# Environmental assessment of biomass pretreatment and processing towards bionanomaterials and bio-polymers enablers

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Luís Miguel Morais Soares Carlow, Irlanda 14/10/2022

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Resumo

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# Os plásticos convencionais estão a danificar bastante o ambiente, principalmente devido à sua vasta acumulação e emissões de gases com efeito de estufa. Apesar da crescente consciencialização entre a população de países desenvolvidos acerca das desvantagens destes materiais, ainda existem poucas alternativas que possam ocupar eficazmente as diversas funções dos plásticos. Esta dependência de plásticos está atualmente a bloquear uma transição para uma economia circular mais sustentável.

A biomassa lignocelulósica é o recurso natural renovável mais abundante que pode ser utilizado para produzir novos produtos e químicos de valor acrescentado. Uma das suas frações, a lenhina, é normalmente considerada um polímero de baixo valor na indústria de polpa e papel, mas as suas propriedades interessantes conduziram a investigações recentes sobre a possibilidade da sua utilização numa ampla gama de aplicações comerciais. Isto seria atingido através do uso de nanopartículas de lenhina como reforços numa matriz polimérica de forma a criar uma classe de materiais emergentes designados nanocompósitos.

No entanto, a obtenção de nanopartículas de lenhina requere um prétratamento da biomassa para superar a sua recalcitrância e permitir um fracionamento eficiente. Estes pré-tratamentos exigem frequentemente grandes consumos de água, químicos e/ou energia, o que significa que é necessária uma gestão cuidadosa de recursos para garantir práticas sustentáveis. Além disso, não existem muitos estudos de avaliação de ciclo de vida publicados para identificar devidamente os *hotspots* da cadeia de valor da lenhina.

Este relatório de estágio tem como objetivo apoiar o desenvolvimento de nanolenhina como um material de valor acrescentado utilizando uma perspetiva de análise de ciclo de vida. Primeiro, inclui uma revisão extensiva do estado da arte da literatura, compilando as vantagens e desvantagens ambientais de processos de biorrefinação com um foco particular nos pré-tratamentos de biomassa (tratamentos *organosolv* e *hydrothermal*). Pretende também apresentar um caso de estudo de avaliação de ciclo de vida prospetivo de uma biorrefinaria inovadora de nanopartículas de lenhina que aplica um tratamento híbrido *organosolv* – *steam explosion* à matéria-prima de biomassa (palha de trigo).

As conclusões do caso de estudo estão de acordo com as da literatura. A cadeia de valor das nanopartículas de lenhina apresenta alguns *hotspots*, o principal sendo a etapa do pré-tratamento, que é a mais intensiva em termos de energia e químicos. Para melhorar a performance ambiental do processo estudado, é



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recomendado o uso de energia de fontes renováveis e a recuperação e reutilização de químicos tanto quanto possível. Mais estudos de LCA deverão fornecer conclusões mais robustas relativamente à otimização da biorrefinaria.

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# Abstract

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Conventional plastics are heavily straining the environment, mainly due to its vast accumulation and emissions of greenhouse gases. Despite the growing consciousness among population from developed countries regarding the disadvantages of these materials, there are still few alternatives that may effectively take on plastics' diverse roles. This dependency of plastics is currently blocking a transition towards a more sustainable circular economy.

Lignocellulosic biomass is the most abundant renewable natural resource that may be used to produce new value-added products and chemicals. One of its fractions, lignin, is generally treated as a low-value polymer in the pulp and paper industry, but its interesting properties led to recent investigations on the possibility of using it on a wide range of commercial applications. This would be achieved by using lignin nanoparticles as fillers on a polymeric matrix to create a class of emergent materials called nanocomposites.

However, obtaining lignin nanoparticles requires a pretreatment of biomass to overcome recalcitrance and allow for an efficient fractionation. These pretreatments often demand large consumptions of water, chemicals and/or energy, meaning that a careful management of resources is necessary to ensure sustainable practices. Moreover, there are not many published life cycle assessment studies to properly identify the environmental hotspots of lignin's value chain.

This thesis aims to support the development of nanolignin as a value-added material using a life cycle perspective. Firstly, it includes an extensive review of state-of-the-art literature, compiling the environmental strengths and weaknesses of biorefining processes with a special focus on biomass pretreatments (organosolv and hydrothermal treatments). It also presents a prospective life cycle assessment case study of an innovative lignin nanoparticle biorefinery that applies a hybrid organosolv – steam explosion treatment to the biomass feedstock (wheat straw).

The findings of the case study are in line with those from literature. The value chain of lignin nanoparticles presents a few hotspots, the main one being the pretreatment stage, which is the more energy and chemical intensive stage. To improve the environmental performance of the studied process, it is recommended the use of energy from renewable resources and the recovery and reuse of chemicals as much as possible. More LCA studies should also provide more robust conclusions regarding the optimization of the biorefinery.



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# Keywords

Biomass, Lignocellulose, Lignin, Nanolignin, Pretreatment, Hydrothermal, Steam Explosion, Organosolv, Ultrasonication, Life Cycle Assessment

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# Abbreviations

- AFEX Ammonia Fiber Expansion
- DES Deep Eutectic Solvent
- DSC Differential Scanning Calorimetry
- EFB Empty Fruit Bunch
- EOFP Ozone Formation Potential, Ecosystems
- FEP Freshwater Eutrophication Potential
- FETP Freshwater Ecotoxicity Potential
- FFP Fossil Fuel Potential
- FT-IR Fourier Transform Infrared
- FU Functional Unit
- GHG Greenhouse Gas
- GPC Gel Permeation Chromatography
- GWP Global Warming Potential
- HMF Hydroxymethylfurfural
- HOFP Ozone Formation Potential, Humans
- HTPc Human Toxicity Potential, Cancer
- HTPnc Human Toxicity Potential, Non-cancer
- HTP Human Toxicity Potential
- IL Ionic Liquid
- IRP Ionizing Radiation Potential
- ISO International Organization for Standardization
- ISQ Instituto de Soldadura e Qualidade
- KL Kraft lignin
- LCA Life Cycle Assessment
- LCI Life Cycle Inventory
- LCIA Life Cycle Impact Assessment
- LHW Liquid Hot Water
- LNP Lignin nanoparticle
- LOP Land Occupation Potential
- MEP Marine Eutrophication Potential
- METP Marine Ecotoxicity Potential



- NMR Nuclear Magnetic Resonance
- NMVOC Non-Methane Volatile Organic Compounds
- ODP Ozone Depletion Potential
- OS Organosolv
- PMFP Particulate Matter Formation Potential
- PT Pretreatment
- SB System Boundary
- SE Steam explosion
- SOP Surplus Ore Potential
- SPORL Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose
- TAP Terrestrial Acidification Potential
- TEP Terrestrial Eutrophication Potential
- TETP Terrestrial Ecotoxicity Potential
- TGA Thermogravimetric Analysis
- WCP Water Consumption Potential
- WEP Water Eutrophication Potential

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# 1. Introduction

## 1.1 Framework

This thesis was written within the scope of the curricular unit Internship of the master's degree in Environmental Sciences and Technology study plan of the Faculty of Sciences of the University of Porto (FCUP). All work was developed at Instituto de Soldadura e Qualidade (ISQ) in Vila Nova de Gaia over the full extent of the academic year under the direct supervision of Helena Monteiro (ISQ) and virtual supervision of Prof. Joaquim Esteves (FCUP), Teresa Mata (INEGI) and António Martins (FEUP).

The central theme of this thesis is in the context of Bio-based nanoMaterials Community (BIOMAC), a Horizon2020 project which aims to establish an Open Innovation Test Bed to develop and upscale production of nano-enabled bio-based materials. The different pilot lines cover the entirety of the value chains, starting at biomass fractionation and finishing with the end-product that may be applied in many industry fields (automotive, agricultural, food packaging, construction and printed electronics). BIOMAC main goal is to boost and sustain an European bioeconomy. This project is only possible through international cooperation among several partners, namely ISQ, responsible for the assessment of the environmental impacts associated to the value chains of the studied products.

We aimed to support the development of a lignin nanoparticle value chain. Lignin is an undervalued fraction of lignocellulosic biomass, which is widely abundant, renewable and offers many potential applications that are, thus far, reliant on conventional plastic materials. There are, however, few studies considering the extraction of this biomass fraction and, throughout this work, we address this knowledge gap.

The goal of this work was to evaluate the environmental impacts in a case study of the lignin value chain. On an earlier phase of the internship, to overcome the difficulty in retrieving data from project partners, a literature review was conducted aiming to understand the main biomass pretreatment processes that can be used, and to compile data from previous life cycle assessment studies applied to such processes. On a second stage, given the scarcity of data, we carried out a life cycle assessment of the studied value chain using a prospective approach.

This work obeyed the following logical sequence: on Chapter 1, besides the framework, it is provided a general explanation of the unsustainable situation we are currently living in and of the necessity of a global transition towards more sustainable practices. Here, lignocellulosic biomass presents itself as a potential candidate to propel such transition.

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On Chapter 2, it is provided a state-of-the-art literature review of lignin as a lignocellulosic fraction, pretreatment processes, existing LCA studies and a brief summary at the end. Chapter 3 describes the materials and methods used in the case study. On Chapter 4, it is presented the results of the case study. Chapter 5 serves as a conclusion to the overall work. This thesis represents an attempt to close the knowledge gap regarding the environmental burdens of biomaterials production by means of an LCA.

#### 1.2 Biorefineries for lignocellulosic biomass

For decades, petrochemical activities have been endorsed by world politicians boosting economies and enabling global technological advancements and citizens welfare. Oil supply is at the core of modern economies, but it is getting increasingly difficult and expensive to extract and produce, mainly due to the need for additional infrastructures investments and operational costs [1, 2]. Also, as oil is becoming insufficient considering global population's needs and the environmental impacts associated with the exploitation of fossil resources, challenging times lie ahead, being necessary to look into alternative industrial solutions.

On the other hand, it is currently consensual that the use of renewable resources is essential to decarbonize the economy and lignocellulosic biomass presents itself as an opportunity to contribute to this objective. This is the world's most abundant renewable resource to produce new added-value materials such as chemicals and biopolymers, known to have a wide range of potential uses. Not only is it widely available, but also presents itself as a low-cost alternative to petrochemical resources that may be able to avoid competition with food crops. It is expected that the sustainable production of biopolymers will thwart the growing concerns of fossil resources dependency [3].

While plentiful, biomass is not unlimited, meaning that its processing should be as efficient as possible to avoid land use competition [4]. Due to natural resources depletion and the aforementioned negative environmental impacts of fossil resources, biorefineries have been given more and more attention. Biorefineries have been consistently touted as a possible path to a low carbon and circular economy [5].

In order to ensure that biorefineries are a viable, reliable and more sustainable alternative compared to petrochemical refineries, and for them to gain traction, their supply chains must be economically and environmentally competitive [6]. Besides, the growing scientific knowledge concerning lignin physicochemical properties has enabled the development of innovative materials produced from lignin resources [7, 8]. This can

be understood as an opportunity for biorefineries to take lignocellulosic biomass components and maximize their respective valorization and head towards more sustainable industrial practices, which will hopefully accelerate a much-needed green transition. Not only does this make sense from an environmental perspective, but it is also logical from an economic point of view [9]. Fostering lignin use as a source of value-added products, rather than treating it as a by-product and burning it for energy recovery, can be a cost-effective way to operate a biorefinery [9].

Nonetheless, it is not certain that lignocellulosic biomass and derived chemicals are sustainable, as biorefineries include complex process systems that require energy, water and chemicals to be used as solvents or catalysts that may hinder environmental performance [10, 11]. An LCA perspective, as incorporated in this review, is a much-needed addition to the state-of-the-art discussion. Moreover, most LCA studies focus on cellulose instead of lignin valorization. For lignin, there is still little information regarding its synthesis at industrial level and end uses [12].

#### 1.3 Synthetic and bio-based polymers

Synthetic polymers such as polyethylene, polypropylene, polystyrene, polyvinylchloride, polyethylene terephtalate and polyurethane, commonly referred to as plastics, are usually made by polymerization of oil or gas derived monomers and addition of diverse chemical additives [13]. With seemingly almost infinite applications, ranging from manufacturing of key materials to construction, automotive, agriculture, packaging and electronic sectors, they represent a reliable product in our daily lives. It is, therefore, difficult to conceive a future in which plastics are not a part of [14]. In fact, global plastic production volume has been steadily increasing ever since the industry birth, back in the 1950s, roughly reaching 370 million tonnes in 2018 [15].

Most of the above-mentioned conventional plastics can be manufactured from renewable feedstocks, such as sugarcane or maize, but remain non-biodegradable materials [16, 17]. This way, plastic waste accumulation may still arise at the end of the value chain [17]. Ultimately, such a system is not sustainable and we, as a society, may need to rethink our practices and attitude towards conventional plastics.

Therefore, it is important to monitor the degradation behaviour of biopolymers and their environmental impacts, before promoting their large-scale application [18]. Also, a product's circularity performance may not match its environmental performance. To be proven as more sustainable than synthetic alternatives, bio-based polymers should be analysed from a life cycle perspective. Addressing sustainability issues demand to reflect about the whole value chain of a product, because environmental



impacts may arise in different life cycle stages. Thus, the life cycle of plastics from raw material extraction to transportation, manufacturing and waste treatment should be taken into account. At the end of its life cycle, plastic incineration for energy recovery will further increase the greenhouse gas (GHG) emissions, which can be understood as a deviation from the Paris Agreement target set at COP 21 of keeping global temperatures rise below 1.5 °C, compared to pre-industrial levels, by 2100. While GHG emissions are soaring due to combustion of fossil fuels, the resulting climate changes are also turning natural carbon sinks, such as forests, ineffective in their role to sequester carbon and may even reverse and start acting as another carbon source [19].

Additionally, plastics directly or indirectly released into oceans will undermine its ability to act as a carbon sink, as pollution caused by microplastics may compromise phytoplankton's capability of  $CO_2$  fixation and its transportation to the deep sea by zooplankton [20]. Conventional plastic degradation in marine environment may take place under specific circumstances, such as solar UV-induced photodegradation reactions, thermal reactions, polymer hydrolysis or microbial degradation. However, most of these reactions are ineffective and plastic degradation rate is extremely low, due to recalcitrant properties, and thus plastics will accumulate in water bodies [21]. Plastic presence in oceans comes with ecological, societal and economic impacts from local to global scale, which may be irreversible to a certain extent. The consequential reduction in ecosystem services provision affects human health and puts in question the sustainability of fisheries and aquaculture with clear economic costs [22]. Microplastics, originated from the fragmentation of large plastics, are persistent in the atmosphere and, even though the full magnitude of their impacts in human health is not well established, they can disrupt oxygen transport in biological organisms by reacting with hemoglobin after reaching the lungs [23]. Microplastics also alter the soil function, microbial communities and affect plant growth, depending on its concentration and exposure time, jeopardizing agricultural practices [24]. Thus, the phenomenon of plastic waste accumulation puts natural ecosystems and world economies under pressure, being prudent to monitor plastic degradation in water, air and soil.

It is, therefore, necessary to find alternatives to traditional plastics that present a faster degradation rate and lower impacts resulting from their elimination, turning a linear economy into a circular one [25, 26]. Globally, public perception towards conventional plastics is overall negative and consider it to be a serious environmental issue. Particularly in developed countries, people have started to properly dispose of plastics, while also being receptive to reduce its use and to transition to bioplastic materials. People have become more aware that plastic waste negatively impacts the



environment and human health [27, 28]. However, covid-19 pandemic can be considered a setback in respect to transitioning towards an environmentally friendly economy. The relaxation on single-use plastics bans is likely to have re-induced a throw-away culture onto the consumer and increased plastic waste generation [29]. As such, green materials such as bioplastics could even play a role in the fight against future pandemics [29]. A circular economy would promote sustainability by adopting product ecodesign, preventing waste and demanding products re-use at the end of their life cycles. These measures are meant to empower consumers, save companies money, boost economy, while protecting the environment by improving security of raw materials supply and reducing total annual GHG emissions. The efforts now lie on the design of more sustainable polymers based on renewable feedstocks, in order to replace conventional polymers based on increasingly depleted fossil resources and to, hopefully, break free from plastic dependency [30].

Green Chemistry [31] aims at designing chemical processes and products in order to reduce or halt the use and manufacturing of dangerous substances by defining a set of principles. Similarly, Green Engineering [32] holds its own set of principles that provide a structure for engineers and scientists to compromise in projecting new materials, products, processes and systems that are beneficial to both human and environmental health. When applied together, they have the potential to improve the performance and public acceptance of new green designed technologies, for instance those at nanoscale [33].

On the other hand, despite the recent bloom of literature describing procedures for extracting lignocellulosic biocompounds, there is still a lack of uniformized studies to properly measure the environmental impacts of such extraction and prove their environmental benefits. This gap, allied to misinformation and lack of transparency, may hinder the confidence of companies in scaling up and adopting more sustainable extraction solutions to replace conventional ones. In such context, LCA methodology reveals itself useful since it can provide relevant information regarding the environmental performance of a product or different product alternatives. It is, to date, one of the most consensual methods to quantitatively assess the environmental impact of a product or process throughout its life cycle. However, since assumptions widely vary among studies, the comparison of results to withdraw generic conclusions on most sustainable practices is challenging and difficult to perform.

Hence, the literature review and case study presented here aim to address these gaps and shed light over LCA studies insights concerning biomass pretreatment processes inherent to biorefineries that can be used for lignin extraction, which have seldom been addressed. The knowledge gathered both in the review and case study



sections will allow a more comprehensive and transparent identification of the main challenges and opportunities that can stand before biomass valorization, while pointing out the limitations of previous studies.

# 2. Literature review

# 2.1 Lignin

# 2.1.1 Lignin physicochemical properties

Lignocellulosic biomass is an abundant and renewable resource from plants, mainly composed of polysaccharides, cellulose and hemicelluloses, and an aromatic polymer, lignin. Plant cell walls are primarily composed of lignocellulose (**Fig. 1**), whose main constituents cellulose (25-45 wt%), hemicellulose (20-40 wt%) and lignin (10-25 wt%) are chemically bonded [34]. Each of these components can be recovered, in varying amounts, from a wide spectrum of renewable raw materials, be it hardwood, softwood, grass and agricultural or forest wastes [35]. The lignin content varies depending on the biomass type, being for example, 17-24 wt% in grass, 18-25 wt% in softwood and 27-33 wt% in hardwood.



**Fig 1.** Enlarged view of a (a) lignocellulosic material, showing the (b) plant cell and the (c) plant cell wall's structure: cellulose in light green, hemicellulose in darker green, lignin in blue (author's own creation).

Lignin's main function is to act as a cement, maintaining the plant cell wall structural integrity and mechanical strength by chemically binding both cellulose and hemicellulose through covalent and hydrogen bonds, forming lignin-carbohydrate complexes [36]. In fact, it cannot be found naturally detached from cellulose and hemicellulose. It is also responsible for the cell's swelling ability and water balance, nutrients transport in plants tissues and shows antimicrobial properties that allow for healthy plant growth [37, 38]. Lignin even regulates the atmospheric carbon concentration by balancing its production/degradation rate, thus greatly influencing earth's carbon cycle [39].

To be able to develop new sustainable materials, using lignin as feedstock in biorefineries, it is fundamental to adequately describe and understand lignin



physicochemical properties. Several analysis techniques may be employed to characterize lignin according to its molecular structure and composition [40]. For example, Carbon-13 (13C) Nuclear Magnetic Resonance (NMR) may be used for analysis of the lignin structural characteristics, including degree of condensation, along with moieties, such as protonated functional group (aromatic C–H), oxygenated functional group (aromatic C–O) and condensed functional group (aromatic C–C) [41]. Similarly, Phosphorus-31 (31P) NMR is useful for the quantification of free hydroxyl groups (OH) in lignin [42]. Gel Permeation Chromatography (GPC) is an analytical method that in this case, is helpful to determine lignin molecular weight distribution [43]. Fourier Transform Infrared spectroscopy (FT-IR) allows for the estimation of lignin's chemical composition and functional properties [44]. Thermogravimetric Analysis (TGA) is a destructive technique that can be used to monitor lignin's mass variation as a function of temperature, producing a degradation profile [45]. Differential Scanning Calorimetry (DSC) can be applied to further study the thermal behaviour of lignin and determine its glass transition temperature (Tg) [46].

Using these, and other complementary techniques, it is possible to understand that lignin is an amorphous phenolic polymer that comprises three distinct monomeric units or monolignols that undergo a process of lignification, in which they are crosslinked to yield its ultimate polymeric structure [39]. These aromatic units are: guaiacylpropane or G-unit (derived from coniferyl alcohol), syringylpropane or S-unit (derived from sinapyl alcohol) and p-hydroxyphenylpropane or H-unit (derived from p-coumaryl alcohol) [39].

Chemical structures of cellulose, hemicellulose and lignin, showing three monolignols and a typical  $\beta$ –O–4 linkage found in lignin are presented in Figure 2.



Fig 2. Chemical structures of: (a) cellulose (adapted from [47]), (b) hemicellulose (adapted from [48]), (c) lignin, with typical  $\beta$ -O-4 linkage, and (d) three monolignols of lignin (adapted from [49]).

Lignin biosynthesis can be separated into three distinct phases: (1) biosynthesis of monolignols, (2) transport, and (3) polymerization. Monomers are produced in cytoplasm through a complex chain of reactions involving deamination, hydroxylation, methylation and reduction. Then they are exported to the secondary cell walls to be polymerized with the aid of two enzymes: peroxidase and laccase [50]. Enzymatic polymerization of substituted phenols prompt the formation of different linkages and functional groups, such as hydroxyl and methoxyl [51]. Monolignol proportion and type of inter-unit linkages show some degree of variety, depending on plant species, the illumination conditions, climate, ecological location, growth, nutrition, tissue, presence of functional groups and even the extraction process [9, 52]. For example, softwoods mainly consist of G units, hardwoods essentially of G and S units, and grasses contain all the three units of H, G and S [53]. Typically, the monolignols inter-unit linkages found in lignin are 5–5',  $\beta$ –1,  $\beta$ – $\beta$ , 4–O–5 and  $\beta$ –O–4, with  $\beta$ –O–4–aryl ether bonds being the most common among all other linkages in most plant species [54]. This clearly indicates that lignin physicochemical characteristics are source-dependent [55].

## 2.1.2 Lignin potential applications

The pulp and paper industry is one of the main players in biomass conversion and a traditional source of lignin, which is often extracted in a hemicellulose rich mixture known as "black liquor" and burnt to generate electricity and heat [56]. Of the 70-100 million tonnes of lignin produced annually at these facilities, only about 2% are converted into value-added materials, such as dispersants, adhesives, surfactants or antioxidants in plastics and rubbers [9, 57]. By 2030, it is expected that the annual production volume will reach 225 million tonnes [58]. This means an intensification of waste of resources and environmental pollution [59]. Valorizing lignin obtained from paper industry as byproduct, using cost effective technologies is, therefore, essential to guarantee that this industry and second generation biorefineries remain economically viable and stable [9].

This is the most plentiful aromatic biopolymer on earth and the overall second most abundant biopolymer in plant tissues. The availability in the biosphere of this renewable polymer is estimated at more than 300 billion tons [60]. It also possesses interesting traits, including its nontoxic nature, stiffness, biodegradability, thermal stability, high carbon content and antioxidant properties, which led lignin to become a recent target for biomass valorization in industrial biorefineries [61]. Until then, it was treated as a low-value material.

Lignin was discovered in 1838 and, in the past decades, its potential applications have been investigated [62]. However, it was only recently that ligninbased products have emerged, with the push of governments around the world to search for more sustainable alternatives to fossil resources and to ensure biodegradability [63]. Nanoscale synthesis was first described in 2012 and, since then, LNP have drawn interest due to their biotechnological potential in a wide range of industrial processes [64, 65].

Wood-based products are commonly used in construction industry, but the main marketed adhesives and binders used on particleboards and plywoods come from fossil resources and many contain carcinogenic formaldehyde. Lignin, despite its structural complexity, can be effectively separated from black liquor to serve as a source for the production of bio-based adhesives for mineral wool in substitution of toxic phenolic resin [66]. This way, it is possible to design and manufacture low-cost, non-toxic and biodegradable green insulation panels to be used in the construction sector, while remaining competitive in terms of performance with conventional products [67]. However, fundamental research is needed to overcome some lingering difficulties, such as poor water resistance, when compared to fossil-based wood adhesives [68].

Despite all technical and economic issues holding back the adoption of lignin adhesives in the composite wood panels industry, its utilization is believed to be feasible [69].

Lignin can also be used in replacement of polyether polyol to produce polyurethane, a valuable chemical that may chemically modify asphalt binder [70, 71]. This lignin-based polyurethane, due to its benzene ring, shows satisfactory adhesion properties, protecting asphalt from continuous exposure to UV radiation and can even surpass the low and high temperature performances of petroleum-based asphalt binders [72, 73]. Moreover, this product is more environmentally friendly, once lignin has the ability to make it biodegradable without compromising the properties of derived products [74].

Although lignin, naturally, does not possess epoxide groups, it can cure epoxies to produce a resin used to fabricate bio-based surface coatings that display effective water resistance and protection against high temperatures [75-77]. Good antioxidant activity, the biocompatibility of its nanofibers, and their fine mechanical properties promise to make a reality the use of lignin-based products for biomedical or healthcare purposes, such as wound dressing [78-80].

As a binder, it is possible to combine lignin with silica or alumina to obtain hybrid lignin $-SiO_2$  or lignin $-Al_2O_3$  fillers, respectively, to improve thermo-mechanical properties of abrasive tools [81-83].

In the automotive industry, it can be used as an alternative to costly traditional carbon fiber precursors to fabricate lightweight and eco-friendly vehicle components [84-86]. Aware of the expanding market of electric vehicles, lignin can also be exploited in energy storage systems to produce lithium-ion battery anodes and cathodes with comparable electrochemical performance to traditional electrodes, with reduced material usage and cost [19, 87, 88]. However, lignin's role in lithium-ion batteries is not limited to being an active electrode, as it can also operate as a binder and even as an electrolyte [88, 89]. Due to its properties, lignin-based binders in lithium-ion batteries grant stability to the electrode and avoid capacity losses [90].

Although in an early stage of development, lignin may largely contribute to boost environmental performance and mechanical properties of 3D printing parts, as it is already used as a raw-material for two types of additive manufacturing technologies [91-93].

Lignin may also be effectively used as a carbon source in flame-retardants systems, preventing the growth of fire or slowing it down. These materials are renewable and eco-friendly with exceptional properties, regarding both fire and smoke suppression [94-96].

In the food industry, there has been a growing demand for natural antioxidant active packaging, as it presents several advantages over direct addition of antioxidants to the food [97]. In particular, these packages are expected to allow for a reduction in environmental impact, minimize food loss and contamination and reuse of by-products [98]. Low molecular weight and phenolic hydroxyl groups in lignin grant it antioxidant activity [99]. As such, there is interest in using LNP antioxidant properties to fabricate products for food industry. Also, LNP may be used as filler in polylactic acid (PLA) films, making use of its ability to inhibit the growth of Gram negative bacteria, to produce food packages with antibacterial properties [100]. Thus, incorporating lignin derivatives in food packaging films warrants that food can safely remain edible for longer, avoiding oxidation of fats.

Lignin may be incorporated into materials to enhance the properties of a vast array of products and assure they are biodegradable, conceivably making these materials a substitute for conventional plastics used in many industrial applications [101]. At nanoscale, materials behave differently from their bulk counterparts. This is due to the relatively larger surface area, which possibly makes them more chemically reactive, and because of the quantum confinement effects that alter their optical, electrical and magnetic behaviour [102]. This opens the door for the manufacturing of new materials as it is the case of nanocomposites. These are composites made of, at least, one nanometric building block such as one-dimensional nanotubes, twodimensional lamellar nanostructures, or three-dimensional nanoparticles, often referred to as fillers, and a polymeric matrix [103]. A minimum of 5 wt% nanofiller content in the matrix appears to be the threshold for a significant improvement in physical and mechanical properties [104]. Nanoscience and nanotechnology are concepts that were first proposed by the Nobel laureate Richard Feynman in 1959 [105] and that Maghrebi et al. [106] later defined as "the targeted and controlled synthesis/manipulation of materials, structures, devices and systems with accuracy/feature size of approximately 1-100 nm and preferably 2-50 nm". Nanotechnologies have been applied to almost every science field to generate various products with commercial applications.

Some of several potential market applications for lignin are illustrated in Figure 3.





Fig 3. Some lignin potential applications in various economic sectors.

Despite lignin-based products being seen as promising solutions to conventional plastics, there is still much research and development to be done for their uptake in various sectors. Current knowledge regarding lignin physical and chemical properties is still insufficient to maximize its valorization potential. For that to be possible, more research is needed to better understand the polymeric structures of lignin [9].

Deepening the understanding of lignin's recalcitrant behaviour and structural heterogeneity will allow the optimization of its recovery processes in biorefineries and prove its feasibility [107]. Also, an efficient fractionation process that makes a second-generation biorefinery conceivably profitable is yet to be achieved [9]. During fractionation, lignin  $\beta$ -O-4 bonds break and carbon-carbon (C–C) bonds tend to form. Then, low weight lignin fractions tend to condensate and that causes lignin to lose its reactive functional groups [39]. Further studies are needed regarding the purification of monomeric products, milder conditions of lignin depolymerization, more promising solvents and catalysts and improvements regarding lignin's reactivity [39, 108].

#### 2.2 Pretreatment processes

#### 2.2.1 Lignin separation from lignocellulosic biomass

In order to be used for different applications, lignin needs to be separated from the other constituents of lignocellulosic biomass. Lignin's complex chemical structures and wide molecular weight distribution results in heterogeneously arranged properties, hence limiting added-value applications [109]. Therefore, a selective depolymerization is a prerequisite for producing economically valuable chemicals from lignin, as a renewable raw material. However, the structural and chemical heterogeneity and complexity of lignin often complicate its separation from cellulose and hemicellulose, making it difficult to shape an industrial scale valorization process into reality [110].

This recalcitrance can be overcome by subjecting biomass to a given pretreatment, so that pure individual lignocellulosic fractions can be obtained. The goal of any given pretreatment is to break the lignin seal and disrupt cellulose's crystalline structure to make it more accessible to the enzymes responsible for converting carbohydrate polymers into fermentable sugars. Hence, the resulting streams can be further valorized [111]. A pretreatment would, in theory, allow the extraction of bulk lignin and its continuous processing into lignin nanoparticles that could be, then, incorporated in a polymeric matrix to produce a given biomaterial.

The design and development of a chemical process, enabling efficient lignin depolymerization, while maintaining its economic viability in a truly holistic biorefinery, is thus a great challenge [112]. Therefore, the selection of a pretreatment method should follow a few principles that include: (1) produce digestible solids, increasing monosaccharide yield; (2) avoid degradation of monosaccharides derived from cellulose and hemicellulose; (3) reduce the formation of inhibitors that may put subsequent steps at stake; (4) recover lignin for further valorization, and (5) minimize heat and power requirements in order to be cost effective [113]. In addition, to guarantee the sustainability of a pretreatment, the process should operate at mild conditions, use low-toxic reagents with high recyclability, low-profile reactors and, once again, reduce the use of water and energy [114].

Li et al. [115] published an article in which they addressed the advantages and disadvantages of various processes for lignin extraction from biomass, such as: base and acid-catalyzed depolymerization techniques (including the use of non-conventional solvents ionic liquids), pyrolysis, hydroprocessing, oxidation, gasification and liquid phase reforming. These authors provided a detailed description of those conversion strategies and analysed the respective technical lignins in terms of their yield, impurities, linkage type prevalence and other aspects that influence the biorefinery feasibility. These authors stated that using formic and acetic acid to fractionate biomass and retrieve lignin for further valorization has a negligible impact on the environment, and that aiming towards lignin-based aromatic products may turn out to be a sustainable transition from the petrochemical industry [115].

Upton et al. [70] review focused on the conversion of isolated lignin into various products such as monolignol-derived products (e.g. polyurethanes, polyesters, epoxide and phenolic resins, hydrogels, polyamides and lignin-derived chemicals), including vanillin, vanillic acid and vanillic alcohol, and also analysed lignin chemical degradation. The authors claimed that methods capable of using lignin as a source for the synthesis of new materials without additional degradation would be more



environmentally friendly. These authors also identified an emerging biorefining method, organosolv (OS), as having interesting environmental performance [70].

Sun et al. [116] analysed new approaches for lignin extraction through oxidative and reductive depolymerization, acid-catalyzed depolymerization, biochemical transformation of lignin and their potential applications into thermosets, thermoplastics, and compounds with pharmacological activity. An overview of value-chains starting from raw lignocellulose to potential final application of lignin-derived monomers was provided. Environmental considerations were limited to assertions that OS has advantages over kraft or sulfite processes, that electrochemical oxidation is preferable over chemical oxidants and the authors also stated that the synthesis of some products used biorenewable and environmentally benign solvents [116].

#### 2.2.2 Kraft pulping

Kraft pulping is the most widely used method at industrial scale, to obtain different streams of lignocellulosic components. However, biomass processing can be done in several ways, often broadly categorized as physical, chemical, physicochemical and biological techniques. Physical techniques include milling, pyrolysis, extrusion and microwave irradiation (see section 3.6). Chemical pretreatment types include, for example, OS (see section 3.4), ionic liquids (see section 3.5), acid and alkali pretreatment (see section 3.6). Physicochemical pretreatments of biomass are, in particular, hydrothermal (SE and LHW) (see section 3.3), ammonia fiber expansion, wet oxidation and sulfite pretreatment (see section 3.6). Biological pretreatment is succinctly discussed in section 3.6 [117].

The Kraft process was first developed in the 19th century and created relatively more resistant paper than other existing processes that could be used for a wide variety of applications [118]. In the pulp and paper industry, where kraft process is the dominant method to produce paper, lignin present in the resulting black liquor typically contains inorganic sulfur [119]. Sulfur content is a determining factor for the lignin categorization on the market. Since most of the lignin found on the market contains sulfur, this limits its potential applications, as it comes with greater environmental impacts and possesses an unpleasant and potentially harmful odor when compared to emerging sulfur-free lignin [120]. Second generation biorefinery process, in turn, is coming out as a source of sulfur-free lignin, and free from other impurities, allowing it to be used for producing valuable products [9]. OS pretreatment has environmental and economic advantages over conventional processes such as kraft. This is because the recovered lignins are sulfur-free with low molecular weight and the purest of all



industrial lignin [121]. Also, the structure of OS lignin (comprising parameters such as molecular weight and type of linkages) resembles more closely the structure of native lignin than kraft or other sulfur-containing lignin [39]. Hydrothermal pretreatment of lignocellulosic biomass is another sulfur-free process that is discussed in this article, addressing its advantages, limitations, role in today's biomass pretreatment landscape and foreseeable future.

The Kraft process is composed by three main steps: (1) digestion or biomass cooking; (2) separation between pulp and black liquor, and (3) recovery of chemicals for reuse [118].

In the first step of kraft pulping, digestion, biomass is cooked in white liquor, which is a mixture of sodium hydroxide (NaOH) and sodium sulfide (Na<sub>2</sub>S), dissolving lignin and some hemicellulose, a process conducted under a batch or continuous system. Gases are removed from the digester and the remaining chemical mix after cooking (black liquor) is transferred to the blow tank. Sodium soaps formed from resin acids and fatty acids are removed from the black liquor and treated with acid to generate as byproduct tall oil, also called liquid rosin or tallol, a viscous yellow-black odorous liquid. Before the second phase, washing, the uncooked fibers are separated from the rest of the pulp. From the blow tank, pulp is washed with water, removing weak black liquor. There are different methods to wash the pulp, all targeting the highest removal efficiency, being the most common the rotary vacuum washer. The black liquor is then sent to the chemical recovery process, starting with evaporation to remove the excess water. There are two methods to conduct evaporation, either by direct contact with the recovery boiler and subsequent black liquor oxidation or by indirect contact. Oxidation is needed to get rid of odorous compounds and to convert sodium sulfide (Na<sub>2</sub>S) to sodium thiosulfate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>). Following evaporation, black liquor is sent to the recovery boiler where it is burned to provide heat to generate steam. Inorganic chemicals, such as sodium sulfide (Na2S) and sodium carbonate  $(Na_2CO_3)$ , originate a smelt in the furnace that is discharged to a dissolving tank where green liquor is produced by soaking the smelt with water at high temperature. This green liquor, in turn, is released into a causticizer and calcium hydroxide  $(Ca(OH)_2)$  is added to sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) to convert it to sodium hydroxide (NaOH) and precipitate calcium carbonate ( $CaCO_3$ ). The precipitate is converted into calcium oxide (CaO), which then reacts with water to produce calcium hydroxide (Ca(OH)<sub>2</sub>) needed in the causticizer [118].

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Fig 4. Flowsheet of kraft pulping process of biomass (adapted from [118]).

Lignin is the main by-product of the kraft pulping process. Despite its primary use in the pulp and paper industry as energy generator, kraft lignin (KL) can be valorized and has been applied in polymers as filler to enhance their adsorption, structural and morphological properties [122]. Nonetheless, KL demonstrates limitations concerning its valorization possibilities, due to the high degree of impurities like sulfur [123]. For example, adding KL to polyurethane foams increases their strength up to 23% (w/w), but still not as much as OS lignin [124]. It is possible to produce vanillin, a valuable chemical, from KL [125]. This process, however, results in relatively small yields (~9%) and room for improvement with respect to the selected solvent [125]. Also, KL production is highly sensitive to market fluctuations, thus being essential a well-planned supply chain design model to narrow the uncertainty surrounding the feasibility of the process [126]. In respect to the use of catalysts in the kraft pulping process, NaOH may be a viable option as it is easily recovered, boosting the efficiency and economic performance of the biorefinery [122].

### 2.2.3 Hydrothermal pretreatment

To be sustainable, a biorefinery needs to integrate valorization solutions for all the biomass components in its process, which is not yet the case for lignin [114]. Choosing the most suitable biomass pretreatment is essential to make it possible a more comprehensive use of biomass components. Purely physical pretreatments are often ineffective in the complete digestion of lignocellulosic biomass, due to its complex polymeric network. In turn, a physicochemical pretreatment, such as hydrothermal pretreatment, provide an adequate conversion of biomass into digestible products. Hydrothermal pretreatment has recently made exciting progresses, encouraging industries to, at least, consider this technique on commercial scale. The two main types of hydrothermal pretreatment are liquid hot water (LHW) that uses water in its liquid state, and steam explosion (SE) that utilizes water in gaseous state to treat biomass, both work at high temperatures. For roughly 100 years, LHW and SE have been used to pretreat biomass to generate different products, such as paper, structural materials and biochemicals [127]. Water will serve as catalyst, since under this high temperature conditions, water shows acidic properties [128]. Overall, hydrothermal pretreatment is considered safer, less expensive and more environmentally friendly than other pretreatment methods. This is because it does not use toxic and corrosive chemicals, such as acid catalysts, whose downstream treatment normally requires significant costs. By simply using water as the reaction medium, through this process it is possible to disrupt the lignocarbohydrate matrix and solubilize hemicellulose.

In the steam-explosion (SE) pretreatment, lignin is obtained from biomass, such as wood, after being subjected to high severity conditions (high temperature and pressure) followed by a quick decompression to atmospheric levels [129]. The SE process can be used to treat a wide variety of lignocellulosic biomass, this way generating different products, depending on the type of feedstock used. The use of a catalyst is not absolutely necessary, given the fact that hydrolysed hemicellulose releases acetic and other organic acids that will assist in the cleavage of ester and ether bonds of the lignocarbohydrate matrix [130].

SE pretreatment, as Hongzhang and Liying [131] described, takes place in a reaction vessel of a given volume. Optimal conditions must be guaranteed to extract the maximum of each lignocellulosic fraction. In the case of wheat straw, this is achieved at 34% moisture, 1.5 MPa pressure and 4.5 min time [131]. Right after being steam exploded, air-dried pretreated biomass is washed in countercurrent four consecutive times, at 75 °C of temperature, to extract hemicellulose. Following washing, the mixture is heated and filtered. Purification of the strong liquor can be



achieved with the aid of a chelating ion exchange resin at room temperature. The severity factor establishes the temperature and residence time of the extraction conditions and is calculated as follows:

$$R = t \times exp\left(\frac{T - 100}{14.75}\right) \tag{1}$$

where t is the residence time (min) and T the hydrolysis temperature (°C) [132]. The fiber obtained is then treated with ethanol at alkaline conditions to extract lignin and cellulose. Cellulose is removed through vacuum filtration and the resulting liquor is distilled to recover ethanol to be reused as solvent in the previous extraction step. Cellulose crystallinity can be evaluated through total crystallinity index (TCI) and lateral order index (LOI). After distillation, the process requires that the pH of the concentrated liquor is reduced to precipitate lignin. Precipitated lignin is then dissolved in acid, centrifuged, washed to remove any odor, dried and purified. Purification can be achieved by dissolving dry lignin into ethanol, mixing the solution with ether to promote sedimentation of fine lignin that, in turn, can be washed, dried and milled.

A hydrothermal pretreatment may also be subcategorized into LHW. This process is similar to SE, as it also uses water under increased temperature, but usually from 130°C to 240°C, and pressure to keep water in liquid phase instead of using steam. This can be done in a batch or co-current reactor, a flow-through reactor or in a counter-current reactor [133]. In a batch reactor, water and biomass are held and heated together during a certain amount of time, while in a flow-through reactor biomass remains immobilized while hot water flows on top of it. In counter-current reactors a flow of hot water flows in the opposite direction to that of the biomass flow.

Morales et al. [134] described various scenarios of an integrated biorefinery using LHW or autohydrolysis as pretreatment for lignocellulosic biomass. First, biomass is hydrolysed at 179 °C for 23 min, which are the optimal conditions for oligosaccharides extraction. The reactor was then cooled to 70 °C and the mixture is filtered through vacuum, separating the liquid fraction (rich in oligosaccharides) from the solid fraction, which is washed with water afterwards. Then, it comes the delignification phase that is accomplished via six distinct paths, three of which use OS treatment in a two-step hybrid treatment (discussed in section 3.4.3) and the other three are accomplished through alkaline treatment. The alkaline treatment is carried out at 121 °C for 90 min in an autoclave with a sodium hydroxide (NaOH) solution. The resulting mixture is, once again, vacuum filtered to separate black liquor from delignified solids. The solids are washed, resulting in a cellulose rich fraction, and black liquor is precipitated by acidification with sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) to obtain lignin [134].



Fig 5. Flowsheet of the hydrothermal (SE) process for the biomass pretreatment (adapted from [131]).

#### Liquid hot water

LHW reaction medium is low cost, as it just uses (subcritical) water, not requiring washing and neutralization of pretreated biomass, nor wastewater treatments, which results in the economic feasibility of the process. The material used for the assembly of the reactor is also inexpensive and feedstock biomass does not require to be preprocessed to reduce its size. The reduced operational and capital costs of LHW make it an attractive alternative for biomass treatment at industrial scale. Regarding its functional goals, LHW is able to satisfactorily disrupt the lignin and cellulose structures and hydrolyse hemicellulose with relatively high recovery and purity [127, 128]. Different types of biomass were analysed under different LHW operating conditions in order to identify the ideal pretreatment conditions for each of them, showing that temperature has a larger influence on the pretreatment severity than residence time, as noted by an increase of residual lignin on biomass surface when temperatures exceed 200 °C [135]. Temperature and the flow rate of the process, impact cellulose degradation and biomass dissolution, respectively [132].
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Comparably, batch reactors have less demanding energy and water requirements to operate, but hemicellulose sugar yields and cellulose digestibility is inferior in respect to flow-through and counter-current reactors. A batch process is also more sensitive to pretreatment severity than other configurations [127]. A flow-through process allows for continuous removal of dissolved components, avoiding lignin condensation, but results in a diluted sugar stream that hinders an efficient fermentation [127]. Another advantage of a flow-through process is that the inhibitor concentration in the hydrolysates is low. There are, however, some inconveniences, including higher-energy consumption and larger amount of wastewater compared to batch processes, which may impact the environmental performance of the process, and insufficient removal of lignin from both hard and softwood [128, 135]. Furthermore, the structural effects that this type of system have on biomass and its components are still not fully comprehended (e.g. in comparison to a standard batch reactor) and are difficult to test at laboratory scale [133].

The hydrothermal pretreatment's main disadvantage is the production of inhibitory compounds that hinder subsequent steps in a biorefinery [130]. The role of a catalyst may be of significance in the LHW. An alkali catalyst, as sodium hydroxide (NaOH), is capable of boosting the yield of glucose relatively to the absence of a catalyst [136]. Also, controlling the pH using a catalyst can limit the formation of toxic inhibitors, such as furfural ( $C_5H_4O_2$ ) and hydroxymethylfurfural (HMF) ( $C_6H_6O_3$ ), thus assuring an efficient enzymatic saccharification of cellulose for formation of monomeric sugars [128]. Despite the use of a catalyst not being absolutely necessary, an alkali catalyst may play an important role in the overall process efficiency by maintaining the optimized pH value [136]. Nevertheless, products still tend to repolymerize and condensate [137].

#### Steam explosion

There are some parameters that can be optimized in the SE process to improve its efficiency. For instance, using catalysts such as carbon dioxide (CO<sub>2</sub>), sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) or sulfur dioxide (SO<sub>2</sub>) will aid in creating the acidic conditions necessary to activate several hydrothermal reactions [130]. One way to improve the economic efficiency of the SE process is to recycle the liquor that will, in turn, reduce the amount of water needed [131]. If conditions are optimized, high yields of pure cellulose and hemicellulosic sugars can be obtained and formation of inhibitory compounds minimized. In comparison, LHW is less sensitive to the severity of the pretreatment [127]. SE also significantly reduces the ash content of biomass, even more than traditional kraft process [138]. Lignin and hemicellulose removal increases the efficiency of hydrolysis and saccharification, by making the biomass porous [130].



While this method produces hydrolysable cellulose and fermentable hemicellulose, it does not result in an effective fractionation of different streams of cellulose, hemicellulose and lignin. In addition, lignin separated from hemicellulose is often too degraded to be used in added-value applications and is, instead, burned as a source of energy to feed the process [139].

Prasad et al. [140] aimed to perform, simultaneously, an LCA and an analysis of the overall product yield of various pretreatment methods of biomass. The pretreatments were: LHW, SE, diluted acid and OS. They concluded that LHW, in this case, was the most technically effective method as it yielded twice as much sugar than any of the other three processes, converting ~91% of glucan and ~82% of xylan [140].

#### 2.2.4 Organosolv pretreatment

OS is an attractive alternative to the conventional kraft process that allows the extraction of pure lignin from the lignin-carbohydrate complex. This comprises a treatment of plant-tissues based on an aqueous solution of organic solvents, with or without the addition of a reaction catalyst, depending on the selected process temperature. This results in three different process streams: cellulose, lignin and hemicellulose [141]. The OS fractionation is based on the partial solubility of lignocellulosic polymers into a given solvent [123]. Cellulose and lignin are recovered as a solid fraction and hemicellulose is dissolved in water [139]. Typically, the OS fractionation is either alcohol or organic acid-based and the process can be performed under different conditions of pressure and temperature.

The OS pretreatment of biomass is a process that dates back to more than a hundred years ago, when the delignification of wood was achieved using ethanol and hydrochloric acid [139]. In 1931, the use of aqueous ethanol to obtain undegraded lignin was proposed as a more efficient process when compared to kraft or sulfite pulping [139, 142]. However, this process has not yet been used on an industrial scale. Some of the setbacks that currently frustrate attempts to widely implement OS fractionation on an industrial scale are the health and environmental risk, toxicity and high cost associated with solvents.

In OS pretreatment, the main components of lignocellulosic biomass (cellulose, hemicellulose and lignin) are separated into three different streams via a treatment in aqueous solution with organic solvents (alcohol or acid), normally at high temperatures and pressures. A ketone can also be used, as there are reports of using it as solvent in OS [143]. Cellulose is recovered as a solid fraction and hemicellulose and products derived from sugar degradation are present in the soluble fraction, such as furfural and



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HMF inhibitors [139]. Water is added to promote the precipitation of lignin that is recovered as a high-purity precipitate, after being diluted and filtered and that, once extracted, is washed and dried [139, 144]. In OS, the organic solvent used in the pretreatment may be reused, after being recovered by evaporation [144]. A broad spectrum of organic solvents has been investigated to assess their potential for selective solubilization of lignin, including alcohols (ethanol, methanol and butanol) [145] and organic acids (acetic acid and formic acid) [146] with or without catalysts. Formic acid fractionation is usually performed under high temperatures and pressure. Nonetheless, conditions may be adjusted to lower temperatures and atmospheric pressure, but, on the other hand, more time is needed to complete the treatment [147]. The pretreatment can be autocatalyzed when performed at higher temperatures (around 185-210 °C) as released organic acids act as catalysts themselves to aid in the rupture of the lignin-carbohydrate complex, but catalysts may also be added to increase the rate at which lignin is removed and lower the temperature necessary for the reaction to occur. These may either be an acid, a base or a salt. Most commonly, the catalyst used in standard ethanol or methanol OS treatment is a mineral acid, such as hydrochloric acid, phosphoric acid or sulfuric acid [148]. However, organic acids, such as oxalic, salicylic and acetylsalicylic acids can also be used [142]. Moreover, a variety of catalysts have been tested alongside ethanol and methanol in OS pretreatment, such as magnesium, magnesium sulfate, calcium/barium chloride/nitrate, sodium bisulfate and sodium hydroxide [149]. Alkaline catalysts, like sodium hydroxide (NaOH), can also be used to aid OS treatment [150]. Commonly used biomass feedstocks including pine, poplar, switchgrass and *Miscanthus* have been treated by a combination of ethanol, as the solvent, and sulfuric acid ( $H_2SO_4$ ) as the catalyst [139].

In alcohol OS pretreatment, whether the solvent is ethanol or methanol, four different stages take place: (1) the linkages between lignin and hemicellulose are hydrolysed by cleavage of 4-O-methylglucuronic acid ester bonds and the same happens to internal lignin bonds, once  $\alpha$  and  $\beta$ -aryl ether linkages are cleaved. This leads to the solubilization of both lignin in stable fragments and hemicellulose; (2) glycosidic bonds of hemicellulose and, to some extent cellulose, are cleaved; (3) formation of monosaccharides' degradation products, such as furfural (C<sub>5</sub>H<sub>4</sub>O<sub>2</sub>), HMF (C<sub>6</sub>H<sub>6</sub>O<sub>3</sub>), formic acid (HCOOH) and levulinic acid; (4) lignin condensation begins to take place through a set of complex reactions that happen more vigorously in the case of the pretreatment being acid-catalyzed [139].

In the case of acid OS pretreatment, using formic acid as solvent,  $\beta$ -O-4 linkages break with a series of consequences: increase of phenol hydroxyl, condensation of lignin through formation of stable C–C bonds, and formylation of lignin

side chain. Lignin condensation reaction is deemed unavoidable during acidic OS fractionation, affecting lignin recovery process, biodegradation processes and lignin-based materials [123, 151].

OS pretreatment, as described by Laure et al. [152] is achieved using ethanol as solvent in a 1:1 ratio to water. The first phase of the process is pulping, which consists of a batch digester with forced circulation, containing the lignocellulosic biomass operated under elevated temperature (180 °C) and pressure (18 bar). Sulfuric acid, optionally, can serve as catalyst. The biomass may be hydrothermally pretreated beforehand in a two-step hybrid process. The pulped biomass is diluted and dewatered and cellulose is hydrolysed with separation of the lignin residue from the liquid. Lignin precipitation is either achieved through addition to water and filtration or by ethanol evaporation. Then, it is washed with water and dried in vacuum. In another batch, ethanol is recovered from resulting filtrates of lignin precipitation and washing liquors to be reused again.



Fig 6. Flowsheet of the organosolv process for the biomass pretreatment (adapted from [152]).

Lignin recalcitrance and low reactivity are obstructing its valorization and, hence, the very profitability of biorefineries. Once OS pretreatment allows the efficient utilization of all major lignocellulosic biomass fractions, it can potentially be attractive from an economic standpoint. This method is able to effectively fractionate lignocellulosic into three individual streams (cellulose and lignin in the solid fraction and hemicellulose in the liquid fraction) [139]. Resulting lignin is recovered in its undegraded form and can potentially be used as a high-quality source for added-value materials and chemicals, thus being a more efficient use of lignocellulosic feedstock when compared to traditional techniques [123]. OS lignin has low molecular weight

(lower than lignin obtained from physical pretreatments, for example), which increases its solubility, has low ash content and is rich in aromatic rings, is sulfur-free and the purest of all industrial lignin, including kraft [121, 123, 138].

Cellulose obtained via OS fractionation shows a low degree of polymerization and alterations in its crystalline structure, which leads to increased cellulose recovery and enzymatic digestibility [139]. Up to 90% of dissolved lignin can be recovered from the liquor, following precipitation and washing and an encouraging conversion rate of cellulose to glucose (86%) via enzymatic hydrolysis can also be achieved [152].

In OS, hemicellulose is hydrolysed into soluble components, such as oligosaccharides, monosaccharides and acetic acid and degradation products, including furfural and HMF, form and, despite some of these secondary coproducts may be fermentation inhibitors, they can all be precursors of valorized products [139].

To ensure the economic sustainability of a biorefinery that uses OS as pretreatment, the solvent must be recovered in order to avoid high costs, for instance those inherent to waste water treatment [152]. Thus, ethanol recovery and reuse is fundamental to avoid further inputs of makeup solvent. Solvent recovery can be very close to 100% (up to 98% and 96% of ethanol and methanol, respectively, can be recycled), but ethyl glucosides that bind ethanol to carbohydrate components of lignocellulosic biomass tend to hinder the complete recovery of ethanol [153-155]. Nonetheless, those same ethyl glycosides are partially decomposed during solvent recovery by distillation [154]. The removal of solvents used in OS from the system is a necessity from a technical point of view, because they hinder enzymatic hydrolysis, fermentation and the growth of microorganisms. So they are usually drained from the reactor, evaporated, condensed and recycled [141].

The Hildebrand solubility parameter ( $\delta$ ), calculated using Equation 2, can be used to provide a numerical estimate of the degree of solubility of lignin or other polymers [156].

$$\delta = \sqrt{\frac{\Delta H \nu - RT}{Vm}}$$
(2)

where  $\Delta Hv$  is the heat of vaporization, R is the universal gas constant, T the absolute temperature and Vm the molar volume. The closer the  $\delta$ -value of an organic solvent medium is to that of lignin, greater solubility of lignin is observed, thus leading to an increased degree of delignification [156].

The most commonly used and studied extraction method of OS lignin is based on low boiling ethanol/water pulping, the Alcell® being an example [157]. This process uses a 50% ethanol solution to extract lignin under elevated temperatures and



pressure [158]. Ethanol is a low cost chemical and is easily recovered through distillation, but using high-boiling organic solvents allow for pretreatment to occur at atmospheric pressures, reducing energy requirements, which is a significant advantage [159]. As an alternative to standard ethanol fractionation, butanol (C<sub>4</sub>H<sub>10</sub>OH) is an effective solvent, given its hydrophobicity [160]. The limited miscibility of butanol in water makes it possible to concentrate hemicellulosic sugars in the aqueous layer, cellulose in the solid fraction and lignin in the butanol layer, thus resulting in an efficient separation of all streams. Organic acids, such as acetic acid and formic acid too offer effective fractionation of biomass and selective removal of lignin [161]. Even though the  $\delta$  -value of acetic acid is similar to that of lignin, it is not as widely used in biomass pretreatment as organic alcohols due to its corrosive nature. Formic acid has a lower boiling point than acetic acid and, therefore, is considered promising for the OS treatment. Additionally, it is a weak and volatile acid, which allows it to be directly recycled for further fractionation [159].

As previously mentioned, acetone can be yet another alternative to ethanol to serve as a solvent in OS treatment of biomass. The process described may be carried out at low temperatures and, compared to ethanol-based treatment, obtained lignin closely resembles native lignin (with a ~67% solubilization), while high yields of cellulose and monomeric hemicellulose sugars are achieved. Some lignin is lost to the surface of the equipment due to inefficient washing, however it is a promising method that is scalable and upgradable [143]. Physicochemical characteristics of the solvent, namely hydrogen bonds between solvent and lignin, are crucial to the extraction process and to the final properties of recovered materials [162]. Optimal solvent concentration to achieve maximum lignin solubility was found to be 60-70%, while higher solvent concentrations lead to increased degradation of carbohydrates [163].

It is possible to lower the required temperature that allows the reaction to happen and to increase the rate of delignification by addition of a catalyst [142, 149]. A mild alkali catalytic OS pretreatment, based on a NaOH-methanol mixture, resulted in very high glucose yield (~98%) and extended delignification (~87%), with recovered lignin being highly pure and possessing an evenly distributed molecular weight and high thermal stability [150]. A sequential OS fragmentation approach that uses ethanol as solvent and different-stage catalysts has also been developed. This method ends up producing high-quality LNP, while also significantly increasing the yield of glucose and xylose [164]. Knowing that the quality of lignin monomers are important to the quality of the end product, OS pretreatment also shows to positively impact lignin-based products, such as adhesives [165].

### 2.2.5 Organosolv-hydrothermal hybrid pretreatment

Increasing the pretreatment severity will enhance the lignin solubility, but in turn it will also degrade a larger proportion of carbohydrates. To achieve the best possible delignification rates, but still avoid sugar degradation, a few studies have proposed a combination of OS with another pretreatment method, such as LHW and SE. Morales et al. [134] proposed one such system. In a first phase of the process, biomass would go through autohydrolysis in a LHW pretreatment, as described in section 3.2.2. In a subsequent stage, lignocelluloses could be treated with the OS technique for 90 min, in a reactor at 200 °C, where ethanol was added in a 70% concentration. Then, the obtained mixture would be filtered by vacuum, obtaining black liquor and a solid fraction rich in cellulose, after washing. Lignin was then precipitated from black liquor by adding two volumes of acidified water, recovered through vacuum filtration and washed with water. The treatment resulted in lignin with high purity (~90%), that could potentially be valorized into various products, and cellulose-rich solids that could be used to produce cellulose nanocrystals or glucose through enzymatic hydrolysis. However, this route was also less efficient than the alternative alkaline delignification pathway [134].

Michelin et al. [166] described a sequential hybrid pretreatment, consisting of both hydrothermal and OS techniques. Using a standard batch reactor, biomass was first dried and milled for size reduction. Then, it was mixed with distilled water in the reactor and heated to 200°C, for a total of 30 min. Afterwards, the reactor was rapidly cooled down. Through filtration, the resulting insoluble solids were separated from the slurry or liquid fraction and, then, washed and dried. Hemicellulose-derived compounds and soluble lignin could be found in the hydrolysate, along with other by-products. Combining LHW pretreatment with OS, using ethanol as solvent, in a single process, resulted in the most effective way to, simultaneously, obtain high-purity lignin with good antioxidant capacity and hydrolyse cellulose [166].

Gullón et al. [167] described a variety of biorefinery approaches, one of which consisted of a combination of autohydrolysis and OS techniques. First, biomass would be hydrothermally treated at 200 °C in a reactor under pressurized conditions. Once completed the autohydrolysis, the reaction media was cooled, and the solid and liquid fractions were separated through centrifugation. Then, ethyl acetate was added to the liquid stream to act as solvent and the organic and liquid fractions were separated via decantation. The organic phase was vacuum-evaporated, resulting in the recovery of the solvent and obtaining an antioxidant-rich extract, while the aqueous phase was concentrated to obtain a stream rich in oligomeric compounds. Meanwhile, the initial

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solid fraction resultant from the autohydrolysis step was delignified to separate lignin from cellulose. This step differs among the described routes, but a particular one uses OS. The chosen solvent was ethanol at a concentration of 50% and the treatment was performed for 90 min at 200 °C. Afterwards, the reactor was cooled down and filtration takes place, separating the delignified solid from black liquor. Lignin was then precipitated from black liquor, filtered and washed until neutral pH and the solid fraction was washed and filtered. Delignification was proved to be more efficient in alkaline treatment than in OS, but of less purity [167].

SE demonstrates a positive saccharification yield, but lacks in fractionation potential. Conversely, OS offers effective delignification, but its biomass deconstruction is a handicap. Matsakas et al. [168], therefore, thought about the possibility of a synergetic effect of these two treatments to grant a hybrid process that incorporates the best qualities that each of its steps has to offer. Results showed that combining OS, using ethanol as the solvent and sulfuric acid as the catalyst, and SE allowed for efficient fractionation of lignocellulosic components (~86% of delignification) and high hydrolysis rate of the solid fraction (~68% with low enzyme load). Moreover, lignin was highly pure, with little ash content [168]. Following publications agreed with these conclusions and warned about the potential applications of this high-quality lignin for various added-value products [169, 170]. Matsakas et al. [171], additionally, performed a technoeconomic assessment of the proposed biorefinery system and concluded that the high steam demand of the process and the degradation of hemicellulosic sugar derivatives negatively impacted the economic performance. Thus, there is room for improvement.

#### 2.2.6 Ionic liquids and deep eutectic solvents

The increased pressure to produce biodegradable materials integrated into a circular economy has pushed forward the search for sustainable pretreatments that enable the valorization of all biomass components. Solvents play a critical role in the pretreatment of biomass. Therefore, developing new ones that simultaneously fulfill their role as solvent and align with the environmental responsibilities of biorefineries has become a priority for researchers [172]. An approach for lignocellulosic biomass pretreatment based on ionic liquid (IL) as solvent has, in recent years, gained interest among academics and industries. Ionic liquid is a molten salt at room temperature that is easily synthesized and recovered, while avoiding the environmental pollution, often associated with traditional organic solvents. Thus, it is often regarded as green solvent [173]. However, it is recommended to use such labels with caution, as ILs may be toxic



to organisms, depending on the length of the alkyl chain of the cations and its head groups [174]. Even though, ionic liquids do offer lower sugars degradation and a selective removal of lignin. This process is still significantly more expensive than subcritical water hydrolysis or LWH, the most favoured process [130]. Normally, the recovery of solvents and the separation of various chemicals used in acidic, alkaline, ionic liquids and other types of treatments is expensive and energy intensive. Ionic liquids can also be used as cosolvents in mild binary systems, consisting of biomass-derived  $\gamma$ -valerolactone (GVL) as the other cosolvent. This is a low cost solution to efficiently dissolve lignin, a potential advantage over standard IL solvation [175].

Nonetheless, an exciting new class of IL has recently emerged, named deep eutectic solvents (DES). Despite their physical properties being similar to those of ILs, chemical characteristics may be significantly different, as the former contain various species of cations and anions, while the latter are mainly composed by only one type of cations and anions [176]. DES are low cost, biocompatible, easier to synthesize, show high biodegradability and, depending on its composition, are non-toxic [177]. Contrarily to typical IL, the higher the treatment temperature is, the less the amount of lignin dissolved by DES [61]. Furthermore, it is possible to incorporate water into the system without compromising DES dissolution performance [61]. They appear to be a promising green and low-cost class of solvents, due to their tunable physicochemical properties and application versatility, including in biomass processing. Nevertheless, their high viscosity and complicated behaviour modelling are issues to account for [178]. As DES are viscous at ambient temperature, the solvent tends to adhere to the biomass and its recovery is more demanding [179].

#### 2.2.7 Alternative pretreatments

Nauman et al. [117] assembled a list of lignocellulosic biomass pretreatments and clustered them into their respective categories. According to these authors, within the class of physical pretreatments, milling [180, 181] is used with the aid of different kinds of devices to reduce the size of lignocellulosic components with the goal of making them more digestible to enzymes. Pyrolysis [182] is used to produce bio oil by thermally degrading lignocellulosic biomass at extreme temperatures (500 – 800 °C). Microwave irradiation [183] carried out at high temperatures in a closed container and in the presence of water, breaks chemical bonds between lignocelluloses, making them more susceptible to enzymatic activity. Mechanical extrusion [184] is a process used to force lignocellulosic material through a die, creating a material with the desired cross-sectional profile. In respect to the chemical pretreatment subcategory, acid

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pretreatment [185] is done in two different methods: it may either be done in a short period of time at high temperatures or have a longer duration at lower temperatures. Different types of reactors have been developed, but all of them must show resistance to the corrosive power of the acids. It has several disadvantages, namely the production of inhibitory products and the expensiveness of the process, making it a less preferable solution, when it comes to biomass pretreatment. Lignocellulosic biomass can also be pretreated with bases in alkali pretreatment [111, 186], which requires more amenable conditions of temperature and pressure, but, in turn, it takes longer to complete.

Ammonia fiber expansion (AFEX) [187] is a physicochemical pretreatment that uses concentrated ammonia as catalyst. It is a similar technique to SE, where biomass is cooked in a pressurized reactor during a given period, at the end of which steam is rapidly released. This method does not remove lignin. Another physicochemical pretreatment is wet oxidation [188]. In this technique either a mixture of oxygen and water or hydrogen peroxide is used to treat biomass at high temperatures in a pressurized environment through induced hydrolysis. It is typically employed to treat wastewater and remediate soil. Sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) [189] is yet another physicochemical pretreatment of biomass. It consists of first removing lignin and hemicellulose fractions with magnesium or calcium sulfite (MgSO<sub>3</sub> or CaSO<sub>3</sub>) at high temperatures and then, milling the obtained biomass to reduce its size. Biological pretreatment [190] treats lignocellulosic biomass with live microorganisms that promote the enzymatic removal of lignin as well as lytic cleavage of both cellulose and hemicellulose [117].



**Table 1.** Alternative pretreatments of biomass clustered according to their classification and reported experiment conditions and target product.

| Technique        | Classification  | Temperature | Pressure | Duration      | Target Product | Reference |
|------------------|-----------------|-------------|----------|---------------|----------------|-----------|
|                  |                 |             |          |               |                |           |
| Milling          | Physical        | <30 °C      | -        | 8 h           | Wood Chips     | [180]     |
| Pyrolysis        | Physical        | 500-950 °C  | 5-20 bar | <1 s - 30 min | Char, oil, gas | [182]     |
| Microwave        | Physical        | -           | -        | 10 - 50 min   | Char, oil, gas | [183]     |
| irradiation      |                 |             |          |               |                |           |
| Mechanical       | Physical        | 60-130 °C   | 780 psi  | 3 - 25 min    | Bioethanol     | [184]     |
| extrusion        |                 |             |          |               |                |           |
| Acid treatment   | Chemical        | 80-120 °C   | -        | 30 - 120 min  | Hemicelluloses | [185]     |
| Alkali treatment | Chemical        | 25-130 °C   | -        | 1 - 13 h      | Glucose        | [111]     |
| AFEX             | Physicochemical | 60-110 °C   | 113 bar  | 5 min         | Glucose        | [187]     |
| Wet Oxidation    | Physicochemical | 185-195 °C  | 12 bar   | 5 -15 min     | Glucose        | [188]     |
| SPORL            | Physicochemical | 180 °C      | -        | 30 min        | Glucose        | [189]     |
| Biological       | Physicochemical | 30 °C       | -        | Weeks         | Glucose        | [190]     |

#### 2.3 Existing LCA studies

This section of the literature review compiles the existing knowledge of LCA studies of the main biorefining processes present in this work. An LCA study is a method of quantitively assess a product or system's environmental impact throughout its life cycle from raw material extraction to final disposal.

## 2.3.1 Kraft pulping LCA

The extensive burning of lignin in the pulp and paper industry may not be the best environmental option. A comparison with alternative valorization chains is needed to support a more holistic biorefinery concept. Nonetheless, the location of the biorefinery process is relevant, because if lignin production is done near pulp and paper industry, the environmental benefits are expected to be greater [191].

Culbertson et al. [192] performed an LCA of lignin extraction using the kraft process. They intended to investigate whether there were any significant changes on the environmental impact of the process if lignin was extracted as another valuable stream. The analysis of the system was conducted in a "cradle-to-gate" boundary, and the functional unit (FU) was set as 1 tonne of produced pulp. Results indicate a reduction in global warming potential of 120 kg per FU, when lignin extraction was accounted for, mainly due to the reduction of fossil fuels and chemicals consumption. Additionally, extracting lignin from kraft pulp reduces the loading of the recovery boiler, contributing to the increase of the process efficiency by 5%, and resulting in a positive

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impact on the process economic performance. Fuel applications of extracted lignin were less preferred over biopolymer applications. However, the incorporation of lignin in wood pellets as a binder, to improve pellet durability, may worsen the environmental performance of the final product [192].

Bernier et al. [193] focused on the life cycle inventory of KL for polymer applications and intended to compare it to the environmental profile of kraft pulp. Even though the authors defined a "cradle-to-gate" boundary for the system, they believe their results to be easily transposable to a "cradle-to-grave" comparison. The defined FU was 1 kg of KL. Results indicated that the main contributor to the environmental impacts of KL production is a particular subsystem (fuel substitution and drying) dependent on natural gas production, even though the impact was lower than that of black liquor. Improvements can also be implemented regarding the chemicals used in the precipitation and washing steps. The authors claim that subsequent transformation steps are relevant to the overall impact of KL polymer applications and, only once these applications are sufficiently developed, it will be possible to compare "cradle-tograve" studies for specific applications. Despite lignin degradation products having potential negative environmental consequences, KL is still recommended over kraft pulp [193].

Putra et al. [194] carried out an LCA to assess the feasibility of substituting wood by empty fruit bunches (EFB) from palm oil, to produce kraft pulp and furfural as co-product. The goal was to quantify the environmental impact of three different scenarios of pulp production, one of which used steam hydrolysis for the cooking of biomass. The system was studied following a "cradle-to-grave" approach and considering 1 kg of produced pulp as functional unit. Results showed that using EFB as feedstock is a more environmentally sustainable and economically profitable way to produce pulp and furfural than woody feedstocks. Results also suggest that the autohydrolysis scenario (the one that used water instead of acid for the prehydrolysis) had better performance, revealing lower impacts in the categories of global warming potential, acidification potential, eutrophication potential and human toxicity potential [194].

#### 2.3.2 Hydrothermal pretreatment LCA

In respect to environmental considerations, LHW is considered a green method for lignocellulosic biomass treatment [135]. It does not require the addition of any corrosive or toxic chemicals, as water can fulfill the role of solvent. As mentioned in section 3.3.3, Prasad et al. [140] performed an LCA to compare the environmental burden of various pretreatment methods. The system boundaries were limited to a cradle-to-gate approach and the authors not only concluded that LHW was the technically preferable pretreatment, but also that it was the best option from an environmental perspective. The least environmentally friendly pretreatment was the dilute acid treatment, mainly due to the length of the process, which required substantial energy to elevate the temperature, ending up topping  $CO_2$  emissions. When it comes to  $CO_2$  emissions are 15 times higher. This is the result of increased electricity consumption of the SE process as it contributed to 88% of total emissions. Regarding the remaining impact categories (eutrophication, acidification and water depletion), OS and the hydrothermal techniques showed similar performances. The authors alerted for the fact that renewable energy sources could, theoretically, lower the impact of energy consumption on  $CO_2$  emissions of both SE and dilute acid pretreatments [140].

Ehman et al. [195] conducted a cradle-to-gate LCA to assess the environmental performance of bio-polyethylene reinforced with a bio-based compatibilizer which was previously treated with LHW. Results were compared with those of a fossil compatibilizer. A reduction in greenhouse gas emissions was achieved when replacing the petroleum-based compatibilizer with a bio-based one (3%), being more significant when increasing the amount of fibers used (18%). Additionally, it also resulted in an increase of the carbon storage of up to ~2.1 kg CO<sub>2</sub>-eq/kg [195].

Cavalaglio et al. [196] determined, by means of an LCA, the environmental impacts associated to a biorefinery employing an acid-catalyzed SE treatment of biomass. The experiment was carried out at pilot-scale using two possible configurations, where one involved a separation between solid and liquid fractions and the other the usage of the whole slurry resultant from the pretreatment. They identified the enzymatic hydrolysis step as a potential hotspot for both configurations, as it either required the usage of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) as catalyst in the separation of the two fractions or of ammonium hydroxide (NH<sub>4</sub>OH) to regulate the acidity of the slurry. Overall, the first configuration offered a normalized reduction of 11% on environmental impacts relatively to the second configuration. In fact, it outperformed the second configuration in all impact categories considered with reductions ranging from 7% to 17%. The authors concluded that SE aided by an acid catalyst is a viable way to produce monosaccharides from biomass and reduce greenhouse gas emissions [196].

Lopes et al. [197] performed a cradle-to-gate LCA analysis of small-scale biorefineries in Portugal and Chile using corn stover and wheat straw, respectively, but



equally pretreated with SE in both cases. Both processes were composed of a series of steps including drying and milling, pretreatment of biomass, enzymatic hydrolysis, fermentation, recovery of products, wastewater treatment, anaerobic digestion and combustion. Both biorefineries had similar results regarding the climate change impact category with a slight advantage of the Chilean biorefinery (~0.80 kg CO<sub>2</sub>-eq/kg vs ~0.76 kg CO<sub>2</sub>-eq/kg, respectively). The latter also outperformed the Portuguese biorefinery in the ozone depletion, marine eutrophication and particulate matter formation impact categories, but showed poorer results in the remaining impact categories: terrestrial acidification, freshwater eutrophication, agricultural land occupation, water and fossil depletion. Overall, the two biorefineries have demonstrated similar impacts, and the disparities identified were credited to the different biomass used [197].

#### 2.3.3 Organosolv pretreatment LCA

By achieving a more comprehensive utilization of lignocellulosic biomass, opposed to conventional techniques such as kraft or sulfite pulping, OS pretreatment is able to address some environmental concerns associated to the bio-based industry [123]. The utilization of appropriate solvents such as ethanol and methanol may lead to a significant reduction of greenhouse gas emissions [198]. Recovering and recycling these solvents and promoting a more complete utilization of biomass can also prove useful in minimizing environmental impacts, even if doing so, the energy requirements will be higher [12, 199].

Teh et al. [200] performed an LCA to compare environmental impacts associated to biorefinery designs, which used birch chips and EFB pretreated with conventional alkali method or OS based on ethanol as solvent and sulfuric acid as catalyst. They used a cradle-to-gate approach to describe the system boundary, limiting the impact assessment until nanolignin production. Homogenization and ultrasonication were the two distinct methods of nanolignin synthesis, and final steps, considered. Results showed that, from all considered pathways, regardless of the selected biomass, OS was the delignification process with the largest global warming potential. This was due to greater electricity expenditure than the other considered routes and to the usage of ethanol as solvent, that generated almost 6kg of CO<sub>2</sub> from an upstream process consisting of the fermentation of biomass. The pretreatment step was, in fact, the biggest contributor to global warming potential, largely surpassing the influence of the final synthesis step. This was also true for human toxicity potential (HTP) and water depletion. In OS routes, significant differences of CO<sub>2</sub> emissions were found between birch chips and EFB routes, as the latter consumed a higher amount of electricity. Results from the assessment of HTP do not show any major differences between pathways, with the exception of alkaline treatment routes of EFB that required a larger amount of chemicals (NaOH and H<sub>2</sub>SO<sub>4</sub>) and, consequently, had a higher HTP impact. These same pathways were responsible for the greatest impact registered for water depletion, as required more water than other pathways by a large margin. Globally, considering the three impact categories, the least environmentally detrimental pathways were feedstock-dependent, indicating that OS may not be the preferrable pretreatment in any given situation. The authors concluded that more LCA studies should be performed, but with a more comprehensive system boundary, following a "cradle-to-grave" approach and a scaled-up valorization process with defined applications of nanolignin, since it is currently little commercialized [200].

Liu et al. [199] work on life cycle assessment of OS biorefinery compared the carbon and energy efficiency and environmental impact between a formic acid pretreatment (Formilline process) and a dilute acid pretreatment. Additionally, a technoeconomic assessment was carried out. Carbon efficiency of the Formilline process  $(\sim 58\%)$  was superior to the carbon efficiency of the dilute acid process  $(\sim 31\%)$ . This was the result of incorporating more carbon into ethanol (0.201 t C), lignin (0.214 t C) and furfural (0.163 t C) instead of concentrating the carbon flow into one single highvalue product as in dilute acid pretreatment's ethanol (0.310 t C). Regarding energy efficiency, even though the Formilline process consumes more energy in total (~633 GJ/h) than dilute acid (~310 GJ/h), it proportionally loses less energy through heat than the other pretreatment, thus resulting in a higher energy efficiency ( $\sim$ 43% and  $\sim$ 36%, respectively). LCA results, on a "cradle-to-grave" basis, show that Formilline process performed better in all assessed impact categories apart from ozone depletion potential (ODP), independently of the applied allocation method (economic, energy or mass) due to the use of chloroform in the furfural extraction step. Across all impact categories, the dominant contributors were chemicals used (glucose, sodium, phosphate and non-ionic surfactants), electricity, process direct emissions (SO<sub>2</sub> and NO<sub>x</sub>) and ash landfill. The techno-economic assessment revealed that the Formilline process route is economically feasible, despite its high capital costs, while the dilute acid process route is unprofitable. Moreover, in both scenarios considered, Formilline process became even more profitable and showed higher reduction potential of CO<sub>2</sub> emissions than the baseline scenario, which was expanded to include the conversion of lignin and furfural to added-value products. The authors concluded that this biorefinery design should be optimized and put to test with various feedstocks [199]. Further studies should be conducted to address distinct biorefinery scenarios and feedstocks, reagents and

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operating conditions of temperature and pressure, evaluating the impacts that arise from scaling up processes to commercial scale [201, 202]. Table 2 summarizes the pretreatment conditions in several LCA studies.

| PT  | T<br>(%C) | t<br>(min) | Solvent | Catalyst | Biomass                      | Product           | FU (kg)           | SB  | Ref   |
|-----|-----------|------------|---------|----------|------------------------------|-------------------|-------------------|-----|-------|
| LHW | 190       | 20         | Water   | Water    | Corn stover                  | Fermentable sugar | 1                 | CG  | [140] |
| LHW | 180       | 30         | Water   | Water    | Sugarcane                    | Bioplastic pellet | 1                 | CG  | [195] |
| LHW | 150       | 180        | Water   | H2SO4    | EFB                          | Dissolving pulp   | 1                 | CG  | [194] |
| LHW | 190       | 30         | Water   | H2SO4    | Corncob                      | Cellulose acetate | 0.01              | CG  | [203] |
| SE  | 190       | 5          | Water   | H2SO4    | Corn stover                  | Fermentable sugar | 1                 | CG  | [140] |
| SE  | 166       | 10         | Water   | H2SO4    | Cardoon<br>stalks            | Fermentable sugar | 1                 | CG  | [196] |
| SE  | 210       | -          | Water   | Water    | Wheat straw /<br>Corn stover | Isobutene         | 1000<br>feedstock | CG  | [197] |
| SE  | 190       | -          | Water   | Water    | Sugarcane                    | Lactic acid       | 1000              | CG  | [204] |
| OS  | 140       | 20         | EtOH    | H2SO4    | Corn stover                  | Fermentable sugar | 1                 | CG  | [140] |
| OS  | -         | -          | EtOH    | H2SO4    | EFB / Birch                  | Nanolignin        | 1                 | CG  | [200] |
| OS  | 180       | 60         | EtOH    | H2SO4    | Spruce bark                  | Lignin            | 1                 | CG  | [191] |
| OS  | 106       | 60         | EtOH    | H2SO4    | Wheat straw                  | Bioethanol        | 1                 | CGr | [199] |
| OS  | 200       | 90         | EtOH    | H2SO4    | Vine shoots                  | Lignin and co-    | 100<br>feedstock  | CG  | [167] |
| OS  | 180       | -          | EtOH    | H2SO4    | Wheat straw                  | Nanolignin        | 1                 | CG  | [12]  |

**Table 2.** Pretreatment (PT) conditions of temperature (T), time (t), solvent and catalyst of different biomass types to yield distinct products according to functional unit (FU) and system boundary (SB) of each study.

LHW = Liquid hot water; SE = SE; OS = Organosolv; EtOH = Ethanol; EFB = Empty fruit bunch; CG = Cradle-to-gate; CGr = Cradle-to-grave \*Organosolv preceded by hydrothermal pretreatment

## 2.3.4 Organosolv-hydrothermal hybrid pretreatment LCA

According to Morales et al. [134], their proposed biorefinery (see section 3.4.3) was in the right direction towards circular economy and a zero-waste objective, while also increasing the value of the residues. Sillero et al. [198] studied the environmental impacts (energy and resource consumption and emissions) of the system proposed that was comprised of three alkaline treatment routes and three OS routes in the delignification phase. The study was conducted at laboratory scale and was based on a cradle-to-gate approach, whereas the autohydrolysis step was common to all six pathways. The biorefinery scenario which presented the lowest environmental impacts did not use OS as means for delignification, but alkali treatment. Results show that, despite applied bleaching treatment having negative impacts on ozone layer depletion and abiotic depletion, due to the usage of chlorine compounds, overall, it presents the



most environmentally conscious method to obtain glucose and lignin. A sensitivity analysis was also conducted to investigate alternative solvents, concluding that ethanol is still preferable over methanol, which is highly biotoxic and not widely documented in literature [198].

Gullón et al. [167], presented a life cycle assessment of a hybrid hydrothermal/organosolv treatment. It intended to evaluate the environmental performance of diverse semi-pilot scale biorefinery routes that led to the valorization of an agro-industrial waste, adopting a gate-to-gate approach. The delignification step was found to be a hotspot of the system, due to the chemicals used in OS, namely ethanol, but consumed less energy than the baseline scenario. Therefore, the authors recommend that alternative solvents be evaluated to potentially soften the impact on the overall environmental performance of the process [167].

**Table 3.** Pretreatment (PT) environmental impacts: WEP (water eutrophication potential), TAP (acidification potential), water depletion, TEP (terrestrial eutrophication potential), FFP (fossil resource scarcity), TETP (terrestrial ecotoxicity), OFP (photochemical ozone formation), ODP (stratospheric ozone depletion) and GWP (climate change).

| PT         | Biomass          | WEP (kg P<br>eq)  | TAP (kg<br>SO₂ eq)                   | Water<br>depletion<br>(m3) | TEP<br>(moles N₂<br>eq) | FFP<br>(kg oil eq)              | TETP (kg<br>1,4-DB eq)  | OFP<br>(kg NO <sub>x</sub> eq)      | ODP<br>(kg CFC-<br>11 eq) | GWP (kg<br>CO₂ eq)      | Ref            |
|------------|------------------|---|--------------------------------------|----------------------------|-------------------------|---------------------------------|-------------------------|-------------------------------------|---------------------------|-------------------------|----------------|
| LHW        | Corn             | 2.60 × 10 <sup>-2</sup>                                     | 7.50 × 10 <sup>-2</sup>              | 1.38 × 10 <sup>2</sup>     | 2.50 × 10 <sup>-1</sup> | -                               | -                       | -                                   | -                         | 9.40 × 10 <sup>-1</sup> | [140]          |
| LHW<br>LHW | Sugarcane<br>EFB | 3.80 × 10 <sup>-4</sup>                                     | 1.78 × 10 <sup>-2</sup>              | -                          | -                       | 3.10 × 10 <sup>-1</sup>         | -                       | 6.78 × 10 <sup>-3</sup>             | -                         | 1.02<br>1.22            | [195]<br>[194] |
| LHW        | Corncob          | 4.11 × 10 <sup>-3</sup>                                     | 1.01 × 10 <sup>-1</sup><br>mol H⁺ eq | -                          | 1.71 × 10⁻¹             | 2.52 × 10 <sup>2</sup><br>MJ eq | -                       | 5.56 × 10 <sup>-2</sup><br>kg NMVOC | 8.46 × 10⁵                | 1.76 × 10 <sup>1</sup>  | [203]          |
| SE         | Corn<br>stover   | 4.70 × 10 <sup>-2</sup>                                     | 4.50 × 10 <sup>-2</sup>              | 2.60 × 10 <sup>2</sup>     | 7.00 × 10 <sup>-2</sup> | - '                             | -                       | -                                   | -                         | 1.43 × 10 <sup>1</sup>  | [140]          |
| SE         | Cardoon          | 1.14 × 10 <sup>-3</sup>                                     | 2.75 × 10 <sup>-2</sup>              | 1.43                       | -                       | 1.64                            | 1.06 × 10 <sup>-2</sup> | 1.65 × 10 <sup>-2</sup><br>ka NMVOC | 7.55 × 10 <sup>-7</sup>   | 6.00                    | [196]          |
| SE         | Corn             | 4.50 × 10 <sup>-1</sup>                                     | 3.33 × 10 <sup>1</sup>               | 1.56 × 10 <sup>1</sup>     | -                       | 1.56 × 10 <sup>2</sup>          | -                       | -<br>-                              | 6.07 × 10⁻⁵               | 7.96 × 10 <sup>2</sup>  | [197]          |
| SE         | Wheat            | 4.60 × 10 <sup>-1</sup>                                     | 3.40 × 10 <sup>1</sup>               | 1.80 × 10 <sup>1</sup>     | -                       | 1.68 × 10 <sup>2</sup>          | -                       | -                                   | 5.01 × 10⁵                | 7.60 × 10 <sup>2</sup>  | [197]          |
| SE         | Sugarcane        | 1.30 × 10 <sup>-1</sup><br>kg PO <sub>4</sub> <sup>3-</sup> | 5.00                                 | -                          | -                       | 1.20 × 10 <sup>2</sup>          | 5.5                     | 1.75 kg<br>NMVOC                    | 4.50                      | 1.60 × 10 <sup>2</sup>  | [204]          |
| OS         | Corn             | 5.30 × 10 <sup>-2</sup>                                     | 2.50 × 10 <sup>-2</sup>              | 1.80 × 10 <sup>2</sup>     | 5.30 × 10 <sup>-2</sup> | -                               | -                       | -                                   | -                         | 9.23                    | [140]          |
| OS         | Birch chips      | -   | -                                    | 4.00 × 10 <sup>-1</sup>    | -                       | -                               | -                       | -                                   | -                         | 4.67 × 10 <sup>2</sup>  | [200]          |
| OS         | EFB              | -   | -                                    | 6.10 × 10 <sup>-1</sup>    | -                       | -                               | -                       | -                                   | -                         | 1.05 × 10 <sup>3</sup>  | [200]          |
| OS         | Spruce<br>bark   | 0.00  | 2.10 × 10 <sup>-1</sup>              | -                          | -                       | -                               | -                       | -                                   | 0.00                      | 2.14                    | [191]          |
| OS         | Wheat            | 1.96 × 10 <sup>-4</sup>                                     | 4.16 × 10 <sup>-3</sup>              | 1.36 × 10 <sup>-2</sup>    | -                       | 1.74 × 10 <sup>-1</sup>         | 7.49 × 10 <sup>-1</sup> | 3.52 × 10 <sup>-3</sup>             | 8.76 × 10 <sup>-6</sup>   | 9.72 × 10 <sup>-1</sup> | [199]          |
| OS         | Vine             | -   | -                                    | -                          | -                       | -                               | -                       | -                                   | -                         | ~1.00 × 10 <sup>3</sup> | [167]          |
| OS         | Wheat            | -   | -                                    | 2.30 × 10 <sup>3</sup>     | -                       | -                               | -                       | -                                   | -                         | 2.00 × 10 <sup>2</sup>  | [12]           |

LHW = Liquid hot water; SE = SE; OS = Organosolv; EFB = Empty fruit bunch; NMVOC = Non-methane volatile organic

compounds

#### 2.4 Literature summary

Regarding the pretreatment processes of lignocellulosic biomass and its constituents, there is still room for much research to be developed to fill several literature gaps. Additionally, it is possible to improve the quality of the lignin produced and it is, therefore, prudent to continue developing new methods to obtain lignin with as few impurities as possible. This is a wise and responsible way to replace petroleum-based chemicals with new sustainable chemicals and materials derived from lignin.

Comprehension of lignin's physicochemical properties is as important as it is understanding the biorefinery processes for biomass processing. Given that hydrothermal pretreatments of biomass, such as LHW and SE, are considered green biorefining methods, minimizing the formation of inhibitory compounds should be a research priority. The catalyst may also be of importance to achieve an efficient delignification. Different types of catalysts may be applied, including alkaline catalysts. Recently, there have been developments regarding a flow-through hydrothermal pretreatment. It may potentially be more economically feasible than a hydrothermal pretreatment using a batch reactor, or even other types of acid-catalyzed pretreatments.

There are many opportunities for future research in OS pretreatment of biomass, including development of efficient solvent and co-product recovery systems and catalysts for lignin conversion to fuels and fuel additives. The effect of preextraction of hemicelluloses and extractive compounds and their contribution for improving process economics should also be investigated. In terms of fundamental research, the changes in cellulose structure and crystallinity during OS pretreatment and their effect on the enzymatic digestibility of the substrate are not fully understood and should be further explored.

Although still in the embryonic stage, the biomass pretreatment methods used in lignin valorization, such as OS and hydrothermal, show encouraging signs that it is indeed possible to scale up production on a sustainable way. We should, nonetheless, keep in mind that, even if these new pretreatments prove to be more environmentally sustainable than others, their economic feasibility still needs to be assessed. Energy and water requirements and chemicals used appear to be the source of the greater portion of impacts associated to these processes, according to LCA studies. There are, however, a few solutions that may help overcome these obstacles or, at least, minimize the environmental impacts. Using energy from renewable resources to feed the plant, recycling water and chemicals and treating the appropriate feedstock in the right operating conditions are mechanisms to push for efficiency and to reduce a



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biorefinery's carbon footprint. Finally, we should favor pretreatment methods and subsequent biomass conversion steps that, in theory, provide the highest possible product yield and quality. Ultimately, more LCA studies are needed for a more comprehensive understanding of the inherent environmental impacts of, not only pretreatment methods, but all steps involved in a biorefinery.

To accomplish that purpose, Chapters 6 and 7 include an LCA case study of LNP on a cradle-to-gate basis, starting at raw feedstock production and ending at nanolignin manufacture as an intermediate product. This research used a hybrid combination of OS and a hydrothermal treatment technique (SE).



# 3. Materials and methods

## 3.1 Methodology presentation

Life cycle assessment (LCA) is an objective methodology to evaluate the potential environmental impacts of a product, process or service, throughout its life cycle, defined by the international standards ISO 14040 [205] and ISO 14044 [206]. It does so by collecting information about all mass and energy flows that are commuted from and to the environment in the product's life cycle under study. It is also useful in identifying specific hotspots of a product's life cycle that may be holding back its sustainability. A complete "cradle-to-grave" life cycle study takes into consideration the whole value chain, starting from the raw materials extraction and upstream processes ("cradle") and finishing with downstream processes and the product's final disposal ("grave").

The LCA methodology comprises four interdependent phases: 1) goal and scope definition; 2) life cycle inventory analysis (LCI), 3) environmental impact assessment (LCIA) and 4) interpretation.

### 3.1.1 Goal and scope

The goal and scope definition presents the purpose of the study, and defines the system boundary for the study, which product life cycle parts will be included, which is the intended audience, and the geographic and temporal scopes. In this step is defined the functional unit, that is a measure of the performance or function of the process system. The LCI and the results of the LCIA should be expressed in terms of the functional unit, as it will simplify results presentation and reduces the dependence of the environmental on processes sizes and capacities. Concerning the scope, in theory an ideal LCA study should include a "cradle-to-grave" system boundary (or, preferably, "cradle-to-cradle"), but in some cases this is not feasible and the study is limited to a "cradle-to-gate" scope. For example, plastics exhibit slow degradation in a natural environment, having instead a vast life span. This means that is difficult to perform a comprehensive LCA, once fundamental data is often lacking, and so most LCA studies of polymers are restricted to a cradle-to-gate boundary. Also, the nature of the LCA study is defined, in particular, if the study is attributive, in which the environmental impacts are directly attributed to the functional unit, or consequential in which the environmental impacts of the changes in the current systems due to the production of a functional unit are determined. Other types of studies can be defined taking into account the goals of the study. Among them one of the most interesting are



the prospective studies, in which the environmental impacts of a product, service or process before it exists and/or is implemented in practice

#### 3.1.2 Life cycle inventory, LCI

In the LCI phase, the practitioner collects the input and output data required to accomplish the goal of the study, in practice an accounting of materials, energy and water consumption, process waste and emissions to air, water and soil. In practice, data from the specific production processes should be used, primary data, and in most studies that is not possible. LCI data is normally complemented with data from other sources, secondary data. An important source are life cycle inventory databases, as for example the Ecoinvent database [207, 208], for the background processes such as the generation of the electricity used in the process system. Depending on the availability of data, information from the literature: scientific articles, reports, patents, among others, may be necessary. Also, process simulation may be considered. This step is perhaps the most time-consuming part of a LCA study, and may require the interaction with different stakeholders associated with the supply chain of a given process, product or service. Depending on the sources and the nature of the process system, in particular for multiproduct processes, allocation procedures may be necessary, to ensure that the LCI is as much as possible linked to the functional unit. This is complex and ambiguous process, as the allocation procedures can have a significant influence in the study results, and care is desired as it is the case of LCA studies of lignin-based technologies [191, 209]. Even though co-product allocation is not recommended by the ISO standard, distinct allocation methods exist to be applied and compared with reference processes, if necessary [205]. Such is the case of mass, volume or economic allocation. Physical allocations may be preferable for lignin production, as economic allocation will most likely produce uncertain results, once price assumptions for such an emergent market may be highly variable [191, 199, 201]. By allocating emissions to each co-product as a percentage of total mass, emissions per mass unit will be equal for all co-products manufactured at the biorefinery. Finally, direct comparisons between studies which use distinct allocation methods are not possible.

#### 3.1.3 Life cycle impact assessment, LCIA

The LCIA step quantifies the environmental impacts based on the data obtained in the LCI and using an environmental impact evaluation methodology deemed suitable for the process system, facilitating a better understanding about the environmental performance of a product system. This stage involves two steps. In the first, the main



environmental impact categories are selected, taking into account the main expected environmental impacts of the process. Based on the LCI, in particular the main items, it is possible to make educated guesses of what those impacts would be. In the second step, a suitable environmental impact evaluation is chosen. Although independent, in practice both steps are grouped together, as most of times the environmental impact categories considered in the methodologies are chosen by default.

Various characterization methods available in the literature and in LCA software. For example, the Tool for Reduction and Assessment of Chemicals and Other Environmental Impacts (TRACI), developed by the U.S. Environmental Protection Agency [210], provides characterization factors for evaluating the environmental impact categories of: acidification; eutrophication; global climate change; human health particulate; human health cancer, noncancer and ecotoxicity; ozone depletion; photochemical smog formation and resource depletion. Overall, there is some degree of uncertainty surrounding results obtained using TRACI that should be acknowledged in order to extract an acceptable conclusion. Another characterization method that can be used for the LCIA is the ReCiPe [211], in which the environmental impacts are evaluated at either the midpoint level (18 indicators), or at endpoint level (3 indicators), in which the midpoints values are combined together, as shown in Fig. 7.



**Fig 7.** Overview of ReCiPe method for LCIA demonstrating lower aggregation impact categories (midpoints) and higher aggregation impact categories (endpoints). Adapted from [211].

Although the aggregation of the midpoint indicators simplifies the results interpretation, that processes increase the uncertainty of the results. The midpoint indicators considered are: particulate matter formation, tropospheric ozone formation, ionizing radiation, stratospheric ozone depletion, human toxicity (cancer), human toxicity (non-cancer), climate change, water use, freshwater ecotoxicity, freshwater eutrophication, tropospheric ozone ecotoxicity, terrestrial ecotoxicity, terrestrial acidification, land use/transformation, marine ecotoxicity, mineral resources and fossil resources. The three endpoint indicators considered are: damage to human health, to ecosystems and to resource availability. It would be relevant to include a dedicated nanoparticle impact indicator in the future [211]. It is worth pointing out that, in many LCA studies, there is a focus on specific impact categories rather than addressing all of them. This means that, in the LCA of certain products, data necessary to evaluate some impact categories may be missing, making comparison efforts difficult.

#### 3.1.4 Interpretation

Finally, in the life cycle interpretation phase, results from LCIA are analyzed together to reach suitable conclusions, and to define actions for improvement taking into the defined goal and scope. As environmental impacts are usually presented per process steps, the higher it is the number of LCAs regarding a given subject, the more consistent and reliable would the conclusions be. Comparing past LCA results is therefore valuable to support practitioners and decision makers [212]. However, the foreground and background assumptions vary quite often among studies, as the environmental impacts evaluation methodologies used, and the task is not always clear.

Comprehensive and comparable LCA studies are needed to estimate the environmental impacts of biorefineries with more confidence, providing valuable insights that will allow for an industrial transition towards a greener future. However, the availability of inventory data is often limited which makes it difficult to carry out LCA studies, blocking comparisons among studies and thwarting any attempt at industrial change [11, 201]. Thus, when searching for the biorefinery configurations that promise to be the most sustainable, preference should be given to those who provide primary data [201]. There is, however, some agreement among LCA studies on what are the main value chain hotspots, opportunities and challenges. For example, purposely cultivating biomass to use as feedstock in biorefineries (first generation), instead of benefiting from biomass residues (second generation), is linked to increased

acidification, eutrophication and heavy metal pollution [213]. Also, higher energy consumption, particularly if it comes from petrochemical sources, can lead to worse environmental performance, as well as intensifying the use of chemical reagents [191, 202, 214]. Therefore, careful energy utilization and integrating renewable energy is essential to minimize the environmental burden caused by biomass conversion processes and push for carbon efficiency [11, 203]. Additionally, it is possible to draw some conclusions regarding the environmental performance of these novel processes of lignin valorization, without spending time and resources upgrading the biorefinery plants [12].

### 3.2 Methodology application

#### 3.2.1 Goal and scope definition

The study targets technological advancements in respect to biomass processing to allow for a more complete valorization of all major fractions by treating lignin as a valuable product. The main goal is to acquire and deepen the knowledge of environmental impacts related to the industrial production of LNP from wheat straw. As the production process does not even exist at a laboratory scale, the LCA can be considered as a prospective study, and the results may be used in the future to assist in more environmentally conscious development of the production process.

Thus, we investigated the environmental impacts of nanolignin value chain using the life cycle assessment methodology under the ISO standards 14040 [205] and 14044 [206]. The study is cradle-to-gate, starting with the cultivation and harvesting of biomass feedstock (wheat straw), ending at the production of LNP. The product utilization, that in many cases will correspond to the incorporation of the nanolignin in other products, and final disposal are not considered, as there is a very significant uncertainty, and it is currently not possible to define adequate scenarios.

Within the product system, all impacts related to material and energy resources were measured according to a functional unit of 1 kg of nanolignin produced from raw material. Impacts attributed to wheat straw production were determined using a mass allocation method (35% w/w), as the remaining portion of wheat refers to grain. Economic allocation is undesirable as, truthfully, nanolignin is still an unmarketable intermediate product that is to be used as filler in a macroscopic matrix.

The study is attributive, in the sense that the environmental impacts will be attributed and expressed as a function of the functional unit. Whenever possible, for the background Portuguese and European conditions will be used.

## 3.2.2 Process description

As stated above, as far as the author knowledge goes, there is no industrial process to obtain nanolignin. Hence, in this work a production system was developed and proposed, based on an extensive analysis of the literature, that is believed to be able to obtain nanolignin with the desired quality for further application.

This study was partially based on the paper published by Koch et al. [12], where LNP were obtained by extracting bulk lignin from wheat straw, a process that inspired our own, which is presented in Figure 8.



Fig 8. System boundary definition, including the life cycle stages considered for the LCA study

The raw biomass material for production of LNP came from the straw of cultivated wheat, which was harvested by a tractor and transported 50 km from a warehouse located near the field to the plant. Transportation to the plant was assumed to be carried out by a diesel truck with a functional unit of 1 ton/km. Prior to the biomass pretreatment step, wheat straw was air dried and milled to reduce its size with a process efficiency of 99% and 98%, respectively. Extraction occurred first at 180 °C for 60 minutes with a solvent mixture of ethanol (95%) and water (60/40 v/v%). Extraction was aided by sulfuric acid catalyst (1% wt). Then, the system was pressurized with steam at high temperatures for 4 minutes and rapidly depressurized to atmospheric levels afterwards. The resulting slurry was then vacuum filtered, resulting in two different fractions: a liquid fraction containing dissolved lignin and a solid fraction, which subsequent processing falls out of the scope of this study. In the next step, centrifugation took place to promote lignin precipitation from the liquid phase and, in parallel, ethanol is partially recovered through distillation to be reused in the pretreatment stage. Ethanol recovery only takes place under the alternative scenario and not under the baseline scenario, where used solvent is discarded as waste. In



each cycle, 98% ethanol was recovered and reintegrated into the system, while the remaining was assumed to be lost as an output stream by evaporation. Lignin yield, after centrifugation, was assumed to be 63% of initial lignin content in wheat straw (18% wt). The final step of this system process was ultrasonic irradiation of lignin to obtain LNP. LNP yield was assumed to be 57% from initial lignin content in wheat straw.

## 3.2.3 Life cycle inventory analysis (LCI)

Life cycle inventory (LCI) data was collected from several sources, including both background and foreground data available from literature [12, 167, 168, 191, 215-217] and databases. Foreground data involved biomass treatment conditions, including temperature, pressure, duration and chemicals used on a biorefinery. In the Ecoinvent v3.8 database, allocation at point of substitution (APOS) was the model selected and conditions based on Europe (RER) were considered, whenever possible. Electricity, however, was supplied through the national energy grid considering the low voltage Portuguese electricity mix as life cycle impacts vary greatly, depending on the location.

The sources of the data by process part are given in Table 4. As the various references considered different functional units when compared to this work, the values obtained from the literature were adjusted to the functional unit used in this work and corresponding mass and energy flows.

| Life cyc                    | le stage  | Source     | Reference |  |  |  |  |  |  |  |
|-----------------------------|---|------------|-----------|--|--|--|--|--|--|--|
| Feedsto                     | ck production:<br>Wheat straw                                       | Ecoinvent  |           |  |  |  |  |  |  |  |
| Organos<br>-<br>-<br>-<br>- | olv–Steam explosion:<br>Energy<br>Ethanol<br>Sulfuric acid<br>Water | Literature | [12]      |  |  |  |  |  |  |  |
| Filtration<br>-<br>-        | :<br>Energy<br>Cellulose-rich fraction                              | Literature | [215]     |  |  |  |  |  |  |  |
| Ethanol                     | distillation:<br>Energy<br>Ethanol                                  | Literature | [217]     |  |  |  |  |  |  |  |
| Centrifuç<br>-              | ge:<br>Energy<br>Lignin   | Literature | [168]     |  |  |  |  |  |  |  |
| Ultrason<br>-<br>-          | ication:<br>Energy<br>LNP   | Literature | [218]     |  |  |  |  |  |  |  |

| Г | abl | е | 4.       | Data | sou | rces |
|---|-----|---|----------|------|-----|------|
|   | abi | • | <b>-</b> | Data | 30u | 100. |



## 3.2.4 Life cycle impact assessment (LCIA)

The LCIA was carried out using the SimaPro v9.3 and Microsoft Excel ® software. Potential environmental impacts were evaluated according to the ReCiPe (2016) method. Main impact categories for the LCIA included:

- Ozone formation ecosystem quality (EOFP) impact category indicates the damage on ecosystems done by the formation of ozone expressed in kg NOx equivalents.
- Freshwater eutrophication (FEP) reflects the discharge of nutrients from the soil to freshwater bodies, measured in kg P eq.
- Freshwater ecotoxicity (FETP) calculates the potential impact of a chemical emitted to freshwater by using the potential impact of 1,4-dichlorobenzene (1,4-DCB) eq as a reference.
- Fossil resource scarcity (FFP) is based on future cost increase of fossil resource, measured in kg oil eq.
- Climate change (GWP), which provides a summary of greenhouse gas emissions with their respective characterization factors that translate to  $CO_2$  eq.
- Ozone formation human health (HOFP) is the change in ozone concentration after emission of  $NO_x$  precursor, expressed in kg  $NO_x$  eq.
- Human toxicity cancer (HTPc) and human toxicity non-cancer (HTPnc) consider the potential impact of toxic substances on human health (for cancer and noncancer diseases, respectively) and are compared with the reference substance 1,4-DCB eq.
- Ionizing radiation (IRP) is the increase in the absorbed dose of radiation, expressed in kBq Co-60.
- Land use (LOP) refers to the relative species loss prompted by land use in m<sup>2</sup>yr crop eq.
- Marine eutrophication (MEP) reflects the discharge of nutrients from the soil to marine water bodies, measured in kg P eq.
- Marine ecotoxicity (METP) calculates the potential impact of a chemical emitted to marine water by using the potential impact of 1,4-DCB eq as a reference.
- Ozone depletion (ODP) quantifies the decrease in stratospheric ozone concentration.
- Fine particulate matter formation (PMFP) considers the change in atmospheric concentration of particulate matter, measured in kg PM<sub>2.5</sub> eq.
- Mineral resource scarcity (SOP) quantifies the extra amount of ore produced in kg Cu eq.

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- Terrestrial acidification (TAP) measures the acidity in the soil by using SO<sub>2</sub> eq as a reference.
- Terrestrial ecotoxicity (TETP) calculates the potential impact of a chemical emitted to the soil by using the potential impact of 1,4-DCB eq as a reference.
- Water use (WCP) evaluates water consumption measured in m<sup>3</sup>.

Although the selection of the environmental impact categories follows the selection of the environmental impact assessment methodology and not an analysis of the LCI, the set of environmental impact categories is adequate. In particular, most of the environmental impact categories, as for example global warming, are consensual and used in most of LCA studies. Moreover, it is expected that energy consumption will be responsible for a significant part of the environmental impacts, and the selected set is particularly suited in that situation. All impact categories are listed below in Table 5, along with their respective LCIA method and measurement unit.

| Impact Category | LCIA method | Unit                      |
|-----------------|-------------|---------------------------|
| EOFP            | ReCiPe      | kg NO <sub>x</sub> eq     |
| FEP             | ReCiPe      | kg P eq                   |
| FETP            | ReCiPe      | kg 1,4-DCB eq             |
| FFP             | ReCiPe      | kg oil eq                 |
| GWP             | ReCiPe      | kg CO <sub>2</sub> eq     |
| HOFP            | ReCiPe      | kg NO <sub>x</sub> eq     |
| HTPc            | ReCiPe      | kg 1,4-DCB eq             |
| HTPnc           | ReCiPe      | kg 1,4-DCB eq             |
| IRP             | ReCiPe      | kBq Co-60 eq              |
| LOP             | ReCiPe      | m <sup>2</sup> yr crop eq |
| MEP             | ReCiPe      | kg P eq                   |
| METP            | ReCiPe      | kg 1,4-DCB eq             |
| ODP             | ReCiPe      | kg CFC-11 eq              |
| PMFP            | ReCiPe      | kg PM <sub>2.5</sub> eq   |
| SOP             | ReCiPe      | kg Cu eq                  |
| TAP             | ReCiPe      | kg SO <sub>2</sub> eq     |
| TETP            | ReCiPe      | kg 1,4-DCB eq             |
| WCP             | ReCiPe      | m <sup>3</sup>            |

Table 5. LCIA impact categories assessed.



# 4. Results

### 4.1 Inventory results

The life cycle inventory data for production of LNP from wheat straw is presented in Table 6, where the mass and energy inputs, and the mass outputs are given. Regarding the energy consumption, it can be seen that organosolv–steam explosion pretreatment is the dominant part, with more than 50% per functional unit, followed by the ethanol distillation and ultrasonification with similar relevance. This shows that the effort to improve the process energy efficiency should start reducing the energy consumption of the pretreatment process.

| Life cvo   | le stage                | Unit | Input                   | Output |
|------------|-------------------------|------|-------------------------|--------|
| Feedsto    | ock production:         |      |                         |        |
|            | Wheat straw             | kg   | 7.89                    | -      |
| Organos    | solv–Steam explosion:   |      |                         |        |
| -          | Energy                  | kWh  | 13.8                    | -      |
| -          | Ethanol                 | ka   | 47.3                    | -      |
| -          | Sulfuric acid           | ka   | $7.89 \times 10^{-2}$   | -      |
| -          | Water                   | kg   | 31.6                    | -      |
| Filtration | า:                      |      |                         |        |
| -          | Energy                  | kWh  | 1.18                    | -      |
| -          | Cellulose-rich fraction | kg   | -                       | 3.08   |
| Ethanol    | distillation:           |      |                         |        |
| -          | Energy                  | kWh  | 5.86                    | -      |
| -          | Ethanol                 | kg   | -                       | 46.4   |
| Centrifu   | ge:                     |      |                         |        |
| -          | Energy                  | kWh  | 8.75 × 10 <sup>-2</sup> | -      |
| -          | Lignin                  | kg   | -                       | 1.18   |
| Ultrasor   | nication:               |      |                         |        |
| -          | Energy                  | kWh  | 4.40                    | -      |
| -          | LNP                     | kg   | -                       | 1.00   |

Table 6. Life cycle inventory data for production of LNP from wheat straw.

#### 4.2 Impact assessment results

The total potential environmental impacts associated with the production of LNP from wheat straw in the baseline scenario and the impacts which arose specifically at certain stages of the value chain (feedstock production, pretreatment and others) were evaluated per functional unit, as presented in Table 7.



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| Table 7. | Environmental | impacts o | f feedstock | production, | biomass | pretreatment | and | overall | nanolignin | life | cycle | for |
|----------|---------------|-----------|-------------|-------------|---------|--------------|-----|---------|------------|------|-------|-----|
| baseline | scenario.     |           |             |             |         |              |     |         |            |      |       |     |

| Environmental impact category | Unit                      | Feedstock production    | Pretreatment            | Others                  | Total                   |
|-------------------------------|---------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| EOFP                          | kg NO <sub>x</sub> eq     | 5.00 × 10 <sup>-3</sup> | 1.56 × 10 <sup>-1</sup> | 3.91 × 10 <sup>-3</sup> | 1.65 × 10⁻¹             |
| FEP                           | kg P eq                   | 4.42 × 10 <sup>-4</sup> | 2.45 × 10 <sup>-2</sup> | 5.67 × 10 <sup>-4</sup> | 2.55 × 10 <sup>-2</sup> |
| FETP                          | kg 1,4-DCB eq             | 1.17 × 10 <sup>-1</sup> | 1.81                    | 1.29 × 10 <sup>-1</sup> | 2.05                    |
| FFP                           | kg oil eq                 | 2.79 × 10 <sup>-1</sup> | 46.4                    | 4.01 × 10 <sup>-1</sup> | 47.1                    |
| GWP                           | kg CO <sub>2</sub> eq     | 2.59                    | 60.6                    | 1.46                    | 64.6                    |
| HOFP                          | kg NO <sub>x</sub> eq     | 4.95 × 10 <sup>-3</sup> | 1.36 × 10 <sup>-1</sup> | 3.88 × 10 <sup>-3</sup> | 1.45 × 10⁻¹             |
| HTPc                          | kg 1,4-DCB eq             | 9.03 × 10 <sup>-3</sup> | 2.14                    | 7.17 × 10 <sup>-2</sup> | 2.22                    |
| HTPnc                         | kg 1,4-DCB eq             | 6.06                    | 31.0                    | 1.35                    | 38.4                    |
| IRP                           | kBq Co-60 eq              | 8.24 × 10 <sup>-3</sup> | 1.07                    | 9.08 × 10 <sup>-2</sup> | 1.17                    |
| LOP                           | m <sup>2</sup> yr crop eq | 8.42                    | 7.95 × 10 <sup>-1</sup> | 3.76 × 10 <sup>-2</sup> | 9.25                    |
| MEP                           | kg P eq                   | 1.62 × 10 <sup>-2</sup> | 4.18 × 10 <sup>-4</sup> | 3.58 × 10⁻⁵             | 1.67 × 10 <sup>-2</sup> |
| METP                          | kg 1,4-DCB eq             | 2.61 × 10 <sup>-2</sup> | 2.38                    | 1.61 × 10 <sup>-1</sup> | 2.57                    |
| ODP                           | kg CFC-11 eq              | 5.84 × 10⁻⁵             | 6.16 × 10⁻ <sup>6</sup> | 5.83 × 10 <sup>-7</sup> | 6.51 × 10⁻⁵             |
| PMFP                          | kg PM <sub>2.5</sub> eq   | 2.39 × 10 <sup>-3</sup> | 5.47 × 10 <sup>-2</sup> | 2.62 × 10 <sup>-3</sup> | 5.97 × 10 <sup>-2</sup> |
| SOP                           | kg Cu eq                  | 1.63 × 10 <sup>-3</sup> | 1.38 × 10 <sup>-1</sup> | 2.56 × 10 <sup>-3</sup> | 1.43 × 10⁻¹             |
| TAP                           | kg SO <sub>2</sub>        | 1.45 × 10 <sup>-2</sup> | 1.58 × 10 <sup>-1</sup> | 7.97 × 10 <sup>-3</sup> | 1.81 × 10 <sup>-1</sup> |
| TETP                          | kg 1,4-DCB eq             | 2.70                    | 169                     | 3.97                    | 176                     |
| WCP                           | m <sup>3</sup>            | 1.16 × 10 <sup>-3</sup> | 5.61 × 10 <sup>-1</sup> | 1.34 × 10 <sup>-2</sup> | 5.75 × 10 <sup>-1</sup> |

The row 'Others' is the sum of impacts from biomass drying and milling, filtration, distillation, centrifugation and ultrasonication stages

The LCA results for the overall value chain of LNP production from wheat straw, under the baseline scenario where no solvent is recovered, are represented in Figure 9. The results indicate that the main contributors originate from the pretreatment stage by a large margin and, in some impact categories (LOP, MEP and ODP) from feedstock production. Under this scenario, impacts coming from other steps are negligible.



Fig 9. LCA results of lignin nanoparticle production, excluding solvent recovery after pretreatment.



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The total potential environmental impacts associated with the production of LNP from wheat straw under the scenario with ethanol recovery and impacts which arose specifically at certain stages of the value chain (feedstock production, pretreatment and others) were evaluated per functional unit, as presented in Table 7.

| Table 8.  | Environmental   | impacts | of feedstock | production, | biomass | pretreatment | and | overall | nanolignin | life | cycle | from |
|-----------|-----------------|---------|--------------|-------------|---------|--------------|-----|---------|------------|------|-------|------|
| solvent r | ecovery scenari | o.      |              |             |         |              |     |         |            |      |       |      |

| Environmental impact category | Unit                      | Feedstock production    | Pretreatment            | Others                  | Total                   |
|-------------------------------|---------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| EOFP                          | kg NO <sub>x</sub> eq     | 5.00 × 10 <sup>-3</sup> | 7.38 × 10 <sup>-3</sup> | 3.91 × 10 <sup>-3</sup> | 1.63 × 10 <sup>-2</sup> |
| FEP                           | kg P eq                   | 4.42 × 10 <sup>-4</sup> | 1.11 × 10 <sup>-3</sup> | 5.67 × 10 <sup>-4</sup> | 2.12 × 10 <sup>-3</sup> |
| FETP                          | kg 1,4-DCB eq             | 1.17 × 10 <sup>-1</sup> | 1.77 × 10 <sup>-1</sup> | 1.29 × 10 <sup>-1</sup> | 4.23 × 10 <sup>-1</sup> |
| FFP                           | kg oil eq                 | 2.79 × 10 <sup>-1</sup> | 1.37                    | 4.01 × 10 <sup>-1</sup> | 2.05                    |
| GWP                           | kg CO₂ eq                 | 2.59                    | 2.80                    | 1.46                    | 6.85                    |
| HOFP                          | kg NO <sub>x</sub> eq     | 4.95 × 10 <sup>-3</sup> | 6.94 × 10 <sup>-3</sup> | 3.88 × 10 <sup>-3</sup> | 1.58 × 10 <sup>-2</sup> |
| HTPc                          | kg 1,4-DCB eq             | 9.03 × 10 <sup>-3</sup> | 1.22 × 10 <sup>-1</sup> | 7.17 × 10 <sup>-2</sup> | 2.03 × 10 <sup>-1</sup> |
| HTPnc                         | kg 1,4-DCB eq             | 6.06                    | 2.12                    | 1.35                    | 9.53                    |
| IRP                           | kBq Co-60 eq              | 8.24 × 10 <sup>-3</sup> | 1.21 × 10 <sup>-1</sup> | 9.08 × 10 <sup>-2</sup> | 2.20 × 10 <sup>-1</sup> |
| LOP                           | m <sup>2</sup> yr crop eq | 8.42                    | 5.72 × 10 <sup>-2</sup> | 3.76 × 10 <sup>-2</sup> | 8.51                    |
| MEP                           | kg P eq                   | 1.62 × 10 <sup>-2</sup> | 4.76 × 10⁻⁵             | 3.58 × 10⁻⁵             | 1.63 × 10 <sup>-2</sup> |
| METP                          | kg 1,4-DCB eq             | 2.61 × 10 <sup>-2</sup> | 2.24 × 10 <sup>-1</sup> | 1.61 × 10 <sup>-1</sup> | 4.11 × 10 <sup>-1</sup> |
| ODP                           | kg CFC-11 eq              | 5.84 × 10 <sup>-5</sup> | 7.70 × 10 <sup>-7</sup> | 5.83 × 10 <sup>-7</sup> | 5.97 × 10⁻⁵             |
| PMFP                          | kg PM <sub>2.5</sub> eq   | 2.39 × 10 <sup>-3</sup> | 4.13 × 10 <sup>-3</sup> | 2.62 × 10 <sup>-3</sup> | 9.13 × 10 <sup>-3</sup> |
| SOP                           | kg Cu eq                  | 1.63 × 10 <sup>-3</sup> | 5.70 × 10 <sup>-3</sup> | 2.56 × 10 <sup>-3</sup> | 9.89 × 10 <sup>-3</sup> |
| ТАР                           | kg SO <sub>2</sub>        | 1.45 × 10 <sup>-2</sup> | 1.24 × 10 <sup>-2</sup> | 7.97 × 10 <sup>-3</sup> | 3.49 × 10 <sup>-2</sup> |
| TETP                          | kg 1,4-DCB eq             | 2.70                    | 8.06                    | 3.97                    | 14.7                    |
| WCP                           | m <sup>3</sup>            | 1.16 × 10 <sup>-3</sup> | 4.89 × 10 <sup>-2</sup> | 1.34 × 10 <sup>-2</sup> | 6.35 × 10 <sup>-2</sup> |

The row 'Others' is the sum of impacts from biomass drying and milling, filtration, distillation, centrifugation and ultrasonication stages.

The LCA results of the overall value chain of LNP production from wheat straw, when ethanol is recovered to be reused in the pretreatment stage, are represented in Figure 10. When solvent recovery is considered, the relative weight of the pretreatment stage on the environmental impacts of the entire nanolignin value chain is significantly reduced and feedstock production, distillation, and ultrasonication leave a more predominant footprint. Nonetheless, biomass pretreatment is still the most environmentally impactful stage of LNP value chain, overall, followed by feedstock production. WCP, in particular, is largely dominated by the pretreatment stage, showcasing that biomass pretreatment consumes much more water than wheat straw production. Pretreatments are still the most determining factor to GWP, but feedstock production is a close second. Too in this case, feedstock production is the main, and almost sole, contributor to LOP, MEP and ODP impact categories. Note that in this case, owing to the reduction of the impact of the pretreatment stage, HTPnc and TAP are mostly influenced from feedstock production. In general, the remaining stages of LNP value chain present less impacts to the environment, but concerning WCP and IRP, distillation and ultrasonication outweigh feedstock production.





Fig 10. LCA results of lignin nanoparticle value chain, including solvent recovery after pretreatment.

LCA results of baseline scenario and of alternative scenario were compared and presented in Figure 11. When comparing both scenarios, it is clear that solvent recovery is an essential mechanism to minimize the environmental impacts of lignin nanoparticle production from wheat straw. Almost every impact category suffered a significant reduction when solvent was recovered for further utilization, apart from ODP, MEP and LOP, where ethanol production is not as influent as other inputs.



Fig 11. Comparison between LCA results of baseline scenario and solvent recovery scenario.



The contribution of LCA results for feedstock production was grouped into wheat straw production and tractor and truck usage, as represented in Figure 12. Across all impact categories the main contributor was wheat straw production, while harvesting by tractor and transportation to the biorefinery by truck were less significant. The only registered exception was HTPc, where tractor and truck usage greatly outweighed the impacts of biomass production. Harvesting and transportation are most significant for TETP, IRP, SOP and WCP impact categories and less significant (or even negligible) for the remaining ones.



Fig 12. LCA results of the feedstock production stage.

LCA results, illustrated in Figure 13, for the pretreatment stage of the baseline scenario, where no solvent was recovered, were assessed by considering electricity, ethanol, water and sulfuric acid as inputs. For every impact category, the main and virtually sole contributor was ethanol production with every other input showcasing no real impacts comparably. Returning to Figure 9, this means that ethanol is the overall greatest hotspot responsible for hindering the life cycle environmental performance of the base case nanolignin production.





Fig 13. LCA results of the pretreatment stage, excluding reutilization of solvent recovered after pretreatment.

LCA results, illustrated in Figure 14, for the pretreatment stage of the alternative scenario were assessed using the same inputs of the baseline scenario (electricity, ethanol, water and sulfuric acid). A drastic change was registered, in respect to the baseline scenario, as in this case the impacts originated from ethanol were greatly reduced. It is the electricity needed to produce thermal energy that mostly influenced the impact categories, followed by ethanol. The exceptions are WCP and FFP, where water and ethanol, respectively, assume the larger impact. Nonetheless, ethanol production is also very significant to all impact categories, whereas water and sulfuric acid presented relatively low environmental impacts.





Fig 14. LCA results of the pretreatment stage, considering reutilization of solvent recovered after pretreatment.

. Life cycle hotspots must be acted upon, if a significant reduction in environmental impacts is to be achieved. This study demonstrated that pretreatments are the main hotspot of the LNP value chain. Most of this impact comes from electricity utilization, which means that environmental impacts may be minimized if the energy mix used in the process is richer in renewable sources instead of fossil ones. Efficient ethanol utilization is mandatory if such a process is to be upscaled to industrial levels, as impacts grow exponentially when solvent is completely discarded as waste after used. Ethanol recovery is a relatively simple process that generates significant environmental benefits and should not be discarded by any biorefinery attempting to use it as solvent in biomass pretreatment. Alternatively, other solvents with a better environmental performance may be used. Feedstock production represents the second largest environmental constraint to the studied value chain. Therefore, more sustainable agricultural practices, with less usage of harmful pesticides and fertilizers, will hopefully lower the impact of wheat straw production. An economic allocation would result in a reduction on the impact of feedstock production, since wheat straw is less valuable than the entire wheat. Also, transport vehicles running on 100% biofuel, electricity or even green hydrogen, may help reduce some environmental impacts associated with biomass transportation to biorefineries. Although these results are in line with what was analysed on earlier chapters of this work, more LCA studies should



be carried out to provide more robust conclusions regarding the life cycle impacts of an LNP biorefinery.


## 5. Conclusions

This work comprised a state-of-the-art review and a case study with the main goal to tackle knowledge gaps regarding the sustainability of biorefining processes. Through the analysis of both the findings from published literature and the original research developed in this thesis, it can be concluded that biomass pretreatments are to be optimized in order to enable the development of a lignin nanoparticle biorefinery. Without detriment to the effectiveness of any of the pretreatments here described, demonstrating a true environmental upgrade from processes applied in traditional refineries is what appeals to an industrial paradigm shift in the first place. While lignin is a renewable polymer with a wide range of potential applications, pretreatments such as organosolv or hydrothermal may be no less harmful to the environment than their conventional counterpart, the kraft process. This research demonstrates that energy and chemicals used along the studied value chain need to be managed as efficiently as possible, but also illustrates the scarcity of data available to further carry out life cycle assessments. In any event, the information collected and compiled here expanded the knowledge of the environmental performance of biorefining processes, allowing the identification of primary challenges faced by biorefineries that wish to carry out a more complete valorization of biomass. There is still room for improvement as energy usage from more sustainable sources and alternative chemicals should be investigated in future research. As demonstrated in the case study, electricity and chemicals are the main contributors of the most impactful stage of lignin nanoparticles value chain and such significant hotspots should be acted upon to improve the ultimate environmental performance of the proposed biorefinery. The solvent, for instance, must be recovered and reused to maximize the efficiency of the process. Future research should also focus on applying the LCA methodology with different approaches from the one applied here, extending the system boundary to cradle-to-grave, for instance, to more broadly assess the environmental impacts related to the valorization of lignocellulosic fractions.



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