

KAIT KAAREL PUSS

Ultrasound processing
of forest biorefinery lignin and
lignin–cellulose mixtures



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Institute of Bioengineering, Faculty of Science and Technology, University of Tartu, Estonia.

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LIST OF ORIGINAL PUBLICATIONS

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The author's contribution to each article is as follows:

- I. The author planned, performed all the experiments and analyzed the results. The author was responsible for the manuscript preparation.
- II. Lead author of the manuscript. Planned and performed all the experiments as well as most of the analysis of results.
- III. Lead author for the manuscript preparation, experimental design and some experiments.

ABBREVIATIONS

HL	– Hydrolysis Lignin
LCM	– Lignin–Cellulose mixture
US	– Ultrasound
MD	– Molecular Dynamics
XRD	– X-Ray Diffraction
HSQC	– Heteronuclear Single Quantum Coherence
NMR	– Nuclear Magnetic Resonance
H	– 4-Hydroxyphenyl
G	– Guaiacyl
S	– Syringyl
LCC	– Lignin Carbohydrate Complex
KL	– Kraft Lignin
PDI	– Polydispersity Index
WRV	– Water Retention Value
SEC	– Size Exclusion Chromatography
HPLC	– High-Performance Liquid Chromatography
PSD	– Particle Size Distribution
SEM	– Scanning Electron Microscopy
UV-Vis	– Ultraviolet-visible Spectroscopy
PDA	– Photodiode array
LD	– Laser Diffraction
APL	– Acid Precipitated Lignin
FT-IR	– Fourier-Transform Infrared Spectroscopy

1. INTRODUCTION

Lignocellulosic biomass is an abundant, renewable resource that can substitute fossil-based materials and chemicals. The term lignocellulose covers all plants and derived waste streams, such as wood and agricultural residues. Its main components are cellulose, hemicellulose, and lignin, which are tightly bound within the plant cell wall. To utilize these biopolymers, biorefineries employ fractionation methods to separate them, allowing each fraction to be further processed.

The individual biopolymers have distinct structures. Cellulose has a uniform molecular architecture made of long glucose-based chains that form a rigid, crystalline structure. In contrast, lignin is an amorphous and structurally heterogeneous polymer. Its monomeric composition varies among biomass sources, and additional variability arises from the extraction method used.

Modern biorefineries aim to valorize all biomass components simultaneously. Because of these milder fractionation approaches – such as the twin-screw extrusion and steam explosion pathways used for birch biomass – the cellulose is recovered as shorter fibers, while the isolated technical lignin retains a structure closer to its native form. This strategy produces a sulfur-free hydrolysis lignin (HL) and a unique intermediate stream called the lignin–cellulose mixture (LCM). To transform these crude intermediates into functional products, downstream methods such as solvent-based solubilization or power ultrasound (US) treatments are used to enhance their properties.

US is a process intensification tool that induces various physicochemical effects in a liquid medium. Depending on the wave frequency, the dominant mechanism shifts between mechanical shear or chemical reactivity. Although power US has been applied to intensify standard biomass extractions and to treat isolated cellulose or lignin, its impacts on complex biorefinery streams remain unmapped. Neither the novel HL nor its corresponding unrefined LCM intermediate has previously been investigated regarding its properties and structural response to acoustic cavitation.

Importantly, mapping the dissolution kinetics of standalone HL provides critical parameters that can be applied to the more complex matrix of the LCM. The insights gained from isolated alkaline and organic solvent screening allow the residual lignin content of the LCM to be modulated. Subsequent US treatment of LCM samples with varied lignin loadings provides a way to study how lignin regulates acoustic energy transfer and fiber fibrillation. These experiments offer new insights into the underlying lignin–cellulose interfacial interactions.

The primary aim of this doctoral study was to analyze the applicability and mechanisms of power US across different processes within a biorefinery context. This dissertation demonstrates that 20 kHz acoustic cavitation can increase HL solubility under mild conditions, drive the mechanical formation of nanoscale cellulose in materials with a high lignin content, and reveal how lignin affects cellulose modification.

2. LITERATURE OVERVIEW

This literature overview first introduces the composition of lignocellulosic wood biomass and the extraction methods for lignin and cellulose. This section gives insight into the current knowledge about lignin solubility in industrially viable solvents and their differences. Finally, it describes the effects of US and its influence on these biopolymers under different conditions.

2.1. Lignocellulosic biomass composition and structure

Wood is a hierarchical lignocellulosic material with a structural complexity extending from the macroscale of the stem down to the nanoscale organization of the cell walls [1]. It exists as a natural fibrillar composite primarily composed of cellulose, hemicellulose, and lignin, which together govern its macroscopic properties. Within this structure, stiff cellulose microfibrils are embedded in a matrix of hemicelluloses and lignin to provide both mechanical strength and biological functionality to the cell wall [2]. Specifically, cellulose imparts tensile strength, hemicelluloses act as flexible macromolecular linker, and lignin offers compressive support, rigidity, and hydrophobicity.

The quantitative distribution and chemical behavior of these polymers dictate the overall performance of the biomass. Cellulose, which accounts for 40–55% of wood by weight, consists of linear chains of repeating D-glucose units that assemble via hydrogen bonding into elementary fibrils (**Fig. 1**) and larger microfibrils. Hemicellulose constitutes around 24–40% of wood, stabilizing the cell wall matrix by forming hydrogen bonds with cellulose while simultaneously facilitating critical interactions with lignin. Lignin typically represents 18–35% of woody biomass [3–5], and its structural integration is further reinforced by proposed covalent linkages to hemicelluloses [6,7].

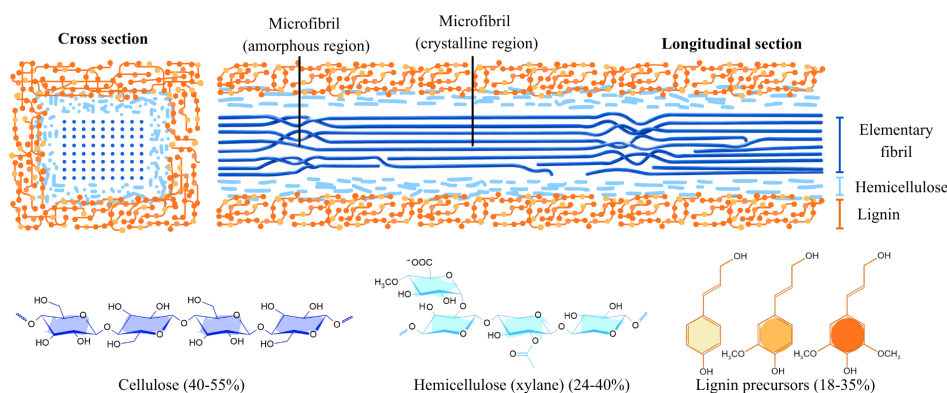


Figure 1. Cross and longitudinal section showing the crystalline and disordered regions of a cellulose elementary nanofibril embedded in lignin and hemicellulose matrix. The three major lignocellulosic components of wood: cellulose, hemicellulose, and lignin. Modified images are inspired by work Chen *et. al* 2020 and Li *et. al* 2019 [1,8].

The lignocellulosic composition varies considerably based on the origin of the biomass. For instance, woody biomass generally exhibits a higher lignin content and a distinct monolignol profile compared to grasses, which typically contains 1–10% lignin (discussed in more detail under Section 2.1.2.) [3]. Furthermore, while the glucose-based structure of cellulose remains uniform, hemicellulose composition is highly species-dependent. For example, xylan-rich hemicelluloses predominate in hardwoods like birch, whereas mannan-rich hemicelluloses are prevalent in softwoods (discussed in more detail in Section 2.1.3.). Consequently, the origin of the biomass strongly influences the physicochemical properties of the isolated fractions and determines their suitability for downstream applications [1,9].

While this unique polymer network gives wood the excellent mechanical performance required for construction and furniture, the industrial processing of timber generates vast quantities of residues. Currently, processing by-products from sawmills and plywood manufacturers are underutilized, serving primarily as low-value energy sources through direct combustion [10,11]. However, these residues represent highly valuable feedstocks for modern biorefineries. By separating the constituent polymers, these side streams can be effectively upcycled into higher-value functional materials and platform chemicals (discussed under Section 2.2.). A comprehensive understanding of biomass composition and structural architecture is therefore a fundamental prerequisite for optimizing the valorization of wood residues, a theme explored throughout this thesis.

2.1.1. Cellulose

As the most abundant structural polysaccharide in terrestrial biomass, cellulose forms the load-bearing framework of the plant cell wall [6,12]. It consists of linear β -1,4-linked glucan chains that assemble into tightly packed, crystalline microfibrils through extensive intra- and intermolecular hydrogen bonding [13]. The structural and functional properties of cellulose vary across plant taxa. In vascular plants, these microfibrils can reach degrees of polymerization exceeding 15,000 glucose units, resulting in high crystallinity and mechanical stability [10]. This supramolecular organization shapes the overall architecture of the cell wall, directly influencing biomass recalcitrance and processability. Consequently, understanding the role of cellulose within this composite structure is essential for efficient biorefinery utilization, especially since its accessibility is modulated by both hemicellulose branching and lignin deposition [7,14,15].

Although the primary chemical structure of cellulose is uniform across species, its functional properties after isolation are dictated by several variables. These key characteristics include fiber length, degree of crystallinity, degree of polymerization, and the purity of the fraction – specifically the content of residual lignin, hemicellulose, and ash remaining after extraction. The target application determines which properties are desirable. For instance, conventional pulp and paper industries require long fibers to achieve high-quality paper structural strength. Conversely, for applications such as oil/water mixture stabilization,

highly fibrillated nanoscale cellulose is preferred. In the latter case, the functional performance relies heavily on the structural duality of cellulose, which exhibits both hydrophilic and hydrophobic surfaces [16]. To evaluate these parameters, the structure and crystallinity of cellulose fibers are extensively characterized using advanced techniques such as X-ray diffraction (XRD), nuclear magnetic resonance (NMR) spectroscopy, and related analytical methods [17–19].

In woody and other vascular plants, the dominant crystalline allomorph is cellulose I β , which features a well-defined crystalline structure characterized by its specific unit cell geometry and crystal plane arrangement [20]. Elementary cellulose fibrils are generally modeled as having a hexagonal cross-section with surfaces that differ significantly in their affinity for water (**Fig. 2**). The (100) and (200) crystal planes are relatively hydrophobic, as the spatial orientation of their hydroxyl groups limits hydrogen-bond formation with the surrounding environment. In contrast, the larger (110), (1 $\bar{1}$ 0), and (010) planes readily form hydrogen bonds and are therefore predominantly hydrophilic [21]. Because macroscopic microfibrils are assemblies of these elementary fibrils, their interactions with water, as well as with the hydrophobic lignin matrix, are governed by the relative exposure and properties of these crystal surfaces [22].

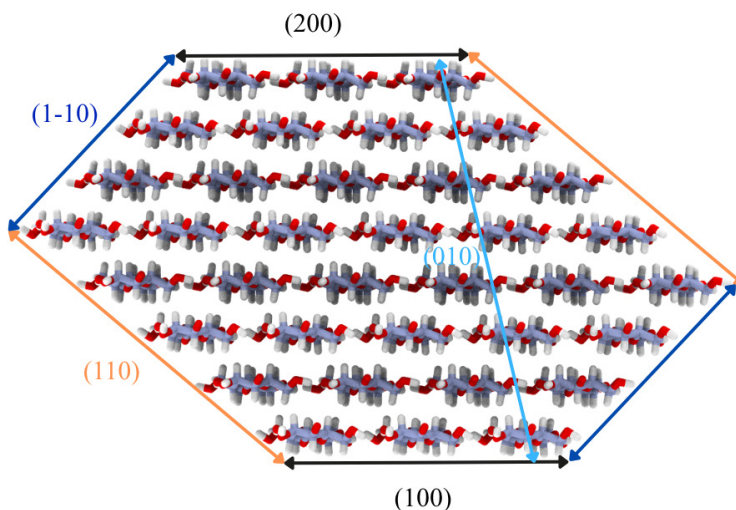


Figure 2. Cross-section of a 36-chain elementary fibril of cellulose I β . The (100) and (200) surfaces of the crystal (black) are the more hydrophobic and the (010) (light blue) represents the most hydrophilic surface. (1 $\bar{1}$ 0) and (110) are more similar to the (010) surface but are less hydrophilic. Hydrogen atoms are shown in white, oxygen atoms in red, and carbon atoms in blue. Model proposed by Ding and Himmel [23] for maize primary cell wall elementary fibril. Modified from [21,24].

This amphiphilic nature of cellulose, exhibiting both hydrophobic and hydrophilic domains, enables the formation of stable intramolecular interactions with lignin within the plant cell wall. Thus, while cellulose appears to be a straightforward linear polymer at first glance, its supramolecular behavior introduces layers of

complexity. Ultimately, when evaluating woody biomass for downstream valorization, cellulose cannot be viewed in isolation; it operates within an intricate, interconnected network of lignin–hemicellulose–cellulose interfaces that create a matrix more complex than its individual constituents.

2.1.2. Lignin

Lignin is widely recognized as one of the most promising renewable sources of aromatic chemicals. Consequently, its valorization has become a major research focus, driving a substantial increase in scientific literature over the past decade [9,25–27]. Within the framework of forest biorefinery, the efficient utilization of lignin is essential for enhancing process sustainability and maximizing the economic value derived from lignocellulosic biomass.

The primary structural building blocks of lignin are p-coumaryl, coniferyl, and sinapyl alcohol, which give rise to the 4-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) subunits in the lignin polymer matrix (**Fig. 3**) [28]. Lignin biosynthesis is a complex process initiated in the plant cytoplasm via the phenylpropanoid pathway, where phenylalanine is converted through a multi-enzyme cascade into H, G, and S monolignol precursors. Following synthesis, the monolignols are packaged into cytoplasmic vesicles that fuse with the plasma membrane, releasing the precursors into the apoplast via regulated transport mechanisms. Once in the apoplast, peroxidases and laccases catalyze the oxidative polymerization of the monolignols [15]. This localized deposition embeds the growing lignin polymer between the cellulose microfibrils and hemicelluloses discussed in Sections 2.1. and 2.2., reinforcing the cell wall matrix to provide compressive support, rigidity, and protection against biochemical and environmental stresses [14,29].

Because monolignol coupling in the apoplast occurs semi-randomly based on local precursor availability, the resulting macromolecule lacks a strictly defined, uniform primary sequence. [14]. Native lignin functions as a complex amorphous polymer, dominated by ether (β -O-4) linkages, alongside carbon-carbon bonds such as β -5 and 5-5 linkages (**Fig. 3**). The linkage nomenclature is based on the aromatic and aliphatic structural components, where the numbers represent the carbon in the aromatic ring and α , β and γ are used for the aliphatic side chain [30,31]. The relative abundance of these linkages and subunits varies across plant taxa, directly influencing biomass recalcitrance. Softwoods (gymnosperms) consist almost exclusively of G units, whereas hardwoods (angiosperms) contain a mixture of G and S units with trace amount of H units. In contrast, grasses typically exhibit a significantly higher proportion of H units [3].

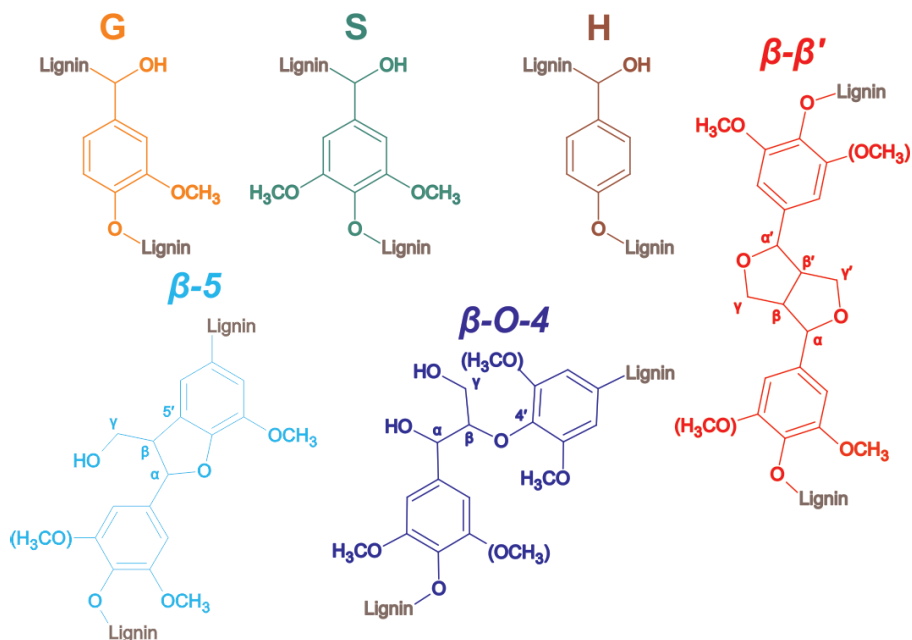


Figure 3. The chemical structure of the main lignin subunits is shown for G, S and H. Main linkage types with lignin subunits are shown, methoxy groups are in parentheses as their presence depends on the specific subunit.

This inherent structural heterogeneity presents a substantial analytical challenge for polymer characterization. NMR spectroscopy represents the state-of-the-art technique for elucidating the structural characteristics of both native biomass and isolated technical lignins [32,33]. Specifically, two-dimensional heteronuclear single quantum coherence (2D-HSQC) NMR allows for the semi-quantitative resolution of overlapping carbon-proton shifts. Resolving the aromatic and aliphatic regions provides critical insights into the composition of polymer [34]. The aromatic region of the spectra is used to calculate the relative abundance of G, S and H subunits, while the aliphatic region quantifies the distinct linkage types, predominantly β-O-4, β-β and β-5, expressed as a percentage or normalized quantity per 100 aromatic units [35–37].

Structural information obtained from experimental characterization can be used to derive statistical descriptors that define the molecular properties of a lignin sample and form the basis for computational lignin models. One such model, shown in **Fig. 4**, was constructed using NMR-derived structural characteristics of a lignin fractionated from a biorefinery HL. Recent advances in computational modeling, particularly classical molecular dynamics (MD) simulations, have provided atomistic insights into the structure, dynamics, and interactions of lignin within complex biomass matrices [38–40]. These topics of lignin–cellulose interactions will be evaluated within this thesis, but much work is still to be done.

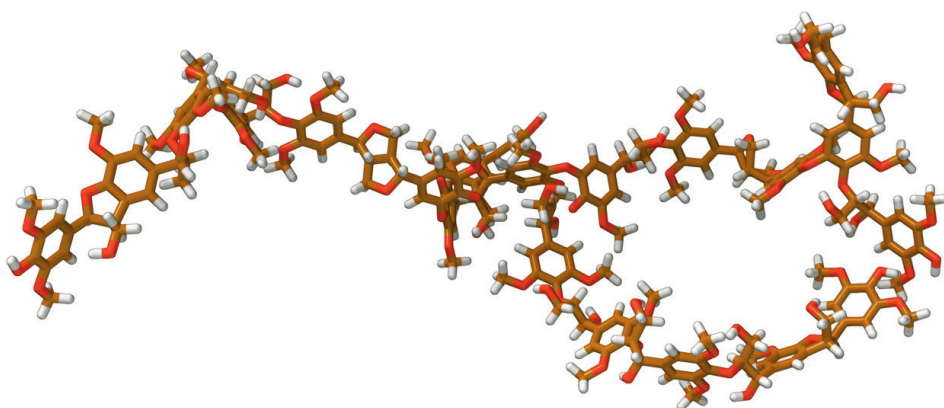


Figure 4. An example of linear lignin polymer. This specific model is representative of hardwood (birch) lignin soluble in aqueous THF (70 wt%) and is composed of 20 subunits, 13 S-, 7 G-units with an S/G ratio of 5.6 (see **Fig. 3** for subunit structures). The molecular weight of this polymer is approximately 4.7 kDa. Brown color designates carbon, red oxygen and white hydrogen atoms. The model was constructed with LigninBuilder [40] plugin and visualized with VMD software [41].

2.1.3. Hemicellulose

Hemicelluloses are a heterogeneous matrix of polysaccharides that contribute to the structural integrity of plant cell walls by associating with cellulose microfibrils and interacting with lignin [42]. Hemicellulose structure includes several distinct polysaccharides such as xyloglucans, xylans, mannans (including glucomannans and galacto-glucomannans), and mixed-linkage β -glucans, which vary by backbone composition, branching patterns, and side-chain substitutions [43]. These structural variations directly dictate the physicochemical properties of the cell wall matrix. For instance, cell walls rich in arabinoxylans have covalent cross-links mediated by ferulic acid esters, which bridge the carbohydrate network to the hydrophobic lignin matrix, thereby enhancing overall biomass recalcitrance [44]. While the existence of these lignin-carbohydrate complexes (LCC) (**Fig. 5**) in native plants remains a subject of ongoing debate within the scientific community, their relevance during and after chemical isolation processes is well established [45–47].

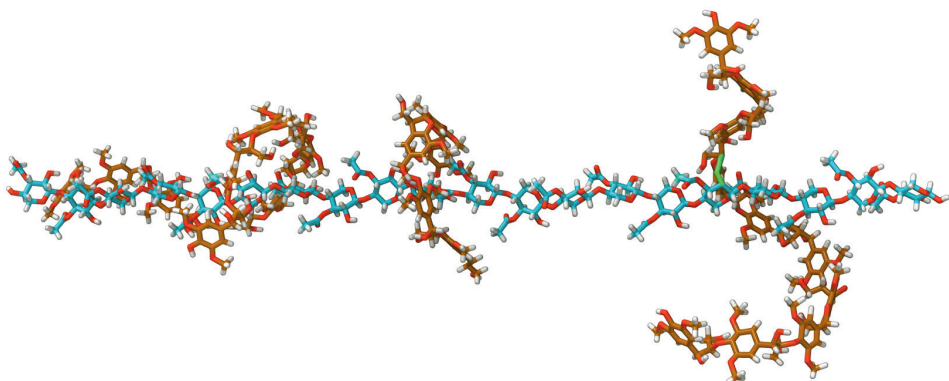


Figure 5. A proposed model of the birch lignin-carbohydrate complex (LCC) containing a xylan and lignin molecules. Brown color designates carbon for lignin and cyan for xylan carbon, red oxygen and white hydrogen atoms. Typical ether bond between lignin and hemicellulose is shown with green. Lignin molecules were generated with LigninBuilder [40] and hemicellulose model with GLYCAM-Web [48]. The models were composed together with SAMSON Connect software (Expert version) [49].

In hardwoods, glucuronoxylans serve as the dominant hemicellulose fraction, characterized by a β -(1 $\rightarrow$ 4)-xylan backbone substituted with glucuronic acid and acetyl groups that modulate solubility and intermolecular interaction [5]. Conversely, softwoods are rich in galactoglucomannans, which play an important role in water retention and mechanical stabilization within the secondary cell wall. As highlighted in Section 2.1., these compositional shifts influence industrial processability, with the highly branched and substituted xylans of hardwoods and grasses introducing steric complexity during enzymatic hydrolysis and pulping, and the glucomannans of softwoods primarily dictating fiber porosity and water interactions [43].

Consequently, traditional chemical pulping and modern biorefining technologies invariably require the depolymerization of hemicelluloses or the dissolution of lignin, both of which involve the cleavage of the underlying polymer structures. Although the individual properties and structural considerations of hemicelluloses are crucial to overall biomass deconstruction, a detailed investigation of this fraction lies outside the scope of the present thesis, which focuses primarily on the core dynamics of the cellulose and lignin fractions.

2.2. Wood-based biorefineries

The growing emphasis on economic, environmental, and social sustainability has accelerated the transition from fossil-based resources to renewable biomass [9,27,50–52]. Although woody biomass has historically been utilized as a low-value fuel for heat and energy production, technological advances in alternative renewable energy sectors, such as solar and wind power, alongside green hydrogen and methanol as energy carriers, are diminishing the role of wood as a primary

energy source [9]. Instead, forest biomass is increasingly recognized as a of raw materials for the production of renewable chemicals, with lignin emerging as one of the most promising macromolecular platforms for renewable aromatic compounds [53–55].

Estonia provides a compelling case study for these emerging biorefining opportunities. Over 50% of the country is covered by forests, supporting a highly developed mechanical wood processing industry. Nevertheless, a substantial proportion of low-grade roundwood and industrial processing residues are currently converted into low-value energy commodities, such as wood pellets, which constitute the Estonian largest export item. It is estimated that 60–70% of harvested wood in Estonia is ultimately combusted for energy production [11]. The abundance of this underutilized resource can stimulate both international and Estonian research initiatives aimed at technologically upgrading low-value forestry biomass into higher-value bioproducts.

Over the past 15 years, substantial research and development efforts have focused on forest biorefining, which aims to convert whole forest biomass into sugars, carbohydrate-based biofuels, lignin and a diverse suite of value-added chemicals [56–58]. Initial paradigms in the forest biorefinery sector focused primarily on converting lignocellulosic sugars into biofuels. However, despite significant technological progress, large-scale commercialization has been constrained by persistent techno-economic barriers. Forest biomass faces distinct commercial disadvantages compared to annual agricultural crops, including slower rotation, lower land productivity, higher harvesting and logistic costs, as well as a highly recalcitrant cell wall. These factors collectively reduce the market competitiveness of forest-derived biofuels relative to first-generation crop-based alternatives [59].

As the biorefinery concept has evolved, strategic focus has shifted towards integrated utilization of all the polymeric components to maximize resource efficiency and process economics. In this context, the valorization of lignin fraction has emerged as both a critical challenge and an opportunity [9,52,58,60,61]. Traditional pulp mills prioritize the extraction of intact cellulose fibers and treat the dissolved lignin as a process byproduct. In conventional kraft pulp mills, this fraction is contained within black liquor and is primarily burned to recover pulping chemicals and generate process energy. In sulfite mills, it is isolated and sold as low-value lignosulfonates. Globally, approximately 98% of industrially isolated lignin is currently combusted for heat and electricity generation [62].

Furthermore, traditional kraft pulp mills generally require massive production capacities exceeding one million tons of cellulose pulp per year to generate sufficient surpluses of process energy and process steam to warrant economic operation. Under these high-capacity conditions, a portion of the dissolved lignin can be recovered from black liquor without compromising the thermal balance of the mill. A prominent industrial example moving toward this paradigm is the Metsä Group bioproduct mill in Äänekoski, Finland, which achieves a production capacity of 1.3 million tons of kraft pulp per year and features integrated infrastructure for future commercial lignin recovery [63].

The highly complex, interconnected structure of the plant cell detailed in the beginning of Section 2 explains why such harsh conditions are traditionally required to isolate pure cellulose fibers. However, these severe pulping conditions heavily modify the native lignin macromolecule, inducing extensive carbon-carbon condensation and introducing structural sulfur contaminants. Consequently, the recovered technical lignin typically requires intensive upgrading before it can be utilized in high-value downstream applications. To address this, several commercial isolation technologies, such as LignoBoost and LignoForce, have been developed to recover lignin from pulp mill side streams [64–67].

In contrast to traditional pulping, novel biorefineries offer stepwise separation of all major biopolymers under significantly milder conditions. The benchmark for successful modern biorefining relies on fractionating and valorizing the highest possible weight percentage of the biomass into functional materials. [56]. The very first industrial-scale facility operating under this concept is beech-based biorefinery of UPM in Leuna, Germany, which commenced operations 2025 [68]. This facility converts hemicellulose and cellulose sugars into bio-monoethylene glycol and bio-monopropylene glycol, while isolating functional, sulfur-free lignin for use as a renewable functional filler in rubber and plastic industries.

Concurrently, a novel forest biorefining model is emerging wherein lignin and microcrystalline cellulose are targeted as the primary high-value outputs, while the remaining carbohydrate streams are treated as secondary coproducts [56]. These high-purity lignin and cellulose streams exhibit immense market potential for downstream application in performance plastics, structural adhesives, and specialty papers. The only industrial-scale, birch-based biorefinery utilizing this specific paradigm is Fibenol, located in Estonia [69]. Because the research presented in this thesis focuses specifically on the intermediates and final products derived from this novel fractionating process.

The central pretreatment step in this birch-based biorefinery utilizes a twin-screw extrusion system (**Fig. 6**), where wood chips undergo intensive mechanical shear combined with high-temperature steam explosion and dilute acid catalyst. This integrated thermo-mechano-chemical process efficiently deconstructs the rigid cell wall matrix, yielding a processable slurry for subsequent fractionation of free saccharides from hemicellulose. The dwell time of the biomass within the extruder is short, lasting approximately 20 seconds, rendering this an efficient and continuous pretreatment method. Following the extraction of the water-soluble hemicellulosic sugars from the slurry, the remaining insoluble suspension is a lignin–cellulose mixture (LCM).

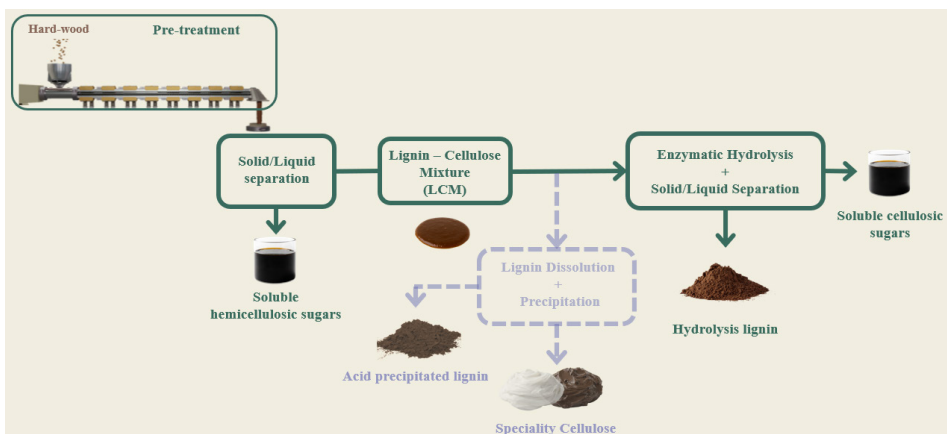


Figure 6. Illustrative overview of the biorefinery process, including a twin-screw based pretreatment with downstream solid-liquid separation of soluble hemicellulosic sugars and LCM. The LCM is enzymatically treated to convert cellulose into soluble saccharides and through a solid-liquid separation HL and sugars can be isolated. Alternatively, through LCM solubilization an alkali soluble lignin can be obtained through down-stream precipitation, and cellulose is in the alkali insoluble residue. Through bleaching a white cellulose can be isolated.

To date, LCM has rarely been utilized or characterized directly as a standalone biorefinery product. Instead, it is typically subjected to downstream processing to separate the cellulose and lignin fractions into conventional product streams (**Fig. 6**). One established pathway involves the enzymatic hydrolysis of the cellulose into glucose, leaving behind an insoluble solid fraction classified as HL [9]. Alternatively, lignin can be selectively solubilized from the LCM under alkaline conditions, isolated via acid precipitation, and dried into a fine technical lignin powder. The remaining cellulose-rich solid residue can then be processed into specialty cellulose through bleaching sequences [70].

Each of these downstream processing methods lead to distinct structural modifications on both the cellulose and lignin fractions. For example, while traditional pulp and paper processes (*e.g.* kraft) preserve a long cellulose fiber length to maximize paper strength (as discussed in Section 2.1.1.), the mechanical shear within the twin-screw extrusion system significantly reduces the cellulose fiber length. Conversely, while traditional chemical extractions severely alter and condense the native aromatic network (as discussed in Section 2.1.2.), the twin-screw extrusion and subsequent enzymatic processing pathways largely preserve the chemical structure of the native lignin within the resulting HL fraction. Therefore, introducing greater operational flexibility into biorefinery processing parameters and intermediate outputs unlocks novel scientific and development opportunities [9].

2.3. Downstream processing of biorefinery products

The biorefinery serves as an initial step to fractionate the biomass into crude intermediate streams and crude products. To isolate well-defined, functional end-materials, targeted downstream processing is essential. These downstream strategies can alter macromolecular properties through physical property modification, chemical conversion, or solvent-based separation governed by specific polymer features.

This thesis primarily investigates the potential of ultrasound (US) as an advanced biorefining tool, focusing on its capacity to facilitate the dissolution of HL and the modification of the LCM. Exploiting these US-assisted pathways may provide the opportunity to modify lignin solubility and produce different lignin fractions, as well as LCM materials with tailored physicochemical properties. The following section provides a brief overview of previous work on the use of these technologies in the valorization of lignocellulosic biomass.

2.3.1. Lignin solubilization and fractionation pathways

For most high-value downstream applications, technical lignin requires targeted macromolecular modification or narrow fractionation to overcome the baseline limitations of the raw polymer mixture [71]. The inherent structural heterogeneity of lignin, coupled with its limited solubility in many solvents and highly poly-disperse molecular properties, has motivated the development of diverse fractionation strategies [72–75]. Historically, lignin has been fractionated by selective precipitation from kraft black liquor at specific pH value [76]. More recently, membrane-based separation has emerged as an alternative approach, employing ceramic ultrafiltration membranes to separate solubilized lignin fractions according to molecular weight. Although effective in principle, membrane ultrafiltration is severely constrained by the poor baseline solubility of certain technical lignins, alongside severe membrane fouling and the high capital and operating expenses associated with industrial ultrafiltration systems [77,78].

Among available processing alternatives, solvent-based fractionation has proven particularly effective for producing isolated lignin fractions with tailored, homogeneous physicochemical properties [72,79]. The process operates under relatively mild conditions and benefits from lower capital equipment costs, minimized chemical consumption, and high potential for closed-loop solvent regeneration. The efficiency of solvent-based dissolution is governed by both the origin of the biomass and the primary isolation technology employed upstream. For instance, organosolv lignins exhibit high solubility in the specific organic media used during their primary extraction, whereas industrial kraft lignin (KL) displays its highest solubility in highly alkaline aqueous systems [80,81].

Alkaline treatment represents one of the most established and robust methods for technical lignin solubilization and fractionation, used extensively at both at laboratory and industrial scales [72,80]. In aqueous alkaline media, dissolution is primarily driven by the deprotonation and subsequent ionization of phenolic

hydroxyl groups. This induction of negative charge density increases the local hydrophilicity of lignin, enabling dissolution into the aqueous phase. The overall yield and efficiency of alkaline solubilization are closely regulated by the specific alkali counterion, its concentration, and the overall alkali-to-lignin mass ratio, which collectively dictates the degree of deprotonation, the structural integrity of the lignin fragments, and the final dissolution thermodynamics [80].

The impact of different alkali counterions have been investigated for industrial KL [80]. Empirical studies demonstrate that lithium hydroxide (LiOH) yields optimal dissolution performance, even though kraft pulping and the recovery lines are strictly sodium-based (Na^+) systems. While complete macromolecular dissolution can be achieved using various hydroxides (e.g., LiOH, NaOH, and KOH), the minimum required stoichiometric concentration varies significantly, showing a clear trend where smaller cations lead to better dissolution kinetics and yields [80]. This counterion effect is largely attributed to the superior spatial accessibility to the smaller solvent shell to the rigid polymer network and its enhanced electrostatic capacity to unfold the lignin macromolecules.

The dissolution or aggregation of lignin is also strongly dictated by the specific properties of pure organic solvents and their mixtures, enabling precise control over macromolecular unfolding or self-assembly [82]. Computational MD simulations confirm that spatial conformation of lignin expands or compacts dynamically based on local solvent shell polarity and hydrogen-bonding capacity, varying significantly between poorly matching and optimized organic solvent systems [39].

To improve the chemical homogeneity and purity of isolated technical lignins, organic solvent-water mixtures are increasingly utilized. For aqueous ethanol (EtOH) systems, the specific solubilization thresholds and the resulting macromolecular properties of the isolated fractions are highly sensitive to the solvent-to-water ratio and the structural state of the initial technical lignin [77,83]. Unlike standard biomass extraction, organic solvent systems utilizing aqueous EtOH or tetrahydrofuran (THF) are deployed directly on pre-isolated technical fractions rather than whole biomass to achieve narrow-purity streams [83–85].

These binary systems enable selective dissolution of specific fractions based on molecular weight and functional group density. The solvent polarity and the water-to-organic solvent ratio were shown to be critical process parameters that fundamentally shape both the overall solubility yield and the chemical and physical characteristics of the dissolved lignin fractions [85]. Specifically, it has been shown that highest lignin solubilities are consistently achieved in aqueous organic solvent mixtures with a 60–80% co-solvent content [72,74,75,83,84].

Recently, the author of this thesis also co-authored a publication on the topic of lignin fractionation in aqueous EtOH and THF solvents, where a similar relationship between HL solubility and co-solvent concentration was observed [86]. As a general thermodynamic trend, an increase in overall solvent dissolution capacity correlates with a simultaneous increase in both the relative molecular weight (M_w) and the polydispersity index (PDI) within both solvent mixtures. However, aqueous THF exhibited a higher total solubility for HL (**Fig. 7A**) and

dissolved a fraction of lignin with a broader molecular weight distribution compared to aqueous EtOH (**Fig. 7B**). For both organic solvents, the highest solubilities and M_w values are around 70–80 wt% of co-solvent content. Furthermore, MD simulations presented in the publication highlighted that both water and the organic co-solvent play cooperative, essential role in the solvation shell stabilization of HL. Additionally, the chemical properties of the isolated fractions showed a distinct preference based on the solvent type and mixture composition. Quantitative ^{31}P NMR spectroscopy revealed that a higher water content in the binary mixture preferentially isolates fractions with a higher total hydroxyl ($-\text{OH}$) content, leading to higher total OH (mmol/g) content in the lignin [86]. These insights demonstrate that discrete, functionally adjusted lignin fractions can be systematically engineered simply by manipulating the thermodynamic parameters of the binary solvent media.

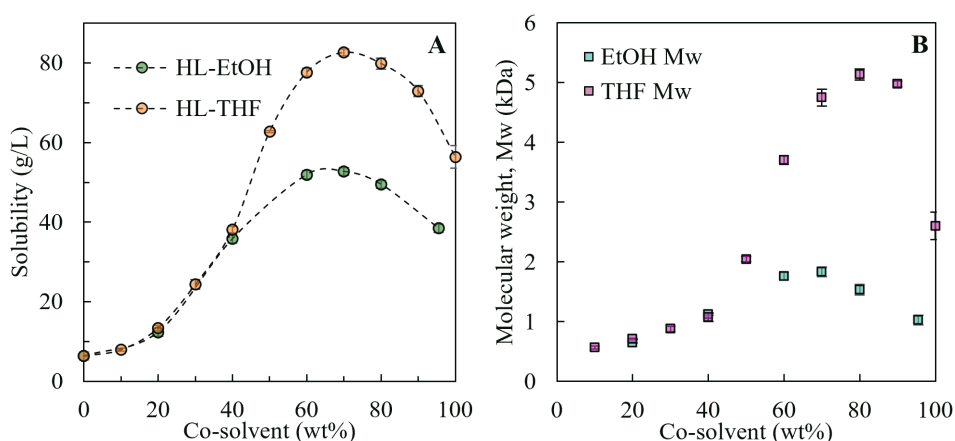


Figure 7. (A) HL solubilities in water-EtOH (green circles) and water-THF (orange circles) mixtures with varying co-solvent content. (B) Relative molecular weights of the soluble lignin fractions in water-EtOH (cyan square) and water-THF (purple square). Within this work, a solid-to-liquid ratio of 1:10 was utilized during HL fractionation [86].

The HL solubilization results were used in this study to selectively solubilize lignin from the LCM, with the goal of lowering the lignin content based on the chemical characteristics. Since HL is isolated from LCM via enzymatic cellulose hydrolysis, therefore the baseline solubility of HL and the properties of the resulting fractions can be considered representative of lignin fractionated directly from the unaltered LCM using identical solvent mixtures. Ultimately, evaluating how the lignin content influences the effects of US on the LCM network, alongside mapping the impacts of acoustic cavitation on the underlying lignin–cellulose interfacial interactions, constitutes the primary scientific objective of this thesis.

2.3.2. Theoretical principles of ultrasonic energy and acoustic cavitation

US is a mechanical acoustic wave, typically spanning the frequency range of 10 kHz to 20 MHz. It is generated by piezoelectric transducers that produce alternating compression and rarefaction cycles when propagated through liquid media [87,88]. Depending on the specific operating frequency, acoustic power, and application method, the transmission of US energy drives several distinct secondary phenomena: thermal heating, acoustic cavitation, acoustic streaming (micro-mixing), nebulization, and homolytic radical formation (**Fig. 8**). While these effects coexist to varying degrees in most US treatments, their relative prominence is fundamentally governed by the applied frequency and power density.

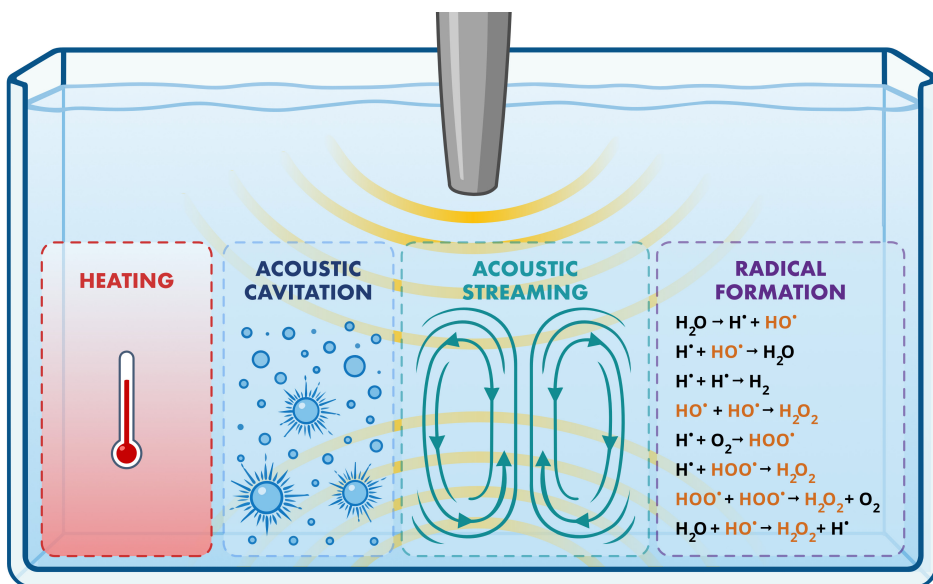


Figure 8. Multi-modal physicochemical phenomena generated by an ultrasonic probe system in liquid media. The propagation of acoustic energy drives localized thermal heating, transient pressure-induced cavitation, micro-mixing via acoustic streaming, and homolytic radical formation. Figure adapted from [89].

In high-frequency US applications (>150 kHz), primary chemical effects dominate due to accelerated sonochemical radical formation. This occurs via the sonolysis of water molecules, yielding peroxides and other highly reactive oxygen species, which can promote chemical reactions or cleave labile C-O linkages within organic polymers [90–92]. Conversely, low-frequency “power ultrasound” (typically 20–80 kHz) minimizes radical formation and instead channels acoustic energy into mechanical effects, driven primarily by acoustic streaming and transient cavitation [87,88,93,94].

The primary mechanism for this mechanical process intensification relies on the propagation of oscillating pressure waves, which induce the nucleation,

growth, and violent collapse of cavitation microbubbles. Under suitable resonant conditions, the rapid implosion of these bubbles generates localized “hot spots” characterized by transient, extreme conditions, including temperatures approaching ~5000 °C and pressures reaching ~50 MPa. When cavitation bubbles collapse near solid-liquid interfaces, such as suspended biomass particles, the asymmetric confinement forces the generation of high-velocity microjets (>100 m/s) and powerful shockwaves. These phenomena induce intense micro-convection currents that eliminate boundary-layer resistance, thereby drastically enhancing energy, mass, and heat transfer across all US applications [95].

The efficiency of cavitation bubble formation and subsequent implosion is highly governed by the physical properties of the solvent matrix, most notably its viscosity, surface tension, and vapor pressure. This implies that the choice of US energy transfer parameters, acoustic power, and operating frequency must be precisely tailored to match the specific properties of the liquid medium and the required processing results [91,94].

Because of this adaptability, US technology has emerged as a powerful tool for process intensification across a wide range of industrial sectors, including chemical synthesis, pharmaceuticals, environmental engineering, and biomass valorization [90,96–99]. The distinctive capacity of acoustic cavitation to induce both mechanical and sonochemical effects has enabled its application in diverse processes such as emulsification [100–102], crystallization [103], extraction [104,105], cell disruption [106], and catalytic transformations [107–109]. In chemical and pharmaceutical industries, US enhances reaction rates, yields, and selectivity by promoting superior transfer and mixing under comparatively mild, non-destructive reaction conditions, contributing to more sustainable and energy-efficient processes [110].

Furthermore, within fields such as food processing and environmental remediation, US has been effectively utilized at scaled-up industrial systems for the extraction of bioactive compounds, degradation of pollutants, and wastewater treatment, where the cavitation-driven phenomena facilitate the breakdown of complex matrices or contaminants [26,111,112]. Given its proven scalability and adaptability across these diverse sectors, US is increasingly recognized as a key enabling technology for the development of next-generation biorefineries, aimed at enhancing the efficiency of biomass fractionation, conversion, and the extraction of high-value products [89].

Within the scope of this thesis, the experimental focus is restricted on the probe-type sonication system, operating within the power US regime at a fixed frequency of 20 kHz. At this frequency, physical and mechanical disruption are maximized, which is desirable due to its proven capacity to enhance energy and mass transfer, micro-fracture lignocellulosic frameworks, and intensify heterogeneous processing streams. Beyond frequency and electrical power, the physical dimensions of the transducer probe represent a critical operational variable; the specific tip size and horn geometry alter the spatial distribution of the applied forces and directly dictate the hydrodynamic and cavitation behavior of the used mixtures.

2.3.3. Ultrasonic treatment for lignocellulosic biomass and isolated products

In the context of forest biorefineries, power US has been increasingly explored as a process intensification strategy to enhance the fractionation and downstream conversion of lignocellulosic biomass. Several studies have demonstrated that US can be applied either as a standalone pretreatment or as an assisting method to improve the efficiency of conventional processes such as delignification, enzymatic hydrolysis, or solvent extraction [89,96,105,113]. The intense localized mechanical disruption induced by acoustic cavitation aids in opening the highly compact, rigid plant cell wall matrix. By micro-fracturing the structural layers, sonication facilitating solvent penetration and enzyme accessibility to the embedded cellulose fibers, while concurrently promoting the boundary-layer mass transfer required for accelerated lignin solubilization or depolymerization [3,109,114].

However, the direct US treatment of raw, untreated woody biomass exhibits severely limited effectiveness. As established in Section 2.1., native wood possesses a highly dense, recalcitrant polymeric matrix. The mechanical forces of cavitation alone are insufficient to overcome this macro-scale structural resistance without prohibitive, commercially unviable energy inputs [115].

Among US wood biomass fractionation technologies, the Bio-Sep/Sonichem (UK) process is one of the few patented systems demonstrated at the pilot scale [116]. This process combines US-assisted organosolv fractionation with an oxalic acid catalyst to separate wood into cellulose-rich fibers, isolated lignin, and hemicellulose-derived sugars [117,118]. Within this patented system, sonication is applied in a batch or semi-continuous mode, which is often combined with traditional thermal digestion. In this configuration, US acts primarily as a process intensification step that promotes disruption of the biomass matrix. While this technological advancement provides unique opportunities to obtain a variety of high-purity lignocellulosic fractions using acoustic energy, the system is constrained by prolonged processing times and multiple required downstream separation steps. Consequently, significant engineering challenges remain regarding total process time and high energy consumption. Nevertheless, this is a promising development which shows that US technology can be scaled up for the valorization of wood biomass.

Because of these limitations, far more promising and energy-efficient results are reported when US is directed toward structurally open or pretreated polymers. Substrates such as partially delignified biomass, isolated technical lignins [119,120], or pre-extracted cellulose streams [101,121,122] permit unhindered transmission of acoustic energy, resulting in pronounced structural disruption or macromolecular depolymerization. For instance, US-assisted alkali pretreatment has been shown to significantly increase lignin removal and cellulose accessibility, leading to improved enzymatic saccharification yields compared to alkali treatment alone. This synergy is attributed to the capacity of acoustic microstreaming to overcome mass transfer limitations while generating transient localized conditions that promote the chemical cleavage of the intermolecular ester and ether bonds anchoring the lignin matrix to the carbohydrates [123].

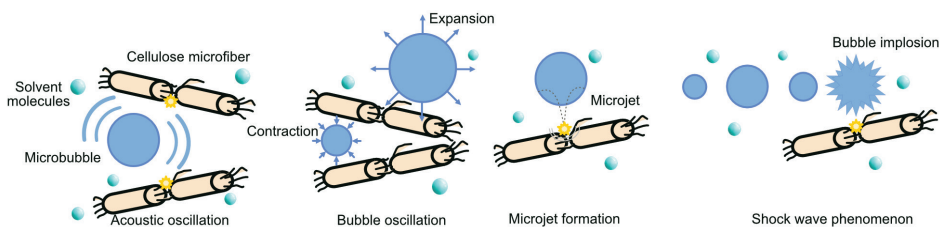


Figure 9. Visualization of cavitation effects in liquid media for cellulose fibers. These include the main effects that alter cellulose polymers via cleavage or the disruption of the crystalline structure.

Beyond pretreatment, power US has been successfully utilized to facilitate nanocellulose production from both purified cellulose and pre-extracted biomass side streams, where the mechanical shear forces assist in the top-down fibrillation and size reduction of cellulose fibers (**Fig. 9**). However, despite these promising laboratory-scale achievements, the widespread commercial integration of US within forest biorefineries is still hindered by challenges regarding process scalability, acoustic attenuation in high-solid mixtures, overall energy efficiency, and sonochemical reactor design [89].

Due to the challenges related to using US treatment on untreated wood biomass, HL and LCM were chosen to investigate the behavior under US. As the complex wood structures have been broken down, sonication could lead to interesting results for these materials.

3. AIMS OF THE STUDY

This doctoral dissertation is based on three peer-reviewed articles that evaluate the effectiveness of ultrasound (US) in upgrading various intermediate fractions generated within modern forest biorefineries, specifically hydrolysis lignin (HL) and the intermediate lignin–cellulose mixture (LCM), as a process intensification tool. Although the effects of acoustic cavitation on conventional technical lignins are well documented, those on novel HL (**Publication I**), which is isolated via continuous twin-screw extrusion and subsequent downstream processing, have not yet been established. Furthermore, the direct conversion of LCM as a standalone biorefinery feedstock has not been widely investigated. The main aim of this study was therefore to gain a deeper understanding of the structural and interfacial interactions of the two main biopolymers, cellulose and lignin, by using targeted US treatment (**Publications II and III**). To achieve these general objectives, the specific research aims were formulated as follows:

In **Publication I**, we determined the optimal thermodynamic parameters and dissolution kinetics of HL in aqueous NaOH solutions. The study quantified the benefits of US treatment in accelerating mass transfer, reducing chemical consumption and modulating the physicochemical properties of the solubilized fractions by introducing 20 kHz acoustic cavitation. Ultimately, we elucidated the mechano-chemical and sonochemical mechanisms driving US-mediated solvation in mild alkaline media.

In **Publication II**, we investigated the mechanical impact of US power directly on the unrefined LCM in water. The study examined how acoustic streaming and shear forces affect the rigid carbohydrate structure when there is a high level of native lignin present. Key objectives included optimizing the overall dry matter loading (wt%) to maximize acoustic energy propagation and tracking the resulting fiber fibrillation within the cellulose framework.

In **Publication III**, we modified the chemical composition of the LCM matrix in order to investigate the interfacial interactions that drive the biopolymer network. Discrete molecular weight lignin fractions were selectively isolated from the LCM framework using solvent-based extraction to assess the effect of varying residual lignin content on the efficiency of US-induced nanocellulose material formation. This links surface morphology and hydration capacity to the responsiveness of the material to acoustic forces.

4. MATERIALS AND METHODS

Due to the major differences between HL and LCM, the analysis of results required different approaches. Briefly, gravimetric methods were employed to determine solubility of lignin for HL and LCM, as well as the water retention value (WRV) of LCM. The structural and macromolecular characterization of the isolated soluble HL fractions was performed using size exclusion chromatography (SEC) and NMR spectroscopy.

The chemical composition of HL and LCM was quantified using the Klason lignin method, while the carbohydrate profile was determined via high-performance liquid chromatography (HPLC). US-induced morphological, structural, and texturing changes within LCM materials were analyzed using laser diffraction (LD), scanning electron microscopy (SEM), and X-ray diffraction (XRD).

4.1. Ultrasonic processing of hydrolysis lignin and lignin-cellulose mixtures

A consistent US system was employed across **Publications I–III** utilizing a Bandelin Sonopuls HD 4200 US homogenizer operating at a fixed frequency of 20 kHz with a maximum power output of 200 W. The acoustic energy was delivered to the liquid media via an immersion probe. For the experiments in **Publications I and II**, a titanium horn tip (model TT213) and in **Publication III** a titanium probe (model TS103) was used.

In **Publication I**, the US irradiation was applied during the dissolution phase of HL in aqueous NaOH solutions to accelerate solvation kinetics. Conversely, in **Publications II and III**, the processing focus shifted to investigating the downstream mechano-chemical effects on the LCM framework. Across all experimental designs, the acoustic energy transfer was systematically controlled by varying the US amplitudes, sonication residence times, and initial dry matter loadings of the raw feedstocks, as detailed in the respective publications.

4.2. Dissolution, fractionation, and characterization of hydrolysis lignin

Aqueous alkaline systems were established as the primary media for optimizing lignin solubilization. The dissolution behavior of HL was investigated by varying the NaOH/HL mass ratio alongside the overall dry weight percentage (wt%) of the solution. In all experiments the solubilization was carried out for 30 min, before any US treatment. Power US was introduced during the dissolution stage to enhance overall solubility yields under mild alkaline conditions.

Lignin solubility yields were determined gravimetrically from the insoluble residues and validated in solution via ultraviolet-visible (UV-Vis) spectroscopy. Spectroscopic quantification was performed at a characteristic wavelength of 280 nm, utilizing a calibration curve constructed from fully alkali soluble acid-precipitated lignin (APL). Following dissolution, the soluble HL fraction was separated from the remaining insoluble lignin by centrifugation, and the dissolved lignin was recovered from the liquid phase through controlled precipitation with dilute sulfuric acid (H_2SO_4). This APL was subsequently dried and used for further SEC and NMR characterization.

The relative molecular weight distributions (M_w – weight-averaged molecular weight and M_n – number-averaged molecular weight) and polydispersity indices ($\text{PDI} = M_w/M_n$) of the solubilized HL fractions were determined using a Shimadzu Prominence SEC system equipped with a photodiode array (PDA) detector operating at 280 nm. The molecular weight-based separation was achieved using sequential column system consisting of PSS MCX columns (10 000 Å and 1000 Å pore sizes, 8×300 mm) protected by a matching MCX pre-column (5 µm particle size, 8×50 mm). The analysis was conducted under an isocratic flow rate of 0.6 ml/min utilizing a 0.1 M NaOH mobile phase at ambient temperature with a total runtime of 50 min and an injection volume of 10 µl. The molecular weight calibration curve was established using sodium polystyrene sulfonate salt standard spanning M_w range of 246 to 100 000 Da. Data collection and data analysis was carried out with LabSolutions software.

The structural and functional group compositions of the isolated lignin fractions were elucidated using quantitative ^{31}P NMR [124] and 2D HSQC NMR spectroscopy [125]. The ^{31}P NMR profiling provided quantitative insight into the distribution of aliphatic and phenolic hydroxyl (–OH) functional groups, while the 2D HSQC experiments revealed the underlying inter-unit linkages and monomeric subunit distributions. All spectral acquisitions were performed on a Bruker Avance-III 700 MHz spectrometer with specific pulse sequences and sample preparation parameters detailed in **Publication I**.

4.3. Lignin–cellulose mixture treatment and characterization

The experimental framework of **Publications II** and **III** centered on evaluating the physical impacts of US on the LCM, determining the effects of lignin content and defining the interfacial boundaries between the two biopolymers. The structural alterations were quantified using the analytical techniques outlined below.

The particle size distributions (PSD) of the untreated and sonicated LCM suspensions were measured via LD using Microtrac S3500 Bluewave particle size analyzer. To ensure reproducible dispersion and minimize particle agglomeration, the instrument is fitted with sample circuit system, which allows all samples to be measured directly (wet form) in deionized water.

The WRV was utilized as a gravimetric indicator to assess the structural openness, swelling behavior, and hydration capacity of both the original and US-treated LCM, serving as an indirect measure of solvent accessibility to the cellulose fibers. To determine the WRV, the control and sonicated suspensions were centrifuged under standardized conditions to remove excess water. The wet mass of the compacted sample was recorded, and the pellet was freeze-dried to a constant weight. The final WRV was calculated as the mass ratio of retained water to dry LCM matter ($\text{g}_{\text{water}} / \text{g}_{\text{dry LCM}}$), following the exact protocols described in **Publications II** and **III**.

The chemical bonds and functional groups of the dried LCM samples were analyzed using Fourier-transform infrared (FT-IR) spectroscopy on a Perkin-Elmer Spectrum BXII spectrometer and KBr disk method. Samples were prepared at a uniform solid concentration of 1 wt% relative to the KBr (total disk mass of 100 mg). The disks were pressed under 10 t and FT-IR spectra were recorded over a spectral range of 4000–600 cm^{-1} by averaging 16 scans at a spectral resolution of 4 cm^{-1} .

To evaluate the top-down morphological fiber fibrillation, and microstructural changes induced by US treatment across the different LCM configurations, the dried substrates were analyzed by SEM. In **Publication II** the material was coated with a thin Pt by layer using a Leica EM SCO 500 coater. High-resolution surface imaging was performed on a Zeiss EVO15MA SEM, equipped with an Oxford X-Max with backscattered electron detector, with digital micro-graphs captured using SmartSEM software (version 6.05). In **Publication III** the samples were coated with 7 nm layer of Au using a SC7640 Auto/Manual High Resolution sputter coater (Quorum Technologies Ltd, East Sussex, England) using 1.5 kV 15 mA for 90 s. Imaging was performed with a Nova NanoSEM 450 (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with a CBS detector. Measurements were made with an electron beam energy of 5 kV, with a dwell time of 10 μs .

The crystalline structure, crystallinity index and apparent crystallite size of the cellulose framework were determined with XRD using Bruker D8 Advance diffractometer. The instrument was operated with Cu-K α radiation, 2.5° Soller pulses, and LynxEye detector. Diffractograms were collected across a 2 θ scanning range of 3–73° with a step size of 0.017° and a cumulative exposure time of 178 sec per data point. Qualitative phase identification was conducted using Bruker DIFFRAC.EVA software linked to the ICDD PDF-5+ (2024) database. Quantitative structural assessment, including polymorph ratio extraction and crystallite dimension calculations, was performed with the full profile analysis software Topas 6 (Bruker, Germany).

5. RESULTS AND DISCUSSION

This section provides an overview of the main experimental results compiled from the 3 peer-reviewed research publications that make up this doctoral study. Although each paper represents an independent investigation, they are fundamentally interconnected through the application of power US and their shared focus on materials generated from a birch-based biorefinery. Our initial investigation evaluated the dissolution behavior and macromolecular properties of high-purity HL. As this particular technical HL had not been studied before, it was essential to establish its fundamental solubility as a standalone polymer (**Publication I**). It was hypothesized that these baseline characteristics would directly translate into the more complex, multicomponent LCM. However, it was found that direct US processing did not increase the alkaline extraction efficiency of lignin from LCM with sonication (unpublished data).

Consequently, the focus of this research shifted toward evaluating how the structural network of LCM transforms with acoustic cavitation (**Publication II**) and determining the role of the surrounding lignin network during this process (**Publication III**). To understand how lignin content affects these acoustic trends, the LCM was selectively delignified using the aqueous solvents tested in our group [86]. Collectively, these studies evaluate the applicability and effects of US across compositionally distinct forest biorefinery-derived material streams.

5.1. Non-ultrasound and ultrasound solubility of hydrolysis lignin in alkaline solutions

5.1.1. Ultrasound-enhanced solubilization of hydrolysis lignin

This section discusses the findings presented in **Publication I** while focusing on the critical operational variables that govern alkaline solubilization. It also addresses how US can be used as a non-destructive method to enhance the mass transfer kinetics and final yield of the soluble lignin fraction of HL in aqueous alkaline media.

The alkaline dissolution parameters were initially investigated using mild NaOH systems, which were adapted from the established bases used in KL solubilization [80]. In the context of kraft pulping, lignin is dissolved at high temperatures using NaOH concentrations that are ten times higher [3]. However, the twin-screw extrusion and steam explosion pathway used to produce the HL in this study preserve a core structure that is more native-like than heavily degraded and condensed KLS. Therefore, identifying the specific solubility boundaries of HL was a critical preliminary step.

The overall dissolution efficiency of technical lignin is driven by the total solid loading and the alkali concentration. Although aqueous alkali is a good solvent for different types of technical lignin, their dissolution behavior varies significantly depending on the biomass origin and extraction method [3,80]. Furthermore, even

within highly effective alkaline media, dissolution efficiency is routinely limited by the high molecular weight of the lignin polymer and its severe thermodynamic tendency to form macro-aggregates. US offers a potential process intensification strategy by introduction acoustic cavitation effects. These effects can mechanically break down macromolecular aggregates, enhance solvent accessibility, and accelerate mass transfer, all without the need for severe temperatures or harsh chemical additives [89].

To characterize the solubility profile of HL, experiments were conducted at ambient temperature across a range of aqueous NaOH concentrations, both without and with US treatment. Sonication was applied using a 20 kHz immersion probe system at a constant power output of 30%, using a Bandelin Sonopuls HD 4200 device equipped with a titanium tip (model TT213). This was applied for residence times of up to 60 minutes. The resulting HL solubility yields were quantified gravimetrically from the insoluble residues, while the isolated supernatant fractions were characterized for relative molecular weight distribution by SEC and structural configurations using quantitative ^{31}P and 2D-HSQC NMR spectroscopy.

The solubilization results of HL in 0, 0.5, 0.75, 1.0, and 2.0 wt% of NaOH aqueous solutions with and without US treatment are listed in Tables 1 and 2 of **Publication I**. Two solid-to-liquid ratios (S/L), 100 and 150 grams of HL per L of NaOH solution, were studied. To compare the effect of alkali dosage while accounting for differences in NaOH concentration and HL solid loading, the HL dissolution yields (%) obtained at S/L ratios of 100 and 150, which were plotted as a function of the NaOH/HL mass ratio ($\text{g}_{\text{NaOH}}/\text{g}_{\text{HL}}$). This parameter represents the amount of alkali available for lignin deprotonation and enables direct comparison of dissolution efficiency across different experimental conditions (**Fig. 10**).

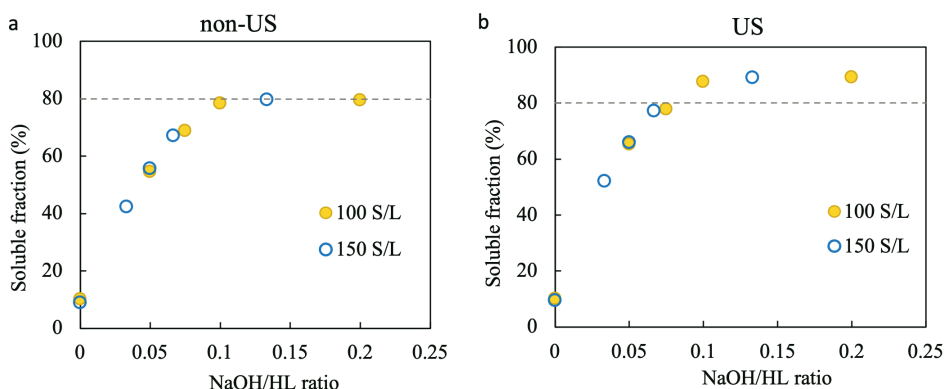


Figure 10. Lignin solubility in aqueous NaOH is shown at 100 and 150 S/L ratio, based on the overall alkaline concentration in respect to lignin (g of NaOH/g of HL). a) lignin solubility with no US applied, it reaches about 80% from the total material, b) lignin solubility with US treatment at 60 min, the overall solubility is increased to 90%.

The experimental data shows, that although the general dissolution trends of HL align with KL solubilities [80] and solubility increases with an increased NaOH/HL ratio, complete dissolution of HL is not achieved under the tested conditions. HL exhibits a maximum solubility of approximately 80 wt% at 0.1 NaOH/HL ratio and further increase in alkaline loading does not increase the solubility further (**Fig. 10a**). It is important to note, that the insoluble fraction contains not only lignin but also cellulose and enzyme residues from the biorefining process, some of which remain insoluble under alkaline conditions, as detailed in **Publication I**. Notably, while US-assisted solubility of HL shows the same tendency as without US, there was an increase in the maximal HL solubility, up to 90% (**Fig. 10b**). Such high solubility cannot be achieved under extended conventional mixing times or at higher temperatures.

To better understand US effect on HL dissolution process, the solubility enhancement was monitored during irradiation. Samples were taken at time intervals from the HL-NaOH solution mixtures, and the corresponding lignin concentrations with US (C_{US}) and without sonication (C_{non}) were estimated via UV-vis spectroscopy. The ratio of the respective concentrations (C_{US}/C_{non}) was considered as the effect of US on solubility enhancement during sonication (**Fig. 11**). Numerical corresponding values of C_{US}/C_{non} can be found in **Publication I** (Supplementary data, Table 4S).

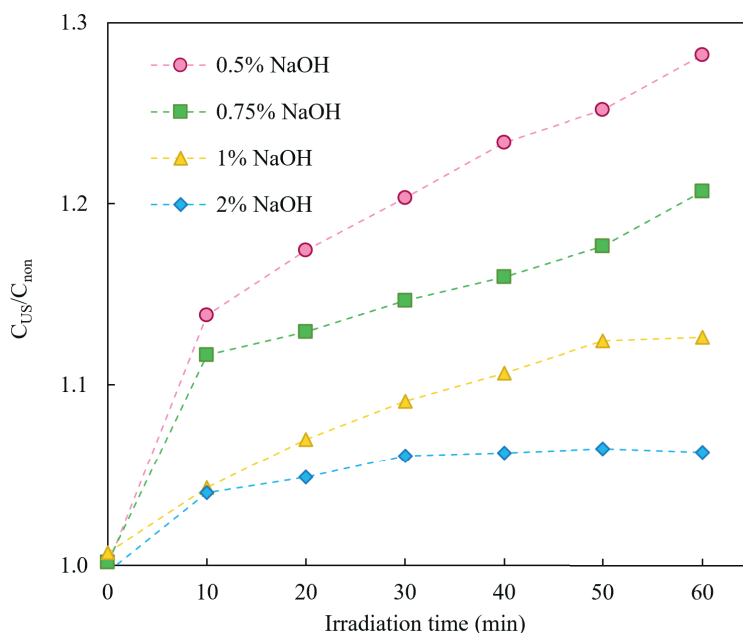


Figure 11. US-enhanced solubilization of HL from 0–60 min at 150 S/L ratio at 25 °C with 0.5–2% NaOH (corresponding to 0.03–0.13 NaOH/HL ratio). The results are expressed as a concentration ratio (C_{US}/C_{non}) to illustrate the relative solubility enhancement during sonication.

As shown in **Fig. 11** the C_{US}/C_{non} is strongly dependent on the irradiation time. In all NaOH solutions, the sharpest increase of solubility happens within the first 10 min of sonication. At 0.13 NaOH/HL ratio the C_{US}/C_{non} ratio reached a maximum after approximately 30 min, with no further significant increase upon prolonged irradiation. In contrast, in lower NaOH/HL ratio conditions solubility continued to increase progressively throughout the sonication period, after 60 min the increase was up to 30%. These results suggest that US rapidly dissolves a fraction of HL that remains insoluble under conventional mixing. Notably, this effect was not observed in pure water, indicating that alkaline conditions are essential to initiate US-enhanced dissolution.

5.1.2. Molecular and structural characterization of dissolved hydrolysis lignin

Due to its heterogeneous polymeric structure, technical lignin is commonly characterized by its M_n , M_w , and PDI. These parameters describe its molecular weight distribution and are typically determined by SEC, which is widely used to evaluate technical lignins [126], lignin fractions [83], and US-induced structural changes [120].

In this study, SEC was used to characterize the dissolved HL fractions. The M_n , M_w , and PDI were determined as a function of the NaOH/HL ratio (**Fig. 5** in **Publication I**). Under conventional mixing, both M_n and M_w increased with higher alkali dosage, indicating that higher NaOH concentrations promoted the dissolution of larger lignin macromolecules. Similarly, with US treatment the increase in solubility also increased the molecular weight averages. These effects were the most pronounced at low NaOH/HL ratios.

These results indicate that the primary contribution of US to the dissolution process is driven by mechano-acoustic effects, which improve the solvent accessibility and release of large lignin molecules from the heterogeneous solid phase. Although mild sonochemical degradation of dissolved lignin may occur during prolonged irradiation [114,127], the enhanced dissolution of larger lignin molecules remains the dominant effect under the investigated conditions.

It is proposed that selective release of larger macromolecules would not occur without sonication. Lignin molecules with a higher molecular weight are likely to interact intramolecularly, associate with each other, or bind to residual cellulose fractions through secondary hydrophobic surface interactions or hydrogen bonding [16,38,128]. In a static system, these associations could restrict the accessibility of phenolic hydroxyl groups to the solvent, preventing the deprotonation necessary for the polymer to enter the aqueous phase. The shockwaves and microjets from acoustic cavitation effectively overcome these physical boundaries.

The structural properties of the dissolved HL fractions were analyzed from the corresponding acid-precipitated lignin samples using 2D-HSQC and quantitative ^{31}P NMR spectroscopy. The results are summarized in Tables 3 and 4 of **Publication I**.

The 2D-HSQC analysis confirmed that the studied birch HL exhibits typical hardwood lignin characteristics, with S units dominating over G units [129]. As this method also characterizes the major linkage types, it was shown that there was minimal change for relative β -O-4 linkage abundance of the soluble fraction whether it was solubilized with or without US. However, increasing the NaOH/HL ratio leads to the dissolution of lignin with lower β -O-4 abundance. This confirms that the enhanced lignin solubility is primarily governed by physical dispersion and disaggregation rather than extensive chemical alteration. However, sonication of only the dissolved HL did result in a slight decrease in β -O-4 linkages, thus indicating that prolonged US treatments could cleave labile linkages in lignin.

^{31}P NMR analysis showed that lignin fractions dissolved at lower NaOH concentrations contained higher amounts of aliphatic and phenolic hydroxyl groups than those dissolved under stronger alkaline conditions. US treatment increased the overall hydroxyl group content of the dissolved fractions, most likely due to improved release of hydroxyl-rich lignin macromolecules from the heterogeneous solid phase and, to a lesser extent, sonochemical cleavage of ether linkages. Overall, the NMR analyses confirmed that US intensifies lignin dissolution while largely preserving the native structural features of HL under the investigated processing conditions.

5.1.3 Key findings on ultrasound-enhanced hydrolysis lignin solubilization

Findings from **Publication I** show that US intensifies HL dissolution primarily through physical dispersion and macro-aggregate disaggregation mechanisms, rather than chemical cleavage. This is consistent with the known physical effects of acoustic cavitation in heterogeneous systems [95]. Intense, localized turbulence and microjets break down macroscopic assemblies to maximize surface area contact without altering the underlying chemical structure of the polymer.

This process intensification approach demonstrates the potential of combining US with mild alkaline solubilization for more efficient and selective processing of HL. This framework provides a sustainable, low-temperature pathway to access higher concentrations of structurally preserved lignin in aqueous systems.

However, while aqueous alkaline conditions are highly effective at dissolving most of the HL polymer matrix, they offer low structural selectivity. To obtain more specific materials, a more selective system is required. Binary organic-water solvent systems can dissolve technical lignin based on its chemical characteristics, even if the overall solubility yield is lower. Within our research group, these binary systems were thoroughly investigated using varying organic solvent-to-water ratios, which altered the isolated lignin properties [86]. The insights gained from both the alkaline dissolution results of **Publication I** and these organic solvents extractions provided the input required to delignify the unrefined LCM material. This allows the assessment of how lignin content and delignification alter LCM acoustic responsiveness.

5.2. Ultrasonic treatment of biorefinery lignin–cellulose mixture

5.2.1. Establishing ultrasonic processing conditions for lignin–cellulose mixture

Within this section the findings of **Publication II** are discussed, wherein the US treatment conditions were established in correlation with the total solid loading as well as characterization of a high lignin content cellulose material. Based on **Publication I**, it was proposed that US treatment could lead to increased solubilization of lignin from the LCM, which would help to develop a novel delignification strategy for this mixture. However, exploratory alkaline delignification with US treatment for the LCM resulted in the formation of a highly viscous mass, with no improvement in the final solubility yield of the lignin fraction compared to conventional mixing (unpublished data). As this combination of alkaline and US processes proved unfeasible, the research shifted towards evaluating the physical and structural changes caused by US in pure aqueous LCM suspensions under neutral conditions. This work identified the optimal batch sonication conditions in terms of total solid loading and prompted a more thorough investigation into the effects of lignin on US treatment, which are detailed further in **Publication III**.

The LCM in this thesis comprised of approximately 40 wt% lignin and 50 wt% cellulose, which is a much more complex mixture in comparison to HL used in **Publication I**. To maximize the technological and economic feasibility of downstream processing, initial optimization trials targeted high total solids loadings. However, when US treatment was evaluated at an initial solid loading of 8 wt%, a highly viscous gel was produced within the first 10 minutes of processing. The rapid induction of this high viscosity severely limited acoustic energy transfer and restricted cavitation propagation within the liquid medium (**Fig. 12**).

To determine a viable operational threshold, the total solids content of LCM was varied alongside sonication time and acoustic amplitude while tracking the onset of gelation. These optimization procedures established a baseline treatment time of 20 minutes, requiring any selected solid loading to remain fully flowable and acoustically active throughout the entire irradiation cycle. Consequently, a total solid loading of $\leq 4\%$ LCM was selected for the experiments in **Publication II and III**.

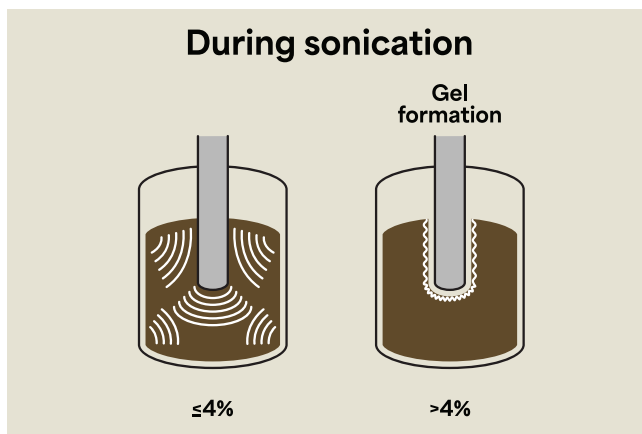


Figure 12. Schematic illustration of US treatment of LCM at solid loadings below (left) and above (right) 4 wt%. At solid loadings below 4 wt%, US waves propagate effectively throughout the liquid phase during the entire treatment period. In contrast, at solid loadings above 4 wt%, US energy transmission ceases after short sonication time due to the formation of a gel-like layer around the sonication probe, resulting in loss of direct contact between the probe and the LCM dispersion.

The experiments done in this thesis were in a batch system, where the restraint of total solid content was identified. It may be possible to overcome this with a flow-through cell, which could potentially alleviate these mass transfer challenges by forcing the high-viscosity slurry into direct contact with the transducer probe. However, such continuous systems were outside of the scope of this thesis.

5.2.2. Characterization of lignin–cellulose mixture with and without ultrasonic treatment

FT-IR spectroscopy was used to assess if any alterations of the chemical structures within LCM are occurring during US treatment. The data showed that there were no measurable changes in chemical bonds or functional groups (Fig. 3 in **Publication II**). Consequently, the analytical focus shifted to investigating the physical and morphological transformations induced by US using WRV, PSD, XRD and SEM.

PSD was determined by laser diffraction (LD) analysis to evaluate the effect of US on the physical disintegration of the LCM. This is a powerful indicator of how pure nanocellulose (e.g., CNF or CNC) [130] or nanolignin [98] evolves during its preparation with sonication and also shows manufacturing efficiency. Although LD cannot distinguish between cellulose, lignin, and their aggregates in heterogeneous suspensions, it enables rapid analysis of untreated aqueous suspensions and is well suited for comparing relative changes in particle size induced by US under identical sample compositions [131].

The untreated LCM material exhibited a broad PSD (2–400 μm), reflecting the heterogeneous nature of the material and the presence of large aggregates.

Following the introduction of 20 kHz power US, a significant structural breakdown was observed within the first 5 minutes of irradiation, characterized by a rapid accumulation of fine particles in the 0.02–3 μm range (Fig. 5 of **Publication II**). Increasing the sonication time progressively increased the volume fraction of sub-micron particles. This physical transition is primarily driven by the cavitation-induced fragmentation of the largest particles (30–400 μm), most likely through the disruption of cellulose fiber bundles or larger lignin–cellulose aggregates. The most significant reduction in volumetric particle size was achieved at the lowest solid loading of 0.5% LCM. Following a 20-minute sonication period, 50% of suspended particles were reduced to below 1 μm , although a population of larger structural fragments exceeding 10 μm was still retained within the matrix (**Fig. 13**). Despite the high lignin content of the LCM, US effectively generated cellulose-rich submicron structures, while a fraction of larger particles remained throughout the treatment. These particles are likely lignin-rich aggregates, as US treatment of HL suspensions resulted in no significant changes in PSD or gel formation (**Publication II**, Supplementary data, Fig. 3S).

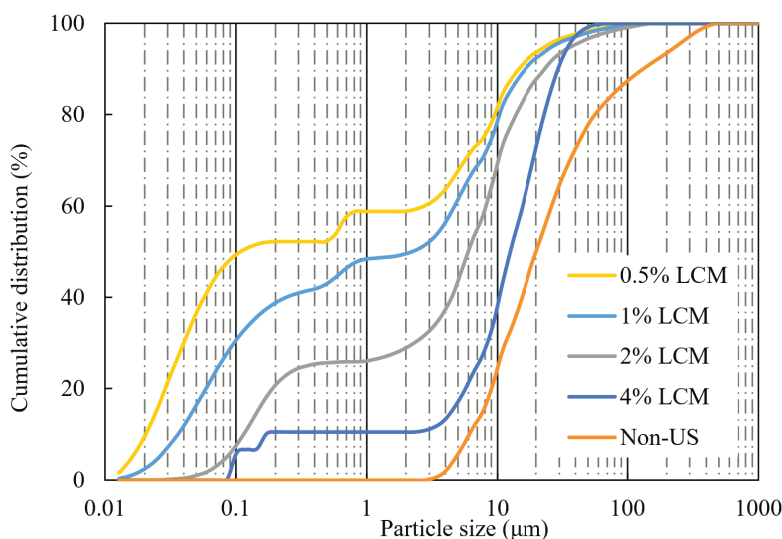


Figure 13. Cumulative particle size distribution of LCM is shown with US treatment at 20 min, with 50% US power applied. The changes of particle size are connected to the overall total solids of the mixture.

The extent of particle size reduction depended strongly on the LCM solid loading. Increasing the solid content reduced the accessibility of acoustic energy and limited the efficiency of particle fragmentation, demonstrating that effective US processing requires sufficient energy transfer throughout dispersion.

Previous studies have demonstrated that the intense shear forces and acoustic microstreaming generated by US effectively disrupt cellulose crystals, providing a route for the production of microfibrillated or nanocellulose [121,122,132].

However, contemporary literature contains very limited information regarding the acoustic processing of materials that natively contain high concentration of lignin with cellulose polymers.

The changes in particle size are also reflected in the material properties characterized by the WRV, which indirectly shows the hydrogen bonding between cellulose and water. WRV is widely used to assess the water-holding capacity of cellulosic materials and provides indirect information about fiber fibrillation and specific surface area [133,134]. An increase in WRV correlates with greater water accessibility to cellulose fibers and amorphous regions. Untreated LCM exhibited a constant WRV of approximately 2.5 ± 0.2 g of water per g of dry material across the investigated total solids, indicating that the initial WRV was independent of LCM concentration. US treatment substantially increased the WRV, demonstrating enhanced water accessibility to the cellulose-rich matrix because of particle fragmentation and fibrillation [135]. To evaluate the magnitude of these changes, the US amplitude was varied from 10% to 50% while holding the processing time constant at 20 minutes. At all LCM concentrations, the WRV reached a plateau after 20 min of sonication at 30% of US power, indicating that further energy input produced only marginal improvements. However, the magnitude of increase in WRV depended strongly on the solid loading of the LCM, with dilute suspensions exhibiting the greatest enhancement. From 4 wt% to 0.5 wt% of LCM, the change in final plateau was approximately twofold (**Fig. 14**). This finding suggests, similar to PSD analysis, that the efficiency of US is governed not only by applied acoustic energy but also by the accessibility of suspension to acoustic cavitation.

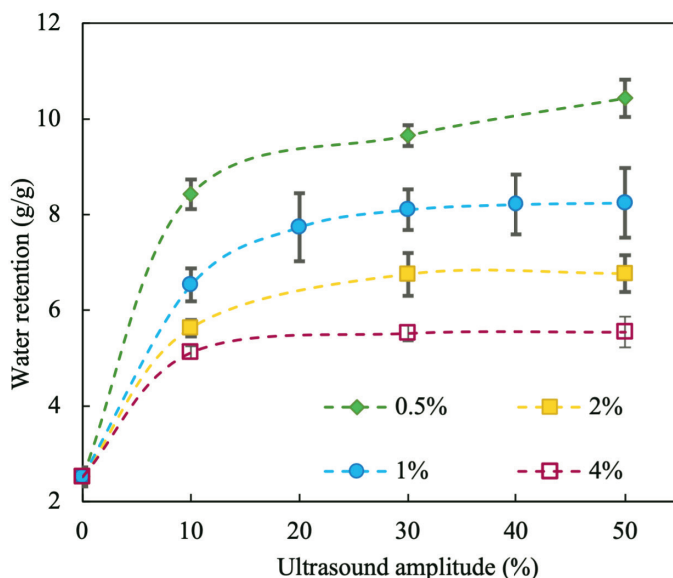


Figure 14. The WRV is shown for LCM with a solid loading from 0.5–4% treated by US with varied amplitude from 0–50% and at 20 min of sonication (TT213 tip; 20 kHz). The overall increase of water adsorption plateau is determined by the solid loading with lowest yielding the best results in a batch process.

These findings demonstrate that US can be used as a standalone treatment to disrupt high lignin-content LCM, producing stable dispersions and partially nanofibrillated cellulose–lignin materials. Thus, US-assisted processing provides a simple and efficient route for producing novel functional lignocellulosic materials directly from biorefinery intermediate streams, without prior separation of cellulose and lignin. However, further research is required to determine how the specific volume and chemical properties of the lignin fraction influence these US-assisted structural changes. This thesis established a controlled, stepwise delignification of the raw LCM as the ideal approach for isolating and analyzing these biopolymer matrix interactions, which are detailed further in the following section.

5.3. Varying lignin content in lignin–cellulose mixture and investigation of the sonication effects

5.3.1. Delignification and characterization of lignin–cellulose mixtures

In this section the results of **Publication III** are discussed, which connects the insights from **Publication I** and **II** by combining HL solubilization principles with the US treatment of the unrefined LCM in water. The focus of **Publication III** was to gradually reduce the lignin content of the LCM to gain a better understanding of the effect of lignin content has on the US performance.

To investigate the role of lignin loading in the US processing of LCMs, controlled delignification was performed using three distinct media: 70 wt% aqueous EtOH, 70 wt% aqueous THF, and a dilute NaOH solution. These solvents systems were selected based on our research group previous study on HL dissolution [86] and the results are briefly discussed in the Section 2.3.2. Both 70 wt% EtOH and THF exhibited maximum HL solubility at this concentration; however, THF dissolved more lignin and preferentially extracted higher-molecular-weight fractions. Although 2D-HSQC NMR confirmed similar lignin subunits and interunit linkages in both fractions, ³¹P NMR showed that the EtOH-soluble lignin contained more hydroxyl groups and was more polar. Consequently, the residual lignin in LCM is expected to be more hydrophobic after EtOH treatment than after THF treatment. In contrast, aqueous NaOH is an effective lignin solvent that enables extensive delignification of LCM (**Publication I**).

Based on composition analysis, all solvent systems delignified LCM in a stepwise manner (**Table 1**). The LCM delignified with EtOH, THF and NaOH aqueous solution are defined as eLCM, tLCM and nLCM, respectively. THF achieved greater lignin removal than EtOH, whereas alkaline treatment resulted in the highest degree of delignification, reducing the lignin content to approximately 15 wt%. These results confirmed that solvent treatment provides an effective approach for preparing LCMs with systematically varied lignin contents with minimal alterations to the cellulose fraction.

Table 1. Composition of the non-treated LCM and the solvent-treated LCMs. The values of Klason lignin and cellulose contents are presented in the table and expressed on a dry mass basis with standard deviations.

Fraction symbol	Delignifying solvent	Klason lignin (%)		Total lignin (%)	Cellulose (%)
		AIL (%)	ASL (%)		
LCM	–	38.3 ± 1.1	2.1 ± 0.3	40.4 ± 1.3	50.2 ± 1.6
eLCM	70 wt% EtOH	26.1 ± 2.4	1.1 ± 0.4	27.1 ± 2.0	63.9 ± 2.6
tLCM	70 wt% THF	20.3 ± 0.6	0.8 ± 0.1	21.1 ± 0.6	69.1 ± 0.6
nLCM	0.8 wt% NaOH	14.6 ± 0.2	0.6 ± 0.2	15.2 ± 0.4	75.3 ± 0.9

The structural integrity of the prepared LCMs was further evaluated by FT-IR spectroscopy. All of the FT-IR spectra of LCMs are shown and interpreted in Fig. 1 of **Publication III**. No significant alterations in the crystalline cellulose fingerprint region were observed after solvent treatment, indicating that the delignification procedures primarily removed lignin without inducing major structural modifications to the cellulose component. Consequently, the solvent-treated LCMs provided suitable materials for investigating the influence of lignin content on the US-induced structural and physicochemical transformations discussed in the following sections.

5.3.2. Effect of ultrasonic treatment of varied lignin content lignin–cellulose mixtures

To investigate the influence of lignin content on US processing, untreated and solvent-delignified LCMs were sonicated under identical conditions while maintaining a constant cellulose concentration (0.3 wt%). Based on optimization studies (**Publication II** and Section 5.2.1.), a low solid loading (0.5 wt% LCM) was selected to maximize acoustic energy transfer and facilitate direct comparison of materials with different lignin contents. It is important to note that, although this study also used US at 20 kHz, it employed a different sonication probe, cell and higher intensity compared to those used in **Publication II** (see Section 4.1.). Nevertheless, the directions and kinetics of the US effects and the morphology and physical properties of the resulting LCM materials were similar, as can be seen below.

Visual assessment of the suspensions after 72 h of storage (**Fig. 15**) demonstrated that both lignin content and sonication strongly influenced dispersion stability. Solvent-delignified LCMs exhibited progressively improved suspension stability with decreasing lignin content, indicating that lignin removal increased the accessibility of hydrophilic cellulose surfaces and promoted stronger interactions with water. US treatment further enhanced dispersion stability, particularly during the first 10 min of irradiation, consistent with increased fibrillation and improved colloidal stability of the cellulose-rich particles. For all the materials stable dispersions were achieved with short irradiation times, 5 min, which did not occur with the raw LCM even at 20 min of sonication.



Figure 15. Solution stability of LCM, eLCM, tLCM and nLCM with varied US treatment from 0–20 min at 0.3% cellulose content, which means varied total loading due to e-, t-, nLCM having less lignin in the mix and thus more cellulose. The stability of water dispersion after 72 h of storage is shown.

Despite the pronounced changes in the physical behavior of the suspensions, FT-IR spectroscopy revealed no significant differences between untreated and sonicated samples, irrespective of the degree of delignification (**Publication III**, Supplementary Materials, Fig. 1). The characteristic absorption bands of both lignin and cellulose remained unchanged, including the cellulose crystallinity region, indicating that the relatively short sonication treatment did not induce measurable chemical modification of either polymer. Consequently, enhanced suspension stability and subsequent changes in material properties can be attributed predominantly to physical and morphological effects of acoustic cavitation rather than to sonochemical reactions. These findings demonstrate that lignin content plays a critical role in determining the colloidal behavior of LCMs during US processing, while the underlying chemical structures of lignin and cellulose remain largely preserved under the investigated conditions.

The influence of lignin content on the US-induced physicochemical modification of LCMs was evaluated using WRV and PSD analyses. WRV was used as an indirect measure of cellulose fibrillation and water accessibility, whereas LD provided information on the extent of particle fragmentation during sonication. Together, these complementary techniques enabled assessment of how lignin affects the response of LCMs to acoustic cavitation.

Without US treatment, progressively delignified LCMs exhibited higher WRVs, demonstrating that lignin removal increased the accessibility of hydrophilic cellulose surfaces and enhanced water uptake. However, the WRVs were small, increasing from 3 to 6.4 for raw LCM and nLCM, respectively (**Fig. 16**). Although the change in VRW is relatively small, the trend correlates with the results of the LCM suspension stability in **Fig. 15**.

US further increased the WRV of all materials; however, the magnitude of this effect strongly depended on the residual lignin content. In the case of the original LCM, the WRV reached a maximum value (WRV of about 10 $\text{g}_{\text{water}}/\text{g}_{\text{dry LCM}}$) after 5 min of sonication and remained unchanged thereafter, which is consistent with the results in **Fig. 14** with 0.5 wt% LCM. These are remarkable results, as they show similar physical properties despite the US experimental conditions being very different. The US frequency and lignin content were the same in these two experiments. Therefore, it can be assumed that the maximum WRV is determined by the lignin content. Consequently, nanofibrillation of cellulose

under US can continue without significantly impacting the WRV at the selected short irradiation times. Still it would be expected that the US treatment is most effective for pure cellulose, and this has been researched comprehensively by other authors [121,132,136].

The greatest increase was observed for the extensively delignified material, for nLCM, WRV is highest after 20 min and is almost 10 times higher than that of the not sonicated nLCM sample (6.4 and 59.7, respectively). A similar continuous increase in WRV with respect to sonication time is shown by all other solvent-delignified LCMs, but a near plateau is also approached after 20 min. These findings indicate that lignin restricts the structural rearrangements and fiber swelling induced by US, while delignification facilitates the development of a more open and hydrated cellulose network.

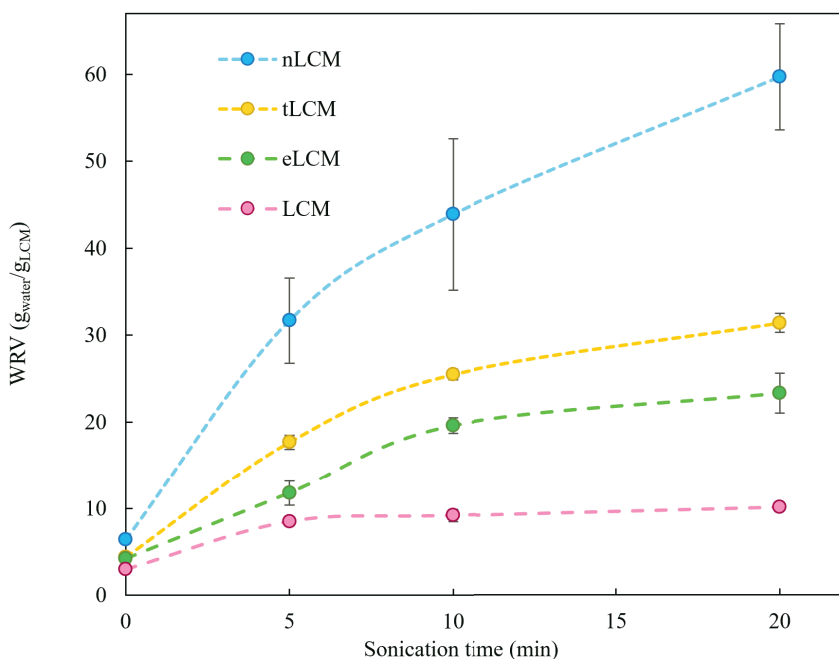


Figure 16. WRV ($g_{\text{water}}/g_{\text{cellulose}}$) for LCM, eLCM, tLCM and nLCM with equalized cellulose contents plotted against the sonication time (TS103 tip with 20% US amplitude, 20 kHz). Errors are present for all samples, but the dots overlap them due to small differences between experiments.

To further evaluate the influence of lignin on the response of LCMs to US, the WRV was correlated with the lignin mass fraction ($m_{\text{lignin}}/(m_{\text{lignin}} + m_{\text{cellulose}})$) of the solvent-treated materials (**Fig. 17**). Without sonication, WRV increased as the lignin content decreased, indicating that lignin removal enhanced the accessibility of hydrophilic cellulose surfaces and improved water uptake (**Fig. 17A**). While EtOH- and THF-treated LCMs correlated linearly with WRVs, the NaOH-delignified material showed a substantially higher value, suggesting that both the degree of delignification and the nature of the residual lignin influence the water-holding capacity of the cellulose-rich matrix. **Fig. 17B** illustrates a comparable

correlation between WRV and lignin mass fraction following 20 minutes of sonication; however, the effect of US becomes more evident as the lignin content reduces. These results suggest that the amount and characteristics of lignin both influence the US modification of LCM. Although WRV alone cannot fully describe the structural changes induced by US, it provides a useful indicator of how lignin content and lignin chemistry influence the physicochemical response of LCMs and can therefore be used to optimize material properties for specific applications.

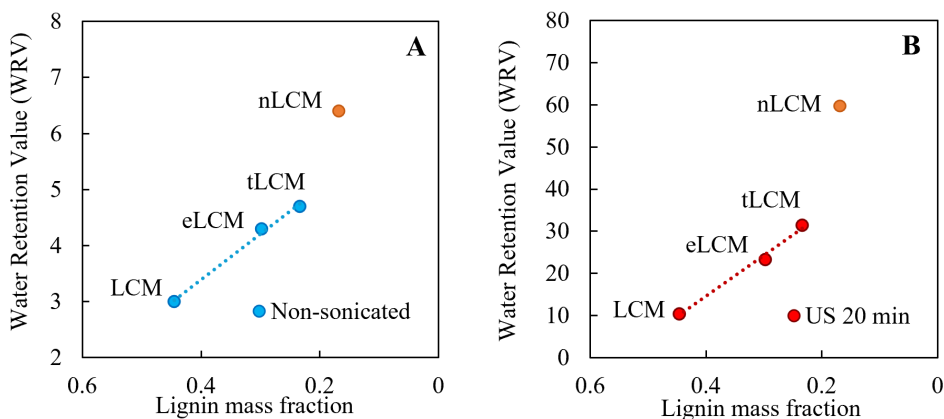


Figure 17. WRV ($\text{g}_{\text{water}}/\text{g}_{\text{cellulose}}$) of LCM, eLCM, tLCM, and nLCM plotted against lignin mass fraction, which is calculated with equation $m_{\text{lignin}}/m_{\text{lignin}} + m_{\text{cellulose}}$. The dependence depicted in panel (A) is for non-sonicated LCMs, and in panel (B) for LCMs after 20 min of sonication.

5.3.3. Morphology and crystallinity of sonicated lignin-cellulose mixtures

The effects of solvent delignification and US treatment on the morphology and crystalline structure of LCMs were investigated using SEM and XRD. As the analysis requires dry materials, then the SEM images are not fully representative of the original wet material. The main reason for this is the hornification effect of cellulose that occurs during water removal [132] and alters the morphology of the visualized materials. However, it still provided insights into the delignified LCMs. SEM analysis demonstrated that progressive lignin removal altered the surface morphology of the untreated LCMs. The native material exhibited large heterogeneous particles covered by an amorphous lignin-rich layer, the cellulose or wooden structural particles seem to be masked by the lignin, whereas increasing degrees of delignification progressively exposed the underlying cellulose-rich structures (see **Publication III**, Fig. 6). In **Fig. 18**, the comparison between unaltered LCM and nLCM is shown, the lignin content is lowered from 40% to 15%. The extensively delignified nLCM displayed well-defined surfaces with minimal amorphous coating, indicating that lignin removal improved the accessibility of the cellulose framework. These observations are consistent with the increased suspension stability and WRV observed for the delignified materials.

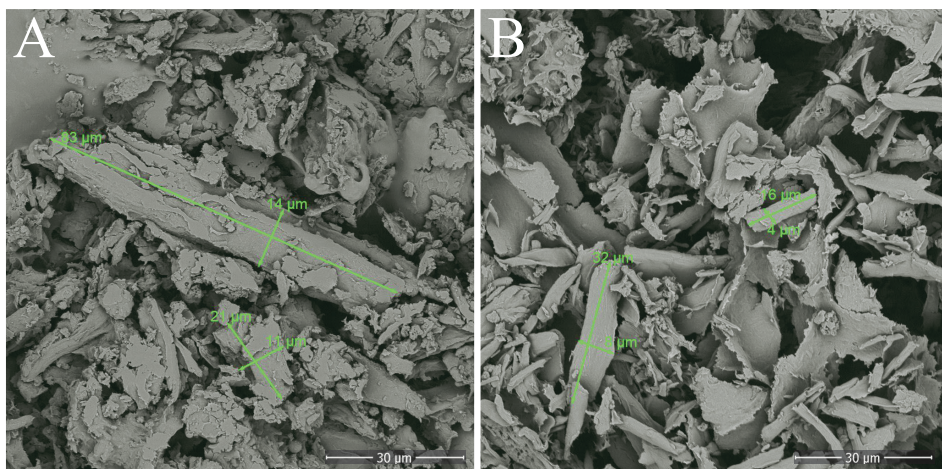


Figure 18. SEM micrographs of (A) LCM and (B) NaOH delignified (nLCM) at 2000 $\times$ magnification.

US treatment caused extensive physical defibrillation of all LCMs irrespective of lignin content (see **Publication III**, Fig. 7). In **Fig. 19** the sonicated materials of LCM and tLCM are shown, where the large lignocellulosic particles are fragmented into layered fibrillar structures composed of cellulose nanofibrils, confirming that acoustic cavitation effectively disrupted the macroscopic fiber network. Although the sonicated LCM no longer contains elongated particles with sharp edges, it is still less homogeneous and contains jagged bumps and rounded formations, which can be linked to the higher lignin content. Progressively delignified LCMs exhibited increasingly homogeneous fibrillar morphologies. The NaOH-delignified nLCM showed the highest degree of fibril separation, with distinct nanoscale cellulose bundles becoming visible after sonication. These morphological changes explain the substantial increase in WRV, and suspension stability observed following US treatment.

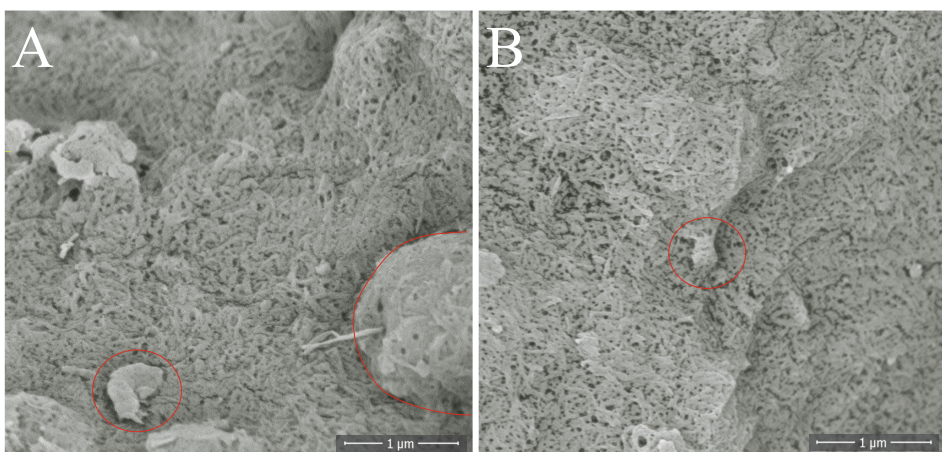


Figure 19. SEM micrographs of (A) LCM and (B) THF-delignified (tLCM) after 20 minutes of sonication at 50 000 $\times$ magnification.

All materials exhibited diffraction patterns characteristic of cellulose I β , with the main reflections at approximately 14.5°, 16.5°, and 22.5° (2 θ). Neither solvent delignification nor 20 min of sonication caused a transformation of the cellulose I β polymorph, indicating that the fundamental crystalline structure of cellulose was preserved during processing. With XRD measurements the total diffractogram intensities comprise of the amorphous lignin and cellulose as well as the crystalline cellulose. As lignin and cellulose contents are characterized via compositional analysis for all of the LCM materials, it is possible to create a corrected graph for cellulose crystallinity. These results are shown in **Fig. 20**. As expected, removal of amorphous lignin increased the apparent crystallinity of the total LCM material, from 47.2% for the raw LCM to 62.5% for the most extensively delignified nLCM. Nevertheless, the crystallinity of cellulose remains largely unchanged in the solvent-delignified samples, as anticipated. Cellulose is more crystalline in the raw LCM. However, undetermined components may also play a role here, which could be removed with aqueous organic solvents and are amplified by correcting the crystallinity calculation.

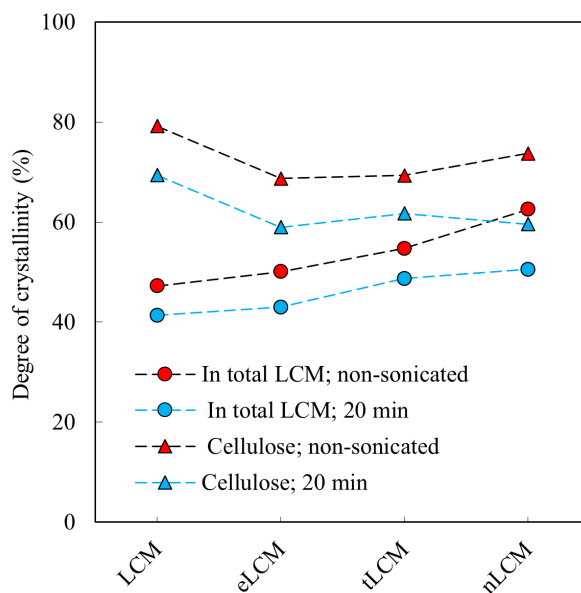


Figure 20. XRD measurement-based calculation of the LCM crystallinity at different lignin contents with and without US treatment (circles), and crystallinity of cellulose where amorphous lignin content has been subtracted (triangles).

In contrast, sonication decreased the crystalline fraction of all LCMs. The average decrease in crystallinity was approximately 6.3% for LCM, eLCM, and tLCM, whereas nLCM exhibited a substantially greater reduction, from 62.5% to 50.5%. The stronger response of nLCM may be associated with its low residual lignin content and consequently greater accessibility of cellulose to acoustic cavitation, although the influence of the preceding alkaline treatment cannot be completely excluded. As suggested by various authors, the decrease in cellulose crystallinity

can be attributed to the intense cavitation forces generated by sonication, which scissor the long cellulose fibers into shorter cellulose nanoparticles and lead to a partial collapse of the crystal structure [137,138]. Importantly, the reduction in crystallinity occurred without a change in the cellulose I β polymorph. Together with the SEM observations, these results suggest that US disrupts and fibrillates the cellulose structure and increases the proportion of less ordered cellulose rather than inducing a crystalline phase transformation.

The XRD results also complement the observed increase in WRV. Cavitation-induced fragmentation and fibrillation can generate new cellulose surfaces, where chains are less ordered than within the crystalline core, thereby increasing the amorphous fraction and the number of hydrophilic surfaces accessible to water. The particularly pronounced changes in nLCM further support the conclusion that residual lignin restricts the accessibility and structural response of cellulose during sonication. Thus, the increase in WRV is associated with US-induced fibrillation and increased cellulose surface accessibility, while the maximum extent of this response is strongly influenced by the lignin content of the LCM.

5.3.4. Key findings of ultrasonic treatment of biorefinery lignin–cellulose mixtures

Overall, the results from **Publication II** and **III** demonstrate that US primarily induces physical and morphological changes in biorefinery LCM while preserving the main chemical structure and cellulose I β polymorph. The efficiency of US treatment depended strongly on solid loading and lignin content, as lower solid loadings improved acoustic energy transfer, while decreasing lignin content enhanced cellulose fibrillation, water accessibility and suspension stability. While lignin did not prevent US-induced fragmentation and fibrillation of cellulose particles, it did restrict the accessibility and hydration of newly exposed cellulose surfaces. US therefore provides a promising approach for the direct physical modification of lignin-containing biorefinery streams, as it does not require the complete separation of lignin and cellulose.

5.4. Considerations at the molecular level on the effect of ultrasonic treatment on lignin–cellulose mixtures with varying lignin content

The combined WRV, SEM, and XRD results indicate that the response of LCMs to US is governed by both cellulose fibrillation and the accessibility of cellulose surfaces, which is strongly influenced by lignin content. Sonication caused extensive fragmentation and longitudinal fibrillation of the cellulose-rich structures, producing CNF-like morphologies in all LCMs. Although SEM showed relatively similar fibril dimensions after 20 min of treatment, the increase in WRV differed considerably between the materials and became progressively greater as

the lignin content decreased. Thus, fibrillation alone cannot explain the observed differences in water retention.

This behavior can be interpreted in terms of the molecular organization of cellulose I β and its interactions with lignin. To gain a deeper, it is crucial to consider the atomistic structure of cellulose in the discussion. In Literature overview in **Fig. 2**, the unit cell of cellulose elementary fibril is depicted, and the outer faces of hexagonal cell are designated according to literature [20,23,139,140]. As cellulose faces (100) and (200) cannot form hydrogen bonds they are thought of as hydrophobic. On the other hand, (110), (1 $\bar{1}$ 0) and (010) faces are considered hydrophilic. Thus, elementary cellulose fibrils contain surfaces with different hydrophilicities.

US is proposed to scissor cellulose fibers along the cross-section at the amorphous region and along longitudinal direction for the crystalline part [137,138]. Taking into account the structure of cellulose elementary fibril, US-induced fragmentation and fibrillation generate additional cellulose surface area available for interaction with water, which in turn could explain the sharp increase in WRV of LCMs.

Recent simulations of lignin–cellulose interactions in water show that the adsorption of large lignin polymer is energetically favored mostly on the hydrophobic face of cellulose. However, due to the amphiphilic nature of lignin, interactions between the hydrophilic face and lignin are still possible [16,38]. This may explain the lower effect of sonication on the WRV of lignin-rich LCM. At high lignin contents, lignin can partially cover cellulose surfaces and compete with water for hydrophilic interaction sites, thereby limiting water accessibility and WRV. The lignin content of the LCM is altered stepwise with about a 6% reduction between e-, t- and nLCM. However, the US response of WRV has a major increase from t- to nLCM, which indicates that not only the overall content but also the properties of lignin play an important role. The particularly high WRV of nLCM, even before sonication, may additionally reflect increased cellulose accessibility following NaOH treatment or, more simply, the substantially lower amount of residual lignin available to restrict cellulose–water interactions.

6. CONCLUSIONS

This thesis investigated the acoustic effects and applicability of power ultrasound (US) treatment on hydrolysis lignin (HL) and unrefined lignin–cellulose mixtures (LCM) derived from a wood biorefinery process. Particular emphasis was placed on analyzing the biopolymer matrix and the interactions between the interfacial within these multicomponent materials. The work explored the dissolution parameters of HL under mild alkaline conditions and evaluated direct US processing as a standalone tool to disrupt the LCM intermediate. Furthermore, it examined how varying the lignin content and its macromolecular characteristics regulates US-induced structural and morphological changes within the cellulose framework.

In **Publication I**, US significantly enhanced the solubilization of HL in aqueous NaOH solutions, increasing lignin solubility by 30% under mild conditions without inducing substantial depolymerization. The enhanced solubility was associated with the disaggregation of HL aggregates, supporting the use of US as a process intensification tool for lignin solubilization under mild alkaline conditions.

Publication II extended the investigation of US to untreated LCM, showing that even in mixtures with high lignin, US enabled the formation of stable aqueous dispersions and induced the disruption of cellulose structures. However, the efficiency of cellulose disruption could have been limited by the presence of lignin, where the surrounding lignin matrix could have restricted the transfer of cavitation-induced mechanical effects to cellulose.

In **Publication III** progressive delignification increased cellulose accessibility to water and enhanced the response of LCMs to sonication. US induced particle fragmentation and cellulose nanofibril-like structures across all lignin levels while the cellulose I β polymorph remained unchanged, indicating that lignin did not prevent fibrillation. However, improvements in water retention and suspension stability increased as lignin content decreased. The most extensively delignified material exhibited the highest hydration capacity. Thus, lignin content primarily influenced the accessibility and hydration of cellulose surfaces.

Beyond the individual findings of the three studies, the work establishes a link between lignin fractionation and subsequent US treatment of multicomponent biorefinery materials. The insights gained from the dissolution of isolated HL were successfully transferred to the unrefined LCM, where selective delignification provided a precise mechanism to control the intermediate composite matrix prior to US treatment. In this way, lignin fractionation and US treatment functioned as complementary processing approaches within the overall experimental framework. Overall, the findings demonstrate that lignin characteristics and total material composition are the dominant variables governing the response of biorefinery-derived materials to US treatment and should therefore be considered in the development of further processing strategies.

Future research should focus on translating these batch laboratory findings into continuous and scalable processes, identifying potential application areas for these materials, and further investigating the functional properties arising from lignin–cellulose interactions.

7. SUMMARY IN ESTONIAN

Puidubiorafineerimise ligniini ja ligniini-tselluloosi segude ultrahelitöötlus

Käesolevas doktoritöös uuriti ultraheli (UH) mõju ja kasutamise võimalusi tööstuslikust puidu biorafineerimisest saadud materjalide töötlemisel. Töö keskendus kahele kasepuidu töötlemisel saadavale materjalile: hüdrolüüsi ligniinile (HL) ning ligniini-tselluloosi segule (LCM). Uuriti HL-i lahustumist leeliselistes tingimustes ning UH mõju LCM-i füüsikalistele ja struktuursetele omadustele. Lisaks hinnati, kuidas ligniini sisaldus ja omadused mõjutavad UH põhjustatud struktuurseid ja morfoloogilisi muutusi tselluloosis.

Töö esimeses osas uuriti HL-i lahustumist NaOH vesilahustes. Tulemused näitasid, et ligniini lahustuvus sõltus eelkõige NaOH ja ligniini suhtest, kus maksimaalne lahustuvus oli 80% ning UH rakendamisel 90%, kusjuures madalamatel NaOH kontsentratsioonidel suurendas UH ligniini lahustuvust kuni 30%. Puhtas vesikeskkonnas ultraheli ligniini lahustuvust oluliselt ei mõjutanud. Suurim lahustuvuse muutus toimus töötamise esimese kümne minuti jooksul, kuid madalamate NaOH kontsentratsioonide korral jätkus lahustumise suurenemine ka pikema töötamise vältel.

Lahustunud ligniinifraktsioonide molekulmassi ja struktuuri analüüs näitas, et UH soodustas suurema molekulmassiga ligniini lahustumist. UH-töötamise käigus ei täheldatud märkimisväärset β -O-4 sidemete lagunemist, mis näitas, et töötlus ei põhjustanud märkimisväärset ligniini depolümerisatsiooni. Tulemused viitasid sellele, et lahustuvuse suurenemine oli seotud eelkõige HL-i aglomeraatide lõhkumisega. Seega toimus UH leeliselise ligniini lahustamise protsessi intensiivistava meetodina.

Töö teises osas uuriti UH mõju töötlemata LCM-ile, mis sisaldas ligikaudu 40% ligniini ja 50% tselluloosi. UH-töötlus vähendas materjali suuremate osakeste mõõtmeid ning suurendas väiksemate, sealhulgas alla ühe mikromeetri suuruste osakeste osakaalu. Samuti suurenes märgatavalt materjali veesiduvus, mis viitas tselluloosi struktuursetele muutustele ja hüdrofiilsete pindade suuremale ligipääsetavusele veele. Kõige tugevam UH mõju saavutati madala kuivainesisalduse korral. Kõrgema tahke aine sisalduse (>4%) juures moodustus töötlemise käigus geeljas materjal, mis piiras UH energia ülekandumist segusse. Tulemused näitasid, et tselluloosi omadusi on võimalik UH abil muuta ka kõrge ligniinisaldusega segus, mis osutab võimalusele töödelda selliseid biorafineerimise vahevooge ilma ligniini ja tselluloosi eelneva eraldamiseta.

Töö kolmandas osas kasutati HL-i lahustuvuse teadmisi LCM-i selektiivseks delignifitseerimiseks, kus kasutati nii orgaanilisi kui ka aluselisi lahustisüsteeme, mille abil vähendati ligniini sisaldust ja muudeti materjali jäänud ligniinifraktsioonide omadusi. Neid materjale kasutati UH töötamise mõju uurimiseks, kus selgelt joonistus välja ligniini sisalduse tugev mõju. Madala ligniini sisaldusega materjalides toimus kõige suurem muutus nii osakese suuruse, veesiduvuse omadustes kui ka tselluloosi kristallstruktuuris.

Tulemused näitasid, et ultrahelitöötuse efektiivsust ei määra ainult ligniini kogus, vaid ka ligniini fraktsioonide molekulaarsed omadused. Suurema molekulaarsusega ligniin võib katta tselluloosi pindu ning piirata vee ligipääsu tselluloosile.

Kokkuvõttes näitas doktoritöö, et UH sobib HL-i lahustumise intensiivistamiseks ning ligniini-tselluloosi segude füüsikaliste ja struktuursete omaduste muutmiseks. UH mõju sõltub oluliselt lahusti koostisest, tahke aine sisaldusest ning töödeldava materjali koostisest, sealhulgas ligniini sisaldusest ja omadustest. Tulemused näitavad, et neid tegureid tuleb arvestada ligniini rikaste biorafineerimise vahevoogude edasiste töötlemisstrateegiate väljatöötamisel. Edasistes uuringutes tuleks keskenduda laboratoorsete tulemuste ülekandmisele pidevatesse ja skaleeritavatesse protsessidesse, võimalike rakendusvaldkondade väljaselgitamisele ning ligniini-tselluloosi vastastikmõjudest tulenevate funktsionaalsete omaduste edasisele uurimisele.

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2010–2013 Tartu Kivilinna Gümnaasium

Erialane teenistuskäik:

2019–... Fibenol Imavere OÜ
2026–... Lignova projektijuht
2023–2026 Lignova müügiinsener
2019–2023 Ligniini teadlane ja tehniline spetsialist
2022–2026 Tartu Ülikool, Tenoloogainstituut, nooremteadur
01.2022–02.2022 Synlab Eesti OÜ, qPCR laborispetsialist

Teaduspublikatsioonid:

Puss, K. K., Palmiste, E., L., Treiberg, N., Vider, H., Pikker, S., Faustova, I., & Salmar, S. Effect of lignin content on ultrasound-induced nanocellulose formation in biorefinery lignin–cellulose mixtures. *Polymers*, 2026, 18(14), 1697. <https://doi.org/10.3390/polym18141697>
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- Örd, Mihkel; Puss, Kait Kaarel; Kivi, Rait; Möll, Kaidi; Ojala, Tuuliki; Borovko, Irina; Faustova, Ilona; Venta, Rainis; Valk, Ervin; Kõivomägi, Mardo; Loog, Mart (2020). Proline-Rich Motifs Control G2-CDK Target Phosphorylation and Priming an Anchoring Protein for Polo Kinase Localization. *Cell Reports*, 31 (11), ARTN 107757. <https://doi.org/10.1016/j.celrep.2020.107757>

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