

# Highly effective fractionation chemistry to overcome the recalcitrance of softwood lignocellulose

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## ABSTRACT

The efficient fractionation and thus production of individual biomass components are pivotal processes in the biorefinery concept. However, the recalcitrant nature of lignocellulose biomass, especially in the case of softwood, is one of the main obstacles to the wider application of biomass-based chemicals and materials. In this study, the use of aqueous acidic systems in the presence of thiourea was studied for the fractionation of softwood in mild conditions. Despite relatively low temperature (100 °C) and treatment times (30–90 min), notable high lignin removal efficiency (approximately 90 %) was obtained. Chemical characterization and the isolation of minor fraction of cationic, water-soluble lignin indicated that the fractionation proceed via nucleophilic addition of thiourea to lignin, resulting in dissolution of lignin in acidic water in relatively mild conditions. Besides high fractionation efficiency, both fiber and lignin fractions were obtained with bright color, significantly elevating their usability in material applications.

## 1. Introduction

Lignocelluloses, especially wood that accounts for 75 % of terrestrial above-ground biomass stores (Erb et al., 2018), are primary sustainable replacements for fossil fuel-based energy and materials. Because of their wide availability, wood, and wood residues (e.g., sawdust) have great importance in energy production, the construction sector, and the pulp and paper industry. Wood and its main biopolymer components, carbohydrates (cellulose and hemicelluloses) and lignin, have also great interest as sources of green chemicals, fuels, polymers, and novel nanomaterials. However, the recalcitrant nature of lignocellulose significantly hampers the utilization potential of biomass in many applications.

Biorefinery concepts, where the individual wood components would be separated selectively without their severe modification and degradation, have become more common in recent years. There are several fractionation methods proposed or taken into practice that can be divided into two principles: dissolving lignin from wood (delignification) to leave the carbohydrates (mainly, cellulose) in a solid fraction or

dissolving the carbohydrates to form a lignin-rich residue (Schutyser et al., 2018). An example of the former is the traditional sulfate (Kraft) process, which is the principal method for the production of high-quality cellulose pulp. Acidic hydrolysis used in cellulosic ethanol production is an example of the latter. In both cases (similarly as in most other methods), carbohydrate fraction is the target product and lignin is seen as an obstacle to efficient utilization of carbohydrates (Ding et al., 2018). Harsh processing conditions that are needed to break covalent crosslinks between lignin and hemicelluloses as well as noncovalent interactions between wood constituents during the fractionation cause irreversible degradation and condensation reactions in lignin (Renders, den Bosch, Koelewijn, Schutyser, & Sels, 2017). Consequently, lignin darkens severely and loses its reactivity, which limits its valorization potential. The carbohydrate fraction is also darkened due to the residual lignin. Nonetheless, several innovative techniques have recently been disclosed that allow the fractionation of lignocellulose in relatively mild conditions, including the use of ionic liquid and deep eutectic solvents (DESs) (van Osch et al., 2017), green organic solvents (e.g.,  $\gamma$ -valerolactone (Alonso et al., 2017)), and acidic hydrotropes (Zhu, Chen, &

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Cai, 2021).

Despite the significant scientific progress in biomass fractionation, there is still a need for a method that can be considered to be universally efficient for a wide variety of lignocellulose. Particularly, there are only a few processes that can efficiently delignify and fraction softwood, as the chemical structure, as well as lignin and hemicellulose arrangement, makes softwood a more recalcitrant structure compared, for example, with hardwood (Shuai et al., 2016; Todaka, Kowhakul, Masamoto, & Shigematsu, 2019). However, softwood is the dominating wood type in the Northern hemisphere (Galbe & Zacchi, 2002) and, globally, is the main source of timber, which creates a significant amount of softwood-based side stream (e.g., sawdust) that is currently underutilized (mainly burned to generate energy) because of the lack of efficient delignification methods.

Delignification processes rely on the chemical alteration of carbohydrate–lignin complex by chemically breaking the covalent bonds (hydrolysis) accompanied many times by the introduction of novel functionalities. Due to the nucleophilic properties, sulfur-based chemicals are widely used in delignification processes as they can react with lignin, resulting in depolymerization or chemical modification. In the sulfate process, alkaline sodium sulfide (Gellerstedt & Lindfors, 1984) is the main delignification chemical. Conversely, the acidic sulfite process is based on the action of sulfite and bisulfite ions (Windeisen & Wegener, 2012). Some novel methods, such as the use of reducing thiol (Klinger et al., 2020) and dithionite (Brienza et al., 2021) treatments are proposed as alternative, sulfur-based approaches for depolymerization of lignin but are rarely used in the delignification of lignocellulose biomass. Bearing in mind the nucleophilicity of sulfur-based chemicals as well as hydrogen bond acceptor properties of lignin, it was hypothesized that using chemistry where the active chemical, thiourea, is both nucleophile and hydrogen bond donor could assist lignin dissolution in relatively mild conditions. The use of mild conditions allows the isolation of both carbohydrate and lignin fractions with good yield and high molecular weight.

In this study, softwood sawdust was treated with aqueous acidic thiourea solution at 100 °C at different times, and the fiber morphology, degree of polymerization (DP), and chemical constitution of solid carbohydrate fraction were analyzed. Hydrogen peroxide bleaching was used to demonstrate the bleachability of the carbohydrate fraction. Furthermore, the lignin fraction was isolated and analyzed. Finally, a tentative mechanism of acidic thiourea fraction was proposed.

## 2. Materials and methods

### 2.1. Materials

Spruce sawdust was obtained in the never-dried form (approximately 50 % dry matter content) from Keitele Forests Oy and stored at −24 °C and thawed before use. For the fractionation of sawdust, thiourea was obtained from Sigma Aldrich and 37 % HCl solution and ethanol (96 %) from VWR Finland.

### 2.2. Acidic thiourea fractionation of softwood sawdust

The acidic thiourea fractionation was performed in a manner inspired by the production of (furan-2-yl)methanethiol by thiourea (2-FURFURYL MERCAPTAN, 1955). In the Scott bottle, 40 ml of water was mixed with 36 ml of 37 % HCl, followed by the addition of 30.4 g of thiourea. The addition of acid to water is a highly exothermic reaction, and acid should always be added to water. The addition of thiourea to acidic water is an endothermic reaction, and strong mixing or mild heating is required for complete dissolution.

After thiourea was completely dissolved, never-dried sawdust (8 g as dry) was added at room temperature and mixed well. The reaction bottle was tightly closed with a cap and placed in an oil bath at 100 °C, and the reaction mixture was stirred with a magnetic stirrer for 30, 60, or 90

min. After the desired reaction time, the bottle was removed from the oil bath and allowed to cool for approximately 5 min. Then, the reaction mixture was poured into a beaker containing 200 ml of ethanol and mixed well. The solid residue was filtrated, washed two times with 100 ml of ethanol, and dried in an oven at 60 °C overnight.

The water-soluble lignin fraction was isolated by mixing 2 g of dried solid residue with 200 ml of deionized water for 2 h at room temperature, followed by filtration and washing twice with 50 ml of deionized water. Approximately 0.3 g of water-soluble lignin was obtained after evaporation of water. The water-washed solid residue was stored at 4 °C without drying for further processing and analysis.

Nonwater-soluble lignin fraction was isolated from the acidic thiourea–ethanol mixture by adjusting pH to around 8 with 20 % NaOH solution. Lignin was allowed to precipitate overnight, filtrated, and washed with 100 ml of ethanol and 500 ml of deionized water. Lignin was dried in a 60 °C oven.

Organic solvent soluble lignin was obtained by dissolution of dried nonwater-soluble lignin in 20 % NaOH solution followed by precipitation by adjusting pH to approximately 3. Precipitated lignin was then filtrated and washed with water until neutral. Lignin was dried in a 60 °C oven.

### 2.3. Bleaching of acidic thiourea-treated softwood fibers

The bleaching of the solid residue of acidic thiourea-treated sawdust was done using an alkaline hydrogen peroxide solution. In the Scott bottle, 180 ml of 4 % NaOH in water was mixed with 18 ml of aqueous H<sub>2</sub>O<sub>2</sub> solution (30 %), followed by the addition of 3 g of dried solid residue. The bottle was then placed in an oil bath at 70 °C and allowed to react under mixing for 1 h. Afterward, the bottle was removed from the oil bath and 50 ml of water was added. Then, the mixture was filtrated, and the solid was washed with 1 l of water. The solid was collected and stored at 4 °C.

### 2.4. Characterizations

Fiber dimensions were measured with Valmet FS5 Fiber analyzer that complies with ISO 16065-2 standard. Length weighted fiber length, width, and fines content (based on the projected area of particles having length <0.2 mm) were used to characterize a fiber fraction after thiourea treatment. The DP was determined using the cupperethylenediamine method according to the ISO 5351 standard. The morphology of the fibers was illustrated using a Zeiss Ultra Plus field emission scanning electron microscope (Germany). Lignin, carbohydrate, and extractive contents of original and acidic thiourea-treated softwood were determined using the National Renewable Energy Laboratory method, and nitrogen and sulfur contents were analyzed using the PerkinElmer CHNS/O 2400 Series II elemental and LECO CS-200 carbon-sulfur analyzers, respectively. Chemical characterizations of fiber and lignin fractions were performed with DRIFT using Bruker Vertex 80v spectrometer (USA). Water-soluble and alkaline treated lignin were analyzed using a Bruker Ascend 400 MHz NMR spectrometer. The samples were dissolved in DMSO-*d*<sub>6</sub> and placed in 5 mm NMR tubes. <sup>1</sup>H and HSQC spectra were recorded under ambient air temperature using Bruker standard parameters PROTON and HSQCETGP, respectively. The raw data were processed and analyzed using ACDLabs Spectrum Processor 2019.1.1 program. The quantitative determination of the molecular weight of the products has been performed using Agilent 8890 gas chromatography equipped with an HP-5MS Ultra Inert column from Agilent (model: USR577054H). All the experiments and characterizations were done in duplicate and error is presented as standard deviation.

### 3. Results

#### 3.1. Acidic thiourea treatment of softwood sawdust

The softwood fractionation was investigated using aqueous HCl as the acid medium and thiourea as the nucleophile; hydrogen bond donating active reagent and the overall concept of the fractionation process is presented in Fig. 1. Thiourea is a urea analog with carbonyl oxygen replaced with sulfur. Despite the structural similarities, thiourea has different chemical behavior when compared with urea. For example, thiourea occurs in two tautomers, namely, thione and thiol forms (Allegretti, Castro, & Furlong, 2000), and thiourea can be alkylated to sulfur atoms by the reaction with alkylhalide. In alkylation, thiourea acts as a nucleophile, and the reaction results in the formation of isothiuronium salt. Alkylation of thiourea is one route to obtain alkylthiols as alkaline hydrolysis of isothiuronium salts results in the formation of thiol (Ouellette & Rawn, 2014). Besides the thiolation of alkylhalides, the thiourea has been used for direct modification of furfural alcohol to produce mercaptofurfural after the hydrolysis of isothiuronium derivate (2-FURFURYL MERCAPTAN, 1955). Aside from being a good nucleophile, thiourea is also a good hydrogen bond donor, which is demonstrated in many organocatalytic reactions where substituted thioureas have been used to accelerate the reactions (Parvin, Yadav, & Choudhury, 2020). Furthermore, thiourea and its derivatives have been studied as hydrogen bond donors in DESs (Mukesh, Mondal, Sharma, & Prasad, 2014; Sharma, Mukesh, Mondal, & Prasad, 2013).

The spruce sawdust used as raw material consist of various sized particles ranging from few hundreds of micrometers to several millimeter long irregular shaped particles (Fig. 2a and b). During the reaction, the initial heterogeneous mixture of sawdust and fractionation solution gradually become homogenous dispersion of fibers and most of the visible sawdust particles disintegrated during the first 30 min. The scanning electron microscopy (SEM) images show that large and randomly shaped sawdust particles disintegrated into individual fibers during the reaction (Fig. 2b and e). The separation of fibers can be assumed to be due to the dissolution of lignin. However, unlike during many lignocellulose fractionation processes, the color of the sawdust was not darkened. Actually, the fiber fraction exhibited even slightly more whitish when compared with the original sawdust.

#### 3.2. Chemical constituents

The mass yield of solid fraction after 30 min reaction times was 50 % and the yield gradually decreased to approximately 42 % when reaction times were increased to 90 min (Table 1). In all the samples, glucose was the main sugar fraction (81 % of total mass), and xylose and mannose are found to be a minor fraction, whereas all the galactose of the original wood was observed to dissolve during the treatment. Dissolution of hemicelluloses is typical for acid-based systems, as hemicellulose is more prone to acid-catalyzed hydrolysis when compared with cellulose. Moreover, extractive content decreased significantly during the acidic thiourea treatment.

Although some carbohydrate fractions were dissolved, the main fraction removed by acidic thiourea treatment was found to be lignin, since already after 30 min, approximately 87 % of original lignin was removed and the residual lignin content in the cellulosic fraction was approximately 7 %, which equates to the typical unbleached sulfate and sulfite pulp (Brännvall, 2017). The increase in the reaction time showed a further decrease in lignin content to approximately 4 % at 90 min reaction time. The removal of lignin is also in agreement with infrared spectroscopy results (Fig. 1c and f) as most of the peaks related to lignin were observed to disappear. Only the small shoulder at approximately 1601, 1510, and 804  $\text{cm}^{-1}$ , related to aromatic skeletal vibration and C—H out of plane bending of lignin (Horikawa et al., 2019), indicates the slightly incomplete delignification.

Unlike many recently published alternative green methods for biomass fractionation/delignification, acidic thiourea treatment had an outstanding ability to delignify softwood, which is shown to be a more cumbersome raw material than hardwood and nonwoody lignocellulose. For example, the removal efficiency of lignin from softwood (pine) with aqueous *p*-toluenesulfonic acid was reported to be approximately 42 % after 90 min at 80 °C, whereas from hardwood (poplar) it was 80 % at the same temperature after only 15 min reaction time (Li et al., 2020). Moreover, the loss of cellulose from pine was approximately 20 %, being significantly higher than the acidic thiourea treatment (4 %–9 % after 30–90 min). In the case of fractionation of Douglas fir with choline chloride-lactic acid DES at a temperature of 145 °C, the lignin removal efficiency of 58 % was found after 9 h reaction, whereas the efficiency of 78 % was achieved for poplar after 6 h (Alvarez-Vasco et al., 2016).

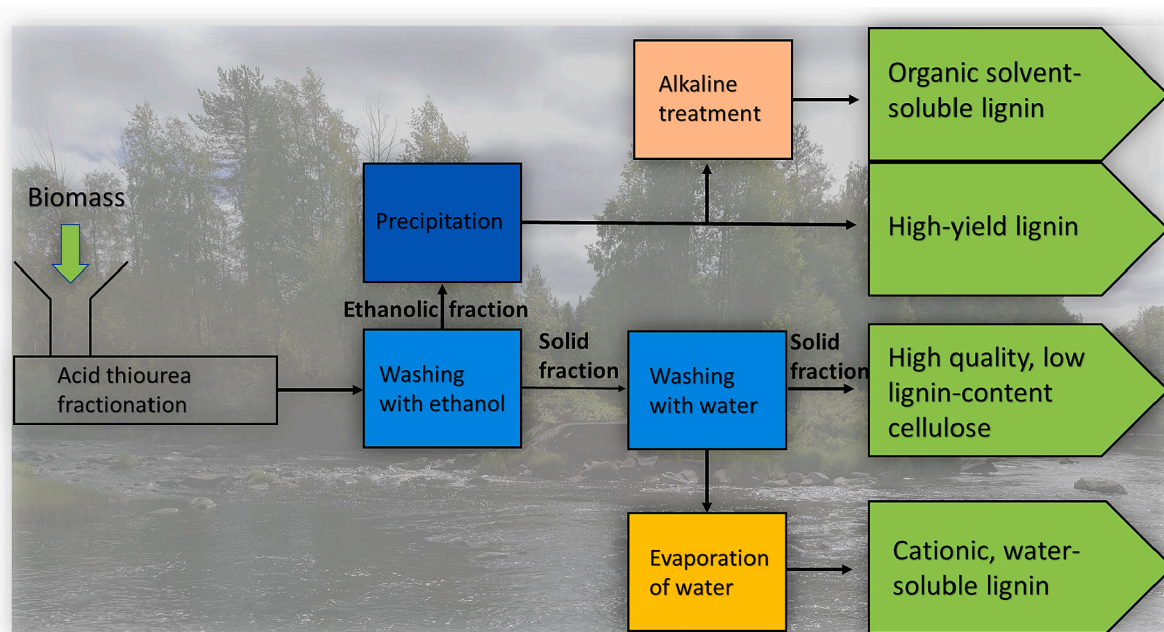
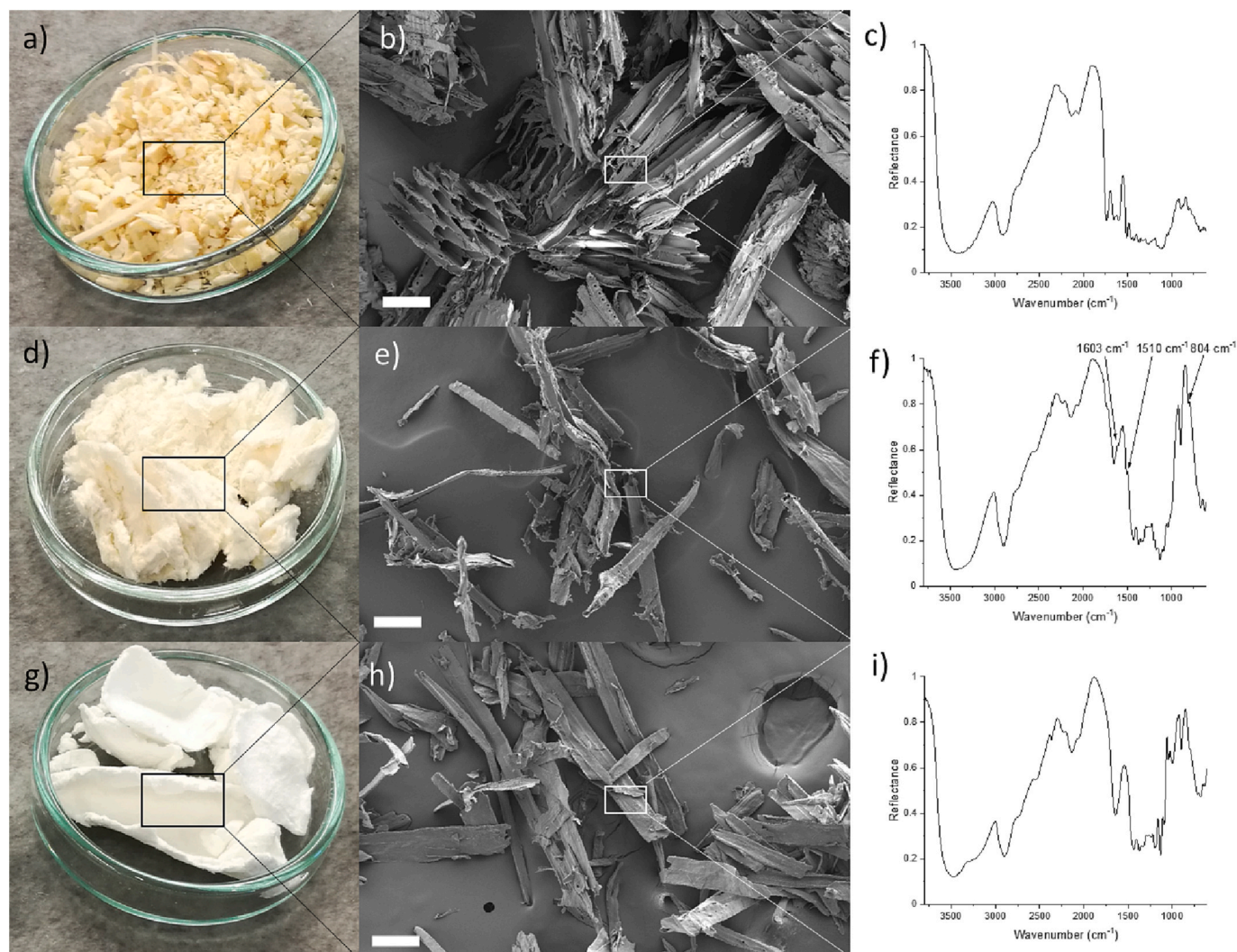


Fig. 1. Flowchart of the acidic thiourea fractionation process.





**Fig. 2.** Photograph (a, d, and g), SEM (b, e, and h), and FTIR (c, f, and i) spectra of original, acidic thiourea-treated, bleached acidic thiourea-treated softwood sawdust (scale bar in SEM images is 100 µm). The shoulders of the residual lignin peaks are marked in the spectrum of acidic thiourea-treated softwood sawdust.

**Table 1**

Chemical composition of the original wood and solid fractions after acidic thiourea treatment.

Sample	Mass yield (%)	Chemical constituents						Removal percentage				
		Glucose (%)	Xylose (%)	Galactose (%)	Mannose (%)	Extractives (%)	AIL <sup>a</sup> (%)	Glucose (%)	Xylose (%)	Galactose (%)	Mannose (%)	Lignin (%) <sup>a</sup>
Softwood	–	41.9 ± 0.1	6.9 ± 0.1	3.3 ± 0.2	12.9 ± 0.2	2.1 ± 0.0	27.9 ± 0.1	–	–	–	–	–
30 min	50	80.7 ± 0.0	3.2 ± 0.2	n.d. <sup>b</sup>	7.4 ± 0.0	0.5 ± 0.0	7.4 ± 0.4	3.7	77.1	100	71.6	86.8
60 min	46	86.5 ± 0.4	3.0 ± 0.0	n.d. <sup>b</sup>	4.9 ± 0.4	0.2 ± 0.0	6.2 ± 0.4	5.7	80	100	82.7	89.9
90 min	42	90.5 ± 0.9	0.9 ± 0.0	n.d. <sup>b</sup>	2.5 ± 0.1	0.1 ± 0.0	3.6 ± 0.2	9.4	94	100	92	94.6
60 min <sup>c</sup>	42	87.0 ± 0.2	1.4 ± 0.0	n.d. <sup>b</sup>	4.5 ± 0.2	0.1 ± 0.0	0.7 ± 0.1	13.0	91	100	85.6	99.0

<sup>a</sup> Acid insoluble lignin.

<sup>b</sup> Not detected.

<sup>c</sup> Bleached.

Similarly, by using the same DES for hydrothermally pretreated poplar and pine at 130 °C with 3 h treatment time, the removal rates were 71 % and 26 %, respectively (Tian et al., 2022). Furthermore, with a low-cost acidic ionic liquid (*N,N*-dimethylbutylammonium hydrogen sulfate), 66 % of lignin could be removed from pine after 22 h at 120 °C (Gschwend

et al., 2019), whereas removal of lignin from nonwoody biomass (*Miscanthus × giganteus*) was 93 % at same conditions (Gschwend, Malaret, Shinde, Brandt-Talbot, & Hallett, 2018).

More efficient chemical treatments for softwood delignification that match the acidic thiourea treatment are also introduced in literature.

However, many previous methods are either not green, or the processing is not simple. Acidic sodium chlorite delignification can be used to obtain cellulosic pulp for example from pinecone with few percentages of residual lignin (Rambabu, Panthapulakkal, Sain, & Dalai, 2016). However, the use of a large quantity of halogen-based oxidizers is undesirable due to the formation of toxic side products (halogenated organic components). Peracetic acid has been proven highly efficient delignification method for softwood to produce white holocellulose with low lignin and high hemicellulose contents. Nonetheless, the peracetic acid method requires either a multistep process (i.e., several consequent treatments) (Yang, Berthold, & Berglund, 2018) or a long reaction time (Westin, Yang, Svedberg, Grundberg, & Berglund, 2021). Moreover, noteworthy, even in the case of peracetic acid delignification, softwood is more resistant to delignification when compared with hardwood (Kundu, Samudrala, Kibria, & Bhattacharya, 2021). Furthermore, to the authors' knowledge, the isolation of lignin from spent liquor may be challenging; at least, it has not been reported thus far. Recently, 87 % removal of lignin from pine was achieved with benzyltrimethylammonium chloride-formic acid DES at 150 °C after 120 min treatment time (De Santi, Galkin, Lahive, Deuss, & Barta, 2020).

### 3.3. Morphology and DP of cellulose-rich fraction

Table 2 lists the changes in fiber dimensions and respective DP during 90 min reaction at 100 °C. After 30 min, the fiber length was 0.61 mm and was decreased to 0.44 mm after 60 min but did not essentially change after 90 min, whereas the fiber width remained essentially unchanged up to 60 min but a significant drop was observed after 90 min reaction time. The fines fraction percentage gradually increased when the reaction time was increased.

The highly acidic conditions contribute to fiber fragmentation to some extent, but the short fiber length mostly originates from raw material as the wood is exposed to strong cutting during the sawing. Although the length of the fibers is shorter than the kraft processing of sawdust, where the length is reported to be over 1 mm (Korpinen & Fardim, 2009), the amount of fines is at a similar level, that is, 40 % after 60 min reaction. This suggests, rather, that the finer sawdust has a smaller particle size used in our experiments than the abnormal fragmentation of pulp being responsible for shorter fiber length. Conversely, the fiber width after acidic thiourea treatment was found to be higher than commercial cellulose pulps (Korpinen & Fardim, 2009; Li, Sirviö, Haapala, & Liimatainen, 2017; Strand et al., 2017) indicating that the solvent system results in strong swelling of the fibers that is retained even after washing.

Although it is apparent that highly acidic conditions cause the alteration of fiber morphology by cutting and erosion to produce short fibers with formation fines, the DP of the cellulosic fraction was found to be relatively high. The DP of the sample after 30 min acid thiourea treatment was found to be 2270 (Table 1), being similar to bleached kraft pulp from black spruce (DP of 2230) (Chakraborty, Sain, & Kortschot, 2006). When the reaction time was increased from 30 to 90 min, the DP was reduced from 2270 to 1090. Although it is evident that prolonged treatment time at acidic conditions results in severe hydrolysis of cellulose fraction, the DP of cellulosic fraction after 90 min treatment is still high and is in line with commercial dissolving pulp (DP

of 1400) (Sirviö, 2019) produced by acidic sulfite process.

Because of the short length, fibers obtained from sawdust after acidic thiourea treatment might not be suitable for traditional paper making, where a longer fiber length is desirable (McCowan, 1993). However, noteworthy, paper sheets produced directly from acidic thiourea-treated sawdust had good mechanical strength (tensile strength and modulus of 42 MPa and 8 GPa, respectively). Additionally, it can be envisioned that cellulose fraction would be suitable for the production of cellulose-based filaments (e.g., textile fibers) or regenerated films and membranes, where high molecular weight and mild color are advantages. Additionally, short fiber length is an advantage in nanocellulose production, since long, entangled fibers might block the devices (Sirviö & Lakovaara, 2021) used to mechanically disintegrate cellulose into nanosized constituents.

### 3.4. Bleaching of acidic thiourea-treated softwood fibers

During the industrial wood pulping (i.e., sulfite and sulfate process), the darkening of the fiber fraction is observed despite the removal of most of the lignin (Viikari, Suurnäkki, Grönqvist, Raaska, & Ragauskas, 2009). Because of the dark color of the fibers, a bleaching step is often required, increasing both the environmental burden and the cost of pulp production due to the use of hazardous and expensive halogen-based oxidants or multistep bleaching with various bleaching chemicals. Likewise, several novel delignification methods result in the formation of dark-colored fiber fractions that may no longer be bleachable. The dark color of the fiber fraction is due to the chemical alteration of lignin structure (e.g., condensation) and introduction of heteroatoms to lignin structure as well as the formation of strongly colored pseudo-lignin from carbohydrates (humin-like compounds (Aarum, Devle, Ekeberg, Horn, & Stenström, 2018)).

Contrary to most existing delignification processes, cellulose fraction directly after acidic thiourea treatment had good brightness with a slight yellowish tint, therefore being suitable as such in many applications where full whiteness is not a request (e.g., printable packaging boards). However, to investigate the possibility to produce white fiber fraction, halogen-free hydrogen peroxide bleaching was studied.

After peroxide bleaching of the sample treated for 60 min in acidic thiourea, bright fiber-like materials were obtained (Fig. 2g and h). The yield of the bleached fibers was 92 wt%. The brightness (ISO 2470-1 standard) of the non-bleached cellulose fraction was 57 %, being notable higher compared to cellulosic pulp obtained by acid-hydrolysis delignification of hardwood ( $\leq 28$  %) without bleaching (Ma, Hirth, Zhai, & Zhu, 2020), but a bit lower than the brightness of holocellulose softwood pulp (68 %) delignified with peracetic acid (Yang et al., 2018). After peroxide bleaching, the brightness of the cellulose fraction obtained by acidic thiourea treatment notable increased to 84 %, demonstrating good bleachability.

The chemical composition has slightly altered during the bleaching (Table 1). Xylose content further decreased to 1.7 %, whereas a slight increase in the glucose content was observed. Most notably, the amount of acid-insoluble lignin dropped from 6.2 % to 0.7 %. The reduction of the lignin content is backed by the disappearance of the peaks related to lignin in the IR spectrum of bleached samples (Fig. 2i). It is possible that after alkaline peroxide treatment some of the lignin is more susceptible to acid-catalyzed hydrolysis and approximately 3 % of acid-soluble lignin was observed. The acid solubility of the residual lignin could be used to further decrease the lignin content closer to 0 via an acidic washing step. Bleaching decreased the DP to approximately half of the original acidic thiourea-treated fibers. It is probable that chosen bleaching conditions to establish maximum bleachability were too harsh causing cellulose to be hydrolysed. Optimization, especially regarding to the dosage of the peroxide, should be studied to prevent the depolymerization of cellulose.

**Table 2**

Fiber properties and DP of the acidic thiourea-treated softwood.

Treatment time	Length [mm]	Fiber width [μm]	Fines (<0.2 mm) [%]	DP
30 min	0.61 ± 0.01	48 ± 2	28 ± 0	2270
60 min	0.44 ± 0.01	52 ± 1	40 ± 0	1650
90 min	0.41 ± 0.00	17 ± 1	47 ± 1	1090
60 min (bleached)	0.42 ± 0.0	46 ± 0	39 ± 0	820



### 3.5. Lignin fractions of acidic thiourea-treated softwood

After the acidic thiourea treatment, intensively yellow spent liquor was obtained (contrary to the black liquor obtained from kraft pulping) (Fig. 1S). When the pH of the solution was adjusted to mildly alkaline, rapid precipitation of lignin was observed. The color of the washed and dried lignin was lightly yellowish/grayish (Fig. 3a). The mass yield of the precipitated lignin after 60 min acidic thiourea treatment was 111 % of the theoretical yield calculated from the amount of original lignin removed from the wood. The over 100 % yield of lignin fraction indicates that either some carbohydrate fractions were precipitated along with lignin or chemical modification occurred during the fractionation. No carbohydrates were observed in the lignin sample, but a significant amount of nitrogen (5.0 wt%) and sulfur (11.1 wt%) were measured (no nitrogen and sulfur were detected in the original wood). Because of the nucleophilicity of thiourea, the formation of cationic isothiuronium salt of lignin is assumed to take place during the acidic thiourea treatment, leading to the dissolution of lignin in an acidic medium (see the subsequent section for chemical analysis of lignin and fractionation mechanism discussion). During the adjustment of pH to alkalinity, some of the isothiuronium groups are hydrolyzed to a thiol. Based on the elemental analysis, the isothiuronium and thiol group content of precipitated lignin were 1.8 and 1.7 mmol/g, respectively. When considering the isothiuronium and thiol group content, the yield of lignin is 90 % of the theoretical yield calculated from the removed lignin. The yield of lignin is in line with the yield of kraft softwood lignin (Zhu & Theliander, 2015) and significantly higher than lignin isolated from Japanese cedar (approximately 30 %) by acid-catalyzed polyethylene glycol solvolysis (Nge, Takata, Takahashi, & Yamada, 2016). With aqueous 4-chlorobenzenesulfonic acid, 46 % lignin yield from pine was reported after 40 min at 80 °C (near-completely isolation of lignin from hardwood was observed in the same conditions) (He et al., 2021).

The precipitated lignin was found to be insoluble in neutral water and organic solvent. The insolubility might be due to the presence of both the hydrophobic backbone of lignin, preventing the solubility in water, and hydrophilic isothiuronium groups, in turn lowering the solubility in the organic solvent. However, lignin was soluble in a concentrated aqueous alkaline solution. The color of the lignin solution in alkaline turned intensively red and after precipitation with adjustment of pH to acidic, red-colored lignin was obtained (Fig. 3b). The change in color indicated the chemical transformation of lignin during the alkaline treatment. The alkaline treated lignin was found to be soluble in organic solvents such as dimethylsulfoxide and *N*-methyl-2-pyrrolidone.

The formation of isothiuronium-derived lignin during the acidic thiourea fractionation was further supported by the isolation of yellowish-colored, water-soluble lignin fraction from the solid residue (Fig. 3c). Initial washing of cellulosic fraction was conducted with ethanol, which removed most of the dissolved fraction of lignin. The

water-soluble lignin fraction was obtained by washing the cellulosic fraction with water. The water-soluble lignin was positively charged with a charge density of 2.75 meq/g. As the surface of natural wood is slightly anionic (Singh, Mishra, Banerjee, & Sharma, 2011), the high positive surface charge of water-soluble lignin is due to the presence of a cationic charge introduced during the fractionation process. Furthermore, charge density of the most common industrial lignin, kraft lignin and lignosulfonates are reported to be  $-0.67$  and  $-1.52$ – $2.25$  meq/g at pH of 10.5 (Oveissi & Fatehi, 2015), indicating unique characteristic of water-soluble lignin fraction obtained in this study.

### 3.6. Chemical characterization and molecular weight analysis of lignin

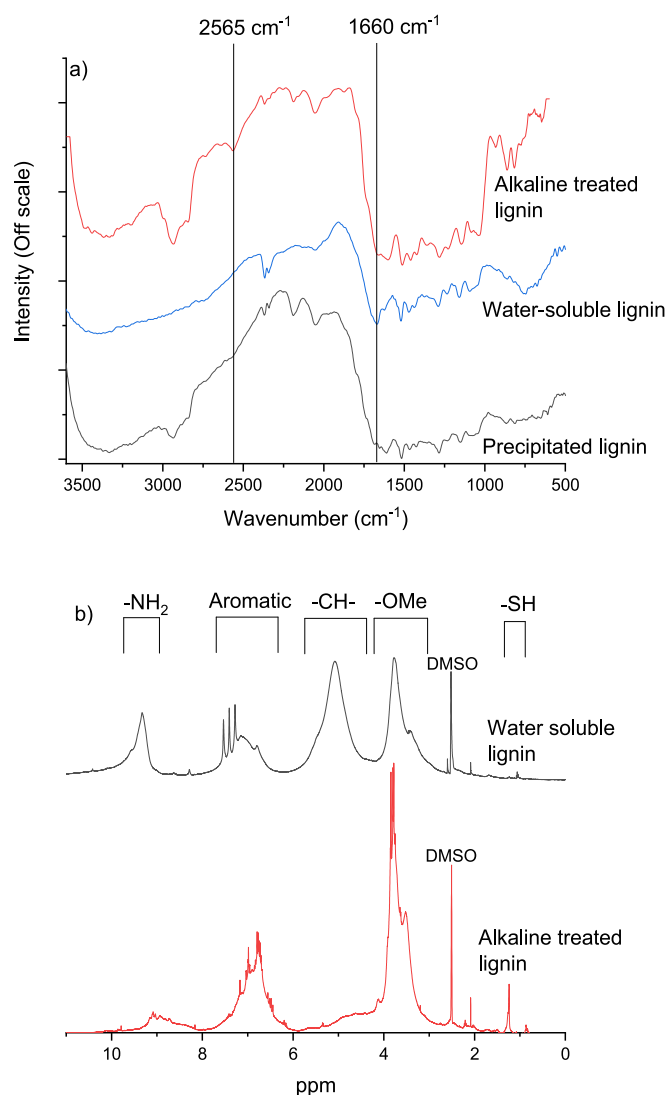
The diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy of precipitated, water-soluble, and alkaline treated lignin showed typical peaks associated with lignin (Horikawa et al., 2019) (Fig. 4a). The most notable difference between spectra is the appearance of S–H stretching of peak thiol at  $2565\text{ cm}^{-1}$  in the spectrum of alkaline treated lignin (Rajalingam et al., 2010). The small shoulder of SH stretching of a peak can be seen in the spectrum of precipitated lignin, whereas no peak related to the thiol group can be seen in the spectrum of water-soluble lignin. Furthermore, the intensity of the peak at approximately  $1660\text{ cm}^{-1}$  in the spectrum of water-soluble lignin appears to be higher, which could be due to the presence of the isothiuronium group ( $\text{NH}_2$  bending (Hemalatha et al., 2006)). However, due to the overlapping with C=O stretching of conjugated carbonyl peak of lignin, no direct conclusion can be made.

The  $^1\text{H}$  nuclear magnetic resonance (NMR) spectra of both water-soluble and alkaline treated lignin showed typical peaks of lignin at approximately 6–7.5 ppm, related to aromatic hydrogens, as well as the peak of methoxy groups at approximately 3–4.5 (Fig. 4b) (Nagy, Kosa, Theliander, & Ragauskas, 2010). The most notable difference between the two spectra is the strong peak at 9.3 in the case of water-soluble lignin, being associated with the protons of isothiuronium functionality (Foreiter et al., 2013). The presence of isothiuronium peaks indicates that the cationicity of water-soluble lignin is due to the reaction between lignin and thiourea to form isothiuronium functionality. The notable reduction of peak related to the isothiuronium group as well as the appearance peak of thiol at 1.2 ppm indicates that alkaline treatment results in conversion of the isothiuronium group into the thiol. In the spectrum of water-soluble lignin, a strong peak is observed at 5 ppm and the intensity of this peak is significantly reduced in the spectrum of alkaline treated lignin, indicating that the presence of this peak is due to the isothiuronium functionality, most likely  $-\text{CH}-$  attached to sulfur. However, further studies are required to fully identify the nature of this peak.

The cross-peak of the main linkage unit of lignin, arylglycerol- $\beta$ -aryl ethers ( $\beta\text{-O-4'}$ ) of guaiacyl unit, should appear at approximately 71.0/4.8 ( $\delta_{\text{C}}/\delta_{\text{H}}$ ) (Crestini, Lange, Sette, & Argyropoulos, 2017). However, no



Fig. 3. Visual appearance of isolated lignin fractions: a) precipitated lignin, b) alkaline lignin, and c) water-soluble lignin.



**Fig. 4.** a) DRIFT spectra of precipitated, water-soluble, and alkaline treated lignin. S–H stretching and NH<sub>2</sub> bending peaks of thiol and isothiuronium group are marked with a line; b) <sup>1</sup>H NMR spectra of water-soluble and alkaline treated lignin in DMSO-d<sub>6</sub>. The –NH<sub>2</sub> and –CH<sub>2</sub>– of water-soluble lignin are related to isothiuronium groups, whereas the –SH group of alkaline treated lignin is related to the thiol group formed during the alkaline hydrolysis of the isothiuronium group.

such a peak was observed in the HSQC spectrum of water-soluble lignin, indicating that these units are either cleaved or converted into the isothiuronium group. Indeed, a large number of peaks can be seen in the aliphatic region in HSQC, which could be related to the isothiuronium groups (peaks in the aliphatic region can be somewhat compared with the liginosulfonate bearing novel peaks in this region (Sáez-Jiménez et al., 2016)). Most of the peaks in the aliphatic region disappear in the spectrum of alkaline treated lignin (the most dominant peak of the methoxy group at 56.60/3.77 can be seen in both spectra), further indicating that the hydrolysis of the isothiuronium group occurred during the alkaline treatment. However, more studies, for example, using model compounds should be conducted to get a better understanding of the chemical structure of modified lignin obtained using acidic thiourea fractionation.

The weighted average molecular weight ( $M_w$ ) of both alkaline treated and water-soluble lignin fractions was approximately 40,000 g/mol (Table S1). The  $M_w$  of lignin obtained here was higher than that of milled wood lignin from spruce (23,500 g/mol) and was close to the

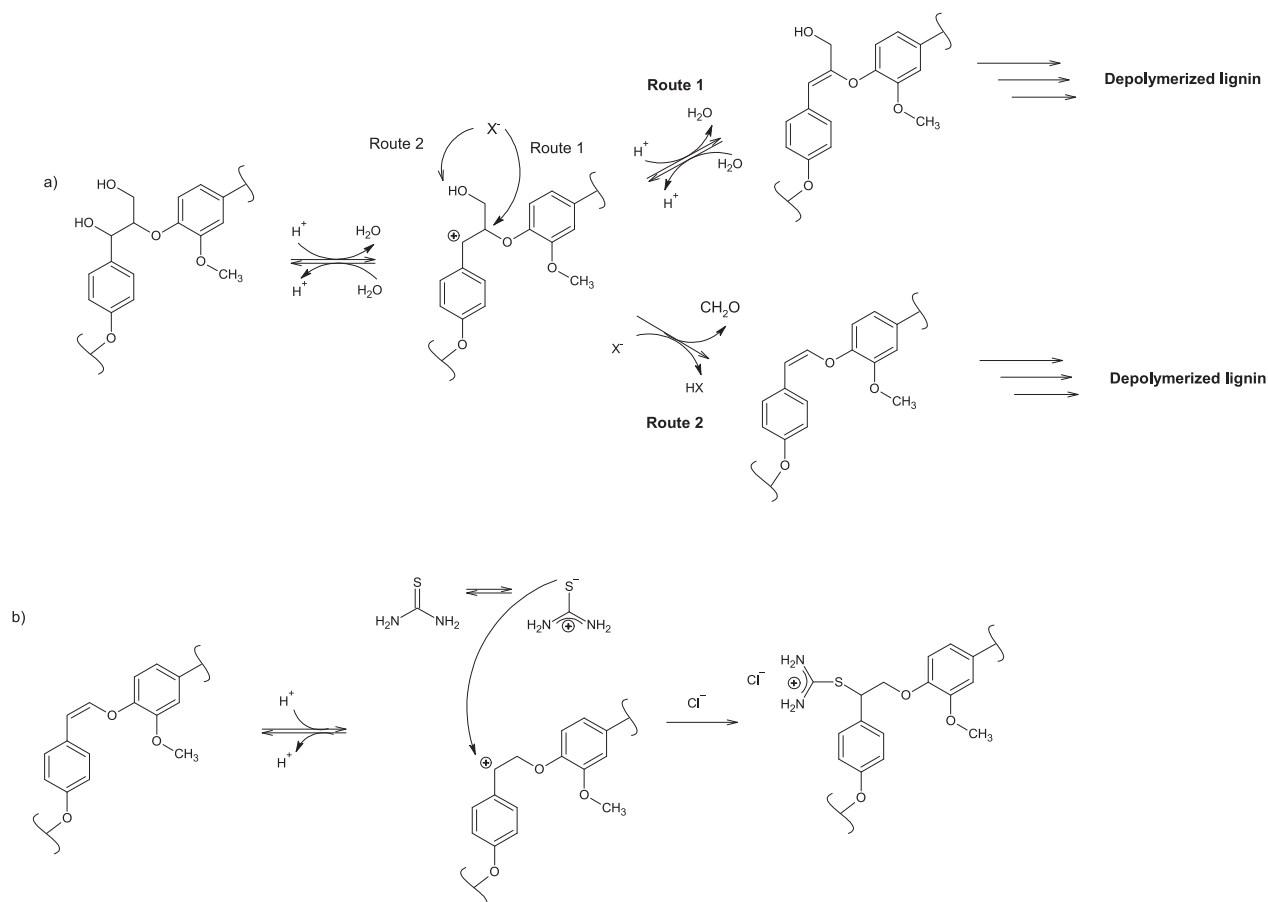
cellulolytic enzyme lignin (53,850 g/mol) (Tolbert, Akinoshio, Khunsapat, Naskar, & Ragauskas, 2014). The  $M_w$  of the spruce sodium liginosulfonate has been reported to be in a similar range (35,000–51,000 g/mol) (Hemmilä, Hosseinpourpia, Adamopoulos, & Eceiza, 2020) compared with both alkaline treated and water-soluble lignin from acidic thiourea treatment. By the use of protic ionic liquid, lignin fraction (27 % of whole isolated lignin) with  $M_w$  of 75,200 g/mol has been obtained (Chambon et al., 2020). Conversely, typical  $M_w$  of kraft softwood lignin can be significantly lower (6000  $M_w$ ) (Crestini et al., 2017). The polydispersity index of both samples obtained by acidic thiourea treatment was found to be high (7.5), indicating that large heterogeneity in the molecular weight of lignin. A high polydispersity index is typical for lignin isolated for example using enzymatic methods (Tolbert et al., 2014). Generally, it is notable that despite the highly acidic conditions, the molecular weight of lignin obtained by acidic thiourea treatment is high, and no extensive hydrolysis of lignin occurred, which is most likely because of the nucleophilic property of thiourea. In the following section, a discussion of the possible mechanism of delignification is presented.

### 3.7. Mechanism of the delignification of acidic thiourea

In nature, lignin is a complex heteropolymer of different aromatic repeating units (mainly sinapyl, coniferyl, and *p*-coumaryl alcohols), with a highly branched and crosslinked structure (Katahira, Elder, & Beckham, 2018). Covalent crosslinking bonds in lignin include carbon–carbon bonds, which are generally assumed to be inert in normal conditions (i.e., have low reactivity) and thus cannot be easily cleaved. Especially, softwood lignin is rich in carbon–carbon linkages, which largely explains the low delignification efficiency of several studied systems toward softwood. Other chemical bonds presented in lignin are cyclic and acyclic ether, which are more prone to basic and acidic hydrolysis, although severe conditions (e.g., high acidity and temperature) are required to hydrolyze carbon–oxygen bonds. Besides the crosslinking between lignin molecules, lignin is covalently attached to the carbohydrates (mainly hemicelluloses) by ester and ether bonds further hindering the delignification of lignocellulose. Compared with ether bonds, ester bonds are more easily hydrolyzed in alkaline or acidic conditions.

The delignification of lignocellulose biomass mainly relies on the alteration of lignocellulose constituents, for example, via degradation (hydrolysis), to enable the dissolution of lignin due to the decrease in molecular weight. The cleavage of the β-O-4 ether bond is proposed as the main lignin degradation mechanism in both aqueous HCl solution (Lundquist, Lundgren, Danielsen, Haaland, & Svensson, 1972; Yokoyama & Matsumoto, 2008) and other acidic solvents, such as DESs (Alvarez-Vasco et al., 2016). In the case of acidic delignification, the cleavage of the β-O-4 bond occurs via protonation of the hydroxyl group followed by the elimination of water molecules to produce benzyl carbocation (Fig. 5a). The proton abstraction by the conjugated base then results in the formation of various intermediates, ultimately leading to the cleavage of the bond between lignin molecules, and the formation of phenolic and carbonyl group-containing units. The cleavage of benzyl ether link in lignin–carbohydrate complex similarly takes place, that is, by protonation of ether oxygen, which will then result in cleavage of the bond between carbohydrates and lignin.

Besides the hydrolysis of lignin, the formation of carbocation can result in condensation/repolymerization of lignin (Funaoka, Kako, & Abe, 1990; Li et al., 2018). The electron-rich aromatic groups of lignin can act as a nucleophile, resulting in the formation of new carbon–carbon bonds between lignin molecules, making lignin more calcitrant toward hydrolysis. The condensation significantly decreases the dissolution rate of lignin and can contribute to the coloration of naturally bright lignin (Tran, Phan, & Lee, 2021). The 2-naphthol has been studied as a carbocation scavenger to suppress the condensation of lignin (Li, Henriksson, & Gellerstedt, 2007). Moreover, during sulfite cooking, the



**Fig. 5.** a) Mechanism of the acid catalyst cleavage of the  $\beta$ -O-4 ether bond of lignin according to literature (Lundquist et al., 1972; Yokoyama & Matsumoto, 2008) and b) tentative mechanism of acidic thiourea-based modification of lignin via Route 2.

sulfite ion is acting as a nucleophile, and thus a carbocation scavenger, resulting in the formation of liginosulfate (Sjostrom, 1993). Although the nucleophilic addition of sulfate ion into lignin can prevent the cleavage of linkage between lignin molecules (the lignin-carbohydrate complex, conversely, is cleaved (Deshpande et al., 2018)), the introduction of charged groups results in the dissolution of lignin (Deshpande, Sundvall, Grundberg, Henriksson, & Lawoko, 2022).

Based on the above-mentioned carbocation formation mechanism and nucleophilic properties of thiourea, the delignification mechanism of acidic thiourea is proposed (Fig. 5b). During the reaction, hydrochloric acid act as a strong acid-producing carbocation into the lignin structure. As shown in Fig. 5a, proton abstraction can take place in two routes, leading to the formation of two different enol ethers. Based on the current results, no clear preference for either of the routes can be concluded, and the formation of the isothiuronium group via Route 2 is demonstrated in Fig. 5. By the action of acid, enol ether is then converted into carbocation and followed by a reaction with thiourea. It has been proposed that the reaction between furfural and thiourea proceeds via an ionic reaction between carbocation of furfural and ionic tautomer of thiourea (Kofod, Veibel, & Vister, 1953), and the reaction between lignin and thiourea might take place via a similar route. However, other reaction mechanisms, such as direct condensation of hydroxyl group and isothiuronium ion (a protonated form of thiourea in acidic solution) or alkylation of thiourea via halide intermediate have been proposed in the case of furfural (Frank & Smith, 1946), and thus further mechanism studies are required to get a better understanding about the reaction between lignin and thiourea.

The nucleophilic reaction of carbocation with thiourea results in the formation of isothiuronium groups that has a positive charge in an

acidic medium. The cationic charge increases the hydrophilicity of lignin, which in turn enhances the solubility of lignin in an aqueous acidic solvent. The formation of isothiuronium lignin can therefore be considered to be analog to the formation of liginosulfate during sulfite cooking. The high delignification efficiency at notable lower temperature and shorter reaction time by acidic thiourea treatment compared with sulfite cooking is most likely due to the high concentration of nucleophilic agent (thiourea) in the cooking solution. Furthermore, thiourea is a hydrogen bond donor, which can interact with oxygen-containing groups of lignin acting as a hydrogen bond acceptor. The hydrogen bond interaction between thiourea and lignin can enhance the dissolution of lignin in the reaction solution. It can also be postulated that excess hydrolysis, and thus, a decrease in the molecular weight of lignin is prevented due to the scavenging of hydrolytically labile carbocation by the thiourea. Additionally, the relatively low temperature and reaction time might allow avoiding the hydrolysis of both lignin and cellulose fractions.

It should be noted that when the treatment was conducted without the addition of thiourea (an equal mass of water was added instead), very dark wood particles were obtained. The use of urea in a place of thiourea reduced the coloration of the wood, observed in the case of pure hydrochloric acid. However, the color of wood after acidic urea treatment was darker compared with starting wood. Additionally, the morphology of the wood remained close to its original state after acidic urea treatment, and no similar fiber separation was observed compared with acidic thiourea treatment. These results strongly indicate that thiourea is essential for the delignification process.



#### 4. Discussion

This study shows that acidic thiourea exhibits high lignocellulose fractionation potential for softwood, which is comparable with industrial pulping methods, surpassing most of the novel delignification systems introduced recently. The clear advantage of acidic thiourea treatment is its high efficiency and the production of both light-colored cellulosic and lignin fractions directly via single-step treatment. It was demonstrated that the bleaching cellulose fraction with hydrogen peroxide is enough for obtaining full-bleached cellulose pulp. Even without bleaching pulp could be suitable for example in printable packaging boards. Because of its recalcitrance structure, there do not exist many effective fractionation methods for softwood. Further optimization of reaction conditions is envisioned to elevate the feasibility of acidic thiourea in the fractionation of any type of lignocellulose biomasses: softwood, hardwood, and nonwood.

Some of the shortcomings of acidic thiourea treatment include the use of a high concentration of volatile HCl, and the effect of the acid concentration on the fractionation efficiency should be evaluated. Additionally, the use of other, less volatile acids such as sulfuric acid should be investigated. However, the chloride-ion might have a role in the delignification process. It has been shown that in presence of strong acid, the chlorinated  $\beta$ -O-4 ether-intermediate was formed in choline chloride-based DES (Lopes, Gomes, Coutinho, & Silvestre, 2020). Although chlorinated intermediate has not been observed in aqueous HCl (Yokoyama & Matsumoto, 2008), the role of chloride ion in acidic thiourea treatment cannot be excluded. Furthermore, the volatility of the HCl allows the regeneration of the acid on an industrial scale (McKinley & Ghahreman, 2018).

Thiourea is low-toxic (LD50 > 2000 mg/kg for rats) and biodegradable chemical (International Programme on Chemical Safety, 2003). However, it is irritating, can be hazardous to aquatic environments over the long term, and is suspected to have reproductive toxicity; thus, care should be taken when working with it. Therefore, the effect of the amount of thiourea in the solvent systems should be thoroughly studied. Nevertheless, as it was postulated that the hydrogen bonding ability of the thiourea takes a crucial role in the fractionation process, the excess hydrogen bond donor might be needed for efficient fractionation. However, it might be possible to replace some amount of thiourea with urea, which could serve as a hydrogen bond donor, whereas thiourea would still act as a nucleophile to produce isothiuronium functionality. Urea-based DESs have previously been shown to swell cellulose fibers (Li et al., 2017; Sirviö, Visanko, & Liimatainen, 2015); thus, urea could help the fractionation process, although it might not be capable of removing lignin from softwood in an acidic solution as was indicated in this study. Additionally, the biomass percentage in the solvent systems is relatively low, approximately 7 %, and should be increased. In this work, low biomass percentage was used mainly to maintain efficient mixing of the solution. Due to the fibrillation and swelling of the wood in acidic thiourea, the solution becomes almost gel-like after approximately 30 min at 100 °C, and the mixing with standard laboratory equipment becomes cumbersome. However, this issue should easily be circumvented using high-consistent reactors.

Paying attention to the above-mentioned shortcomings in the optimization of the fractionation method presented here, it is foreseen that cellulosic fraction produced by acidic thiourea treatment could be used to produce many cellulose-based materials, such as films (Hyden, 1929; Nguyen et al., 2020) and fibers for textiles (Wang, Lu, & Zhang, 2016). Additionally, cellulose fraction can be used as a sustainable alternative to producing low-lignin-containing cellulose nanomaterials (research currently ongoing). Residual lignin could provide UV protection (Liu et al., 2020; Xia et al., 2021) and when desired, colorless cellulose nanomaterial is easily obtained by mild bleaching. Isolated lignin fractions could be used as filler in composites (Haridevan, Evans, Ragauskas, Martin, & Annamalai, 2021) as the color of the lignin fraction was notably lighter than lignin obtained, for example, via the kraft process.

Furthermore, thiol groups of alkaline treated lignin possess unique reactivity and could be used for example in thiolene chemistry to produce different lignin-based materials (Liu & Chung, 2017). Additionally, cationic lignin fraction could be used in water treatment (isothiuronium groups are used in commercial chelating ion exchange resins (Lloyd-Jones, Rangel-Mendez, & Streat, 2004)). In future studies, the isolation of cationic lignin with higher yield should be investigated.

#### 5. Conclusion

Aqueous acidic thiourea was found as highly efficient fractionation method for softwood to obtain mildly colored cellulose fiber and lignin fractions at relatively mild conditions. Very high lignin removal percentage is notable as softwood is generally recognized as more resistant toward novel fractionation methods. The fractionation is assumed to take place via formation of isothiuronium-modified lignin via nucleophilic addition on thiourea to lignin, which enables the dissolution of lignin in acidic reaction solution. The formation of isothiuronium minimize both degradation and condensation of lignin during the fractionation, which in turn results in production of high molecular weight lignin and prevents the darkening carbohydrate and lignin fractions.

#### CRediT authorship contribution statement

Juho Antti Sirviö: Conceptualization, Investigation, Writing – original draft. Marja Mikola: Carbohydrate analysis, Reviewing. Juha Ahola: Carbohydrate analysis, Reviewing. Juha P. Heiskanen: NMR analysis, Writing – reviewing and editing. Svitlana Filonenko: Molecular mass analysis, Writing – reviewing and editing. Ari Ämmälä: Writing – reviewing and editing.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.carbpol.2023.120815>.

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