Supporting Information

Lignocellulose Saccharification Historical Insights and Recent Industrial Advancements towards 2nd Generation Sugars

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The chemical structure of wood

The chemical composition of wood is broadly reviewed in literature [1] [2] [3] [4] [5] [6]. Overall, dry wood has an elemental composition of around 50% carbon, 44% oxygen, 6% hydrogen, and trace amounts of nitrogen compounds and inorganics. Rowell *et al.* [1] classified the chemical composition of wood in macromolecular structures or high-polymeric compounds, accounting for 97–99%, and low molecular weight substances found in the remaining 1–3% of the wood (Figure S1). Some minor polymeric compounds like starch and pectins are also included in the macromolecular group, while proteins account for at most 1% of the wood cells and are mainly found in the non-wooden parts of the stem [5].

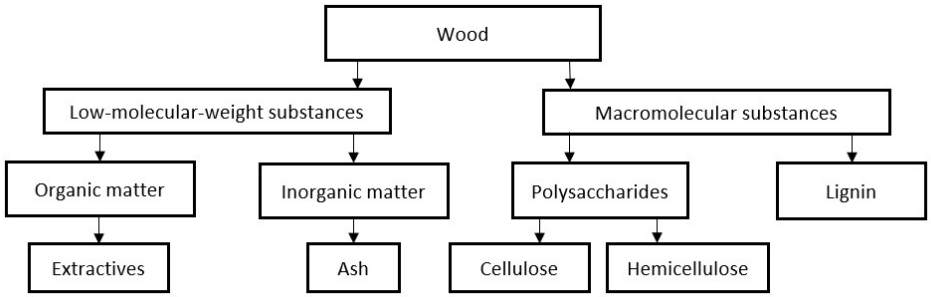


Figure S1. Chemical structure diversification of the wood materials based on molecular weight.

In the macromolecular category, three main biopolymers are present: cellulose, hemicellulose, and lignin (Figure S1). The proportion of each component varies significantly depending on the tree type and growth conditions. Lignin content ranges from 18-35% w/w of the wood and is distinct from the other two polymers due to its highly branched, amorphous, and three-dimensional structure containing large amounts of phenolic substituted moieties, typically acting as a binder for cellulose and hemicellulose [7]. The term holocellulose refers to the carbohydrate-containing material in the tree, essentially the combined cellulose and hemicellulose. Together, these sugar-based polymers make up around 65-75% w/w of the wood (Figure S1). Generally, softwoods have a higher lignin content, ranging from 26-35%, compared to hardwoods, which contain 18-30% lignin. The distribution of holocellulose in softwoods, hardwoods, and other herbaceous materials such as grasses is detailed in Table S1.

Table S1. Composition of holocellulose in hardwood, softwood and herbaceous plants, from K. Świątek [13].

	Beech Wood (hardwood) [8]	Spruce wood (softwood) [9]	Miscanthus giganteus (herbaceous) [10]
		(wt%)	
Cellulose	36	44	38
Hemicellulose	35	24	24

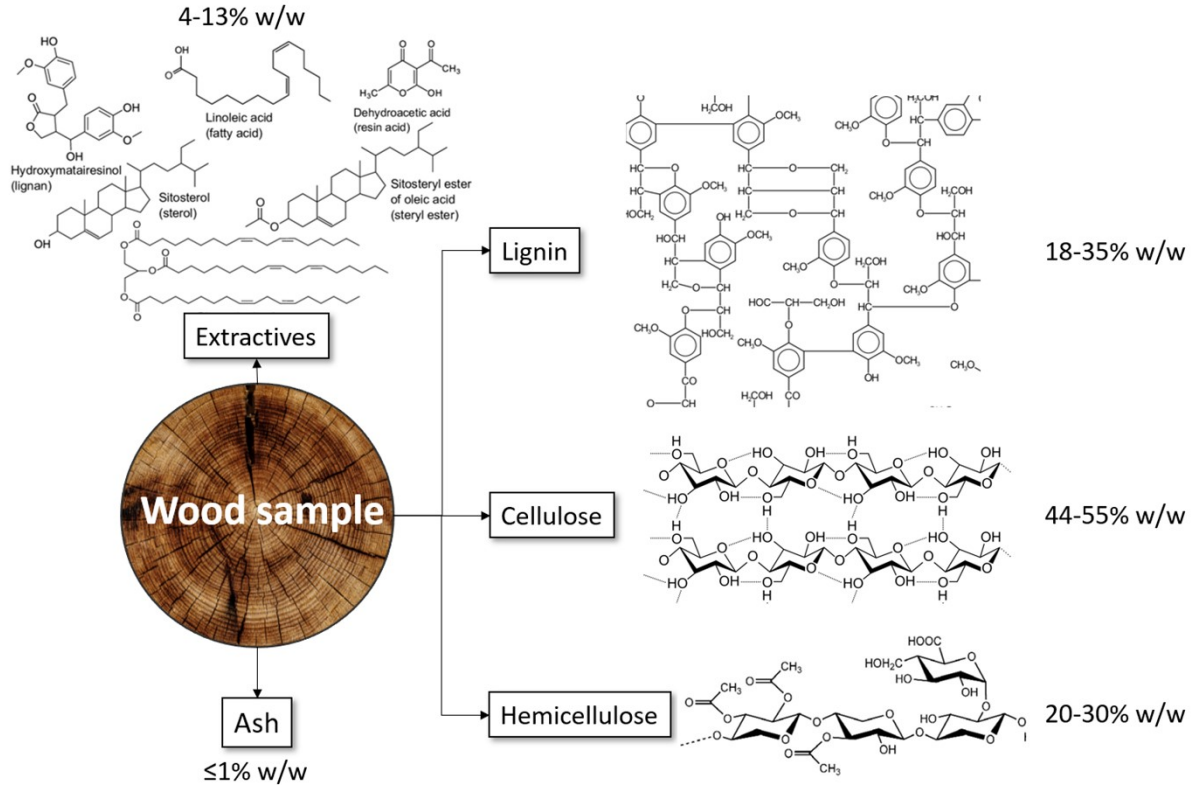


Figure S2. Wood chemical composition.

The low molecular weight substances in wood are extractives and ash. Wood extractives are the non-structural components of wood and depending on the wood constitute around 4–13% of the wood weight [11]. Extractives are typically found in the heartwood inside the trunk, and researchers found these substances help to protect trees against environmental stress [11]. Based on their chemical composition, these extractives can be divided into three major subgroups: substituted phenolic compounds, aliphatic compounds (fats and waxes), and terpenoids [12].

The ashes are the inorganic residue left after the incineration of wood. They typically constitute less than 1% of the tree's weight, with slightly higher amounts found in wood from tropical climates. The ash contains significant levels of calcium (Ca), potassium (K), phosphorus (P), aluminum (Al), silicon (Si), and iron (Fe). High iron content is often associated with trees growing in highly urbanized areas and can sometimes include minor, but potentially toxic, elements such as lead (Pb), cadmium (Cd), zinc (Zn), nickel (Ni), copper (Cu), arsenic (As), mercury (Hg), and chromium (Cr). These metals accumulate slowly as the tree absorbs them from the soil. When wood is burned in fireplaces, it releases these elements, particularly Pb and Cd, into the atmosphere, suggesting that repeated exposure to fireplace emissions could potentially lead to carcinogenesis in humans [13].

Cellulose

Cellulose exists in nature as a densely packed linear macropolymer composed of anhydro-D-glucopyranose units (AGU). The repeating unit of this biopolymer is cellobiose, which consists of two D-glucopyranose monomers linked by β-(1,4)-glucosidic bonds. Each AGU contains

three hydroxyl groups (R-OH): one primary and two secondary, making up more than 30% of its weight [14]. In an alkaline environment, the terminal hydroxyl at the C1 end is in a hemiacetal/aldehyde equilibrium, allowing the AGU to alternate between closed and open structures, which imparts reducing properties to the C1-OH (Figure S3, reducing end). The terminal -OH at the C4 end of the cellulose chain remains a primary hydroxyl group in an alkaline medium, making it the non-reducing end. [15] [16]

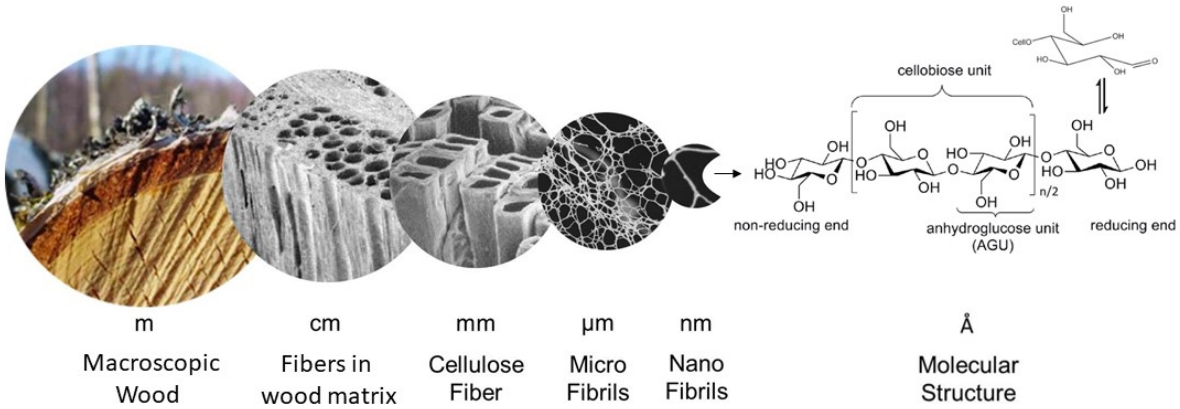


Figure S3. Wood fibers and the anhydro-glucose units inside the cellulose structure.

The chemical properties of cellulose are primarily determined by its composition and structure, while its physical properties result from its degree of polymerization (DP), crystallinity index (CI), and surface area [17] [18]. The degree of polymerization, which indicates the chain length of cellulose, varies depending on the source and treatment of the raw material [19]. As a linear polymer, a higher DP generally correlates with higher molecular weights (MW). For example, cotton has a DP ranging from 6500 to 10000, softwood cellulose around 5000, hardwood cellulose approximately 4000, and agricultural residues such as bagasse and wheat straw have lower DPs around 1000 [17].

Most wood-derived cellulose is highly crystalline and can contain as much as 65–70% crystalline regions, which increases with the age of the tree [20]. The remaining portion has a lower packing density and is referred to as amorphous cellulose. In crystalline regions of cellulose, the cellulose chains are tightly packed together and stabilized by strong and complex hydrogen-bond networks. When the cellulose is hydrolyzed, the amorphous regions are usually easier to depolymerize, while the crystalline regions exert higher resistance to the action of hydrolyzing agents. Cellulose with high DP and CI is more resistant to hydrolysis and shows increased density and tensile strength [21]. The most accessible areas of cellulose for water and microorganisms to access, are usually the non-crystalline sections and the outer surface of the crystalline areas. Sometimes amorphous cellulose can be covered with both hemicelluloses and lignin, i.e. becoming non-accessible cellulose, and thereby more difficult to hydrolyze. Concepts of accessible and non-accessible cellulose are very important in chemical modifications, moisture sorption, pulping, extractions and interactions with chemicals and microorganisms. The drying process of cellulosic materials can alter this accessibility to a great extent [22]. The pores of wet cellulose fibers have been shown to shrink as the moisture content is decreased (an irreversible phenomenon called hornification),

resulting in smaller pore sizes and a narrowed pore size distribution. When three pulp samples, namely never-dried (ND), air-dried (AD) and oven-dried (OD), were tested for enzymatic hydrolysis, it was found that the rate of reaction (hydrolysis) decreased in the order ND > AD > OD.

Following the representation of the cellulose sheets inside the wood in Figure S3, it can be found that cellulose chains stack in the b-direction (Figure S4, b-plane) to form a three-dimensional crystalline structure [23]. In the b-plane the atoms in the axial position are hydrogens. These hydrogen atoms are small, allowing the cellulose layers to pack so close that the van der Waals forces can stabilize this tight packing. This crystalline packing structure is referred to as cellulose I or native cellulose. These properties were first discovered in 1913 by Nishikawa and Ono with the aid of X-ray diffraction [24], and the macromolecular nature of cellulose was proposed by Staudinger in 1920 [25]. The results led to several models of this crystalline unit, of which Meyer and Misch elucidated a model in 1937 that is still valid today [26].

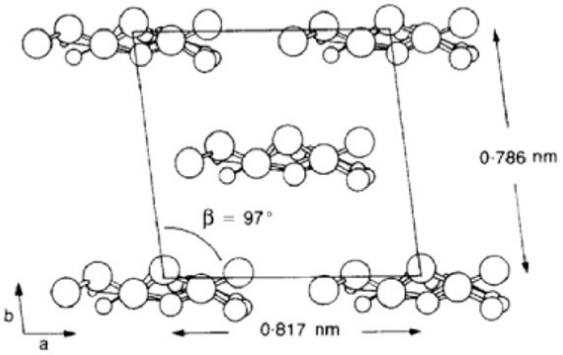


Figure S4. Plane view showing the position of the chains in cellulose. There is hydrogen bonding between chains in the a-c plane, but only van der Waals forces in the b-direction. from *[23]*.

The hydroxyl groups present in the cellulose are involved in a number of intra- and intermolecular hydrogen bonds, creating various crystalline arrangements. Perez and Samain [27] reviewed the structures, or polymorphs, of cellulose, and distinguished the structure of seven different types of cellulose: I_α, I_β, II, III_I, III_{II}, IV_I and IV_{II}. In nature, cellulose is found as cellulose I_α or I_β, and occurs in various ratios according to the source. For instance, the cellulose produced by bacteria or algae is mostly I_α, whereas the cellulose of higher plants (for instance, woody tissues or cotton) consists mainly of cellulose I_β [27].

Matthews *et al.* [28] studied the transformation of cellulose I_α to I_β by heating the first and producing a high temperature phase, which upon cooling transformed into cellulose I_β. To effectively convert cellulose I_α to I_β, the temperature must be held at 260 °C for 30 min in 0.1 M NaOH, or at 280 °C for 60 minutes in an oxygen-free atmosphere [28]. This observation is assumed to be a proof that the I_β phase is lower in free energy than the I_α phase. However, although the assumed difference in free energy might be correct, this observation is not a measurement of relative thermodynamic stability [29].

During the 1840s, Mercer realized that by using strong caustic soda solutions on cotton fabric, the cloth shrank, had better thickness, strength and increased dyeing capacity [25]. It was later determined, that the crystallographic form of cellulose I (α or β) was irreversibly changed to the polymorph of cellulose II [25]. The transition from cellulose I to cellulose II is irreversible, and it implies that cellulose II is a more stable allomorph compared with the metastable cellulose I. An allomorph refers to a different structural form of the same substance. For cellulose, this involves different crystalline structures. The crystallinity index of cellulose I is found to be 84.5%, while for cellulose II is about 50% [30]. This 1840s cellulose transformation eventually was called mercerization after its discoverer. It was a breakthrough discovery for the modification of natural cellulose and cotton textile fibers, which is still used nowadays [16]. Apart from mercerization, cellulose II can also be prepared through a process of cellulose regeneration [31]. Regeneration involves either preparing a solution of cellulose I in solvents like 4-*N*-methylmorpholine-4-oxide (NMMO) or through synthesis of an intermediate derivative, followed by recrystallization [22].

Treatment with liquid ammonia, or with certain amines such as ethylenediamine (EDA), allows for the preparation of cellulose III_I, from cellulose I. Cellulose III_{II} can be obtained from cellulose II. The CI of cellulose III is about 69% and when treated at 260 °C in glycerol it transforms into cellulose IV [28]. Here again, two types exist: cellulose IV_I and IV_{II}, respectively obtained from cellulose III_I and III_{II}. It is generally accepted that cellulose IV_I is a disordered form of cellulose I. Despite identical unit cell parameters, the structures differ in chain polarity: in cellulose IV_I both chains of the unit cell are parallel, whereas in cellulose IV_{II} they are antiparallel. The synthesis and interconversion of the different polymorphs of cellulose can be found in Figure S5.

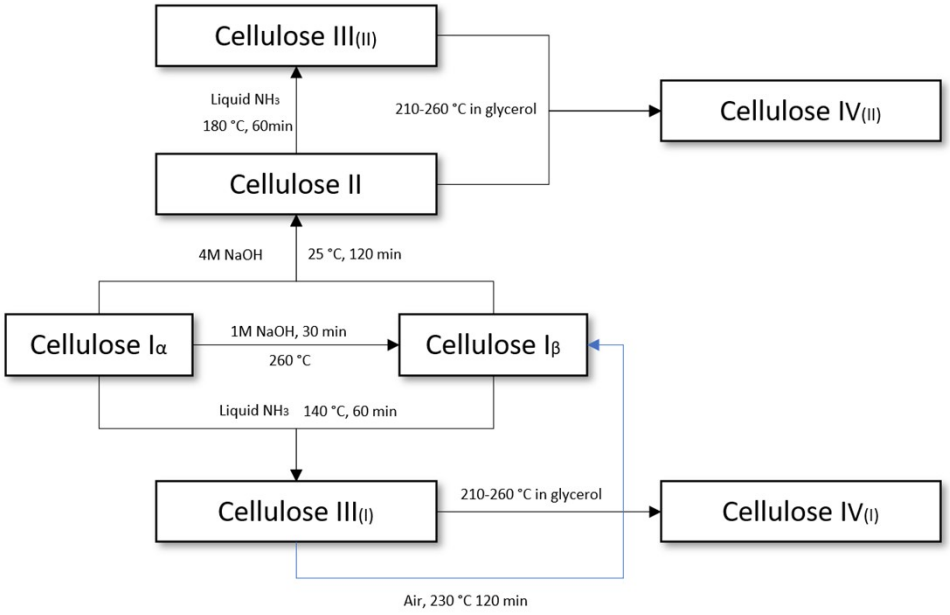


Figure S5. Transformation of cellulose into its polymorphs and relation between them [29] [32] [33] [34].

Ciolacu *et al.* also studied the crystallinity and accessibility of microcrystalline cellulose, and compared it with cotton fiber and spruce dissolving pulp [35]. The starting allomorph of the

cellulose in these materials was cellulose I. In these studies, the crystallinity of cellulose I was measured and transformed into the allomorphs of cellulose II and III to compare and study its accessibility for water. With help of X-ray diffraction it was concluded that for the three materials tested there is a descending order in accessibility found for amorphous cellulose > cellulose II > cellulose III > cellulose I (Table S2, Cellulose I).

Table S2. Comparative studies on the accessibility of cellulosic materials table from Ciolacu [35].

Sample	Accessibility (%)		
	Microcrystalline cellulose	Cotton fibers	Spruce dissolving pulp
Cellulose I	38	44	51
Cellulose II	58	60	64
Cellulose III	48	53	58
Amorphous cellulose	90	92	96

Rowell also reviewed other types of cellulose [1]. One such type, often referred to as Cross and Bevan cellulose, is primarily cellulose I but also contains some hemicellulose. It is extracted by chlorinating wood, followed by washing with aqueous solutions of 3% sulfur dioxide (SO_2) and 2% sodium sulfite ($NaSO_3$) [36]. Another type, known as Kürschner cellulose, is derived using a different extraction method, involving refluxing wood three times for 1 hour with a 1:4 (v/v) mixture of nitric acid and ethanol [37]. This method, however, is less commonly used because it partially degrades the cellulose and the nitric acid-ethanol mixture is potentially explosive. Additionally, Kürschner cellulose contains some hemicellulose [38].

Hemicellulose

The hemicellulose fraction of wood comprises a collection of polysaccharides with lower degree of polymerization compared to cellulose, with an average DP of 100–200 only [39]. A widely accepted role of the secondary cell wall hemicellulose is to, by non-covalent interactions, tether or wrap cellulose microfibrils and act as a link between the cellulose and lignin [39]. Occasionally, ester or ether bonds can form between hemicellulose and lignin, facilitating an effective transfer of shear stress across the cell [23].

The backbone of hemicellulose consists of 6-carbon sugars (hexoses) and 5-carbon sugars (pentoses), along with small fractions of organic acids and pectins [40]. In softwood hemicellulose, mannose is the predominant carbohydrate, whereas xylose is most abundant in hardwood hemicellulose. Figure S6 illustrates the hemicellulose composition, which includes these sugars (pentoses or hexoses), deoxy-sugars, and organic acids. The differences in crystallinity between cellulose and hemicellulose arise from the structural variations of the sugars within the holocellulose material. For example, β-D-galactose and β-D-mannose, both present in hemicellulose, differ from β-D-glucose (the main component of cellulose) only in the steric configuration at C4 and C2, respectively. This difference affects the spatial position of the hydroxyl group on those carbon atoms, shifting from equatorial in glucose to axial in galactose and mannose, making hydrogen bonding and close packing of polymer chains more

challenging for the latter. Consequently, layers of galactose and mannose are more spaced out and easily accessible. Additionally, larger groups like carboxylic groups in β-D-glucuronic and D-galacturonic acid replace hydroxyl moieties in hemicellulosic sugars, further reducing close packing and crystallinity [41]. Hemicellulose also contains acetyl groups in parts of the chain, which facilitate non-covalent interactions with lignin while maintaining compatibility with cellulose surfaces [42]. These acetylated areas account for about 4–6% w/w of hardwoods and 1–2% w/w of softwoods.

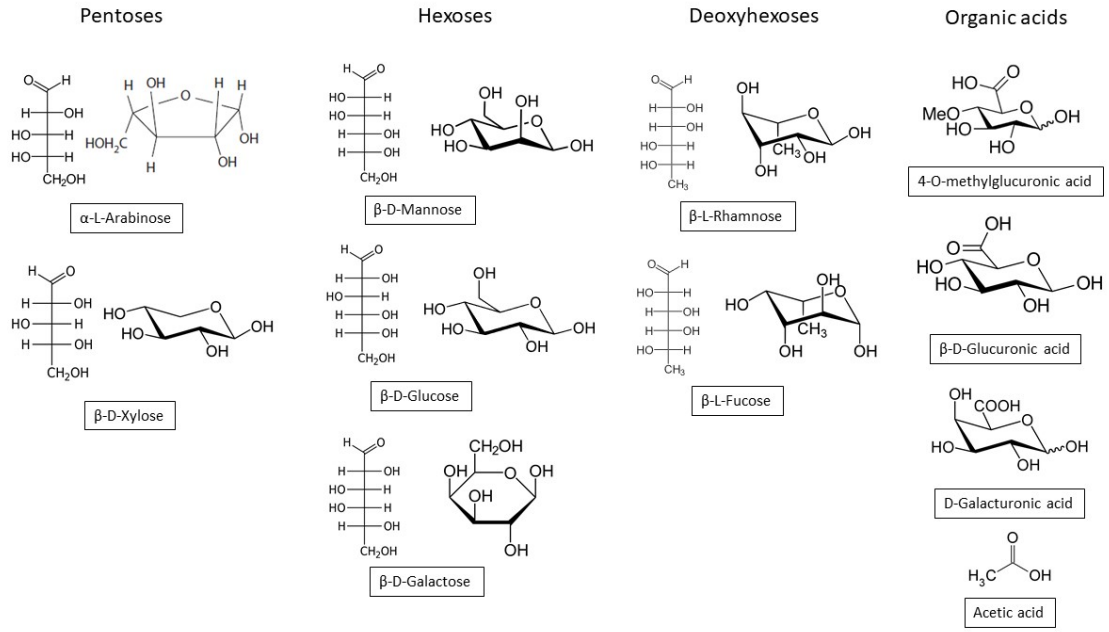


Figure S6. Common sugars and organic acids found in the polymeric complexes of the hemicellulose structure.

The amount, structure and composition of different hemicelluloses varies between species, cell type and location in the cell wall. The different polymers found in hemicellulose were extensively reviewed by Deshavath *et al.* [43], and the structure and proportion for the most common hemicellulose polymers in wood are shown in Table S3 [23].

Table S3. Percentage of the different hemicellulose biopolymers.

Hemicellulose polymer	Occurrence	Percentage in wood(%)	DP
Glucomannan (GM)	Softwood	10-15	200
Arabinoglucuronoxylan (AGX)	Softwood	7-10	100
Galactoglucomannan (GGM)	Softwood	5-8	100
Arabinogalactan (AG)	Softwood	1-5	200
Glucuronoxylan (GX)	Hardwood	15-30	200
Glucomannan (GM)	Hardwood	2-5	200

Glucomannans and galactoglucomannan (or O-acetyl-galactoglucomannans) are the major hemicellulosic constituents of softwoods, accounting for 20–25 wt% of their dry mass, with an average DP of 40-100 [43]. The structure of galactoglucomannan (GGM) consists of a glucomannan backbone, which is made up of β-D-glucopyranose units and β-D-

mannopyranose units connected by β-1,4-glycosidic bonds (Figure S7). This backbone is partially functionalized with α-D-galactopyranose units through α-1,6-glycosidic linkages, whereas the acetyl groups are sometimes attached to the C2- or C3-OH positions of glucose and mannose [43]. This heteropolymer can be categorized in galactose-rich glucomannans, which are usually referred to as galactoglucomannans, and galactose-poor glucomannans which are simply called glucomannans. The molar ratios of galactose/glucose/mannose were found to be 1:1:3 in galactoglucomannans, and 0.1:1:3 in glucomannans [43].

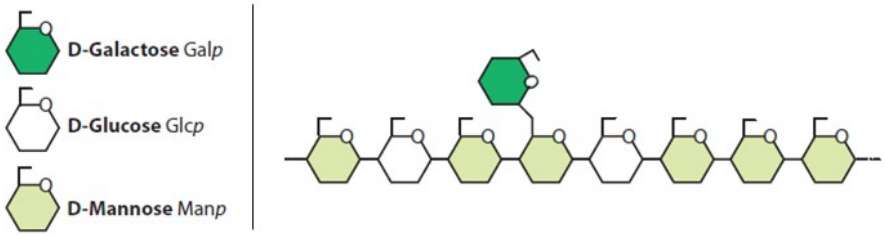


Figure S7. Chemical composition of glucomannans [43].

Arabinoglucuronoxylans or arabino-4-O-metylglucuronoxylans (AGXs) accounts for 7–10% of the dry wood mass. AGXs are composed of D-xylopyranose units linked by β-1,4-glycosidic bonds [43]. Xylans have additional substituents, such as arabinofuranose rings, attached through α-1,3-glycosidic bonds, 4-*O*-methyl-D-glucuronic acid, and/or glucuronic acid residues crosslinked through α-1,2-glycosidic bonds. Acetyl groups can also be found at the xylan C_3 position (Figure S8). The molar ratio of arabinose/glucuronic acid/xylose is around 1:2:9 [43]. The average degree of polymerization of AGX ranges between 50 and 185. Hardwood AGXs are less acetylated than softwoods, but do contain low amounts of galacturonic acid and rhamnose.

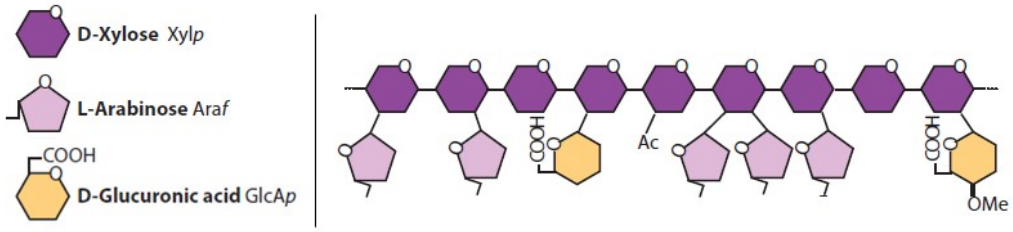


Figure S8. Chemical composition of arabinoglucuronoxylans [43].

Arabinogalactans (AGs) are a type of slightly water-soluble polysaccharide found in hardwood biomass. These polymers feature a highly branched structure, with a backbone composed of galactopyranose units linked by α-1,4-glycosidic bonds, and branching points at the C6 position of most galactose units (Figure S9). Additionally, this backbone is substituted with arabinofuranose residues through α-1,5-glycosidic bonds.

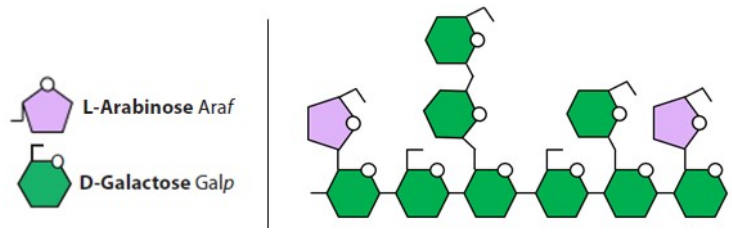


Figure S9. Chemical composition of arabinogalactans [43].

Glucuronoxylans (GXs), or O-acetyl-4-O-methylglucuronoxylans, are the primary hemicellulose components in hardwood biomass, comprising 15-30% of their dry mass [43]. The GX backbone consists of β-D-xylopyranose units linked by β-1,4-glycosidic bonds, with some xylose units substituted with acetyl groups at the C2 or C3 positions [43]. Additionally, these xylose units feature an uronic acid group (4-O-methylglucuronic acid) at the C2 position, connected via an α-1,2-glycosidic bond (Figure S10). These uronic acid side chains confer increased resistance to hydrolysis by acids or microorganisms. Besides the main structural units, GXs may also contain small quantities of galacturonic acid and L-rhamnose [43].

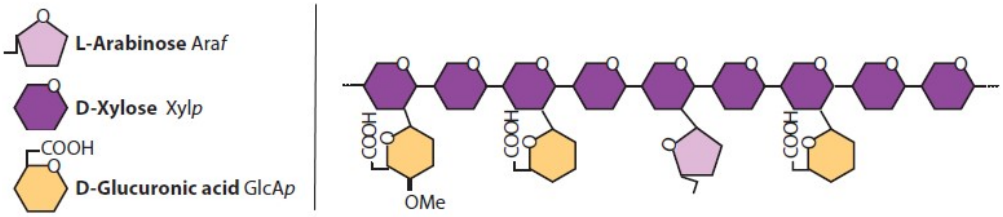


Figure S10. Chemical composition of glucuronoxylans [43].

Xyloglucan (XG) represent about 20% of the primary cell walls in hardwoods and around 10% in softwoods [43]. However, since the fraction of primary cells in comparison to secondary cell wall is very small, the total abundance of XG is low. The XG chain consists of β-1-4 linked glucose units, which are substituted with xylose by α-1-6 linkages (Figure S11). Also, additional sugar units may be present on the xylose, such as galactose and L-fucose, resulting in elongated branches [43].

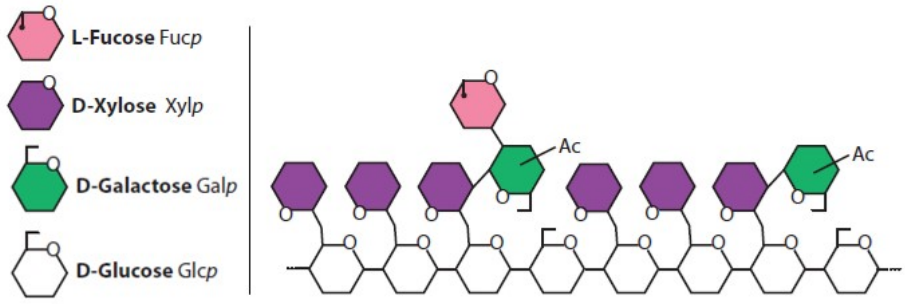


Figure S11. Chemical structure of xyloglucans [43].

Lignin

The word lignin has its origin in the Latin word used for wood (lignum), and it was first used in literature by Schulze in 1865 [44]. Klason, in 1897, put forward the idea that lignin was

chemically related to coniferyl alcohol, and later proposed that lignin is a macromolecular substance connected by ether linkage between coniferyl alcohol units [45].

The structure of lignin is extensively reviewed in literature [45] [46] [47] [48] [49]. Huang, Zakzeski, Ralph, Becker and Hatakeyama are a few of the most recent contributors to the elucidation of the lignin structure. Lignin functions as a structural stabilizer, a water pipeline inside plants, and as barrier against microbial decay [50]. Chemically, lignin is a three-dimensional polymer built up of C–O–C and C–C linkages of mainly three phenylpropane units: *p*-coumaryl alcohol (H), coniferyl alcohol (G) and sinapyl alcohol (S) (Figure S12).

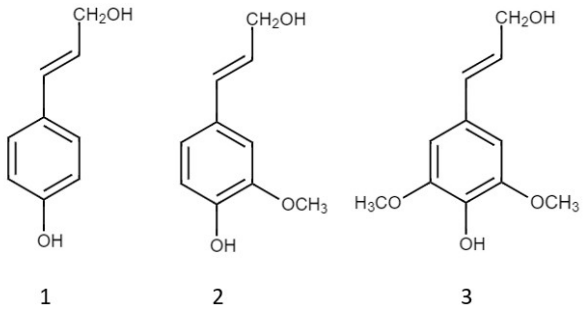


Figure S12. 1 *p*-coumaryl alcohol (H-unit), 2. coniferyl alcohol (G-unit), 3. sinapyl alcohol (S-unit), figure from J. Becker [48].

The amount and proportion of these groups in the structure of lignin changes depending on the wood source. Usually, the amount of lignin decreases in the order of softwoods > hardwoods > herbaceous plants. The lignin content in softwood is between 25–35%, and mainly contains coniferyl alcohol groups (G-unit) and is commonly referred to in literature as *guaiacyl lignin*. Hardwood contains less lignin, around 18–25%, and it is almost equally composed of coniferyl (G-unit) and sinapyl alcohol (S-unit), the rest belonging to the *p*-coumaryl alcohol (H-unit). In literature the S-unit is referred to as *syringyl–guaiacyl lignin* [1] [48] (Figure S13).

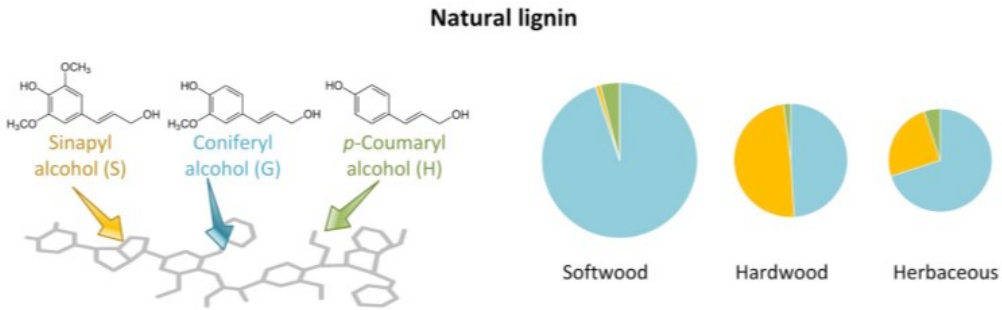


Figure S13. Lignin subunit distribution in lignocellulosic materials, from [48].

Generally speaking, the content of functional groups in softwood lignin is different from that of hardwood lignin [45]. The phenolic hydroxyl, aliphatic hydroxyl, and carbonyl content of softwood lignin is higher than in hardwood lignin, with the latter containing higher amounts of methoxy groups [51]. These groups are primarily connected to each other in both softwood and hardwood in β-O-4 linkage, consisting of approximately 50% of softwood linkages and

60% of hardwood linkages [46]. The identification and quantification of the various structures in lignin is a remarkable challenge even with advanced NMR techniques, since the structure is not constructed as cellulose or proteins by a single key-type linkage [49]. After a comprehensive study of wood lignin, C. Crestini put forward a new view that wood lignin is a series of linear oligomers, rather than a cross-linked network structure [52] (Fig. S14). Because these oligomers are prone to supramolecular association, the molecular weight measured by GPC is much higher than NMR data suggested [45]. Figure S14 and S15 show some of the common and broadly accepted structures for softwood and hardwood lignin [46] [53].

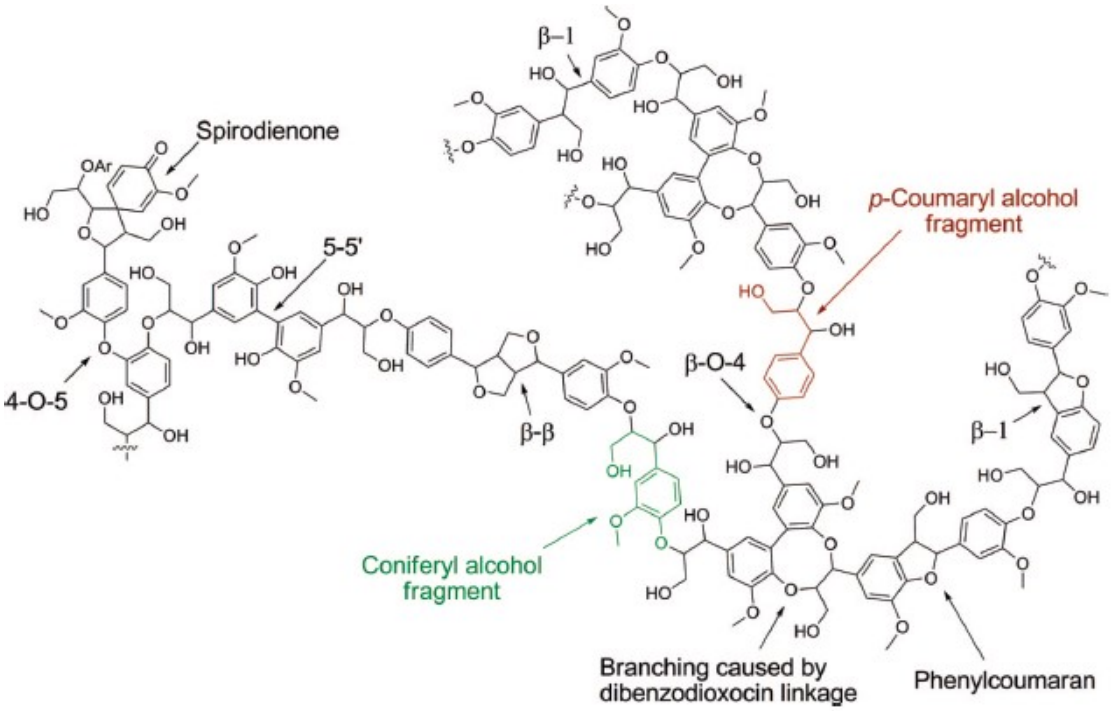


Figure S14. Softwood lignin structure, figure from Zakzeski [46].

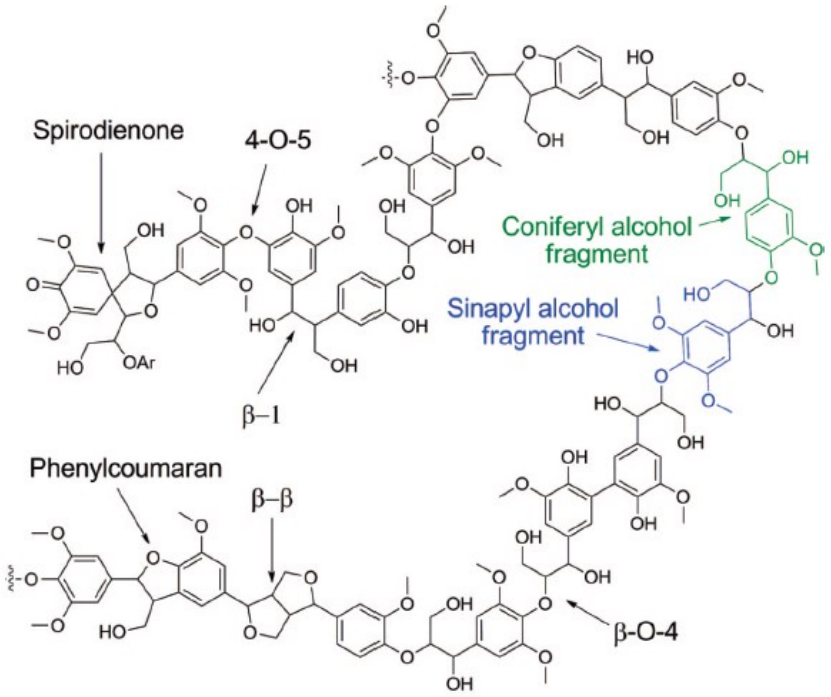


Figure S15. Hardwood lignin structure, figure from Zakzeski [46].

During the chemical treatment of lignocellulosic materials, lignin undergoes condensation reactions, resulting in structures that are more stable under acidic or alkaline conditions compared to the natural lignin found in wood. For example, in the sulfite pulping process, lignin undergoes condensation and incorporates sulfonic acid at the α-position of its side chain [45]. Chlorinated lignin is also found in nature, which points to the action of microorganisms that are able to excrete reactive chloride, which inserts in the structure of the lignin [54] [55]. In order to obtain lignin with a structure similar to the natural lignin in wood, enzymes are used that are able to selectively remove the hemicellulose and the cellulose, leaving the chemical structure of the lignin intact. However, acidic pretreatments (which can alter the chemical structure of lignin) are usually required to enhance the activity of these enzymes in order to make these processes economically attractive [56].

The color in Kraft lignin and sulfite pulping effluent tends to appear brown or red because it contains a variety of chromophoric groups [57] (Figure S16). These groups are unsaturated compounds that absorb light and can be formed in the lignin due to condensation reactions that disruption of the structure. Since every lignin can be virtually different from each other, it means that for similar processes the resultant color of the lignin greatly varies.

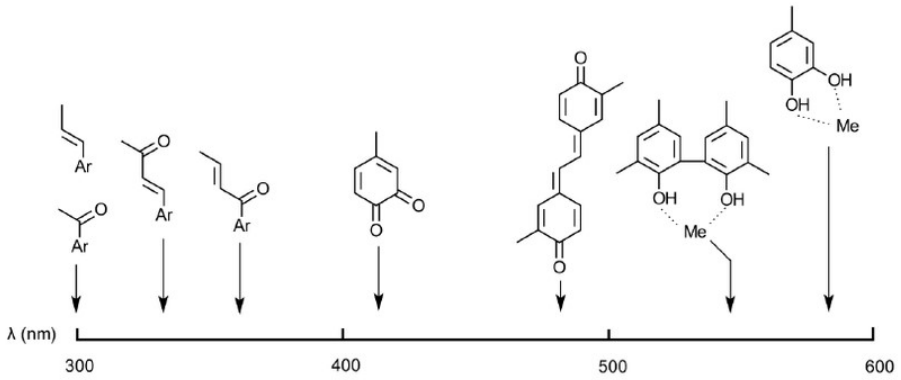


Figure S16. Lignin-related chromophores in lignin pulps and their approximate absorption. Me represents metal ions, figure from Paulsson [57].

The Bergius-Rheinau process

The feedstock for the Bergius-Rheinau process was primarily forestry residues. The wood residues were first shredded into slightly larger diameters than ordinary sawdust and then mixed with sawdust in amounts up to 25% in weight. During the first step, the wood mixture was dried to lower the moisture content from 40 wt% to about 5–10 wt%. This was accomplished by using revolving drums heated with the waste gases from the boiler.

After shredding and drying the mixture, this feedstock was loaded into the diffusers or reactors. In the first design, 7 reactors built of iron and lined with quartz or platinum to resist the action of the HCl were connected in series. For the hydrolysis, a countercurrent system was applied using an aqueous 40 wt% HCl solution at 15–20 °Celsius and a ratio of seven parts of acid to one of wood was fed into the system. The acid solution was introduced at the bottom of the last reactor and it flowed counter-currently through the others connected in series. While the front of the liquor was higher in sugar concentration, this feed always entered reactors with recently loaded biomass. This acid-sugar solution continuously hydrolyzed new material until passing through at least 7 reactors in series, increasing in this way the final sugar concentration in solution. Once the hydrolysis reached completion, two-thirds of the wood in weight was dissolved by the acid and one-third remained as lignin, achieving a nearly quantitative saccharification. After collecting the product hydrolysate from the bottom of the reactors, the lignocellulose residue still contained the acid-sugar solution retained inside the particles. In order to recover these sugars, the wet lignin was repeatedly washed with water and later recovered in a 1-stage evaporator. The lignin could be used as fuel for the plant or briquetted without binders to convert it into charcoal. Its usage in thermosetting plastics was also proposed but no significant utilization of it was ever reported.

The final sugar concentration in the product hydrolysate relied on the number of reactors connected in series. Figure S17 shows that ten diffusers were necessary to achieve a sugar concentration of 30%, which eventually was the goal set for the factory at Rheinau.

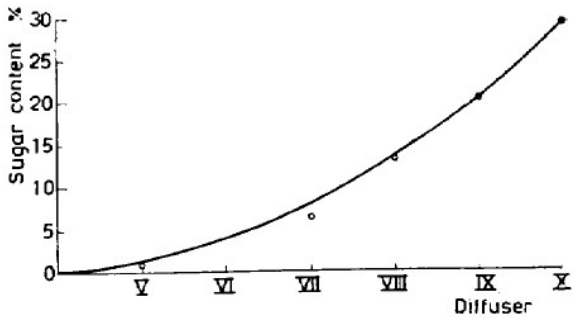


Figure S17. Hägglund plotted the sugar content in connected diffusers or reactors, original figure from [58].

Hägglund also discovered that the concentration of the acid decreased after the consecutive hydrolysis of several reactors. The acid concentration was fed at 40% in weight, and after seven reactor it was found to be around 32%. This decrease in concentration was attributed

to the absorption and interactions of the HCl with the lignin and sugars present in the solution. Hägglund illustrated this behavior in Figure S18.

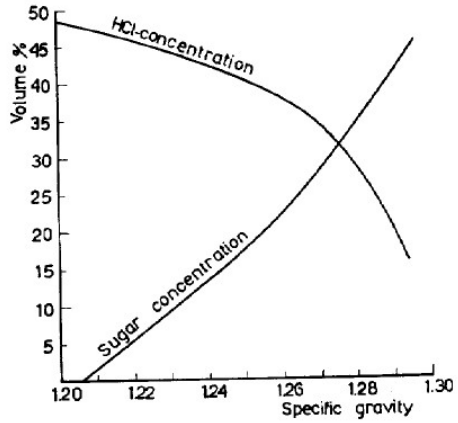


Figure S18. The HCl concentration dropped after hydrolyzing the material inside reactors in series original figure from Ref. [58].

The next step comprised the separation of the acid from the sugars in an evaporator described by Bergius in 1925 [59]. Bergius studied the difficulties in designing a system that could efficiently recover the acid and prevent corrosion of the equipment, even under mild conditions, while minimizing the degradation of the sugars. For that, Bergius designed a distillation system at reduced pressure to first remove the HCl and then condense it by using an immiscible carrier oil at different temperatures (Figure S19).

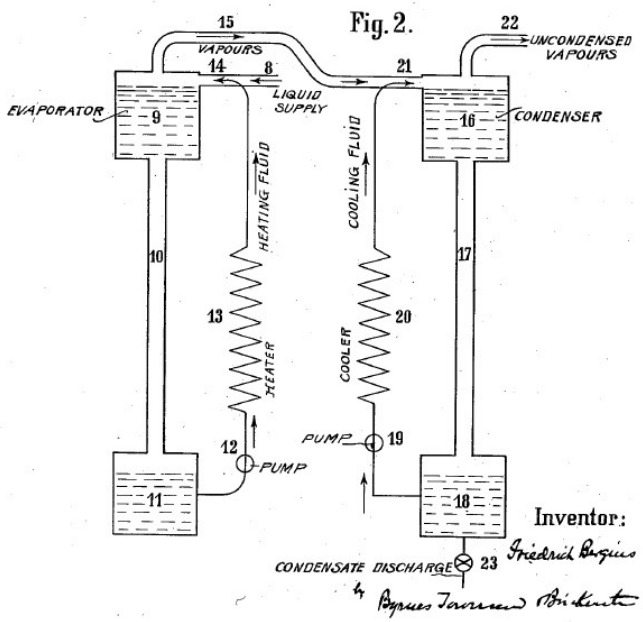
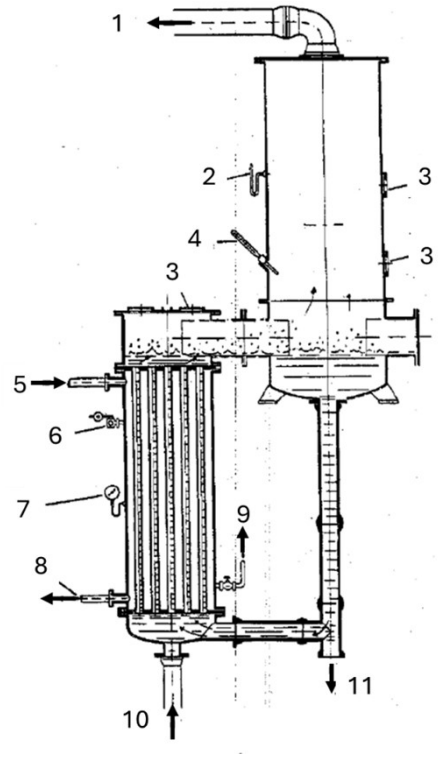


Figure S19. The acid sugar separation as patented by Bergius in 1925, from [59].

Following Figure S19: the hydrolysate entered the evaporator through feeding tube 8 (“Liquid supply”). Carrier oil was pumped through 12 and heated in coil 13 before entering and contacting with the acid-sugar solution in 14. In vessel 9, the HCl and water evaporates from the solution through 15, while the oil residue with the sugars settled by gravity to vessel 11. The sugars deposited and were recovered at the bottom of vessel 11 while the oil was

recirculated and heated again to start a new cycle. The HCl, water and other gases leaving the solution in 9, were mixed in 21 with colder carrier oil and entered the condenser 16 to produce an emulsion of oil and HCl-water that would allow for recovering the acid at the bottom of vessel 18 through valve 23. The oil was then again recirculated through pump 19 and cooled with heat exchanger 20 before being mixed again with a new gaseous stream of acid and water. The gases that were not condensed in 16, could leave the system through conduit 22. Receptacles 9, 11, 16, and 18, as well as tubes 10 and 17 were preferable made of stony material. Bergius noted that the carrying oil was not sufficiently free from water before recirculation and installed a tower filled with calcium chloride to protect coils 13 and 20 from corrosion.

The main challenge lied in avoiding the dangerous effect of the different heat expansion coefficients for the ceramic and iron parts, while at the same time ensuring a good heat transfer. Also, the recovery of the sugars that precipitated in the oil as extremely fined solids is time consuming and challenging. In a more modern design by Bergius, shown in Figure S20, no oil was used and 80% of the HCl was recovered by vacuum distillation using an acid-proof system consisting of evaporators with thin tubes made of a special ceramic material [60]. Following Figure S20, The HCl-H_2O was discharged via 1, the sugar syrup recovered by gravity in 11 and the carrier oil recycled with new hydrolysate entering at 10.

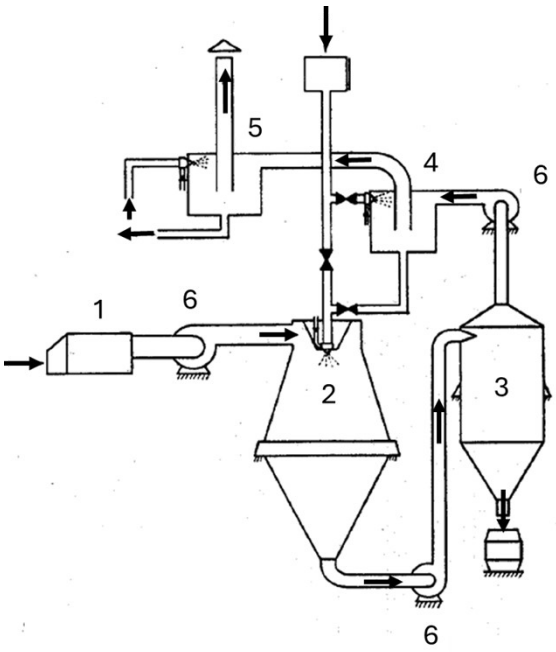


1. Vapor discharge
2. Vacuum gage
3. Sight glasses
4. Thermometer
5. Steam intake
6. Safety valve
7. Manometer
8. Condensate outlet
9. Air exhaust
10. Solution intake
11. Sirup

Figure S20. Tubular evaporator for the acid-sugar separation, adapted from Ref. [60]

The system HCl-H_2O forms an azeotropic mixture of 20:80 wt% (HCl:H_2O) and by means of vacuum distillation only down to the azeotropic mixture could be recovered as pure HCl. After the evaporator, the acidic syrup contained 55 to 65% of sugars and the remaining acid was recovered in a consecutive step by using a spray dryer (Figure S21). The tubular evaporators

were equipped with tantalum-coated boiling tubes and a saturated solution of calcium chloride was sometimes added to break the HCl-H_2O azeotrope.



1. Air heater used to dry sugar concentrate
2. Spray dryer for sugar solution
3. Cyclone for removing dried material from suspension
4. Separator in which the dust remaining in the air from the cyclone is removed by a spray of feed solution
5. Separator in which escaping air is washed free from hydrochloric acid by a water spray
6. Blowers

Figure S21. Spray dryer for the sugar syrup containing HCl, adapted from Ref. [60]

Following Figure S21: The sugar syrup was compressed with air and sprayed with hot air into chamber 2. The finely divided sugar crystals were circulated to a centrifuge in 3 where the sugars were recovered at the bottom. The rest of the gases containing very fine dust moved to a separator in 4 where the dust was removed after spraying fresh feed liquor. The HCl was recovered from these gases after the separator by spraying water and collecting the acidic solution from the bottom. The rest of the gases left the dryer through conduit 5.

The sugar syrup recovered after the spray drying contained around 1–2% HCl, 8% water and 90% sugars. Bergius reported that almost no sugar loss occurred during the drying as every possibility of sugar decomposition due to high temperatures was excluded.

Zechmeister discovered in 1931 [61], that the sugars in the acidic solution after the hydrolysis were mostly forming oligomers of three to six sugar monomers. After spray drying, the still acidic solution was diluted three times its volume with water and heated for about half an hour at 120 °C to post-hydrolyze the oligomers and obtain the final sugar product. Bergius explained this oligomeric sugar mixture could be neutralized with lime and the product directly used as fodder [62].

After post-hydrolysis, the biomass lost 65–70% of its original dried weight when coniferous wood was used in this process, which corresponds with an almost quantitative hydrolysis of the sugar-bearing materials. The sugar distribution in the product hydrolysate was around 60% glucose, 17–21% mannose, 5% galactose, 13–16% xylose and 1% fructose. For every 100 kg of wood fed, about 2 to 2.5 kg of acetic acid and 33 kg of lignin containing resins and humins were obtained. When these sugars were used for fermentation, 100 kg of fermentable sugars yielded about 50 liters (40 kg) of pure alcohol. Xylose and galactose could not be fermented

so they were recovered as residue after distilling the alcohols from the fermentation of the other sugars. This residue was recirculated to a new batch of product hydrolysate and after several runs, the concentration of these non-fermentable sugars was high enough to separate, crystallize and use them as fodder.

Udic-Rheinau process

Several aspects of the original Rheinau process were amended. The hydrolysis was now divided in two separate steps in which different HCl concentrations and temperatures were applied to remove the hemicellulose prior to the main-hydrolysis of the cellulose. After the cellulose hydrolysis, the water-washing of the lignin was adjusted by synchronizing the pumping speed of the water inside the reactor to the diffusion velocity of the acid across the porous lignin residue. In this way, the total amount of water employed during this washing step was significantly reduced and the HCl concentration in the washed-out water remained constant, so it could be directly recycled in a new pre-hydrolysis cycle. By increasing the efficiency of the washing step, it allowed to reduce the water deployed by 50% . Recovery of the HCl was now accomplished using 3 separate distillers operating at different pressures to ensure higher recovery, better recycling and lower overall energy consumption of the system. As a result, in the Udic-Rheinau process the acid losses were claimed to be reduced from 18–20% in the Bergius-Rheinau process to around 5–6%. The post-hydrolysis of the sugar oligomers in solution was now accomplished at lower sugar concentrations. An additional purification step before the crystallization of the sugar was introduced to remove the salts, introduced by the neutralization of the remaining acid or sometimes added to break the HCl-water azeotrope. These changes claimed to lead to final sugar syrups of 90% glucose, reaching 99% after crystallization [63] [64]. A simplified mass balance and overview of the Udic-Rheinau process is shown in figure S22.

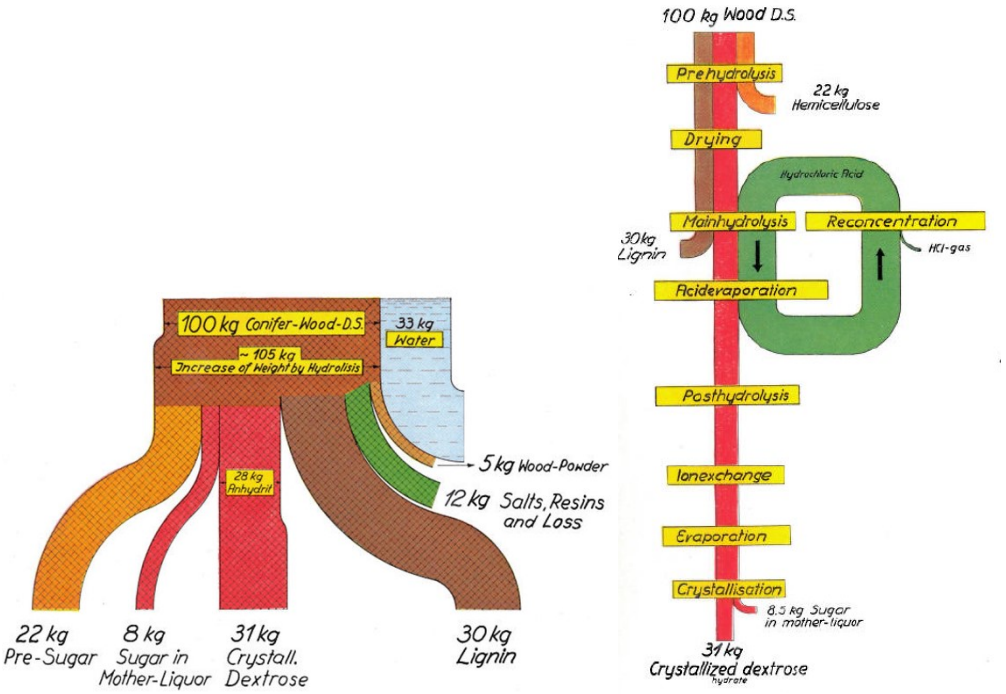


Figure S22. Left. Simplified mass balance of the wood. Right. Recovery of the wood components along the process, original figure from Ref. [63].

In this first modification, the pre-hydrolysis of the Udic-Rheinau process was accomplished inside autoclaves using 1% HCl and temperatures of 130 °C. The sugar solution from the hydrolysis of the hemicellulose was used to obtain sugars for fodder or fermentation. When soft wood was fed into the system, the pre-hydrolysate stream mainly contained mannose and was used for fermentation purposes, obtaining 7.7 liters of alcohol per 100 kg of dry feedstock. When hard wood was fed into the system, the hemicellulosic sugar solution contained xylose which was transformed into furfural, obtaining 9.0 kg of furfural per 100 kg of dry feedstock.

After the pre-hydrolysis, the solid lignocellulosic residue was centrifuged and dried before hydrolysis of the cellulose. The main-hydrolysis was performed in reactors connected in series with a counter-current feeding of 41 wt% HCl at 20 °C. The feeding speed of the acid inside the reactors remained constant, and the front-end of the acid-sugar solution was higher in sugar concentration due to consecutive hydrolyses in the reactors in series. After hydrolysis of the cellulose, the lignin residue was washed with water to recover the acid and sugars retained inside the residual porous material.

In 1958, in a later modification [65], the pre-hydrolysis was carried out with 32 wt% HCl at 20°C. The autoclaves previously used for this step were replaced with the same type of reactors placed in series as those used for the main hydrolysis of cellulose to simplify the process. The main hydrolysis was further accomplished without prior drying the lignocellulosic residue after the pre-hydrolysis, reducing the heat consumption. This modification also allowed for the use of larger amounts of finely divided material, such as sawdust.

The hydrochloric acid was distilled from the hydrolysate product in three consecutive steps at different pressures. In the first step, using a pre-evaporator operating at 0.13 bar, 95% pure HCl gas was recovered from the acid solution. The second evaporator, operating at 0.07 bar, recovered a 30 wt% HCl acid solution, which was then re-concentrated to 41 wt% by feeding the 95% pure HCl gas stream from the first evaporator. This 41 wt% aqueous HCl solution was recirculated to the main hydrolysis reactors to continue the hydrolysis of cellulose. In the last evaporator, steam was injected to reduce the HCl content of the sugar syrup to 3.5%, recovering a gas stream of 10% HCl. Although the injection of steam could potentially lower the sugar concentration, the use of a thin-layer evaporator minimized this effect by shortening the time and reducing the decomposition of sugars. In this way, the steam consumption was reduced from 27.3 tons per hour in the original Bergius process to 8.8 tons per hour.

The continuous performance of most operations in the Udic-Rheinau process enabled considerable heat recovery. The cooling water was recycled several times, and the generated heat, formed during the production of 41 wt% HCl from the distilled 30 wt% HCl solution, could be recovered and reused. After filtering and decolorizing the sugar syrup, a crystallization step was implemented to achieve glucose purities of 99%. By feeding 100 kg of

dry wood, 22 kg of crystalline glucose could be obtained after a single crystallization process. This process also allowed for a more optimal use of finely divided raw material, such as sawdust, which had been mentioned earlier. The recycling of energy and materials in this process was greatly improved by the simultaneous use and recovery of hydrochloric acid from both the pre- and main-hydrolysis steps, thereby significantly reducing acid consumption. The sugars from the pre-hydrolysis were also obtained in a purer, salt-free form and at a higher concentration compared to the runs at the plant in Regensburg.

Pilot tests running the Bergius-Rheinau process

Figure S23 illustrates the hydrolysis section scheme. Reactor 5 was loaded with wood chips and filled with intermediate hydrolysate containing 41 wt% HCl from tank 8 to initiate the batch pre-hydrolysis or hemicellulose hydrolysis. After the main hydrolysis, water was pumped into reactor 1, pushing the hydrolysate through the connected reactors in series. The hydrolysate entering reactor 4 had a higher sugar concentration and gradually became more diluted in sugar and acid by the time it reached reactor 1. Concurrently, fresh 41 wt% HCl was added to reactor 3 to maintain a consistent 41 wt% HCl concentration, thereby starting the main hydrolysis or cellulose hydrolysis. The final product hydrolysate was collected in tank 7. After 5 hours, when the sugar concentration from reactor 4 began to decrease, the intermediate hydrolysate was collected from it. Reactor 6 was then loaded with fresh wood chips, while reactor 5 continued the batch hemicellulose hydrolysis with the intermediate hydrolysate. During this new stage, reactors 1 to 4 remained connected in series, with water pumped into reactor 1 and fresh 41 wt% HCl into reactor 3 [66].

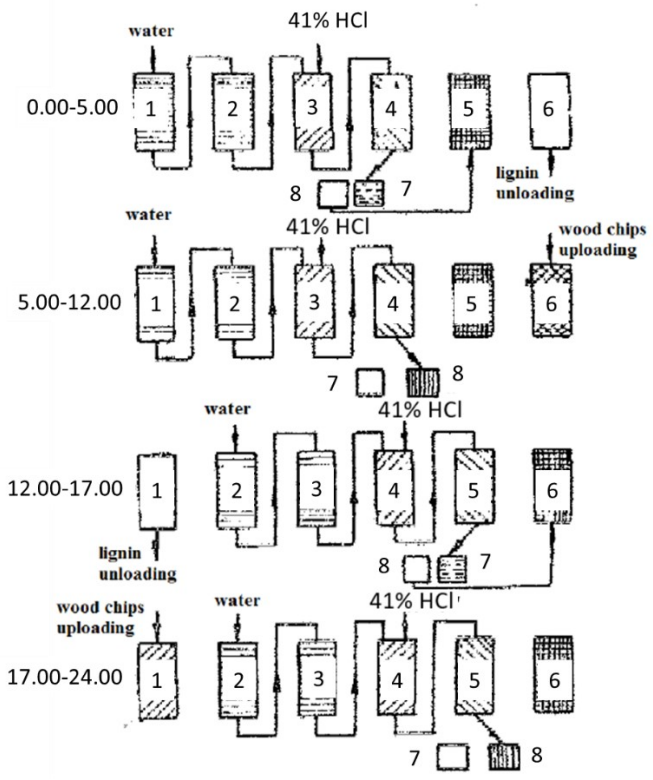


Figure S23. Overview of the Bergius-Rheinau hydrolysis scheme at the Kansk pilot plant, figure modified from Ref. [66].

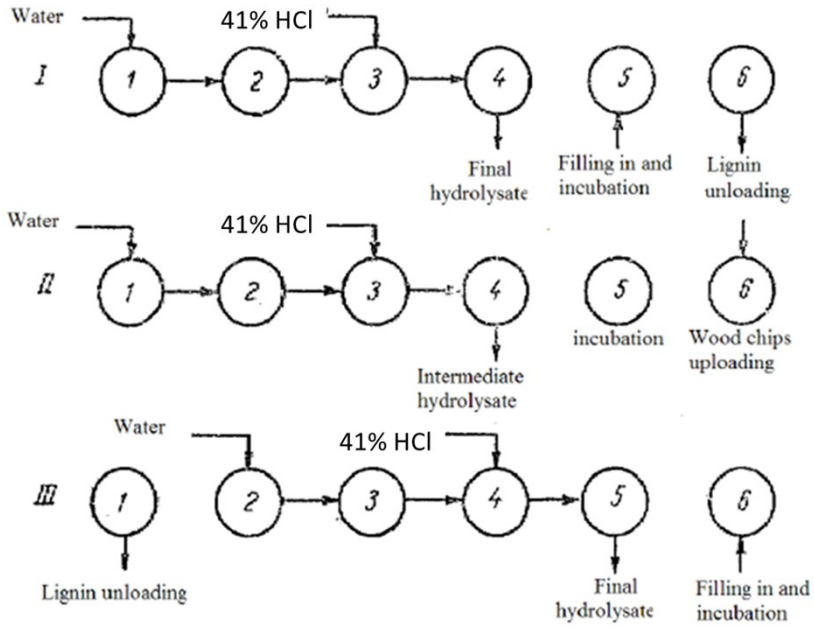


Figure S24. Second iteration of the Bergius-Rheinau hydrolysis process in Kansk, original figure from Ref. [67].

When larger wood chips were used, the number of reactors was increased to seven to extend the hydrolysis time and achieve sufficient sugar yields. Each phase shift lasted 14 hours, so the entire hydrolysis process took 56 hours, including the stationary hydrolysis of the hemicellulose. The ratio of intermediate hydrolysate to wood was set at 5:1. During the hemicellulose hydrolysis (referred to as incubation in Figure S24), there was an increase in sugar concentration by 3.3% and a reduction in HCl concentration by 2.2%. The final hydrolysate contained 17.4% sugars and 31.8% HCl. According to the mass balance, the total sugar yield corresponded to 62.2% of the wood mass, equating to 96% of the theoretical sugar yield. Petkevich [67] reported higher yields when repeating the procedure at a laboratory scale, obtaining sugar yields corresponding to 68% of the wood, with a hydrolysate sugar concentration of 21–22%.

In 1961, a new modification was incorporated into the hydrolysis section [67]. Petkevich observed that separating the hydrolysis of hemicellulose from cellulose could improve the overall efficiency. By effectively hydrolyzing the hemicellulose, the partial disruption of the cellulose structure enhanced its subsequent main hydrolysis with concentrated hydrochloric acid.

For these studies, wood chips were used as feedstock. Six reactors were utilized across three different stages, each lasting approximately 4 hours. An additional step was introduced before the hydrolysis of hemicellulose. During this step, the wood was dried while containing small amounts of hydrogen chloride to partially hydrolyze the hemicellulose and disrupt the crystalline structure of the cellulose, making it more accessible for subsequent hydrolysis. This drying step, performed with 2 wt% HCl at 100 °C for 3 hours, resulted in the partial hydrolysis

of 2–4% of the cellulose into glucose, which was then found in the pre-hydrolysate during the subsequent hemicellulose hydrolysis.

The battery of reactors (Figure S25) used at pilot scale consisted of 2 sections: a hydrolysis and a washing zone. The reactor containing cellolignin, *i.e.* the dried wood chips partially hydrolyzed during the drying step, was filled with intermediate hydrolysate from tank 8 in the same manner as described before to hydrolyze the hemicellulose. After filling reactor 5, a recirculation of the intermediate hydrolysate was performed for 1 h to improve the penetration of the wood material by the acid, and to protect sugars from the rapid temperature elevation because of the release of swelling energy. A 41 wt% HCl solution was still used to hydrolyze the cellulosic component. The final product hydrolysate was collected in tank 7 while intermediate hydrolysate was collected in tank 8. By monitoring the sugar concentration through several cycles, extending the reaction time showed that after 24 h the hydrolyzed feedstock did not contain any polysaccharides and consisted of lignin only.

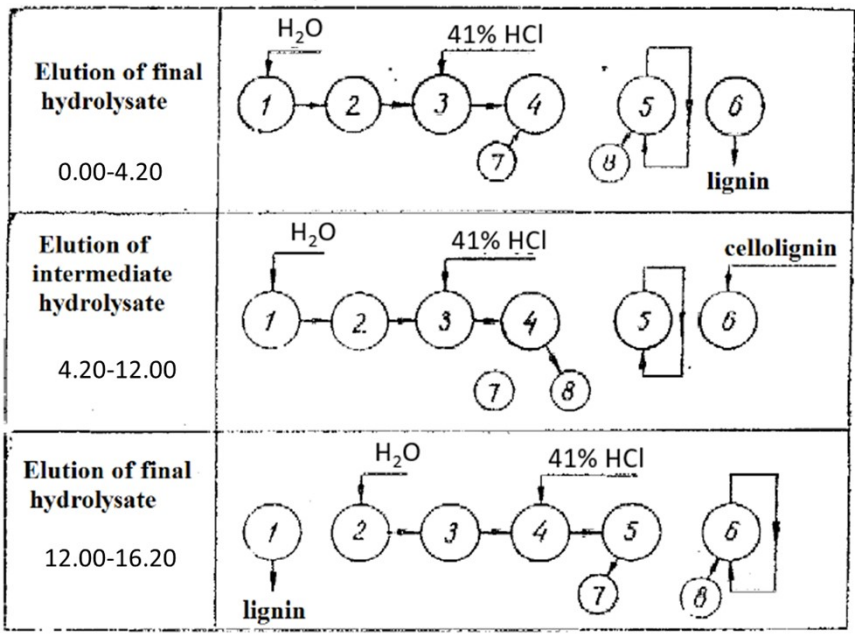


Figure S25. Hydrolysis scheme using (treated) pre-dried wood chips, adapted figure from Ref. [67].

According to the data, the final hydrolysate contained 18.1% (w/w) sugars and 31.7% (w/w) HCl. The yield of sugars in the product hydrolysate constituted 59.2% of the cellolignin (pre-treated wood chips) corresponding to 96% of the theoretical sugar yield. The residual lignin did not contain any polysaccharides. The amount of hydrogen chloride pumped into the battery of reactors as 41.5% acid was equal to 111.7% of the cellolignin (w/w). After drying the lignin, it contained 0.27% HCl, with overall losses of HCl equal to 0.8% of the cellolignin (w/w).

Later in 1961, a different hydrolysis process was tested in the pilot plant located at Kansk [68]. The process was similar to the last modification of the Udic-Rheinau process and the hydrolysis had now three interconnected sections: hemicellulose hydrolysis with 36-38 wt% HCl, cellulose hydrolysis with 41 wt% HCl, and water-washing of the lignin.

The intermediate hydrolysate was made with 37 wt% HCl instead of 41 wt% HCl. The hydrolysis of the hemicellulose began with a stationary phase lasting the first 4 hours, after which the reactor was continuously fed with fresh 37 wt% HCl while the intermediate hydrolysate was collected from the bottom. After 12 hours, the reactor was connected to the others in series, and the hydrolysis of the cellulose with 41 wt% HCl commenced, followed by the water-washing of the lignin residue. The hydrolysis time was extended to 12 hours for the pre-hydrolysis and 44 hours for the main hydrolysis. The sugar concentrations in the pre-hydrolysate and main hydrolysate were 21.21% and 16.39%, respectively. Overall, 65% of the starting wood mass was hydrolyzed, which corresponds to a 94% conversion of the total sugars.

A complete separation of hemicellulose and hexose hydrolysates could not be achieved with this method. The hydrolysates obtained by sequential hydrolyses of pine wood in the reactor battery had the following composition, as a percentage of the total sugar amount:

- Hemicellulose hydrolysate (wt% of total sugars): glucose (49.5%), mannose (21.8%), galactose (6.8%), xylose (12.9%), arabinose (9.2%), with total hexose and pentose content of 77.1% and 22.4%, respectively.
- Cellulose hydrolysate (wt% of total sugars): glucose (78.1%), mannose (11.3%), xylose (4.8%), arabinose (2.8%).

A portion of the cellulose was prematurely hydrolyzed during the hemicellulose hydrolysis. Additionally, hemicellulosic sugars could not be completely washed out from the biomass with 37 wt% HCl. The consumption of concentrated HCl solution increased by 15–20%. It was concluded that to achieve a better separation of hemicellulose and cellulose hydrolysates, the volume of 37 wt% HCl should be increased by a factor of 1.5, which would result in a total HCl consumption increase of 25–30%.

In the last Russian modification published in 1968 [69], the water washing of the lignin was separated from the rest of the continuous process. The total hydrolysis time was reduced from approximately 60 hours to 40 hours, with the main goal of achieving higher glucose purity in the main hydrolysate product from the cellulose fraction. To achieve this, the method of hydrolysis used two different concentrations of HCl to selectively hydrolyze the hemicellulose and the cellulose. A larger volume of 41 wt% HCl was employed compared to previous iterations to minimize the cross-contamination of hemicellulosic sugars in the main hydrolysis product. After the main hydrolysis, the lignin residue was washed with water until the acid concentration at the reactor outlet reached 36.5% HCl. This acid stream was then recirculated and used for the hydrolysis of hemicellulose in a new cycle. These changes significantly reduced the final sugar concentration in the main hydrolysate and increased glucose purity to around 85%. This level of purity was adequate for ethanol production and other industrial applications.

The Virdia process

This technology was studied by Michael Zviely, who published a comprehensive summary of the process in 2013 (Figure S26) [70]. The Virdia pilot plant primarily used pine wood chips as feedstock. Initially, the biomass was treated with steam to remove bark, extractives, tall oil, and ash. Hydrolysis was performed using 42 wt% HCl at low temperatures (10–15 °C) to minimize sugar decomposition products such as furfural and humins. Approximately 98% of the theoretically available sugars in the wood, accounting for about 65% of the dry weight, were hydrolyzed. The acid-sugar mixture was further processed to recover the acid, followed by a secondary hydrolysis to convert oligomers into monomeric sugars. The hydrolysate, containing glucose, mannose, galactose, xylose, and arabinose, was utilized not only for fermentation to produce ethanol but also explored for various other applications including the production of renewable chemicals, materials, and biofuels [70].

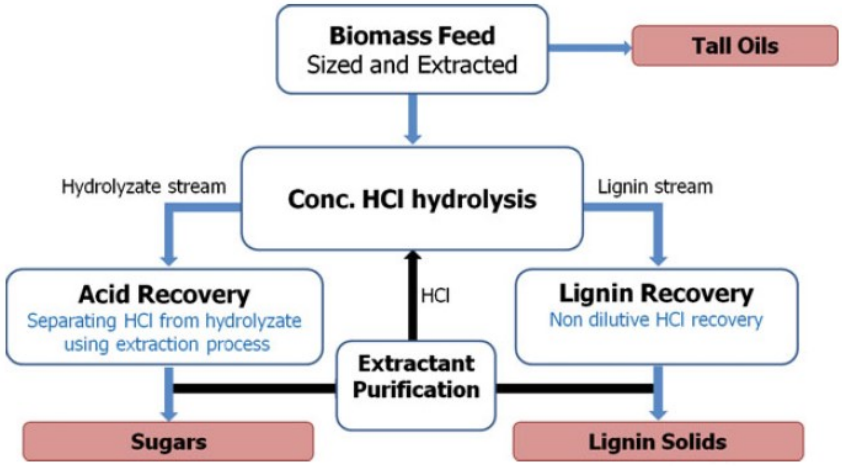


Figure S26. The Virdia process, figure from M. Zviely [70].

An interesting aspect of this study comes with the analysis of the various hydrates that HCl can form with different amounts of water, for example:

- $HCl{\cdot}2H_2O$ or the dihydrate $(H_2O\text{-}H^+\text{-}OH_2)(Cl^-)$
- $HCl{\cdot}3H_2O$ or the trihydrate $(H_2O\text{-}H^+\text{-}OH_2)(H_2O)(Cl^-)$
- $HCl{\cdot}4H_2O$ (fuming HCl).
- The hexahydrate $(H_3O^+)(H_2O)_5(Cl^-)$.

These species were held responsible for the efficient hydrolysis of cellulose [70]. These hydrates are formed mostly at high-HCl concentration in water (40–42 %) and below this concentration, the HCl hydrates required to hydrolyze cellulose drops sharply.

After recovering the HCl through the amine-HCl complexation, the HCl was stripped and recycled, and the lignin residue was recovered, dried and used in several application such as; binders, activated carbon, carbon fibers, fire-retardants, motor fuel, dispersants, sorbents, surfactants, and starting material for vanillin production. An additional by-product of the Virdia process was tall oil, a generic name for a group of compounds which consist of resin

acids, fatty acids, fatty alcohols, some sterols, and other alkyl hydrocarbon derivates. Resin acids occur in pine in a number of isomeric forms having the molecular formula of $C_{20}H_{30}O_2$ and related structures. The most prevalent are abietic- acid types, such as levopimaric, palustric, abietic, and neoabietic acid; and pimaric- acid types, such as pimaric and isopimaric acid.

Enzymatic Hydrolysis

Table S4. Biorefineries using enzymatic hydrolysis to produce biobased chemicals.				
Industry	**Pretreatment process**	**Enzyme loading**	**Sugars recovery**	**Current status of industry**
Sweetwater Energy, US	Dilute acid (single stage)	Moderate	High C5 and C6 sugars in solution	In 2022 it received the final acceptance of its first commercial Sunburst® patented process in Estonia
DuPont	Ammonia and steam	High	High C5 and C6 sugars in solution	Sold the plant to German bioenergy producer Verbio Vereinigte BioEnergie AG, now Verbio SE.
Poet-DSM	Dilute acid (two stage)	Low	C5 sugars recovered separately, C6 sugars in enzyme hydrolysate	POET-DSM paused cellulosic ethanol production
Abengoa	Dilute acid (single stage)	Moderate	C5 sugars recovered separately, C6 sugars in enzyme hydrolysate	Hugoton cellulosic ethanol plant sold out of bankruptcy to Synata Bio, Warrenville, Ill
Beta renewables, Italy	Steam explosion	High	Lower xylose yield recovered separately, C6 sugars during enzyme hydrolysis	Beta Renewables owns and licenses the Prosea® technology. Company closed in 2018.
Raizen, Brazil	Iogen's steam explosion	Moderate	High C5 sugars during pretreatment and C6 sugars recovery during enzyme hydrolysis	Raízen produced 16.5 million liters of 2G ethanol in 2018/19. Plans to scale up its cellulosic ethanol technology over the next 2 years to 40 million liters.
Granbio, Alagoas,	Liquid hot water	Moderate	High C5 sugars during	BioFlex®, the first biorefinery using Novozyme's customized

Brazil			pretreatment and C6 sugars recovery during enzyme hydrolysis of pretreated sugarcane straw	enzyme.
CTC, Piracicaba, Brazil	Steam explosion	Moderate	Moderate C5 sugars recovery and C6 sugars recovery during enzyme hydrolysis	CTC paused cellulosic ethanol production.
Praj industries, India	Dilute acid (two stage)	Not known	Hydrolysis and co-fermentation to ethanol	2G bioethanol pilot plant operating since 2009 'Enfinity' cellulosic ethanol technology has 1 million liters/year capacity.
Clariant, Germany	Hydrothermal	Low	High C5 and C6 sugars in solution to produce ethanol	Completion in 2021 of their first cellulosic ethanol commercial.
Edeniq, US	Colloid mill	Moderate	Moderate C5 sugars recovery and C6 sugars recovery to produce ethanol	In 2020 Edeniq faced lower demand of company's product and closed several facilities.
ICM, US	Dilute acid (one stage)	Moderate	High C5 and C6 sugars in solution to produce ethanol	Annual production of 30 billion liters of Ethanol.
St1 Biofuels, Finland	Particle size reduction	Not known	Hydrolysis and co-fermentation to ethanol	Ethanol commercial plant in Finland.
Celtic Renewables	Solvent fermentation technology	Not applicable	Not publicly detailed	As of 2021, Celtic Renewables launched their first commercial plant in Scotland,

	derived from the ABE process			aimed at converting whisky residues into biofuel.
DSM Poet	Enzymatic hydrolysis following mechanical and acid pretreatment	High	High C5 and C6 sugar recovery to maximize ethanol yield.	As of 2021, DSM and POET announced they are pausing production to focus on technological enhancements and research & development.
Green Plains	High-temperature enzymatic hydrolysis	Moderate to high	Moderate recovering of fermentable sugars for ethanol production.	Green Plains evolved, focusing now on transforming into protein production.

DAWN process

The dried wood chips (hard wood) are fed into PVC Lined reactors, significantly reducing process costs compared to pre-oil crisis versions that utilized tantalum reactors [59]. Each reactor features a bottom outlet equipped with annular sieves for product extraction and a Hastelloy valve, known for its HCl resistance. These valves, being one of the priciest reactor components, are designed with minimal diameter to cut costs. The reactor setup consists of a simulated moving bed configuration, incorporating seven identical reactors arranged in two parallel trains. Each reactor operates in a switch mode, cycling through seven phases of eight hours each (Figure S27).

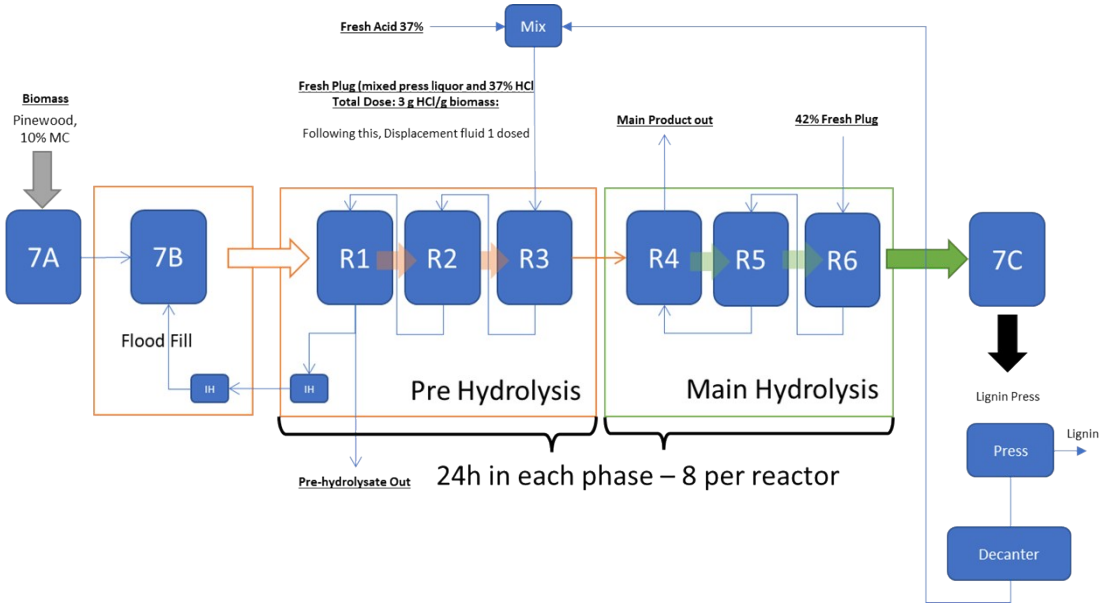


Figure S27. Block flow diagram of the simulated moving bed (SMB) hydrolysis.

The biomass is loaded into the first reactor (7A), the reactor is quickly filled with intermediate hydrolysate (HCl 37 wt% with some hemicellulosic sugars in it, 7B). Then, this reactor becomes R1 and the first block of eight hours starts. After each block of eight hours, every R(X) reactor becomes R(X+1). When R1 becomes R3 after 16 hours, a fresh plug of HCl 37% is fed at the top to ensure completion of the pre-hydrolysis and minimize sugar carry-over between both hydrolysis stages. This fresh-plug of HCl 37% is followed by displacement fluid to collect the sugars across the three pre-hydrolysis reactors (R1 to R3). Then R3 becomes R4 and main-hydrolysate with HCl 42% from R5 and R6 is fed into it, repeating the same process for each block of eight hours and culminating with a fresh-plug of HCl 42% to ensure hydrolysis completion. The pre- and main-hydrolysis composition is shown in Figure S28.

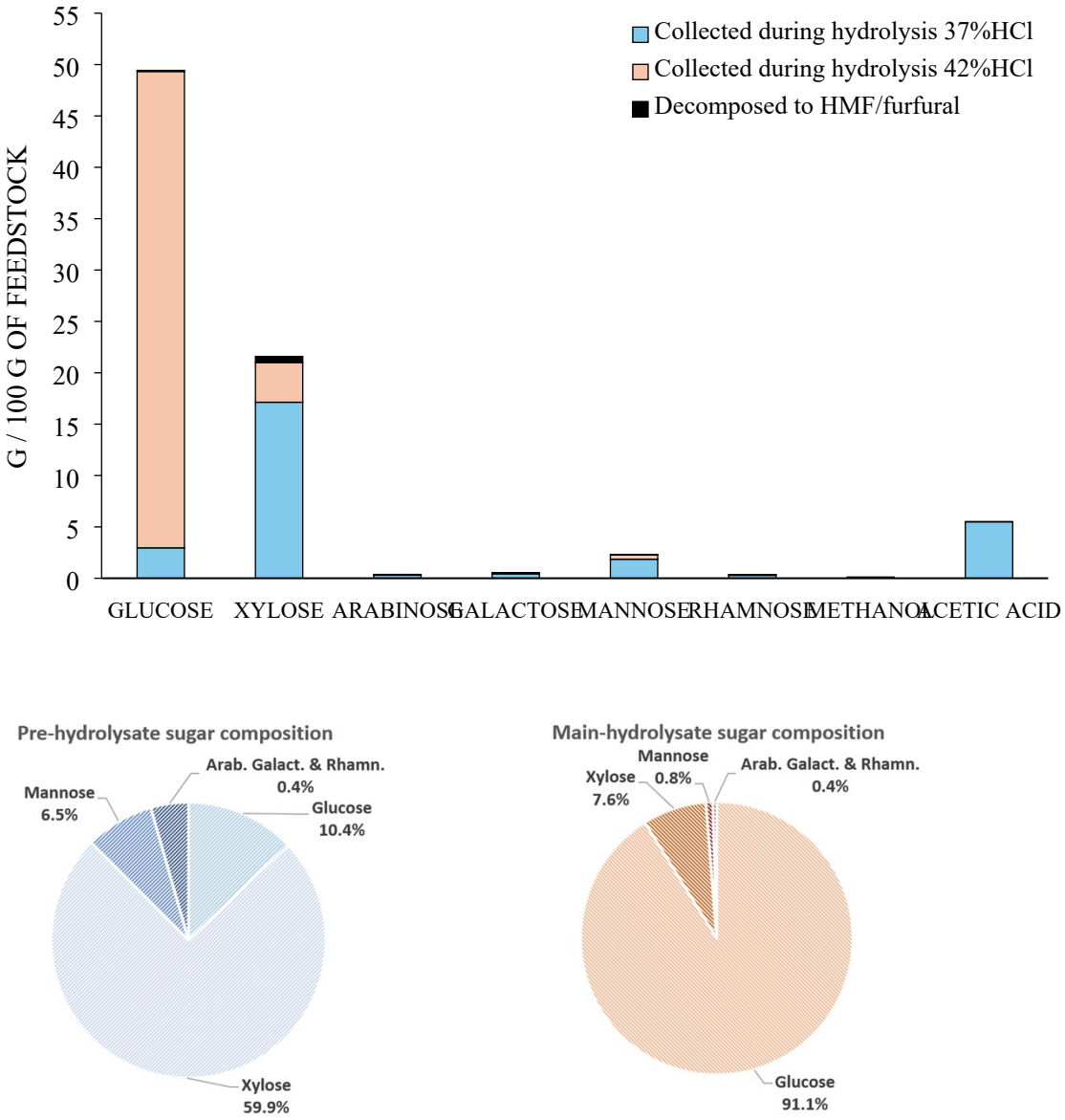


Figure S28. Typical standard product specification after hydrolysis of hardwood. Top: Sugar recovery during the hemicellulose pre-hydrolysis (37wt% HCl) and the cellulose main-hydrolysis (42wt% HCl). Bottom: Sugar composition of the product sugar solutions from the pre- and the main-hydrolysis.

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