignocellulosic residues to be used in SMEs and rural areas

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CELISE: Sustainable production of Cellulose-based products and additives to be used in SMEs and rural areas

Deliverable D1.2. Pre-treatments of lignocellulosic residues to be used in SMEs and rural areas

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Keywords	Mechanical pre-treatments, alkaline hydrolysis, acid hydrolysis, steam explosion, DES

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3. LIST OF ABBREVIATIONS

Table 2. List of abbreviations

Acronym	Definition
AFEX	Ammonia fiber explosion
DES	Deep eutectic solvents
HMF	Hydroxymethylfurfural
IL	Ionic liquids
LCB	Lignocellulosic biomass
LHW	Liquid hot water
PHA	Polyhydroxyalkanoates
SE	Steam explosion

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4. INTRODUCTION

4.1. General purpose of the document

Lignocellulosic biomass is the most abundant and renewable raw material in the world. It is the main constituent of wood as well as of wild and agricultural plants. The term "lignocellulosic" itself reveals the main constituents of the resource - cellulose and lignin, however they are complemented also with other important ingredients, such as hemicelluloses, extractives, proteins, and other organic and inorganic substances, significant for the formation and growth processes of different plants. All these components of biomass can be used for several applications within the biorefinery concept.

Pretreatment and delignification followed by hydrolysis and/or detoxification (fractionation) prior to fermentation is usually required to assure an efficient bioconversion of lignocellulosic biomass into bio-based chemicals, biopolymers, biofuels and energy. Optimization of these stages permits the integral use of the feedstock, increasing the lignocellulosic biorefinery economic margins and the global demand for the use of biomass renewable resources.

Nowadays, most challenges in lignocellulosic fractionation consist of overcoming technical and economic barriers. Ina ddition, depending on the application, several barriers need to be taken into account. In the case of being use for SMEs and/or rural areas, the processing of the biomass can difer from other applications at larger scales. Therefore, a complete understanding of the lignocellulosic pre-treatments of the biomass is one of the challenges of this project.

The manuscript/paper/document offers a review of existing and the most advanced as well as most useful methods of pre-treatment of lignocellulosic biomass and some recommendations about the use of these methods in rural areas and SMEs.

4.2. Role and contribution from partners

The D1.2 is part of WP1 activities and LSIWC leads it. In this case, UC has been the main responsible of this deliverable; however, the deliverable joins all of the activities related to the pre-treatment methods of biomass and the following partners are involved in these activities: UC (Universidad de Cantabria), LSIWC (Latvian State Institute of Wood Chemistry), SGGW (Warsaw University of Life Sciences), WUT (Warsaw University of Technology), IChF (Institute of Physical Chemistry, Polish Academy of Sciences), AUTH (Aristotle University of Thessaloniki), UTB (Univerzita Tomase Bati ve Zline), CVUT (Ceske Vysoke Uceni Technicke v Praze), UL (Univerza v Ljubljani), BESARTE (Besarte Fibre Natural, S.L.U.), StuSci (Student Science, s.r.o.), UCC (Universidad Cooperativa de Colombia), UNACH (Universidad Nacional de Chimborazo), UNL-FICH (Universidad Nacional del Litoral) and Latitud (Latitud-Fundación Laboratorio Tecnológico del Uruguay).

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5. BIOMASS

5.1. Introduction to biomass

Biomass is a renewable and sustainable resource, easy to store and transport, and economically viable [1]. The term biomass refers to wood, short-rotation woody crops, agricultural waste, short-rotation herbaceous species, wood waste, bagasse, industrial residues, used paper, municipal solid waste, sawdust, biosolids, grass, food processing residues, aquatic plants, and algae waste, among other materials [1]. Traditional biomass is produced unsustainably and used as a non-commercial source, typically with very low efficiencies, for cooking in many countries [2]. Biomass can also be classified based on its physical conditions into wet and dry biomass [3]. According to its origin, it can be natural or primary (found in nature without human intervention, e.g., forests) and secondary or dry residual biomass: including by-products of human activities such as agriculture, livestock, forestry, agro-industrial processes, and wood transformation (e.g., pruning wood, sawdust, straw, dry urban waste) [4-5]. Another classification method is based on the sector or activity from which it is obtained, including agricultural, forestry, and industrial biomass [1,5]. According to [3], the proportion of components present in biomass determines the following classification: Lignocellulosic biomass, predominantly composed of plant fibers such as cellulose, hemicelluloses, and lignin (e.g., straw, wood, and energy grasses). This type of biomass is intrinsically linked to the classification into herbaceous biomass (plants with non-woody stems that die at the end of the growing season) and woody biomass (trees and shrubs) [6]. Biomasses rich in sugar with carbohydrates in the form of monosaccharides (mainly glucose or fructose) and disaccharides (sucrose), such as sugar beet and sugarcane. Biomasses rich in starch, with a high proportion of storage polysaccharides, mainly starch and inulin, such as grain cereals (wheat, corn, etc.) and tubers (potato, Jerusalem artichoke, etc.). Biomasses rich in oil, with high lipid content, especially in specific parts, such as rapeseed and some micro- and macroalgae. Biomasses rich in proteins, obtained from plant biomass such as oilseeds (e.g., soybean, sunflower) and legumes (e.g., peas) as well as animal biomass (e.g., pork and fish). Due to their nitrogen content, high cost, and the principle of prioritizing food production, this type of biomass is not feasible for long-term use as a bioenergy raw material [1,3,7].

5.2. Lignocellulosic biomass

The components of Lignocellulosic biomass (LCB) include cellulose, hemicellulose, lignin, lipids, proteins, simple sugars, starches, water, hydrocarbons, and ash [1]. Generally, lignocellulosic biomass (LCB) consists mainly of cellulose (35–45%), hemicellulose (25–30%), and lignin (15–30%), as well as other minor components (15–20%), such as extractives, ash, proteins, and minerals [8]. The structural composition of LCB varies with plant components, even within the same species, biomass type, nutrient conditions, soil fertility, harvesting method, and geographical regions [9]. Cellulose is a polymer composed of glucose monomers linked by 1,4-β-glycosidic bonds, with 7,000 to 15,000 units in a single chain [9]. Hemicellulose is a branched heteropolymer consisting of C5 (xylose and arabinose) and C6 sugars (glucose, galactose, mannose, etc.) with various short branches of O-acetyl and glucuronic acid [10]. Lignin is an integral part of plant cell walls. It acts as a glue, binding polymers to cellulose and hemicellulose, filling the spaces between them [11]. Lignin is the most abundant renewable natural phenolic polymer on the planet, forming a major component of secondary plant cell walls and maintaining the integrity of the cellulose/hemicellulose/pectin matrix [12].

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6. PRE-TREATMENT OF LIGNOCELLULOSIC BIOMASS

6.1. Introduction to the pre-treatments

By combining safe and environmentally low-impact technologies, the use of biomass can provide a renewable alternative to fossil resources. It can establish a new sustainable supply chain for the production of high-value chemicals, including fuels and energy, as well as materials [13]. The development of biomass conversion methods into energy has significantly expanded. The choice of conversion technology is influenced by factors such as the type and quality of the biomass, the final form of energy, environmental regulations, economic conditions, and project-specific factors [14].

Biomass fractionation refers to the conversion of lignocellulosic biomass into its constituent components (cellulose, hemicellulose, and lignin). The processes used can be physical-mechanical, chemical, physicochemical, or biological, depending on the type of biomass and the purpose of fragmentation.

Pretreatment is critical when recovering the cellulose component from lignin-based biomass, as opposed to starches. Lignocellulosic biomass must undergo thorough pretreatment to break the lignin barrier before enzymatic hydrolysis can convert it into fermentable sugars [7]. The natural recalcitrance of lignocellulosic raw materials is a major concern in industrial biorefinery processes. To achieve economic and environmental feasibility, it is essential to employ an effective pretreatment method that enhances cellulose digestibility by breaking the rigid carbohydrate-lignin matrix, reducing particle size, removing hemicellulose/lignin, and increasing specific surface area and porosity, resulting in high sugar recovery after enzymatic hydrolysis [15].

The monomeric sugar released after enzymatic hydrolysis can be directly fermented into biopolymer, i.e., PHA (polyhydroxyalkanoates), or it can be fermented into a chemical precursor for fuel application and/or biopolymer transformation. Several potential pretreatment methods are available to achieve this, including physical (e.g., mechanical), chemical (e.g., using dilute acids [16], alkalis [17], and ionic liquids [18]), physicochemical (e.g., ammonia fiber explosion [19], steam explosion [20], liquid hot water [21], and ultrasonic methods [22]), and biological (fungal and bacterial digestion, or enzymatic processes) [23].

There are various techniques to break down biomass through hydrolysis. This can be done physically, chemically, or biologically [24]. Some of these techniques are used for the removal of physical impurities and activation; however, others are used to obtain energy-producing chemicals in solid, liquid, and gaseous states [7].

6.2. Mechanical pre-treatment

The physical pre-treatment of LCB is a prerequisite before any other pretreatment method.

6.2.1. Milling

This treatment involves polymer crushing to improve surface area, reactivity, reduce size, shape, and segregation of lignocellulosic biomass [25]. The operation includes different mechanical techniques, such as chipping and milling. The basic milling tools explored are the two-roll mill, ball mill, vibratory energy mill, colloid mill, hammer mill, and wet disk mill. Milling efficiency is further improved by modifying the medium, such as using acids, enzymes, and microbial treatments [26].

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The impact of different mills has its own advantages and limitations and can thus be used depending on the application in terms of duration and type. Similarly, disk milling generates more effective fibers after improving cellulose hydrolysis compared to hammer milling [27]. Currently, this pretreatment is applied to herbaceous plants with low lignin content. For example, wheat straw, stalks, and various substrates pretreated via expeller crushing, cutting, and ball processing not only reduce substrate size and polymerization level but also decrease lignin content from 38.5% to 54.43% [28]. Although these methods do not involve chemical consumption, high energy consumption and economic feasibility remain major concerns for these physical methods [29].

6.2.2. Radiation – Ultrasound

Ultrasound, ultraviolet, electron beam, pulsed electric field, gamma radiation, microwaves, and infrared are among the high-energy radiations employed in LCB processing [7]. Ultrasound waves can be used in lignocellulosic deconstruction to improve cellulase accessibility for hydrolysis. Additionally, ultrasonic pretreatment can be combined with acid, alkali, or ionic liquid addition to enhance the process [30].

Ultrasonic pretreatment is based on the principle of cavitation using ultrasonic radiation. Cavitation generates shear forces that break the complex LCB network structure and promote the extraction of desired compounds such as cellulose, hemicellulose, and/or lignin [31].

6.3. Chemical Pretreatments

6.3.1. Alkaline pre-treatment

This is a widely studied chemical pretreatment method based on the solubilization of lignin in an alkaline solution. The reagents used include sodium, potassium, calcium, and ammonium hydroxides. Among these, sodium hydroxide was found to be the most effective [32].

During the entire alkaline pretreatment process, a saponification reaction occurs, causing the cleavage of intermolecular ester bonds between hemicellulose and lignin [27]. This results in the solubilization of lignin and hemicellulose fragments in the alkaline solution and facilitates cellulose-enzyme interaction. In summary, alkaline pretreatment is an effective technique for lignin removal and makes carbohydrates more accessible for use in subsequent processes.

6.3.2. Acid pre-treatment

LCB acid pretreatment is based on the susceptibility of glycosidic bonds between hemicellulose and cellulose to acid. The hydronium ions originating from the acid catalyst cause the breakdown of long cellulose and hemicellulose chains into sugar monomers [33].

Although acid pretreatment significantly improves cellulose digestibility, it is more expensive than other pretreatment procedures, such as steam explosion or lime treatment. High risk budgets, operation and management costs, functional limitations, and environmental impacts restrict the commercial feasibility of dilute acid pretreatment [7].

6.3.3. Ionic liquids and deep eutectic solvents

Ionic liquids (IL) are a relatively new class of solvents with a melting point <100°C, composed of cations and anions. The cations are generally organic, including imidazolium, pyridinium,

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aliphatic ammonium, alkylphosphonium, and sulfonium ions, while the anions include organic and inorganic ions [34].

During the pretreatment process, both cations and anions play a crucial role in the solubilization of cellulose and lignin [27]. Recently, ionic liquids and deep eutectic solvents (DES) have been demonstrated to be non-toxic, biocompatible, and non-corrosive, and their tunable properties classify them as "green solvents" for biomass pretreatment. IL are low-temperature (<100°C), recyclable molten salts with non-flammable thermal stability and low vapor pressure characteristics. They are composed of organic hydrogen-donor cations (e.g., alkylimidazolium, alkylpyridinium, alkylphosphonium, and ammonium) and organic or inorganic hydrogen-acceptor anions [22].

6.3.4. Organosolv process

In this process, LCB is pretreated with organic solvents or their aqueous solutions, which cause the rupture of internal bonds between lignin and hemicellulose, leaving behind relatively pure cellulose residue [27].

The process is often accompanied by the addition of a catalyst to reduce the pretreatment temperature or enhance the delignification rate. Commonly used catalysts include mineral acids (hydrochloric acid, sulfuric acid, phosphoric acid), bases (lime, sodium hydroxide, ammonia), and some salts [35].

6.4. Physicochemical pre-treatments

6.4.1. Steam explosion

Steam explosion (SE) is the most commonly employed and effective pretreatment method, typically combining mechanical forces and chemical effects applied to LCB. In this technique, biomass is subjected to high-pressure saturated steam (0.69–4.83 MPa) at a temperature of 160–260°C, allowing water molecules to penetrate the substrate structure. The pressure is then suddenly reduced, enabling the explosive release of water molecules [27].

For example, SE technology [36] is used as a kind of hydrothermal treatment of wood in a closed reactor under saturated high temperature and pressure steam rapidly opening the reactor at the end of the treatment that creates the explosion effect. As a result of exposure to steam up to 10 min acids (mainly acetic acid) are formed from wood components (mainly pentosans), which start to act as autocatalysts these hydrolyses cellulose and hemicelluloses destructing also lignin bonds, generally ß – O – 4. At the end of the process, by activating decompression, the high-pressure steam expands and breaks down the structure of the rigid wood, turning it into fibre bundles whose surfaces overlap with lignin and hemicellulose destruction products with binder properties. It should be noted that in the SE process, hemicelluloses are partially converted into volatile products, but the remaining part has been modified and lost the ability to absorb water, which significantly improves the moisture resistance of pre-treated biomass. SE pretreatment is used efficiently and competitively in the processing of various lignocellulosic raw materials even without added catalysts, thus increasing the added value of biomass [37,38].

6.4.2. Liquid hot water

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Liquid hot water, LHW, or hydrothermolysis pretreatment is very similar to steam explosion, but as its name suggests, LHW uses high-temperature water (170–230°C) and pressure (up to 5 MPa) instead of steam. Unlike steam explosion, LHW does not require rapid pressure release, and the application of pressure is only to prevent water evaporation. LHW hydrolyzes hemicellulose by releasing its acetyl groups and removes lignin, making cellulose fibers more exposed [27].

6.4.3. Ammonia fiber explosion

Ammonia fiber explosion (AFEX) pretreatment is an advancement over conventional alkaline pretreatment processes [29]. High pressure and specific temperatures cause lignocellulose to swell, and the rapid pressure release disrupts the fibrous structure of the biomass, reducing cellulose crystallinity and thereby improving enzyme accessibility. AFEX pretreatment can be optimized by adjusting four parameters: temperature, purge pressure, water loading, and ammonia loading [39].

AFEX pretreatment partially removes lignin and hemicellulose from lignocellulosic materials but shows better enzymatic hydrolysis results with lower enzyme loading compared to other pretreatment processes [27].

6.5. Biological pre-treatments

Bacterial activity, fungi, and enzymes are used to classify biological pretreatment methods. In contrast to physical and chemical pretreatment approaches, biological pretreatment methods appear to be a promising technology with several advantages, including low energy input, no chemical requirements, and an environmentally friendly operational method [40].

6.5.1. Fungal pre-treatment

According to Nahak et al. (2022) [7], due to cellulose's higher resistance to fungal attack compared to other components, fungi that preferentially decompose lignin and hemicellulose while consuming less cellulose have been studied for fungal pretreatment. This type of fungal pretreatment results in increased cellulose digestibility. The delignification of lignocellulosic biomass through biological pretreatment could be an efficient way to enhance enzymatic hydrolysis and thus obtain more methane gas [8].

6.5.2. Microbial pre-treatment

Unlike fungal pretreatment, which primarily focuses on lignin degradation, microbial associations generally have a greater capacity for cellulose and hemicellulose degradation [7].

6.5.3. Enzymatic pre-treatment

Lignin is the most abundant aromatic polymer, consisting of phenolic and non-phenolic compounds. Some fungi, bacteria, and insects are capable of producing enzymes that digest lignin [27]. There are two families of ligninolytic enzymes that play a crucial role in enzymatic degradation: phenol oxidases (laccase, Lac) and peroxidases (lignin peroxidase, LiP; versatile peroxidase, VP; and manganese peroxidase, MnP) [41].

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7. PRE-TREATMENTS OF LIGNOCELLULOSIC RESIDUES TO BE USED IN SMES AND RURAL AREAS

Tables 3-7 show a summary of the pre-treatments of lignocellulosic residues, together with the advanateges and disadvantages to be used in SMEs and rural areas.

Table 3. Physical pre-treatments in SMEs and rural areas

Pretreatment, characteristics	Advantages	Disadvantages	References
Mechanical: Enhances microorganism and synthetic compound accessibility to cellulose and hemicellulose.	Free from chemical ingestion, increased surface area, low production cost. Eco-friendly methods that rarely produce toxic material.	High energy input, low sugar release, lower commercial viability.	[25,42,43]
Ultrasound: Ultrasonic bath.	Greater porosity, highly efficient at batch scale, dissolves extracts, with heating. Ultrasound use can reduce biomass hydrolysis time by up to 80%, benefiting biofuel production.	High energy input, low sugar release, lower commercial viability. The process is energy-intensive, requiring detailed research to optimize process parameters for large-scale applications.	[30,44,45]

Table 4. A: Chemical pre-treatments in SMEs and rural areas

Pretreatment, characteristics	Advantages	Disadvantages	References
Alkaline pretreatment: Based on lignin solubilization in an alkaline solution.	Effective technique for lignin removal, exposing carbohydrates for subsequent processes.	A major drawback is the recovery of added alkalis, requiring further research. Additionally, alkaline pretreatment is less productive for hardwoods.	[7,27,46,47]
Acid pretreatment: Uses sulfuric acid, nitric acid, hydrochloric acid, and organic acids such as formic acid, maleic acid, and oxalic acid.	>90% hemicellulose dissolution, high enzymatic yield, increases cellulose crystallinity, enlarges pore size, commercially feasible. Further research is needed to explore the advantages of organic acids over mineral acids for LCB pretreatment.	Greater toxin formation, acid neutralization, pseudolignin formation, increased cellulose crystallinity, catalyzed sugar loss due to HMF (hydroxymethylfurfural) and furfural formation. Most concentrated acids are highly toxic and corrosive, requiring high operational and maintenance costs.	[27,46,48-50]

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Table 5. B: Chemical pre-treatments in SMEs and rural areas

Pretreatment, characteristics	Advantages	Disadvantages	References
Ionic Liquids (IL) and Deep Eutectic Solvents (DES): Early studies show that quaternary ammonium salts have been among the most popular precursors for obtaining DES eutectic mixtures. Others are based on imidazolium, pyridinium, pyrrolidinium, ammonium, phosphonium, among others.	Most are recoverable and reusable, non-volatility, non-toxicity, high thermal and chemical stability, and, most importantly, the adjustable nature of their cations and anions. High lignin and hemicellulose solubilization, reduced crystallinity, wide pore size distribution, structural transformation of cellulose crystal, no inhibitor formation, suitable for small-scale use, recyclable green nature, higher sugar release during hydrolysis.	High cost of ionic liquids; recycling remains a major challenge; toxic to enzymes and yeast. Not commercially applicable. Despite their distinctive chemical properties, ILs present major drawbacks of being expensive and toxic to microorganisms and enzymes.	[9,27,48,51-54]
Organosolv process: Various organic solvents, such as methanol, formic acid, ethanol, glycerol, ethylene glycol, ethyl acetate, acetic acid, methyl isobutyl ketone, γ-valerolactone, and acetone, have been used to extract lignin, hemicellulose, and extractives from LCB. A wide range of organic solvents like ethanol, methanol, acetone, organic acid, peracid, and ethylene glycol, or their mixtures with water, have been used for LCB pretreatment.	Highly selective for lignin solubilization without chemical alteration of its structure, increases total surface area, reduces biomass crystallinity, recyclable under optimized conditions. Easy solvent recovery by distillation, solvent recycling for pretreatment, and high-quality lignin utilization as value-added byproducts for industrial applications.	Requires pressurized vessels, high cost, ease of recovery, toxicity, safety (low vapor pressure), and environmental concerns remain a challenge for large-scale applications. The need for pressurized reactors, temperature control, and process optimization are key concerns. Most organic solvents are too expensive and must be recovered as much as possible, which is an energy-intensive process. Additionally, the high flammability and volatility of organic solvents require the pretreatment to be conducted under specially controlled conditions.	[10,29,30, 35,51,55-57]

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Table 6. Physico-chemical pre-treatments in SMEs and rural areas

Pretreatment, characteristics	Advantages	Disadvantages	References
Steam explosion: Superheated steam. LCB undergoes high-pressure physicochemical treatment with saturated steam (0.5–5 MPa) at high temperatures (180–260°C) for a residence time of 2–10 minutes, followed by a rapid explosion to atmospheric pressure.	Low environmental impact, limited chemical use, high energy efficiency, no recycling costs, and total sugar recovery compared to other pretreatment methods. Can be applied directly to ground LCB without using any chemicals.	Promotes hemicellulose solubilization, low oligomer formation under reduced severity, high enzymatic yield, reduces particle size and increases surface area, suitable for any biomass type, commercially feasible for biorefinery.	[9,58-63]
Liquid hot water (LHW), Hydrothermolysis: Hot water, superheated steam. Hydrothermal pretreatment includes treating lignocellulosic biomass at high temperatures (>300°C) and pressures (>22 MPa) using compressed hot water, acidified hot water, and organic solvent/water, which significantly enhances extraction, lignin and xylan solubilization, and improves specific surface area.	No catalyst or chemicals required, minimal toxic material formation, low solvent cost for large-scale applications. Removes lignin, exposing cellulose fibers. High hemicellulose and extractive solubilization, moderate sugar release, decrystallizes biomass crystalline matrix, increases porosity and surface area.	Requires pressurized vessels, harsh condition requirements, and inhibitor formation. The process is energy-intensive due to the large amount of water involved.	[27,64-70]
Ammonia Fiber Explosion (AFEX): Liquid ammonia. Can be efficiently used for biomass with low lignin content, resulting in >90% glucose yield during simultaneous saccharification and fermentation. It has advantageous innovative characteristics such as low temperature, short reaction times, and high enzymatic saccharification and fermentation without detoxification. Ammonia-based pretreatments exhibit a high degree of selectivity for lignin reactivity.	The main advantage of AFEX is the negligible formation of inhibitors compared to other pretreatment methods. Requires pressurized vessels, harsh condition requirements, and inhibitor formation. Ammonia recovery remains a challenge. Not effective for the pretreatment of lignocellulosic biomass with high lignin content.	Recent advancements in pretreatment technology over conventional alkaline pretreatment processes. The cost of ammonia is mostly associated with its manufacturing process and significant application. Environmental concerns about ammonia odor have increased. Ammonia must be recovered and recycled due to its high cost and volatility to reduce overall operating costs and minimize environmental damage.	[7,27,29, 71-73]

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Table 7. Biological pre-treatments in SMEs and rural areas

Pretreatment, characteristics	Advantages	Disadvantages	References
Fungal pretreatment: Process of modifying the physical and chemical composition of lignocellulosic biomass using degradative fungi. White-rot fungi.	Results in increased cellulose digestibility. Fungal pretreatment with white-rot fungi could be a suitable way to reduce washing and inhibitor removal costs during traditional pretreatment procedures.	Low efficiency and long residence times when used alone. White-rot fungi are generally used as whole-cell microorganisms as they are less potent in degrading the cellulose fraction of LCB.	[7,27,74,75]
Microbial pretreatment: Selected microbes from natural habitats perform microbial consortium pretreatment on decaying lignocellulosic biomass as a substrate.	During microbial consortium pretreatment, lignocellulosic sterilization is not required, making it beneficial.		[7]
Enzymatic pretreatment: Uses hydrolytic enzymes.	Saccharification yield observed up to 74.2% for rice straw; 50% lignin removal using laccase as an enzyme in wood.	Due to its high cost, enzymatic pretreatment is rarely used for biogas production.	[76,77]

The main biorefinery techniques involve physical, chemical, thermal, and hydrothermal processes. The main techniques are shown in Tables 1-4, where it is observed that physical and chemical processes determine the use of inhibitors, which present both advantages and disadvantages. Some of these inhibitors are pollutants, raising the dilemma of assessing how viable it is to revalue resources using these techniques or to discard them, especially in rural areas.

Among chemical processes, deep eutectic solvents (DES) and ionic liquids (IL) are emerging as new solvents to replace conventional ones used in the separation of the constitutive elements of waste. Over the past two decades, both IL and DES have gained enormous attention from the scientific community, and the number of articles reported in the literature related to this topic has grown exponentially [78].

Mechanical-physical processes remain an available alternative free from the use of chemicals and the pollution they may generate. Due to the structure of lignocellulosic materials and depending primarily on the key parameters in the pretreatment stages, numerous by-products are generated, acting as inhibitors in fermentation operations [79]. The conversion of lignocellulosic materials into higher-value products requires the separation of the material into its components. Pretreatments range from simple size reduction to more advanced biological or physicochemical processes designed to improve biomass digestibility [80].

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8. CONCLUSIONS

Lignocellulosic biomass (LCB), including agricultural waste, forest residues, and woody biomass, is an abundant (130 billion t/year), renewable, and economical resource that does not compete with the food supply chain.

Lignocellulosic biomass is a promising source of renewable energy and valuable chemicals. This type of biomass can be converted into useful forms of energy and chemicals, through biochemical or thermochemical processes.

Small biorefineries needs a pre-treatment step in order to separate the different fractions of the lignocellulosic biomass. Physical, chemical, physico-chemical and biological pre-treatment can be used.

Depending on the advantages or disadvantages of the pre-treatments, different options can be used in SMEs and rural areas. The operations and the necessary biomass pretreatments align with the principles of the Circular Economy and environmental sustainability.

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9. REFERENCES

1. Martin-Ramos, P. Biorrefinerías basadas en explotaciones agropecuarias y forestales (OCW Unizar) Synthesis and validation of new natural origin antifungals with applications in viticulture: strategies in Ribera del Duero D.O. to Fight Esca View Project Arqueometría View Project. 2017.
2. Goldemberg, J. The Case for Renewable Energies. 2004. Secretariat of the International Conference forRenewable Energies, Bonn.
3. Sánchez, J.; Curt, M.D.; Robert, N.; Fernández, J. Biomass Resources. Role Bioenergy Emerg. Bioeconomy Resour. Technol. Sustain. Policy 2019, 25–111, doi:10.1016/B978-0-12-813056-8.00002-9.
4. CEARE-UBA Proyecto para la promoción de la energía derivada de biomasa (UTF/ARG/020/ARG) Guía para realizar estudios de impacto ambiental de proyectos bioenergéticos. In; 2019 ISBN 9789251324677.
5. Nogués, D.; García-Galindo, D.; Rezeau, A. Energía de la biomasa (Volumen I), 2010. Ed: Universidad de Zaragoza, Prensas de la Universidad de Zaragoza.
6. FAO FAO Forestry Department Wood Energy Programme Food and agriculture organization of the united nations unified bioenergy terminology UBET. 2004.
7. Nahak, B.K.; Preetam, S.; Sharma, D.; Shukla, S.K.; Syväjärvi, M.; Toncu, D.C.; Tiwari, A. Advancements in net-zero pertinency of lignocellulosic biomass for climate neutral energy production. Renew. Sustain. Energy Rev. 2022, 161, 112393, doi:10.1016/J.RSER.2022.112393.
8. Raj, T.; Kapoor, M.; Gaur, R.; Christopher, J.; Lamba, B.; Tuli, D.K.; Kumar, R. Physical and chemical characterization of various indian agriculture residues for biofuels production. Energy and Fuels 2015, 29, 3111–3118, doi:10.1021/EF5027373/SUPPL_FILE/EF5027373_SI_001.PDF.
9. Raj, T.; Kapoor, M.; Semwal, S.; Sadula, S.; Pandey, V.; Gupta, R.P.; Kumar, R.; Tuli, D.K.; Das, B.P. The cellulose structural transformation for higher enzymatic hydrolysis by ionic liquids and predicting their solvating capabilities. J. Clean. Prod. 2016, C, 1005–1014, doi:10.1016/J.JCLEPRO.2015.12.037.
10. Mugwagwa, L.R.; Chimphango, A.F.A. Optimising wheat straw alkali-organosolv pre-treatment to enhance hemicellulose modification and compatibility with reinforcing fillers. Int. J. Biol. Macromol. 2020, 143, 862–872, doi:10.1016/J.IJBIOMAC.2019.09.147.
11. Hamelinck, C.N.; Van Hooijdonk, G.; Faaij, A.P.C. Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle- and long-term. Biomass and Bioenergy 2005, 28, 384–410, doi:10.1016/J.BIOMBIOE.2004.09.002.
12. Huang, C.; Peng, Z.; Li, J.; Li, X.; Jiang, X.; Dong, Y. Unlocking the role of lignin for preparing the lignin-based wood adhesive: a review. Ind. Crops Prod. 2022, 187, 115388, doi:10.1016/J.INDCROP.2022.115388.
13. Pfaltzgraff, L.A.; Clark, J.H. Green Chemistry, Biorefineries and second generation strategies for re-use of waste: an overview. Adv. Biorefineries Biomass Waste Supply Chain Exploit. 2014, 3–33, doi:10.1533/9780857097385.1.3.
14. Bajpai, P. Biomass Conversion Processes. Biomass to Energy Convers. Technol. 2020, 41–151, doi:10.1016/B978-0-12-818400-4.00005-0.
15. Gschwend, F.J.V.; Hennequin, L.M.; Brandt-Talbot, A.; Bedoya-Lora, F.; Kelsall, G.H.; Polizzi, K.; Fennell, P.S.; Hallett, J.P. Towards an environmentally and economically sustainable biorefinery: heavy metal contaminated waste wood as a low-cost feedstock in a low-cost ionic liquid process. Green Chem. 2020, 22, 5032–5041, doi:10.1039/D0GC01241F.

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 101007733

16. Mittal, A.; Vinzant, T.B.; Brunecky, R.; Black, S.K.; Pilath, H.M.; Himmel, M.E.; Johnson, D.K. Investigation of the role of lignin in biphasic xylan hydrolysis during dilute acid and organosolv pretreatment of corn stover. Green Chem. 2015, 17, 1546–1558, doi:10.1039/C4GC02258K.
17. Bensah, E.C.; Mensah, M. Chemical pretreatment methods for the production of cellulosic ethanol: technologies and innovations. Int. J. Chem. Eng. 2013, doi:10.1155/2013/719607.
18. Yang, Y.; Yang, S.; Yao, X.; Kang, Y.; Xin, J.; El-Tantawy El-Sayed, I.; Xu, J.; Lu, X. A Renewable co-solvent promoting the selective removal of lignin by increasing the total number of hydrogen bonds. Green Chem. 2020, 22, 6393–6403, doi:10.1039/D0GC02319A.
19. Da Costa Sousa, L.; Jin, M.; Chundawat, S.P.S.; Bokade, V.; Tang, X.; Azarpira, A.; Lu, F.; Avci, U.; Humpula, J.; Uppugundla, N.; et al. Next-generation ammonia pretreatment enhances cellulosic biofuel production. Energy Environ. Sci. 2016, 9, 1215–1223, doi:10.1039/C5EE03051J.
20. Semwal, S.; Raj, T.; Kumar, R.; Christopher, J.; Gupta, R.P.; Puri, S.K.; Kumar, R.; Ramakumar, S.S.V. Process optimization and mass balance studies of pilot scale steam explosion pretreatment of rice straw for higher sugar release. Biomass and Bioenergy 2019, 130, 105390, doi:10.1016/J.BIOMBIOE.2019.105390.
21. Larnaudie, V.; Ferrari, M.D.; Lareo, C. Enzymatic hydrolysis of liquid hot water-pretreated switchgrass at high solid content. Energy & Fuels 2019, 33, 4361–4368, doi:10.1021/ACS.ENERGYFUELS.9B00513.
22. Rahmati, S.; Doherty, W.; Dubal, D.; Atanda, L.; Moghaddam, L.; Sonar, P.; Hessel, V.; Ostrikov, K. Pretreatment and fermentation of lignocellulosic biomass: reaction mechanisms and process engineering. React. Chem. Eng. 2020, 5, 2017–2047, doi:10.1039/D0RE00241K.
23. Sindhu, R.; Binod, P.; Pandey, A. Biological Pretreatment of lignocellulosic biomass – an overview. Bioresour. Technol. 2016, 199, 76–82, doi:10.1016/J.BIORTECH.2015.08.030.
24. Asgher, M.; Ahmad, Z.; Iqbal, H.M.N. Alkali and enzymatic delignification of sugarcane bagasse to expose cellulose polymers for saccharification and bio-ethanol production. Ind. Crops Prod. 2013, 44, 488–495, doi:10.1016/J.INDCROP.2012.10.005.
25. Zoghlami, A.; Paës, G. Lignocellulosic biomass: understanding recalcitrance and predicting hydrolysis. Front. Chem. 2019, 7, doi:10.3389/FCHEM.2019.00874.
26. Arora, R.; Sharma, N.K.; Kumar, S.; Sani, R.K. Lignocellulosic ethanol: feedstocks and bioprocessing. Bioethanol Prod. from Food Crop. 2019, 165–185, doi:10.1016/B978-0-12-813766-6.00009-6.
27. Baruah, J.; Nath, B.K.; Sharma, R.; Kumar, S.; Deka, R.C.; Baruah, D.C.; Kalita, E. Recent trends in the pretreatment of lignocellulosic biomass for value-added products. Front. Energy Res. 2018, 6, 141, doi:10.3389/FENRG.2018.00141/XML/NLM.
28. Dahunsi, S.O. Mechanical pretreatment of lignocelluloses for enhanced biogas production: methane yield prediction from biomass structural components. Bioresour. Technol. 2019, 280, 18–26, doi:10.1016/J.BIORTECH.2019.02.006.
29. Raj, T.; Chandrasekhar, K.; Naresh Kumar, A.; Kim, S.H. Lignocellulosic biomass as renewable feedstock for biodegradable and recyclable plastics production: a sustainable approach. Renew. Sustain. Energy Rev. 2022, 158, 112130, doi:10.1016/J.RSER.2022.112130.
30. Yang, X.; Cui, C.; Zheng, A.; Zhao, Z.; Wang, C.; Xia, S.; Huang, Z.; Wei, G.; Li, H. Ultrasonic and microwave assisted organosolv pretreatment of pine wood for producing pyrolytic sugars and phenols. Ind. Crops Prod. 2020, 157, 112921, doi:10.1016/J.INDCROP.2020.112921.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 101007733

31. Ravindran, R.; Jaiswal, A.K. A comprehensive review on pre-treatment strategy for lignocellulosic food industry waste: challenges and opportunities. Bioresour. Technol. 2016, 199, 92–102, doi:10.1016/J.BIORTECH.2015.07.106.
32. Kim, J.S.; Lee, Y.Y.; Kim, T.H. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. Bioresour. Technol. 2016, 199, 42–48, doi:10.1016/J.BIORTECH.2015.08.085.
33. Lloyd, T.A.; Wyman, C.E. Combined sugar yields for dilute sulfuric acid pretreatment of corn stover followed by enzymatic hydrolysis of the remaining solids. Bioresour. Technol. 2005, 96, 1967–1977, doi:10.1016/J.BIORTECH.2005.01.011.
34. Yoo, C.G.; Pu, Y.; Ragauskas, A.J. Ionic Liquids: Promising green solvents for lignocellulosic biomass utilization. Curr. Opin. Green Sustain. Chem. 2017, 5, 5–11, doi:10.1016/J.COGSC.2017.03.003.
35. Borand, M.N.; Karaosmanoğlu, F. Effects of organosolv pretreatment conditions for lignocellulosic biomass in biorefinery applications: a review. J. Renew. Sustain. Energy 2018, 10, 033104, doi:10.1063/1.5025876.
36. Quintana, G.; Velásquez, J.; Betancourt, S.; Gañán, P. Binderless fiberboard from steam exploded banana bunch. Ind. Crops and Products 2009, 29, 60–66. doi:10.1016/j.indcrop.2008.04.007.
37. Tupciauskas, R.; Berzins, A.; Pavlovics, G.; Bikovens, O.; Filipova, I.; Andze, L; Andzs, M. Optimization of thermal conductivity vs. bulk density of steam-exploded loose-fill annual lignocellulosics. Materials 2023, 16, 3654. doi: 10.3390/ma16103654
38. Tupciauskas, R.; Orlovskis, Z.; Blums, K.T.; Liepins, J.; Berzins, A.; Pavlovics, G.; Andzs, M. Mold Fungal resistance of loose-fill thermal insulation materials based on processed wheat straw, corn stalk and reed. Polymers 2024, 16, 562. doi: 10.3390/polym16040562
39. El-Naggar, N.E.A.; Deraz, S.; Khalil, A. Bioethanol production from lignocellulosic feedstocks based on enzymatic hydrolysis: current status and recent developments. Biotechnology 2014, 13, 1–21, doi:10.3923/BIOTECH.2014.1.21.
40. Amin, F.R.; Khalid, H.; Zhang, H.; Rahman, S.; Zhang, R.; Liu, G.; Chen, C. Pretreatment methods of lignocellulosic biomass for anaerobic digestion. AMB Express 2017, 7, 1–12, doi:10.1186/S13568-017-0375-4/TABLES/2.
41. Zámocký, M.; Gasselhuber, B.; Furtmüller, P.G.; Obinger, C. Turning points in the evolution of peroxidase–catalase superfamily: molecular phylogeny of hybrid heme peroxidases. Cell. Mol. Life Sci. 2014, 71, 4681, doi:10.1007/S00018-014-1643-Y.
42. Behera, S.; Arora, R.; Nandhagopal, N.; Kumar, S. Importance of chemical pretreatment for bioconversion of lignocellulosic biomass. Renew. Sustain. Energy Rev. 2014, 36, 91–106, doi:10.1016/J.RSER.2014.04.047.
43. Shirkavand, E.; Baroutian, S.; Gapes, D.J.; Young, B.R. Combination of fungal and physicochemical processes for lignocellulosic biomass pretreatment – a review. Renew. Sustain. Energy Rev. 2016, 54, 217–234, doi:10.1016/J.RSER.2015.10.003.
44. Jiao, Y.; Guo, C.; Wang, S.; Guan, S.; Pan, X.; Ma, X.; Zhang, Z.; Zhang, Q.; He, C. Enhancement of converting corn stalk into reducing sugar by ultrasonic-assisted ammonium bicarbonate pretreatment. Bioresour. Technol. 2020, 302, 122878, doi:10.1016/J.BIORTECH.2020.122878.
45. Luo, J.; Fang, Z.; Smith, R.L. Ultrasound-enhanced conversion of biomass to biofuels. Prog. Energy Combust. Sci. 2014, 41, 56–93, doi:10.1016/J.PECS.2013.11.001.
46. Akhlisah, Z.N.; Yunus, R.; Abidin, Z.Z.; Lim, B.Y.; Kania, D. Pretreatment methods for an effective conversion of oil palm biomass into sugars and high-value chemicals. Biomass and Bioenergy 2021, 144, 105901, doi:10.1016/J.BIOMBIOE.2020.105901.
47. Yang, H.; Shi, Z.; Xu, G.; Qin, Y.; Deng, J.; Yang, J. Bioethanol production from bamboo with alkali-catalyzed liquid hot water pretreatment. Bioresour. Technol. 2019, 274, 261–266, doi:10.1016/J.BIORTECH.2018.11.088.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 101007733

48. Zou, R.; Qian, M.; Wang, C.; Mateo, W.; Wang, Y.; Dai, L.; Lin, X.; Zhao, Y.; Huo, E.; Wang, L.; et al. Biochar: from by-products of agro-industrial lignocellulosic waste to tailored carbon-based catalysts for biomass thermochemical conversions. Chem. Eng. J. 2022, 441, 135972, doi:10.1016/J.CEJ.2022.135972.
49. Zhao, J.; Tao, X.; Li, J.; Jia, Y.; Shao, T. Enhancement of biomass conservation and enzymatic hydrolysis of rice straw by dilute acid-assisted ensiling pretreatment. Bioresour. Technol. 2021, 320, 124341, doi:10.1016/J.BIORTECH.2020.124341.
50. Sheng, Y.; Tan, X.; Gu, Y.; Zhou, X.; Tu, M.; Xu, Y. Effect of ascorbic acid assisted dilute acid pretreatment on lignin removal and enzyme digestibility of agricultural residues. Renew. Energy 2021, 163, 732–739, doi:10.1016/J.RENENE.2020.08.135.
51. Zhao, X.; Li, S.; Wu, R.; Liu, D. Organosolv fractionating pre-treatment of lignocellulosic biomass for efficient enzymatic saccharification: chemistry, kinetics, and substrate structures. Biofuels, Bioprod. Biorefining 2017, 11, 567–590, doi:10.1002/BBB.1768.
52. Xia, S.; Baker, G.A.; Li, H.; Ravula, S.; Zhao, H. Aqueous ionic liquids and deep eutectic solvents for cellulosic biomass pretreatment and saccharification. RSC Adv. 2014, 4, 10586–10596, doi:10.1039/C3RA46149A.
53. Chen, H.; Liu, J.; Chang, X.; Chen, D.; Xue, Y.; Liu, P.; Lin, H.; Han, S. A review on the pretreatment of lignocellulose for high-value chemicals. Fuel Process. Technol. 2017, 160, 196–206, doi:10.1016/J.FUPROC.2016.12.007.
54. Brandt-Talbot, A.; Gschwend, F.J.V.; Fennell, P.S.; Lammens, T.M.; Tan, B.; Weale, J.; Hallett, J.P. An economically viable ionic liquid for the fractionation of lignocellulosic biomass. Green Chem. 2017, 19, 3078–3102, doi:10.1039/C7GC00705A.
55. Wei, W.; Wang, B.; Wang, X.; Ling, R.; Jin, Y. Comparison of Acid and alkali catalyzed ethylene glycol organosolv pretreatment for sugar production from bagasse. Bioresour. Technol. 2021, 320, 124293, doi:10.1016/J.BIORTECH.2020.124293.
56. Zhang, Z.; Harrison, M.D.; Rackemann, D.W.; Doherty, W.O.S.; O'Hara, I.M. Organosolv Pretreatment of plant biomass for enhanced enzymatic saccharification. Green Chem. 2016, 18, 360–381, doi:10.1039/C5GC02034D.
57. Amiri, H.; Karimi, K.; Zilouei, H. Organosolv pretreatment of rice straw for efficient acetone, butanol, and ethanol production. Bioresour. Technol. 2014, 152, 450–456, doi:10.1016/J.BIORTECH.2013.11.038.
58. Pielhop, T.; Amgarten, J.; Von Rohr, P.R.; Studer, M.H. Steam explosion pretreatment of softwood: the effect of the explosive decompression on enzymatic digestibility. Biotechnol. Biofuels 2016, 9, 1–13, doi:10.1186/S13068-016-0567-1/FIGURES/7.
59. Bhatia, R.; Winters, A.; Bryant, D.N.; Bosch, M.; Clifton-Brown, J.; Leak, D.; Gallagher, J. Pilot-Scale Production of xylo-oligosaccharides and fermentable sugars from miscanthus using steam explosion pretreatment. Bioresour. Technol. 2020, 296, 122285, doi:10.1016/J.BIORTECH.2019.122285.
60. Baêta, B.E.L.; Cordeiro, P.H. de M.; Passos, F.; Gurgel, L.V.A.; de Aquino, S.F.; Fdz-Polanco, F. Steam explosion pretreatment improved the biomethanization of coffee husks. Bioresour. Technol. 2017, 245, 66–72, doi:10.1016/J.BIORTECH.2017.08.110.
61. Fan, X.; Cheng, G.; Zhang, H.; Li, M.; Wang, S.; Yuan, Q. Effects of acid impregnated steam explosion process on xylose recovery and enzymatic conversion of cellulose in corncob. Carbohydr. Polym. 2014, 114, 21–26, doi:10.1016/J.CARBPOL.2014.07.051.
62. Liang, Y.; Lei, B.; Zhong, H.T.; Feng, Y.H.; Qu, J.P. A Promising screw-extrusion steam explosion pretreatment process: effects on the morphological and structural features of eucalyptus woodchips. RSC Adv. 2016, 6, 109657–109663, doi:10.1039/C6RA24639G.
63. Sharma, S.; Kumar, R.; Gaur, R.; Agrawal, R.; Gupta, R.P.; Tuli, D.K.; Das, B. Pilot scale study on steam explosion and mass balance for higher sugar recovery from rice straw. Bioresour. Technol. 2015, 175, 350–357, doi:10.1016/J.BIORTECH.2014.10.112.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 101007733

64. Bhutto, A.W.; Qureshi, K.; Harijan, K.; Abro, R.; Abbas, T.; Bazmi, A.A.; Karim, S.; Yu, G. Insight into progress in pre-treatment of lignocellulosic biomass. Energy 2017, 122, 724–745, doi:10.1016/J.ENERGY.2017.01.005.
65. Zhuang, X.; Wang, W.; Yu, Q.; Qi, W.; Wang, Q.; Tan, X.; Zhou, G.; Yuan, Z. Liquid Hot Water pretreatment of lignocellulosic biomass for bioethanol production accompanying with high valuable products. Bioresour. Technol. 2016, 199, 68–75, doi:10.1016/J.BIORTECH.2015.08.051.
66. Kothari, N.; Bhagia, S.; Pu, Y.; Yoo, C.G.; Li, M.; Venketachalam, S.; Pattathil, S.; Kumar, R.; Cai, C.M.; Hahn, M.G.; et al. The effect of switchgrass plant cell wall properties on its deconstruction by thermochemical pretreatments coupled with fungal enzymatic hydrolysis or clostridium thermocellum consolidated bioprocessing. Green Chem. 2020, 22, 7924–7945, doi:10.1039/D0GC02546A.
67. Echeverria, C.A.; Ozkan, J.; Pahlevani, F.; Willcox, M.; Sahajwalla, V. Effect of hydrothermal hot-compression method on the antimicrobial performance of green building materials from heterogeneous cellulose wastes. J. Clean. Prod. 2021, 280, 124377, doi:10.1016/J.JCLEPRO.2020.124377.
68. Cao, X.; Peng, X.; Sun, S.; Zhong, L.; Chen, W.; Wang, S.; Sun, R.C. Hydrothermal conversion of xylose, glucose, and cellulose under the catalysis of transition metal sulfates. Carbohydr. Polym. 2015, 118, 44–51, doi:10.1016/J.CARBPOL.2014.10.069.
69. Elliott, D.C.; Biller, P.; Ross, A.B.; Schmidt, A.J.; Jones, S.B. Hydrothermal liquefaction of biomass: developments from batch to continuous process. Bioresour. Technol. 2015, 178, 147–156, doi:10.1016/J.BIORTECH.2014.09.132.
70. Pérez Pimienta, J.A.; Papa, G.; Rodriguez, A.; Barcelos, C.A.; Liang, L.; Stavila, V.; Sanchez, A.; Gladden, J.M.; Simmons, B.A. Pilot-scale hydrothermal pretreatment and optimized saccharification enables bisabolene production from multiple feedstocks. Green Chem. 2019, 21, 3152–3164, doi:10.1039/C9GC00323A.
71. Arshadi, M.; Attard, T.M.; Lukasik, R.M.; Brncic, M.; Da Costa Lopes, A.M.; Finell, M.; Geladi, P.; Gerschenson, L.N.; Gogus, F.; Herrero, M.; et al. Pre-treatment and extraction techniques for recovery of added value compounds from wastes throughout the agri-food chain. Green Chem. 2016, 18, 6160–6204, doi:10.1039/C6GC01389A.
72. Balan, V.; Bals, B.; Da Costa Sousa, L.; Garlock, R.; Dale, B.E. Chapter 5:A short review on ammonia-based lignocellulosic biomass pretreatment. RSC Energy Environ. Ser. 2011, 2011, 89–114, doi:10.1039/9781849732857-00089.
73. McMillan, J.D. Pretreatment of lignocellulosic biomass. 1994, 292–324, doi:10.1021/BK-1994-0566.CH015.
74. Tian, L.; Branford-White, C.; Wang, W.; Nie, H.; Zhu, L. Laccase-mediated system pretreatment to enhance the effect of hydrogen peroxide bleaching of cotton Fabric. Int. J. Biol. Macromol. 2012, 50, 782–787, doi:10.1016/J.IJBIOMAC.2011.11.025.
75. Maurya, D.P.; Singla, A.; Negi, S. An overview of key pretreatment processes for biological conversion of lignocellulosic biomass to bioethanol. 3 Biotech 2015, 5, 597–609, doi:10.1007/S13205-015-0279-4.
76. Dhiman, S.S.; Haw, J.R.; Kalyani, D.; Kalia, V.C.; Kang, Y.C.; Lee, J.K. Simultaneous Pretreatment and saccharification: green technology for enhanced sugar yields from biomass using a fungal consortium. Bioresour. Technol. 2015, 179, 50–57, doi:10.1016/J.BIORTECH.2014.11.059.
77. Rico, A.; Rencoret, J.; Del Río, J.C.; Martínez, A.T.; Gutiérrez, A. Pretreatment with laccase and a phenolic mediator degrades lignin and enhances saccharification of eucalyptus feedstock. Biotechnol. Biofuels 2014, 7, doi:10.1186/1754-6834-7-6.
78. Galbe, M.; Wallberg, O. Pretreatment for biorefineries: a review of common methods for efficient utilisation of lignocellulosic materials. Biotechnol. Biofuels 2019 121 2019, 12, 1–26, doi:10.1186/S13068-019-1634-1.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 101007733

79. Coz, A.; Llano, T.; Cifrián, E.; Viguri, J.; Maican, E.; Sixta, H. Physico-chemical alternatives in lignocellulosic materials in relation to the kind of component for fermenting purposes. Mater. (Basel, Switzerland) 2016, 9, doi:10.3390/MA9070574.
80. V, A.; Carere C, Cicek N, Sparling R, L.D. Advances in biorefineries: biomass and waste supply chain exploitation; 2014; ISBN 9780857095213.

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