Doctoral Thesis No. 2024:61
Faculty of Forest Sciences

Steam Explosion Technology for the Valorization of Softwood Bark

Andreas Averheim

Steam Explosion Technology for the Valorization of Softwood Bark

Andreas Averheim
Faculty of Forest Sciences
Department of Forest Biomaterials and Technology
Umeå

SLU
SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

DOCTORAL THESIS
Umeå 2024

Acta Universitatis Agriculturae Sueciae
2024:61

Cover: Continuous system for steam explosion processing via a vertical reactor. With permission from Valmet. © 2024 Valmet Oyj.

ISSN 1652-6880

ISBN (print version) 978-91-8046-352-2

ISBN (electronic version) 978-91-8046-388-1

https://doi.org/10.54612/a.3rucs3brk1

Swedish University of Agricultural Sciences, Department of Forest Biomaterials and Technology, Umeå, Sweden

Print: NRA, Umeå 2024

Steam explosion technology for the valorization of softwood bark

Abstract

This thesis focuses on the valorization of softwood bark via a steam explosion processing step. A mixed softwood bark from an industrial source was steam exploded in a scalable system for continuous steam explosion. Two pathways for converting the steam-exploded bark into marketable products were investigated.

The first path focused on sugars and bioethanol from the steam-exploded bark via enzymatic hydrolysis and fermentation. By studying enzymatic digestibility for substrates steam-exploded at a variety of conditions (autohydrolysis and dilute acid hydrolysis), it could be determined that conversion into glucose was modest (<57% yield of theoretical) even at high enzyme dosages (10% w/w). Pretreatments with the carbocation scavenger 2-naphthol did not improve the hydrolysis yields, possibly due to the precipitation of water-soluble extractives at harsh process conditions. On the other hand, the fermentability of the hydrolysates was acceptable, even in hydrolysates containing traces of 2-naphthol.

The second path focused on producing activated carbon from bark via steam explosion, activation, and pyrolysis. Results showed that carbon materials that had been steam exploded possessed more pronounced graphitic structure, higher surface area, and additional functionalities than materials that had not been steam exploded. Consequently, they also had a better capacity as adsorbents of the azo dye reactive orange 16.

Finally, blow steam condensates were collected from the steam explosion stage, and the concentrations of some known degradation products were measured with high-performance liquid chromatography. With the help of Raman spectroscopy and modeling using orthogonal projections to latent structures, a solid model for quantification of furfural concentration was obtained, which opens the possibility of improved process monitoring and rapid quantification of this valuable byproduct.

Keywords: activated carbon, adsorption, enzymatic hydrolysis, fermentation, furfural, 2-naphthol, Raman spectroscopy, softwood bark, steam explosion

Ångexplosionsteknik för förädling av barrträdsbark

Abstract

Denna avhandling fokuserar på förädling av barrträdsbark genom en ångexplosionsprocess. En blandad barrträdsbark från en industriell källa ångexploderades i ett skalbart system designat för kontinuerlig ångexplosion. Två vägar för att omvandla den ångexploderade barken till säljbara produkter undersöktes.

Det första spåret fokuserade på socker och bioetanol från den ångexploderade barken via enzymatisk hydrolys och fermentering. Genom att studera enzymatisk nedbrytbarhet för substrat som ångexploderats under olika förhållanden (autohydrolys och svag syrahydrolys) fastställdes att omsättningen till glukos var måttlig (<57% utbyte av teoretiskt) även vid höga enzymdoser (10% vikt/vikt). Förbehandlingar med additivet 2-naftol förbättrade inte hydrolysutbytena, möjligen på grund av utfällning av vattenlösliga extraktivämnen vid hårda processförhållanden. Å andra sidan var fermenterbarheten hos hydrolysaten acceptabel, även i hydrolysat som innehöll spår av 2-naftol.

Det andra spåret fokuserade på att producera aktivt kol från bark via ångexplosion, aktivering och pyrolys. Resultaten visade att kolmaterial som hade ångexploderats hade en mer uttalad grafitstruktur, större ytarea och ökad kemisk funktionalitet än material som inte hade ångexploderats. Följaktligen hade de också en bättre kapacitet som adsorbenter av azofärgämnet reactive orange 16.

Slutligen samlades kondensat från blåsångan från ångexplosionssteget, och koncentrationer av några kända nedbrytningsprodukter mättes med högupplösande vätskekromatografi. Med hjälp av Ramanspektroskopi och multivariat kalibrering erhölls en solid modell för kvantifiering av furfuralhalt, vilket öppnar möjligheten att bättre övervaka processen och snabbt kvantifiera denna potentiella biprodukt.

Nyckelord: adsorption, aktivt kol, barrträdsbark, enzymatisk hydrolys, fermentering, furfural, 2-naftol, Ramanspektroskopi, ångexplosion

Dedication

To my family

“Well done is better than well said.”

Franklin (1737)

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Averheim, A., Larsson, S.H. & Thyrel, M. (2022). Carbocation scavenger pretreatment to mitigate lignin self-condensation in a semi-industrial steam explosion process. Bioresource Technology Reports, 20, 101292. https://doi.org/10.1016/j.biteb.2022.101292

II. Averheim, A., Stagge, S., Jönsson, L.J., Larsson, S.H. & Thyrel, M. (2024). Separate hydrolysis and fermentation of softwood bark pretreated with 2-naphthol by steam explosion. Biotechnology for Biofuels and Bioproducts, 17(1), 102. https://doi.org/10.1186/s13068-024-02552-y

III. Averheim, A., Simões dos Reis, G., Grimm, A., Bergna, D., Heponiemi, A., Lassi, U. & Thyrel, M. (2024). Enhanced biobased carbon materials made from softwood bark via a steam explosion preprocessing step for reactive orange 16 dye adsorption. Bioresource Technology, 400, 130698. https://doi.org/10.1016/j.biortech.2024.130698

IV. Averheim, A. & Thyrel, M. Raman spectroscopy for the quantification of furans in blow steam condensates derived from a steam explosion process (submitted)

Andreas Averheim made the following contribution to the papers included in this thesis:

I-IV. Averheim set the specifications for the preprocessing and supervised the steam explosion trials.

I. Averheim planned most of the work, conducted all the sampling, and performed the enzymatic hydrolysis experiments and all the analyses. Averheim had a prominent role during the data evaluation, wrote the manuscript with help from coauthors' comments, and acted as the corresponding author.

II. Averheim conducted the sampling during the steam explosion trial, executed the enzymatic hydrolysis experiments, and performed parts of the sugar analyses. Averheim participated in the data evaluation, wrote the main part of the manuscript, and acted as the corresponding author.

III. Averheim actively participated in the study's planning, conducted all the sampling during the steam explosion trial, produced most of the carbon materials, studied adsorption kinetics and equilibria, and performed Raman spectroscopy. Averheim also wrote the main part of the manuscript and contributed to its revision.

IV. Averheim conceptualized the study, conducted all analyses except HPLC, performed the evaluation, and wrote the main part of the manuscript.

Abbreviations

AIL	Acid-insoluble lignin
ASL	Acid-soluble lignin
ATEX	Atmosphere Explosible
ATR	Attenuated total reflectance
BET	Brunauer-Emmett-Teller
BTC	Biomass Technology Centre
DoE	Design of experiments
DP	Degree of polymerization
DRIFTS	Diffuse reflectance infrared Fourier transform spectroscopy
EDX	Energy dispersive X-ray spectroscopy
FTC	Fiber Technology Center
FTIR	Fourier transform infrared
G	Guaiacyl
H	P-hydroxyphenyl
HPAEC	High-performance anion-exchange chromatography
HPLC	High-performance liquid chromatography
ISO	International Organization for Standardization
MLR	Multiple linear regression
MWL	Milled wood lignin

NMR	Nuclear magnetic resonance
NREL	National Renewable Energy Laboratory
OPLS	Orthogonal projections to latent structures
PAD	Pulsed amperometric detection
PAH	Polycyclic aromatic hydrocarbon
PCA	Principal component analysis
PCB	Polychlorinated biphenyls
RO16	Reactive orange 16
S	Syringyl
SCAN	Scandinavian Pulp, Paper and Board Testing Committee
SDG	Sustainable Development Goal
SEM	Scanning electron microscopy
WIS	Water-insoluble solids
XPS	X-ray photoelectron spectroscopy

1. Introduction

A sustainable and growing bioeconomy strengthens the renewable segment of a circular economy (European Commission Directorate-General for Research and Innovation 2018) and is a potential step toward some of the Sustainable Development Goals (SDGs) formulated by the United Nations (United Nations 2015; Ferraz & Pyka 2023). The SDGs consist of 17 goals for economic, social, and environmental development, where the bioeconomy is traditionally regarded as a positive contributor to SDG 7, Affordable and Clean Energy; SDG 9, Industry, Innovation and Infrastructure; and SDG 12, Responsible Consumption and Production. The European Commission has adopted an updated bioeconomy strategy that considers the SDGs and involves all sectors that use and produce biological assets (European Commission Directorate-General for Research and Innovation 2018). The strategy, in general, targets a better utilization of currently employed as well as underused resources.

The forestry sector is a significant part of the bioeconomy in the Nordic countries, and paper, softwood-sawn wood, and wood pulp exports account for 16%, 16%, and 14%, respectively, of the world trade (Hannerz & Ekström 2023). Forest production in the boreal biogeographic region of Europe, to which the majority of Finland, Sweden, and part of Norway belong, is dominated by coniferous stands, mainly Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* L. Karst.) (Aldea *et al.* 2021). In Sweden alone, more than 90 million cubic meters of forest are harvested annually, of which more than half are spruce and a third are pine (Skogsstyrelsen 2024).

Around 10-15% of the standing volume consists of bark, which is separated from the logs at the sawmills and pulp industries. This by-product has few uses except as a solid fuel, either internally at the mill sites to

produce process heat or at external heating and power plants (Anerud *et al.* 2020). However, the heating demand is seasonal, and the bark is considered a low-value fuel due to its high moisture content (Kärkkäinen 1976), high ash content, and low net calorific value. The bark can thus be regarded as an underused resource.

Numerous research initiatives have investigated alternate applications for softwood bark, such as thermally upgraded pellets (Erixon & Björklund 2018), fermentable sugars (Normark *et al.* 2014), bioethanol (Kemppainen *et al.* 2012; Frankó 2018), composites (Ngueho Yemele *et al.* 2013), activated carbon (Siipola *et al.* 2020) and a variety of chemical applications for extractive fractions derived from the bark (Valentín *et al.* 2010; Le Normand *et al.* 2014; Jablonsky *et al.* 2017) to mention some. Nevertheless, more research is needed to unlock new value chains fully and position softwood bark as an appreciated resource in a circular bioeconomy.

1.1 Scope of the thesis

The scope of this thesis was delimited to the valorization of softwood bark via a steam explosion preprocessing step. Three sub-conditions further narrowed down the scope:

- The first targeted an improved sugar or bioethanol production via a carbocation scavenger pretreatment, further elaborated in Papers I and II.
- The second sub-goal aimed at producing a biobased carbon material with a high surface area. The carbon material is a higher-value end-product and its applicability as an adsorbent was determined in Paper III.
- The final sub-goal was to develop a rational method for monitoring or quantifying the by-products in the blow steam derived from the steam explosion process, further described in Paper IV.

2. Background

Biorefining could be defined as the sustainable processing of biomass, such as crops, grasses, marine biomass, and biomass residues, into food/feed ingredients, chemicals, materials, and bioenergy (IEA Bioenergy 2022). Lignocellulosic biomass, such as agro-residues, forestry waste, and energy crops, contains chiefly polysaccharides, lignin, and extractives, which could be converted into marketable products within a biorefinery. The literature lists more than 200 biochemicals that could be produced from lignocellulose (Chandel *et al.* 2018). One such product is second-generation bioethanol, and its production via steam explosion, enzymatic hydrolysis, and fermentation (Figure 1) has been frequently researched (Galbe & Zacchi 2012; Robak & Balcerek 2018). Although the technology has been demonstrated in several instances (IEA Bioenergy 2022), additional research is needed to ensure the sustainability and marketability of the product in bulk quantities.

Biobased carbon materials are other biorefinery products that have recently attracted much attention. Activated carbon can be used, for example, as adsorbents and in energy storage systems such as batteries and supercapacitors. Future research within the field may help replace fossil precursors such as coal and graphite and potentially reveal new and improved functionalities (Correa & Kruse 2018).

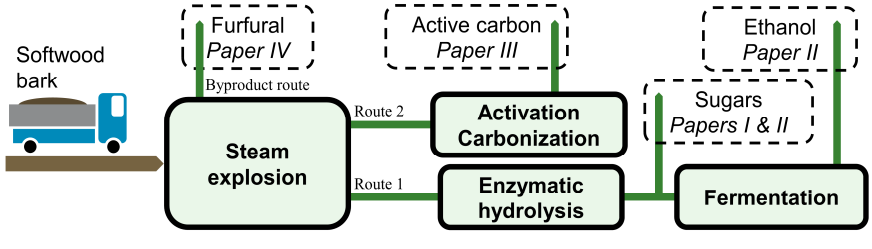

Figure 1. The biorefinery routes investigated in this work.

2.1 Softwood bark

The bark is the outer layer of the tree surrounding the stem and can be divided into a living inner bark (phloem) and dead outer bark (rhytidome). The inner bark contains cells for liquid transportation, nutrient storage and transportation, and mechanical support. In contrast, the outer bark protects the wood tissue from mechanical damage, humidity, and temperature variations (Sjöström 1993).

Bark has a complex chemical composition, containing constituents found in wood but also high contents of soluble constituents such as pectin, phenolic compounds, and suberin (Sjöström 1993). Compositions of Norway spruce bark (Table 1) and Scots pine bark (Table 2) from the literature reveal a total saccharide content of 34.9-45.5% (w/w). The glucan, in particular, can be present in cellulosic and non-cellulosic structures, such as hemicellulose, pectin, and starch. The total lignin content, summarizing acid-insoluble lignin (AIL) and acid-soluble lignin (ASL), is 26.9-44.9%, extractives 13.1-24.0%, and ash 0.9-4.6%. The analysis of both lignin and extractive contents is highly affected by how the analysis is conducted, which explains some of the variations in the reported data. Water-soluble extractives may contribute to the lignin content if not removed properly before AIL analysis (Torget *et al.* 1991), whereas the extractive content is affected by the solvent choices and number of extraction steps (Jablonsky *et al.* 2017).

Table 1. Composition of Norway spruce bark.

	Frankó *et al.* (2018)	**Kemppainen *et al.* (2014)**	**Miranda *et al.* (2012)**	**Neiva *et al.* (2018)**
Arabinan	4.0	4.5	4.2	4.6
Galactan	2.4	2.1	2.0	2.0
Glucan	25.2	27.7	28.2	29.5
Mannan	2.3	2.2	3.9	4.4
Xylan	3.8	3.5	3.9	5.0
AIL	24.4	33.5	26.8	26.1
ASL	5.5	3.3	1.1	0.8
Extractives	24.0	13.1	21.6	17.6
Ash	2.3	3.1	3.3	3.3

Table 2. Composition of Scots pine bark.

	Frankó *et al.* (2018)	**Valentín *et al.* (2010)**	**Miranda *et al.* (2012)**
Arabinan	4.1	4.2	4.9
Galactan	3.0	3.2	2.8
Glucan	20.0	28.8	22.7
Mannan	3.2	4.0	3.0
Xylan	4.6	3.5	3.8
AIL	36.9	44.9	32.9
ASL	4.0	<1.0	0.8
Extractives	19.4	19.3	18.8
Ash	0.9	-	4.6

2.1.1 Cellulose

Cellulose is a polysaccharide composed of cellobiose units linked together via β(1→4) glycosidic bonds. It is the most common polysaccharide found in nature and a vital contributor to the glucan content in softwood bark (Valentín *et al.* 2010; Kemppainen *et al.* 2014). Cellulose is unbranched, unsubstituted, and has a degree of polymerization (DP) in the 800-10000 range (Klemm *et al.* 2005). The molecules are linear and aggregated in sheets through strong hydrogen bonds stocked into fibrils. Native cellulose is found in two configurations, cellulose Iα or Iβ, depending on the stocking pattern. Cellulose Iβ is predominant in lignocellulosic materials (Poletto *et al.* 2013).

Cellulose has crystalline regions with a high packing density and non-crystalline regions. Cellulose in wood is highly crystalline, and for Norway spruce, crystallinity in the range of 51-71% has been reported (Andersson *et al.* 2003).

2.1.2 Hemicellulose

Hemicelluloses are branched heteropolysaccharides with a DP of up to 200. The primary hemicellulose in Scots pine and Norway spruce is galactoglucomannan built up by β-D-glucopyranose, β-D-mannopyranose, α-D-galactopyranose and acetyl, followed by arabinoglucuronoxylan, built up by β-D-xylopyranose, 4-O-methyl-α-D-glucuronic acid, and α-L-arabinofuranose (Sjöström 1993).

2.1.3 Lignin

Lignins are complex, highly branched macromolecules of phenylpropane units. The precursors (Figure 2) *p*-coumaryl, coniferyl, and sinapyl alcohols appear in lignin as p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) generic units, respectively. Functional groups such as phenolic hydroxyl, carbonyl, benzyl ether, and benzyl alcohol are integrated into the lignin structure (Sjöström 1993; Katahira *et al.* 2018). Softwood lignin contains mainly G lignin units, and literature reports an S/G ratio of 0.02 for Scots pine bark (Normark *et al.* 2014).

The lignin units are linked mainly via β-O-4, β-5, 5-5, 4-O-5, β-β and β-1 couplings (Ralph *et al.* 2004). In softwoods and hardwoods, 40-60% of the linkages are of β-aryl ether type (β-O-4). The exact size of the lignin molecule is difficult to estimate as it varies with the extraction method, but typical numbers reported for softwood MWL are in the range of 20600-23500 Da (Sjöström 1993; Katahira *et al.* 2018).

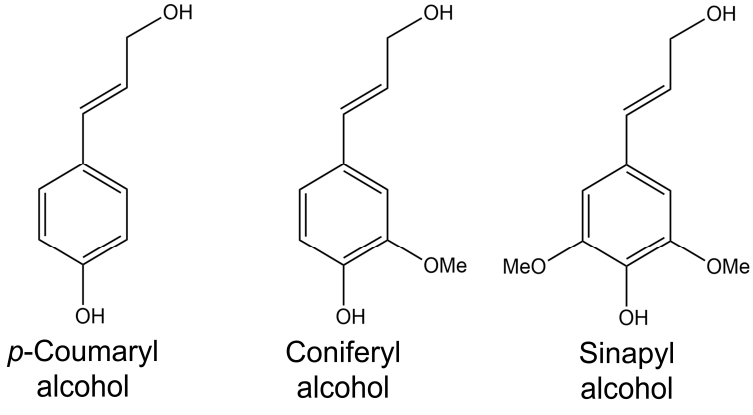

Figure 2. The lignin precursors *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol.

2.1.4 Extractives

Extractives are various compounds that can be removed with different solvents. Softwood bark distinguishes itself by having a high amount of lipophilic and hydrophilic extractives. Lipophilic extractives consist mainly of fats, waxes, terpenoids, and higher aliphatic alcohols. In contrast, hydrophilic extractives consist mainly of polyphenolic compounds such as

stilbenes and tannins, which are important for plant development and protection against damage and infection (Jablonsky *et al.* 2017).

Pectic saccharides can also be recovered with pressurized hot water extractions (Krogell *et al.* 2012). The bark of Norway spruce can contain high amounts of extractable pectin (up to 13%). Pectins are heterogenous polysaccharides composed of D-galacturonic acid, followed by D-galactose, L-arabinose, L-rhamnose, and smaller quantities of other monosaccharides. The molecules can be highly branched, with arabinan or arabinogalactan in the side chains (Le Normand *et al.* 2021).

2.2 Steam explosion

A pretreatment step is commonly employed within a biorefinery as a first step to fractionate lignocellulosic biomass for efficient processing into value-added products. Thus, pretreatment has become a term that includes physical, chemical, physio-chemical, biological methods, and even combinations thereof. Steam explosion is one of the most frequently investigated pretreatment methods (Galbe & Wallberg 2019). Its origin dates to the 1920s when the process was patented for fibreboard production (Mason 1926), and modified versions of the process have been in commercial use ever since.

The steam explosion process is conducted in an environment of saturated steam at temperatures around 170-210 ºC. The process can be divided into two stages: the reaction phase, where the material is subjected to temperature in the presence of subcritical water for a specific time, and the decompression phase, where the material is discharged from the reactor under explosive decompression (Ziegler-Devin *et al.* 2021). The amount of water required for the process is approximately 1.5 kg per kg of dry biomass (Hoang *et al.* 2023). However, dried biomass can be used for some applications (Erixon & Björklund 2018). The process can be operated in batch or continuous mode, where the latter may provide better effectiveness, accuracy, and reproducibility (Zimbardi *et al.* 1999; Hoang *et al.* 2023).

2.2.1 Steam explosion reactions

The predominant reaction during a steam explosion treatment is the degradation of hemicelluloses under the prevailing acidic conditions. The self-ionization of water initiates the reactions, leading to partial deacetylation and depolymerization of the hemicelluloses. The formation of acetic acid and

other degradation products, such as formic and levulinic acid, further lowers the pH and catalyzes the breakage of glycosidic bonds, causing an autohydrolysis of the hemicellulose (Garrote *et al.* 1999). Meanwhile, the process could be reinforced with a mineral or organic acid (Hoang *et al.* 2023), intensifying the hemicellulose degradation reactions, typically reducing the required temperature and time to reach a given hemicellulose depolymerization.

For the autocatalytic steam explosion process, without reinforcement with acid, the severity factor (Overend & Chornet 1987), which relates to carbohydrate conversion, has become an established measure of process intensity. The severity factor, S_0, is a function of process temperature, T (ºC), and residence time, t (min), and calculated according to:

$$S_0 = \log_{10} R_0 \qquad \text{(eq. 1)}$$

$$R_0 = t \times \exp[(T - 100)/14.75] \qquad \text{(eq. 2)}$$

The breakdown of hemicellulose yields water-soluble oligomeric and monomeric sugars, and the pentoses (e.g., arabinose and xylose) and hexoses (e.g., glucose, mannose, and galactose) can be further degraded into furfural and 5-HMF, respectively, as illustrated in Figure 3. As the reactions progress, other degradation products, such as formic and levulinic acid, pseudo-lignin, and humin, are formed (Rasmussen *et al.* 2014). Pseudo-lignin is a reaction product that behaves like lignin during Klason analysis but is derived from sources other than lignin (Sannigrahi *et al.* 2011). Carbohydrates and their degradation products (Aarum *et al.* 2019), as well as extractives (Torget *et al.* 1991), could be potential sources for pseudo-lignin formation.

Lignin is also affected by the process temperature, time, and acidity chiefly through cleavage of β-O-4 ether and other acid-labile linkages (Santos et al. 2013). The lignin degradation gives rise to numerous derivates of cinnamyl alcohols and condensed byproducts such as phenols, carboxylic acid, and oligolignols. Condensation reactions intensify with increased pretreatment severity (Hoang et al. 2023).

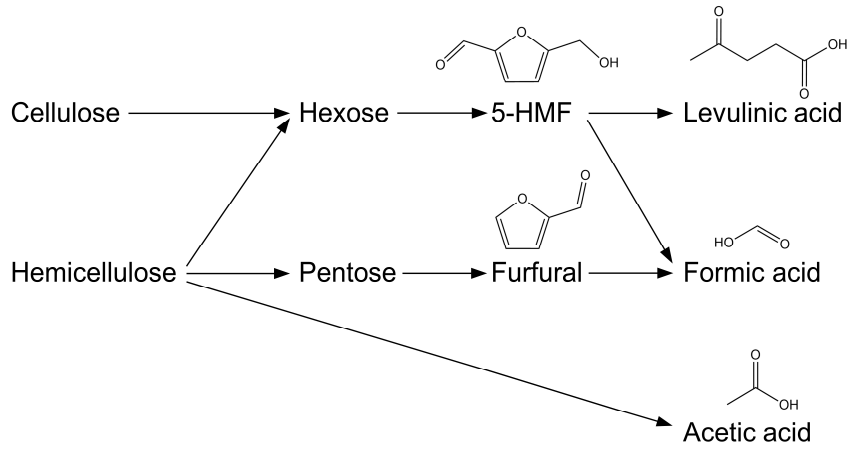

Figure 3. Common degradation pathways for cellulose and hemicellulose during acidic heat treatments (Jönsson *et al.* 2013).

2.2.2 Explosive decompression

The steam explosion process has a mechanical disintegrating effect on the biomass, which, together with the loud bang from instantly releasing the pressure from a batch system, has given rise to the name of the process. However, no actual explosion occurs in the physical sense (Galbe & Wallberg 2019).

During the decompression, pressure is released instantaneously, causing a flashing of water from the biomass interior and a dramatic volume expansion and acceleration of the steam, subjecting the biomass to shear and collisions. Together with the softening of lignin and chemical breakdown occurring during the reaction phase, the mechanical forces during decompression disintegrate the biomass structure (Muzamal *et al.* 2015).

The pressure-release rate is important for the degree of mechanical action during decompression. However, a continuously discharged process releases pressure in milliseconds, facilitating the best possible conditions for explosive decompression.

2.2.3 Furfural

Furfural is a by-product of the steam explosion process that could be sold as a biochemical if purified. The world production of furfural is around 270 kt/a, and it is used for the conversion into furfuryl alcohol (60-70%) or other furan derivatives, in the recovery of lubricants from cracked crude, in the

production of adhesives, and as a flavor compound. Furfuryl alcohol, in turn, is widely used in the chemical industry to produce resins, vitamin C, lubricants, dispersing agents, and food ingredients (Rosales-Calderon & Arantes 2019).

Current furfural production is biobased, primarily using sugarcane bagasse or corncobs as feedstock. The conversion follows reaction pathways like those of the steam explosion process. However, cellulose and lignin are typically only utilized for heat and energy production, the yield from pentoses is below 50%, and the process generates plenty of effluent and is expensive to operate (Rosales-Calderon & Arantes 2019). On the contrary, by utilizing steam explosion technology, furfural may form an additional value stream for a biofuel plant or a biorefinery (Moe *et al.* 2022), supporting the efficient use of biomass.

2.3 Second-generation bioethanol

Second-generation bioethanol is ethanol from non-edible sources such as agricultural and forestry byproducts. The production process typically contains four key steps. Initially, the lignocellulosic material is pretreated, for example, in a steam explosion process, to yield a substrate accessible to enzymes. The second step is enzymatic hydrolysis to convert polysaccharides to fermentable monosaccharides. Finally, the sugars are fermented into ethanol, which is further concentrated and purified (Toor *et al.* 2020).

2.3.1 Enzymatic hydrolysis

Enzymatic hydrolysis utilizes cellulases and potentially other supporting enzymes to digest poly- and oligosaccharides into monomeric sugars in a process operating around pH 5 and at 45-50 ºC (Sun & Cheng 2002). The discovery of cellulolytic enzymes dates back to the Second World War when the fungus responsible for the deterioration of cotton tents and clothing in the South Pacific was isolated by American Scientists. The discovered fungus, *Trichoderma reesei* (today classified as *Hypocrea jecorina*), is now used in the industry to produce commercial enzymes (Sheehan & Himmel 1999).

H. jecorina produces a combination of the cellulases endoglucanases and exoglucanases (cellobiohydrolases), together with β-glucosidases, that

synergistically catalyze cellulose degradation. The endoglucanases act on low-crystallinity regions of the cellulose to cut the cellulose chain, while the endoglucanases remove cellobiose from the chain ends. Finally, the β-glucosidases hydrolyze the cellobiose to glucose (Sun & Cheng 2002).

Cellulases are product-inhibited (Teugjas & Väljamäe 2013), and the amounts of β-glucosidases are limited in enzyme preparations produced from *H. jecorina*. Therefore, modern enzyme cocktails contain a supplemented excess of β-glucosidases (Lynd *et al.* 2002) and other supporting enzymes that help break the lignocellulosic matrix. Xylanases are accessory enzymes that may work synergistically with the cellulases by improving cellulose accessibility and reducing inhibition from xylo-oligomers (Hu *et al.* 2013). Another example is lytic polysaccharide monooxygenases, which are non-hydrolytic enzymes present in modern enzyme cocktails. These can catalyze the oxidative cleavage of polysaccharides (Vaaje-Kolstad *et al.* 2010).

Pretreatment of the lignocellulosic material is essential for attaining high conversions during enzymatic hydrolysis. The pretreatment aims to make the substrates accessible to the hydrolytic enzymes. At the same time, the formation of inhibiting compounds must be kept at tolerable levels (Galbe & Wallberg 2019). Steam explosion, in particular, opens up the biomass structure due to the degradation and solubilization of hemicelluloses. The mechanical effects, in addition, are important for the digestibility of softwood substrates both at high and low severities (Jedvert *et al.* 2012). On the other hand, steam explosion may increase the non-productive binding of cellulases onto lignin-rich residues, decreasing enzyme efficiency (Rahikainen *et al.* 2013). In addition, solubilized aromatic and phenolic reaction products may inhibit enzyme activity (Jönsson & Martín 2016), highlighting the importance of selecting appropriate steam explosion conditions.

2.3.2 Carbocation scavengers

Carbocation scavengers are additives that could be used in the steam explosion process to mitigate lignin repolymerization and improve enzyme accessibility of the derived substrates. The scavengers are nucleophilic compounds like naphthol derivatives (2-naphthol, 2-naphthol-7-sulfonate, 6-hydroxy-2-naphthoic acid, 2-hydroxy-1-naphthoic acid), phenolic acids and mannitol (Liu *et al.* 2024).

2-naphthol as a scavenger has been extensively researched. Under acidic conditions, carbocations (carbonium ions) are formed from benzyl alcohol structures in lignin (Li *et al.* 2007). These may cause cleavage of the β-O-4 linked structures, lignin fragmentation, or further repolymerization (Figure 4) through the reaction with adjacent lignin structures during the formation of C-C bonds (Robert *et al.* 1988). The carbocation scavengers react with and stabilize the carbocations, thus prohibiting further repolymerization reactions (Wayman & Lora 1978).

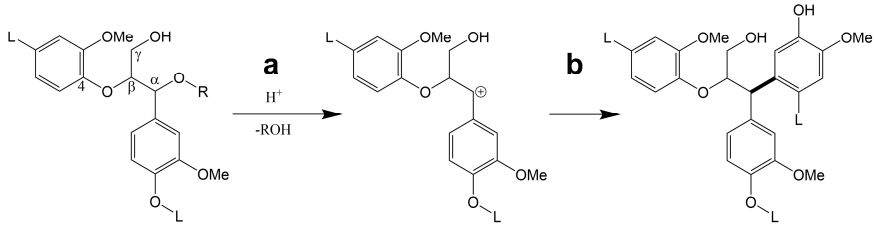

Figure 4. The formation of a carbocation from a β-O-4 linked lignin structure (a) followed by a repolymerization reaction with an adjacent lignin unit forming C-C bonds (b). Adapted from Li *et al.* (2007).

Adding 2-naphthol to autocatalytic or dilute acid pretreatments may increase biomass porosity, decrease lignin surface coverage (Chu *et al.* 2019), reduce inhibitory phenolics (Chu *et al.* 2021), and decrease the lignin surface area (Pielhop *et al.* 2015). It may also stimulate the activity of lytic polysaccharide monooxygenases (Hansen *et al.* 2022). As a result, scavenger pretreatments display a drastically elevated enzymatic digestibility of resulting substrates. For softwoods, such as Scots pine and Norway spruce, the positive effects of the scavenger addition to autocatalytic treatments are especially pronounced at severities above 4.5 (Pielhop *et al.* 2017; Borrega *et al.* 2021; Hansen *et al.* 2022). For dilute acid pretreatments with sulfuric acid, the scavenger's enhancing effect on the enzymatic digestibility of spruce is detectable in a wide range of severities (Pielhop *et al.* 2017). Unfortunately, 2-naphthol is proven toxic to ethanol-producing organisms such as baker's yeast, which limits its potential use in bioethanol processes (Pielhop 2015; Seidel *et al.* 2019).

2.3.3 Fermentation

Fermentation of monosaccharides into ethanol is an ancient technique adopted by humanity thousands of years ago (Liu *et al.* 2018). Still, producing second-generation bioethanol from lignocellulosic hydrolysates poses challenges to ethanol-producing organisms. Baker's yeast, *Saccharomyces cerevisiae*, is the most commonly used organism for fermenting hexoses (e.g., glucose, mannose, and galactose), working optimally at pH 4-5 and 30 ºC temperature. The baker's yeast provides high yields and productivity and has a remarkable ethanol tolerance, developed through millennia of adaptation. However, lignocellulosic hydrolysates contain various other compounds inhibiting microorganisms (Olsson & Hahn-Hägerdal 1996).

Hydrolyzates contain degradation products formed during pretreatment. Carbohydrate degradation creates aliphatic carboxylic acids (mainly acetic, formic, and levulinic acid) and furan aldehydes (mostly furfural and 5-HMF). These are mildly toxic to fermenting organisms but could occur in high concentrations. On the contrary, degradation products from lignin, such as aromatic carboxylic acids and aldehydes, are present at a much lower concentration but possess a potent inhibitory effect (Jönsson & Martín 2016).

To achieve an economically viable ethanol distillation, the fermented broths should contain at least 4% ethanol w/w (da Silva *et al.* 2020). To reach this, 80-100 g/l of fermentable sugars should be present initially, meaning enzymatic hydrolysis should be conducted at around 15% solids w/w or higher. High solid loadings, however, cause impaired mixing during the initial stages of the enzymatic hydrolysis and a concentration of inhibitors, both in the enzymatic and fermentation stages. Thus, conducting investigations at relevant process conditions is essential for proper evaluations of process modifications.

2.3.4 Ethanol from softwood bark

Ethanol production from spruce and pine bark through acidic pretreatment processes, enzymatic hydrolysis, and fermentation is difficult. Although fermentability is not impaired compared to studies of corresponding wood materials (Frankó 2018), the enzymatic hydrolysis yields are lower (Yamamoto *et al.* 2014; Frankó 2018). To some extent, the resistance to enzymatic degradation can be attributed to condensation reactions involving water-soluble polyphenolic extractives found in softwood bark. Removing

these extractives before pretreatment could elevate the enzymatic hydrolysis yields (Kemppainen *et al.* 2012; Frankó 2018). However, additional measures are needed to reach close to full conversion of softwood bark.

2.4 Biobased carbon materials for adsorption

Industrial, agricultural, and domestic activities lead to the pollution of water and environmental ecosystems. Nitrates, dyes, PAHs, PCBs, dioxins, heavy metals, pharmaceutical products, and pesticides are all problematic pollutants, harmful to ecosystems and, in many cases, ultimately, human health. Dyes can reduce photosynthesis in aquatic environments, and some may be carcinogenic and toxic to humans (Vievard *et al.* 2023).

Adsorption is one promising technique for wastewater purification, and biobased activated carbons are potential adsorbents that can be used instead of fossil-based carbon materials. Their porous features and surface functionalities characterize activated carbons. Biobased activated carbons are often meso- and microporous, meaning they have pores with a width of 2-50 nm and <2 nm. The kinetics of adsorption onto heterogeneous solid surfaces is governed by external diffusion in the bulk phase, internal diffusion inside the pores, surface diffusion, and the elementary process of adsorption/desorption (Dąbrowski 2001).

2.4.1 Production of biobased carbon materials

Activated carbon can be produced from biomass through carbonization and activation. The carbonization may, for example, be conducted by pyrolysis at temperatures between 300-900 ºC. The activation step may be chemical or physical. Chemical activation can be performed before or after carbonization using for example, iron chloride, potassium hydroxide, phosphoric acid, sulfuric acid, or zinc chloride. On the other hand, physical activation is performed on already carbonized materials at high temperatures with an activation agent such as steam or carbon dioxide (Vievard *et al.* 2023).

3. Materials and methods

The raw material for the research presented in Papers I-IV was a softwood bark of industrial source, containing predominantly Norway spruce (*Picea abies* Karst. L.) and Scots pine (*Pinus Sylvestris* L.). Utilized processing methods, characterization methods, and statistical and modeling tools are presented in Table 3-5.

Table 3. Overview of the utilized processing methods.

Papers:	**I**	**II**	**III**	**IV**
Raw material preprocessing	X	X	X	X
Steam explosion	X	X	X	X
Enzymatic hydrolysis	X	X		
Fermentation		X		
Activation and pyrolysis			X	

Table 4. Overview of the utilized characterization methods and techniques.

Papers:	**I**	**II**	**III**	**IV**
General biomass properties and composition	X	X	X	X
HPEAC-PAD	X	X	X	
Qualitative and quantitative extraction	X	X		
FTIR spectroscopy		X		
NMR spectroscopy		X		
HPLC		X		X
BET specific surface area			X	
Dye adsorption testing			X	
Raman spectroscopy			X	X
XPS			X	

Table 5. Overview of the utilized statistical and computational methods.

Paper:	**I**	**II**	**III**	**IV**
DoE	X			
PCA-X	X			X
Adsorption isotherm and kinetic models			X	
OPLS				X

3.1 Raw material

Two different deliveries (February 2019 and March 2023) of softwood bark from a pulp mill in the northeastern part of Sweden were used for the work presented herein. The pulp mill's intake of wood consisted chiefly of a mixture of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* Karst. L.), with smaller fractions of Contorta pine (*Pinus contorta* Bol.) and reject wood in the annual mix. Sampling was conducted to avoid the Contorta and reject wood to the largest possible extent.

The mill's debarking station consisted of a defrosting unit and a debarking drum, where the bark was separated from the logs. The bark fraction was then coarsely shredded and dewatered in a bark press, from which the samples were obtained. The collected bark samples had an approximate volume of 50 m^3 each, a total solids content close to 40%, and a stemwood content near 15%.

3.2 Preprocessing and steam explosion

Papers I-IV utilized raw material preprocessing and steam explosion as processing steps. The flowsheet in Figure 5 displays the interlinkage between occasions for sampling, preprocessing, steam explosion trials, and Papers I-IV.

The raw materials were processed at BTC in Umeå to prepare the coarsely shredded bark for the steam explosion pilot. One dried and one undried (as received) assortment of preprocessed material was prepared by shredding the bark in a single shaft shredder (Lindner Micromat 2000, Lindner-Recyclingtech GmBh, Spittal, Austria) followed by a screening of oversized material (Mogensen G-Sizer, Mogensen, Hjo, Sweden). The shredder had a screen size with 30 mm diameter holes, while the screen was equipped with a mesh wire having 14 mm openings. Paper II and III utilized the feedstock

without pre-drying, whereas Paper IV utilized a predried feedstock, and Paper I a combination of each for the steam explosion. Drying was conducted in a flatbed dryer by blowing 40 ºC air through the material bed. As a final preprocessing step, high-density particles such as stones and gravel were removed from the bark in an in-house constructed junk trap mounted to a pneumatic conveying line. The removal was necessary to ensure the stable operation of the continuous steam explosion pilot.

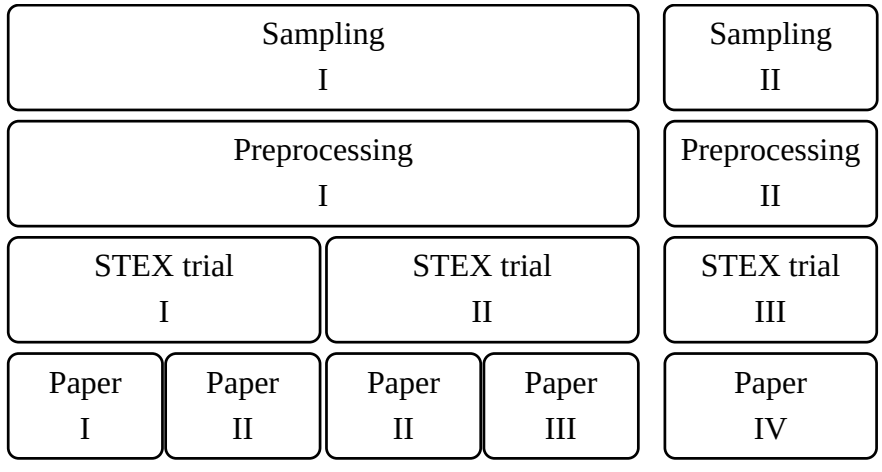

Figure 5. Flowsheet of bark collection, preprocessing, and steam explosion (STEX) trials conducted for Papers I-IV.

Steam explosion treatments were conducted at Valmet FTC in Sundsvall utilizing a pilot scale system for continuous steam explosion (BioTrac, Valmet AB, Sundsvall, Sweden). The system consisted of a raw material buffer bin, conveyors, plug screw, reactor (Figure 6), blow line, and discharge cyclone. In addition, auxiliary systems provided the pilot with saturated steam, compressed air, chemicals, sealing water, and cooling water for condensate sampling.

The pilot was operated at a feed rate of 60 kg/h (dry biomass basis) by intermittently refilling the buffer bin with bark to keep the level at least 50% filled with material. A bottom scraper and a screw at the outlet of the bin controlled the feed rate of material going to the reactor. Calibration of the screw speed to achieve desired mass flow had been undertaken before the trials. Conveyor belts and screws were used for the transportation of biomass between the buffer bin and the reactor. One of these conveyor screws was also used as a dosage point for chemicals. In Papers I and II, an addition of

2-naphthol and acetic acid was investigated. The 2-naphthol was provided as a ground powder (<2 mm) from a gravimetric feeder (K-Tron Loss-in-Weight Feeder, K-ML-SFS24-KT20, K-Tron GmbH, Switzerland), while the acid was supplied as a dilute solution from a positive displacement pump operating under flow control. In Papers III and IV, dilute sulfuric acid was dosed to the system according to the same principle as the acetic acid.

A plug screw feeder was used for injecting the reactor with biomass. The plug screw compressed the bark to a dense plug that worked as a pressure seal, and a conical plug breaker inside the reactor disintegrated the plug into a loosely packed material once more. The reactor was of a horizontal tube reactor type, and the crucial parameters, residence time and temperature, were accurately controlled. An auger conveyor transported the bark through the reactor, and the average residence time (t_A) was set by controlling the rotational speed of the screw. The relationship for ideal residence time (τ_{ideal}) in screw conveyors (Chamberlin *et al.* 2018)

$$\tau_{ideal} = L/(p \times \omega_{sc}) \qquad \text{(eq. 3)}$$

and the conveying efficiency (η)

$$\eta = (t_A/\tau_{ideal}) \qquad \text{(eq. 4)}$$

was combined,

$$t_A = L/(\eta \times p \times \omega_{sc}) \qquad \text{(eq. 5)}$$

to derive the expression for average residence time in the reactor. The reactor characteristics, L, which denotes the section length between the reactor inlet and outlet centreline (Figure 6), and p, which is the screw pitch, were derived from manufacturer drawings. At the same time, tracer pulse experiments had previously been performed to determine conveying efficiency, η (not part of this work).

The reactor temperature was set by setting the pressure in the vessel. The pressure could be achieved by pressure-controlling the injection of saturated steam into the reactor through the two steam inlets at the reactor top and side. A preset flow ratio controlled the division of steam flow through the two inlets.

Finally, material and steam were discharged from the reactor through a pressure-reducing nozzle (8 mm diameter) that accelerated the steam and biomass through a blow line and to a blow cyclone for steam separation. Steam-exploded material was collected directly from the cyclone's material outlet. The blow steam was also sampled in Paper IV by collecting a partial flow from the cyclone's steam outlet. Assisted by vacuum, the partial gas flow was led through a Teflon hose and three 30 cm glass condensers from which a gas condensate with a temperature < 15 ºC was collected at a flow rate of 2-3 kg/h.

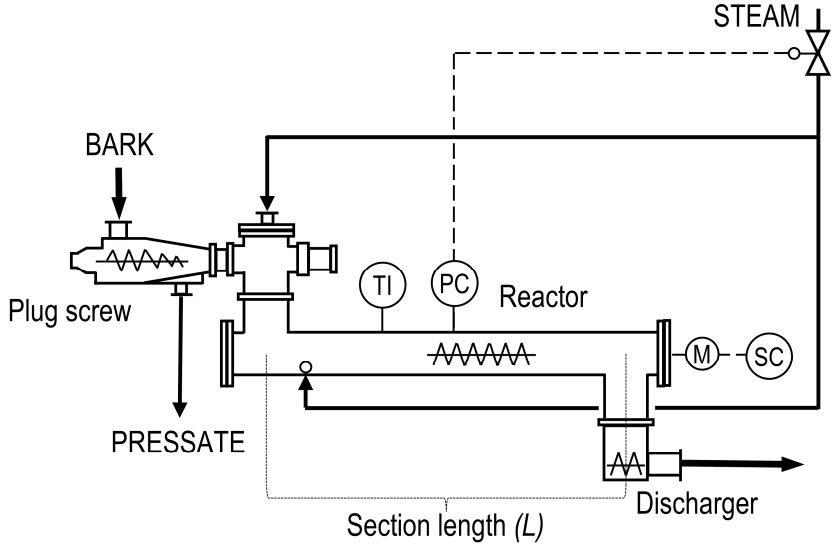

Figure 6. Schematic illustration of the steam explosion reactor, with temperature indication (TI), pressure-controlled steam supply (PC), and rector screw motor (M) with speed control (SC).

3.3 General biomass properties and composition

The standardized methods for the basic characterization of raw material and steam-exploded materials utilized in Papers I-IV are presented in Table 6. ISO standards were selected where applicable. However, the laboratory analytical procedures issued by NREL were, in some cases, better adapted for the analysis of pretreated materials and thus chosen for some analyses.

Table 6. Standardized methods for determination of general biomass properties.

Measurement	Method	Reference
Bulk density	ISO 17828:2015	ISO (2015b)
Particle size distribution	ISO 17827-1:2016	ISO (2016)
Total solids	NREL/TP-510-42621	Sluiter *et al.* (2008a)
Water-insoluble solids	NREL/TP-510-42627	Sluiter *et al.* (2008c)

Table 7 presents the standardized methods used for compositional analyses of raw and pretreated materials in Papers I-III. In Papers I and III, acetone extraction instead of the two-stage water-ethanol extraction proposed by NREL were utilized for the raw material, and the acid-insoluble lignin was not corrected for ash content due to practical reasons. On the contrary, this was done in Paper II entirely according to NREL standards. The ISO standard was preferred before the NREL standard for determining acid-soluble lignin since carbohydrate degradation products may affect UV-vis adsorption at 240 nm (NREL) (Korpinen *et al.* 2014).

Table 7. Standardized methods for compositional analysis.

Measurement	Method	Reference
Acetone-soluble matter	SCAN-CM 49:03	SCAN (2003)
Acid-insoluble lignin	NREL/TP-510-42618	Sluiter *et al.* (2008b)
Acid-soluble lignin	ISO 21436:2020	ISO (2020)
Ash content	ISO 18122:2015	ISO (2015a)
Sample preparation	NREL/TP-510-42620	Hames *et al.* (2008)
Structural carbohydrates	NREL/TP-510-42618	Sluiter *et al.* (2008b)

3.4 Enzymatic hydrolysis and fermentation

Enzymatic hydrolysis was conducted in Papers I and II to evaluate the pretreatment with and without 2-naphthol. In Paper I, the enzymatic hydrolysis was performed as 20 g batches in rotating, 50 ml Falcon tubes. Firstly, a premix was prepared by mixing the steam-exploded material in a kitchen mixer (Bosch MUM8) with milli-Q water to a solid content of ~17-20%, whereafter the pH was adjusted to 5.2-5.3 with dilute KOH.

Premix, citric acid/citrate buffer, milli-Q water, and lastly, enzyme (Novozymes Cellic CTec3 HS, Novozymes, Bagsværd, Denmark) was mixed in a 50 ml Falcon tube to reach a total solid content of 12%, buffer

concentration of 57 mM and the targeted enzyme dose (2-10% on substrate total solids). The final mixing was conducted in a paint shaker (Fast & Fluid SO400, IDEX Corporation, IL, USA), whereafter the prepared tubes were inserted into a rotisserie mixer (Intelli-Mixer RM-2, ELMI) in an incubation oven (Incucell, MMM Group) and kept rotating at 25 rpm and a temperature of 50 ºC for the desired hydrolysis time.

In Paper II, the hydrolysis was conducted as shaking flask experiments in batches of 75 g in an incubation shaker (Infors Ecotron, Infors, Switzerland). Instead of the paint shaker, shaking was set at 250 rpm for the first 4 h and then 150 rpm for the remaining hydrolysis time. Apart from this, the same hydrolysis conditions were applied in Papers I and II.

Sampling was conducted by taking a part of the hydrolysis slurry and diluting it 8-10 times with milli-Q water. The diluted sample was centrifuged, and the supernatant was filtered through a syringe filter and stored frozen for further analysis. Sampling was conducted at the end of the hydrolysis in Paper I and II and on several occasions during the hydrolysis in Paper II. In this case, a minimal sample of 0.5 g was taken from the flasks to avoid shifting the composition of the remaining slurry.

A separate hydrolysis and fermentation with intermediate lignin removal was conducted in Paper II. The remaining hydrolysis slurry was centrifuged, and the supernatant was recovered for the subsequent fermentation tests. The solutions were supplemented with a nutrient solution, the pH was adjusted to 5.5, and rehydrated *Saccharomyces Cerevisiae*, Ethanol Red (Fermentis, Marcq en Baroeul, France), was provided to reach an initial cell concentration of 2 g/L. The fermentations were conducted in a heating chamber at 30 ºC in 30 mL glass flasks equipped with magnetic stirrers and sealed with rubber caps pierced with cannulas to evacuate CO_2. Samples were taken at 0, 4, 24, and 48 h and were frozen if further analysis could not be immediately undertaken.

3.5 Production of activated carbons and dye adsorption testing

Activation and pyrolysis were conducted to produce an activated carbon by chemical activation with $ZnCl_2$ or KOH at a chemical-to-biomass ratio of 1:1 or 2:1 (w/w). The pyrolysis temperature was 600 or 800 ºC. Paper III reports the activation with $ZnCl_2$ at a ratio of 1:1, and the principle for activation,

pyrolysis, and washing of the carbons is described therein. However, washing the materials treated with KOH was undertaken with 0.01 M HCl instead of 6 M.

The carbons were evaluated as adsorbents of the azo dye RO16 using a batch adsorption procedure in Paper III. The kinetics of adsorption, adsorption equilibria, and desorption characteristics were assessed. In addition, the chromophore removals from two synthetic dyehouse effluents were tested. Paper III detailly describes the conditions for testing.

3.6 Measurement techniques and instruments

Chromatography (HPAEC-PAD and HPLC) was an essential technique in this work for quantifying carbohydrates and degradation products formed during the steam explosion treatment. The list of chromatography instruments is presented in Table 8. In Papers I-IV, ion chromatography was used to determine structural carbohydrates in the raw and steam-exploded materials. Furthermore, ion chromatography was used to determine carbohydrate concentrations after enzymatic hydrolysis in Papers I and II and after fermentation in Paper II. HPLC was used for the quantification of 5-HMF and furfural (Papers II and IV), ethanol (Paper II), methanol (Paper IV), 5-methylfurfural (Paper IV), and organic acids (Paper IV).

Various spectroscopic techniques were used for qualitative and quantitative analysis of the steam-exploded materials and activated carbons, and the list of applied instruments is found in Table 8. UV-visible spectroscopy was used for the quantification of acid-soluble lignin content in Papers I and III, whereas it was used for the determination of total aromatic and phenolic content in Paper II. Furthermore, UV-vis was applied to measure the RO16 concentration in Paper III.

FTIR spectroscopy was used in both Paper II and III to identify functional groups in steam-exploded and carbon materials. DRIFTS was applied in Paper II on steam-exploded materials, while ATR-FTIR was used on the carbon materials in Paper III. These carbon materials were further analyzed with XPS to determine the functionalities more clearly, and BET and SEM were used to assess surface morphological features. In addition, Raman spectroscopy was used in Paper III to assess the degree of graphitization for the carbon materials. Raman was also an essential technique in Paper IV,

where models for degradation product concentrations in blow steam condensates from the steam explosion process were based on Raman spectra.

In Paper II, steam-exploded biomass was extracted with cyclohexane/acetone (9:1 v/v) to investigate lignin behavior after pretreatment with 2-naphthol. The extracts were subjected to ^{1}H NMR spectroscopy analysis, while the non-extractable solid residue was analyzed with ^{13}C CP-MAS NMR spectroscopy.

Table 8. List of applied instruments.

Technique	Instrument	Papers
1H NMR	Bruker Avance III HD 600 MHz	II
13C CP-MAS NMR	Bruker Avance III HD 500 MHz	II
ATR-FTIR	Thermo Scientific Nicolet iS5	III
BET surface area	Micrometrics Tristar	III
DRIFTS	Bruker IFS 66v/S	II
Extraction	Soxtec Avanti 2050	I, III
Extraction	Dionex ASE 350	II
HPEAC-PAD	Dionex ICS-3000/CarboPac PA1	I, III
HPEAC-PAD	Dionex ICS-6000/CarboPac PA1	II, IV
HPEAC-PAD	Dionex ICS-6000/SA-10	II
HPLC	Dionex UltiMate 3000/Zorbax RRHT SB-C18	II
HPLC	Agilent 1260 Infinity/Aminex HPX-87H	II
HPLC	Dionex UltiMate 3000/Aminex HPX-87H	IV
Raman	Bruker BRAVO	III, IV
SEM-EDX	Carl Zeiss Evo	III
UV-vis	Agilent Cary UV-100	I, III
UV-vis	BioTek Epoch 2	II
UV-vis	Shimadzu UV-1800	III
XPS	Thermo Scientific ESCALAB 250Xi	III

3.7 Statistics and modeling

Principal component analysis (PCA) was conducted on all complex datasets, such as pilot data (Papers I-IV) and the large set of laboratory data in Papers I and IV. The PCA was done to identify outliers and generate an overview of the data, and it was performed in the SIMCA 17 (Sartorius AG) software. However, basic statistics, such as t-tests, were sufficient to evaluate the lab

data in Paper II. In addition, SIMCA was used to derive calibration models through OPLS modeling of Raman spectra in Paper IV.

In planning the first pilot trial (Papers I and II), DoE was applied. MLR was utilized to evaluate the effect of 2-naphthol and acetic acid additions to the steam explosion process (Paper I). The MODDE Pro 13 software package (Sartorius AG) was used, and the modeling is further described in Paper I.

In handling FTIR and Raman spectra (Papers II-IV), MATLAB R2021b (MathWorks) was the primary tool operated with the assistance of the graphical user interface available from the Vibrational Spectroscopy Core Facility at Umeå University (Gorzsás 2018). The interface had features such as trimming, baseline correction through the asymmetrical least squares method (Eilers 2004), area normalization, and curve smoothening (Savitzky & Golay 1964). Furthermore, peak separation was performed in Paper III to determine peak areas from Raman spectra of activated carbons. The peak areas were used to calculate the degree of graphitization, and the peak separation and area calculation were conducted using the Origin Pro 2020b software (OriginLab).

In Paper III, the adsorption of RO16 onto biobased activated carbons was modeled using conventional models for kinetics (Lagergren 1898; Ritchie 1977; Ho & McKay 1999; Wu *et al.* 2009) and equilibria (Freundlich 1906; Langmuir 1918; Sips 2004). The models were fit to the experimental data through least squares curve fitting using the Levenberg-Marquart algorithm (Levenberg 1944) in the Origin Pro 2020b software (OriginLab).

4. Results and discussion

This section is divided between the potential products investigated: the sugar platform/bioethanol, the activated carbon, and the byproduct furfural, including the methodology for monitoring the degradation products in the steam explosion blow steam. The shredded and screened bark used for investigations in Papers I-III had a bulk density of 150 kg/m^3 and size distribution of 25% < 3 mm, 42% 3-8 mm, 28% 8-16 mm, and 5% > 16 mm. Physical characterization of the bark was not conducted for Paper IV, but preprocessing conditions were the same as in Papers I-III.

4.1 Fermentable sugars and bioethanol from softwood bark

Softwood bark was steam exploded under different autocatalytic conditions. Reinforced autohydrolysis with acetic acid or dilute acid hydrolysis with sulphuric acid was also tested. Most trial conditions were repeated with and without a dosage of the carbocation scavenger 2-naphthol applying design of experiments (DoE). The resulting substrates were evaluated in an enzymatic hydrolysis stage for their conversion into monomeric sugars (Paper I), whereas a deeper investigation tested the fermentability into ethanol (Paper II).

4.1.1 Enzymatic hydrolysis of pretreated substrates

Enzymatic hydrolysis of the pretreated substrates revealed a maximum glucose conversion of 0.57 at the highest enzyme dosages (10% w/w on substrate total solids). This conversion corresponded to a final glucose concentration of 23.3 g/l at an initial solid loading of 12% in the enzymatic hydrolysis stage (~10% WIS). In addition, the hexoses, mannose, and

galactose had a total concentration of 4.1 g/l in the hydrolysate. The best conversion was achieved at 205 ºC, 10 min residence time, and autocatalytic pretreatment conditions.

Autohydrolysis at different temperatures revealed that the difference in glucose recovery between the substrates pretreated at different severities was minor at an enzyme charge of 4% w/w. At enzyme dosages below 4% w/w, glucose concentrations were higher for the substrate pretreated at 190 ºC than 200 ºC (Figure 7). On the contrary, higher enzyme dosages were beneficial at higher severity pretreatments, indicating that adding more enzymes could overcome some inhibition of the cellulases. Magnification of the inhibitory effects with increasing severity could be due to condensation reactions, as well as increased lignin and carbohydrate degradation products in the liquid phase.

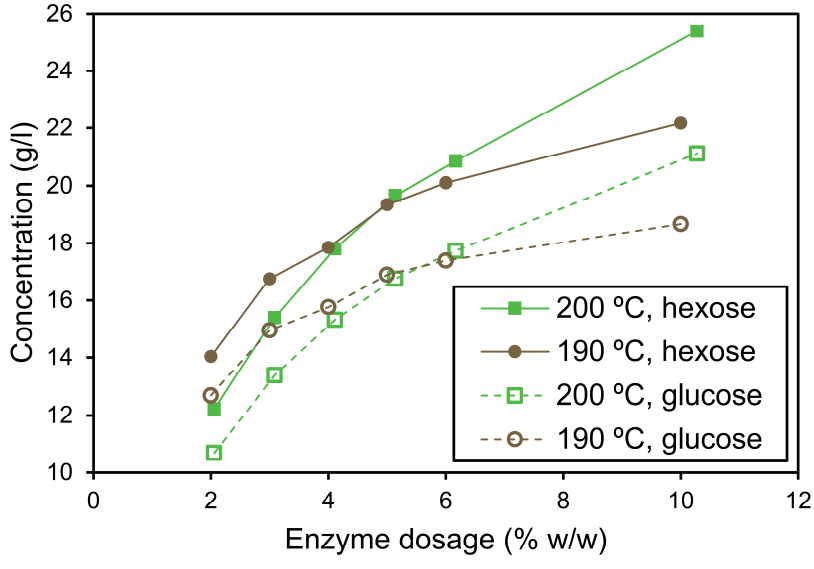

Figure 7. Concentrations of glucose and total hexose (glucose, mannose, and galactose) in hydrolysates versus enzyme dosage expressed in % w/w on substrate total solids. The substrates were unwashed, and pretreatment was conducted at 10 min residence time, 190 and 200 ºC.

As elaborated in Paper I, adding additional acetic acid to the process had minimal impact on the hydrolysis yields. Meanwhile, dilute acid hydrolysis provided substrates that performed somewhat better at the highest enzyme

dosage than the autocatalytically pretreated materials regarding summarized glucose recovery from pretreatment and enzymatic hydrolysis. However, at enzyme dosages of 4% w/w and below, the sulfuric acid treatment was detrimental to the glucose yield, analogous to the findings by Kemppainen (2012). On the other hand, Frankó (2018) stated that gas impregnation with SO_2 positively impacted cellulose's digestibility, attributed to a higher degree of hemicellulose removal and increased enzyme availability. However, SO_2 also delignifies and solubilizes lignin to a larger extent than H_2SO_4 (Wang *et al.* 2018), which cannot be ruled out as a potential cause for the positive effects on the enzymatic digestibility observed by Frankó (2018).

The glucose yields are in the same range as those found in similar studies (Kemppainen *et al.* 2012; Frankó 2018), which confirms that hydrolyzing softwood bark with cellulose-degrading enzymes is problematic.

4.1.2 Ethanol recovery

Fermentation of the hydrolyzates with *S. cerevisiae* (Paper II) led to a hexose yield of 0.45 g/g out of the theoretical 0.51 g/g. Glucose, mannose, and galactose were wholly consumed, indicating that inhibitory effects were minor and could be overcome. The pretreatment was conducted at 200 ºC and 10 min, which can be considered mild conditions for softwood pretreatment, which limits the amount of inhibitors derived from lignin and hemicellulose in the hydrolysate. On the other hand, other inhibitors characteristic of bark, such as dissolved uncondensed tannins, may lead to more pronounced toxicity at low severities (Boussaid *et al.* 2001).

Even though inhibitory effects could be managed, and treatments were conducted at 12% solids loading, the final ethanol concentration was slightly below 10 g/l. Further improvement of the fermentation to maximize yield, such as detoxification or introducing yeast with a xylose-to-ethanol fermenting pathway, would potentially increase this concentration by 2-3 g/l. However, any target of obtaining an ethanol concentration of 40 g/kg to render the process economically feasible (da Silva *et al.* 2020) was far from achieved. Further improvements in the pretreatment and enzymatic hydrolysis would be needed to increase the ethanol concentration substantially. A complete enzymatic conversion of the hexoses in the pretreated materials at a total solid content of 16-17% in the enzymatic stage would be required to attain 40 g/kg ethanol concentration, assuming the ethanol yield could be sustained at the higher solids loading.

4.1.3 Effects of scavenger pretreatment

Scavenger pretreatments of softwood bark were conducted at autocatalytic conditions (severity factor 3.36-3.94). The relatively mild severity conditions were chosen based on the works by Kemppainen *et al.* (2012). Furthermore, initial screenings indicated extensive carbohydrate degradation into furfural at higher severities.

The carbocation scavenger, 2-naphthol, did not elevate the enzymatic digestibility at low or high enzyme dosages for autohydrolyzed substrates (Paper I). The pretreated material displayed a higher AIL and ASL content (on average 1.4 and 0.2% w/w) than references without 2-naphthol, which could indicate the inclusion of the scavenger with the lignin and a stabilization of solubilized lignin. Desired reactions between lignin and the scavenger may have occurred, but the severity was still too low to unlock potential benefits regarding enzymatic digestibility. Several authors revealed a positive effect of the scavenger pretreatment first at severities higher than 4.5 for pine and spruce wood (Pielhop *et al.* 2017; Borrega *et al.* 2021; Hansen *et al.* 2022), which may explain the lack of positive effects.

Pretreatments with sulfuric acid (Figure 8) displayed trends similar to those of autocatalytic treatments. The substrates pretreated with 2-naphthol possessed lower glucose concentrations after enzymatic hydrolysis. The results contradict the literature, where scavenger pretreatments with dilute acid are efficient in a wide range of severities (Pielhop *et al.* 2017). However, bark differs in composition from wood, for example, due to its high amount of water-soluble polyphenolic extractives and pectin, which makes it hard to perform direct comparisons.

A deeper investigation of the pretreated materials by NMR revealed the elevation of peaks possibly related to G-lignin in the scavenger pretreated material. At the same time, NMR indicated pristine 2-naphthol in extracts derived from the pretreated material (Paper II). FTIR conducted on steam-exploded material also revealed an elevation of peaks possibly related to G-lignin for materials pretreated with 2-naphthol. However, only materials pretreated with 2-naphthol and sulfuric acid possessed peaks around 750 and 815 cm^{-1}, indicating 1,2-disubstituted naphthalenes (Hawkins *et al.* 1957), which could be seen as a confirmation that the 2-naphthol had participated in reactions.

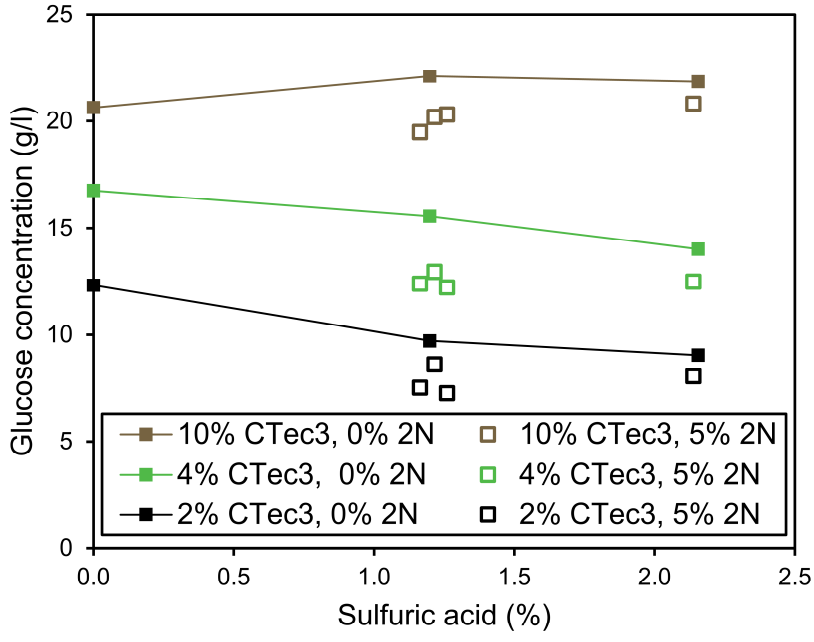

Figure 8. Glucose concentrations in hydrolysates for substrates pretreated at different sulfuric acid concentrations with and without 2-naphthol (2N) and hydrolyzed with varying enzyme loadings (CTec3). Chemical dosages are expressed as % w/w of the biomass total solids at the inlet of the pretreatment and enzymatic stages, respectively.

Although desired reactions may have occurred, some factors prohibit an efficient scavenger pretreatment of bark. At low severities, the cellulase accessibility of the substrates is most likely not good enough due to incomplete degradation of hemicelluloses. The pretreatments catalyzed with sulfuric acid solubilized or degraded most of the bark hemicelluloses. However, condensation reactions under acidic conditions may prohibit enzymatic accessibility and create substances that participate in the non-productive binding of cellulases. If the condensation reactions mainly originate from water-soluble extractives, the scavenger, which prohibits lignin condensation, is less useful.

Adding 2-naphthol as a ground powder to the continuous biomass feed is a simple and scalable method for providing the additive. However, other potentially scalable methods have recently been shown to be more efficient (Seidel *et al.* 2024). A spray-impregnation with 2-naphthol dissolved in ethanol or acetone could provide excellent results while omitting a high

liquid-to-solids usage. However, using flammable solvents calls for additional health and safety measures in these zones (ATEX assessments), which may make the process costly. Therefore, dosing the 2-naphthol as a slurry together with water is an interesting option for industrial applications that provides better results than dry provision of the powder. Regardless, potential applications for bark call for further investigations, likely including the removal of water-soluble extractives and the potential valorization of this by-product.

Finally, fermentation of the substrates pretreated with 2-naphthol showed that yeast inhibition can be overcome if the process severity is kept at 3.94, which can be considered a mild treatment for softwood (Paper II). Since the 2-naphthol is shown to be toxic to *S. cerevisiae* (Pielhop 2015), especially through cross-inhibition in the presence of other known inhibitors (Seidel *et al.* 2019), this is a promising finding. The mild severity and a continuous process that partly separates known inhibitors such as furfural and acetic acid from the substrate facilitated successful fermentation at high solids loading. To conclude, a suitable balance between harsh pretreatment conditions that maximize the scavenger's potential in terms of optimized enzymatic digestibility and mild conditions that provide suitable growth conditions for yeast must be found if bioethanol from softwood bark is the intended product.

4.2 Activated carbon from softwood bark

Bark that was steam exploded at 200 ºC and 10 min with 2.2% H_2SO_4 was chemically activated and pyrolyzed to produce activated carbon. Both KOH and $ZnCl_2$ were evaluated as activation chemicals.

4.2.1 Physiochemical properties

The physicochemical properties of carbon materials relate to their performance in technical applications and provide a deeper understanding of prevailing mechanisms during their production and usage. The degree of graphitization (I_D/I_G) is one measure that provides information about the relation between graphitic structures and disordered structures in the carbons. A lower value means a more pronounced graphitic structure, which is further explained in Paper III.

Most of the tested steam-exploded carbons possessed more graphitic characteristics than reference materials produced without steam explosion (Figure 9). I_D/I_G increased with pyrolysis temperature for materials activated with $ZnCl_2$. Analogously, the higher chemical dosage of KOH and $ZnCl_2$ led to less graphitization. This result was surprising, especially since a high KOH dosage would promote graphitization reactions. Possibly, the presence of other disordered structures unrelated to the D peak at 1360 cm^{-1} complicates the assessment of the graphitization degree, and some caution must be taken before drawing extensive conclusions (Sadezky *et al.* 2005).

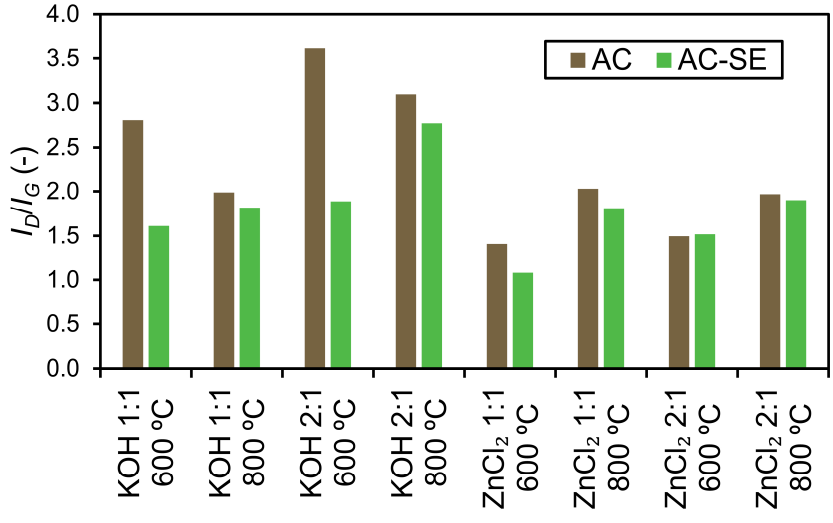

Figure 9. Degree of graphitization (I_D/I_G) for materials activated at 1:1 or 2:1 chemical/biomass ratio with KOH or $ZnCl_2$. AC: activated carbon, reference without steam explosion. AC-SE: steam-exploded activated carbon.

The carbons' porosity features were evaluated by measuring the BET surface area (Table 9). Steam-exploded materials activated with $ZnCl_2$ could develop better porosity features regarding BET specific surface area, microporosity, and pore volume. The increase in surface area was mainly due to the formation of micropores. Meanwhile, the steam explosion did not affect the porosity of materials activated with KOH. The pyrolysis yield (Table 9) revealed that steam explosion increased the mass yield for activation with KOH but decreased the mass yield for activation with $ZnCl_2$.

KOH and $ZnCl_2$ act slightly differently as activation agents. $ZnCl_2$ may act as a dampening agent that promotes the release of volatiles and tar from the biomass interior and facilitates the formation of a meso- and microporous structure. Swelling and dissolution of cellulose are also characteristics of $ZnCl_2$ treatments that help create cavities and increase the surface area. On the other hand, KOH acts on the carbonized matrix and is responsible for the stability and expansion of the carbon layers, leading to a highly microporous structure (Heidarinejad *et al.* 2020).

The treatments with KOH and $ZnCl_2$ also show that the relationships between temperature and surface area were different for the two activation agents. Carbons activated with KOH developed better porosity at 800 ºC and $ZnCl_2$ at 600 ºC (Table 9). Also, the surface area was higher for the material activated with $ZnCl_2$, which was somewhat surprising considering other findings in the literature (dos Reis *et al.* 2022). KOH likely requires additional temperature (e.g., 900 ºC) to fully develop a highly microporous structure caused by a K intercalation process (dos Reis *et al.* 2022). On the other hand, the higher surface area at 600 ºC for material impregnated with $ZnCl_2$ may be due to a more pronounced favoring of carbonization before volatilization at 800 ºC. For example, the carbonization of volatiles inside the pores before they are evacuated from the carbon matrix may lead to pore blockage and shrinkage. Activation of bark with $ZnCl_2$ by dos Reis *et al.* (2021) revealed a higher surface area for carbons produced at 700 compared to 800 and 900 ºC, which is in line with the findings in this work.

Table 9. BET surface area (S_{BET}), percentage of micropores (S_{MICRO}), pore volume, and pyrolysis yield for materials activated with 1:1 KOH or 1:1 $ZnCl_2$. AC: activated carbon, reference without steam explosion. AC-SE: steam-exploded activated carbon. 600/800: pyrolysis temperature (ºC). The fraction of mesopores can be calculated from $S_{MESO} = 100 - S_{MICRO}$.

Sample	S_{BET} **(m^2/g)**	S_{MICRO} **(%)**	**Pore volume (cm^3/g)**	**Yield (% w/w)**
KOH 1:1 AC 600	538	89.0	0.18	13
KOH 1:1 AC 800	1100	92.1	0.38	12
KOH 1:1 AC-SE 600	466	89.1	0.16	19
KOH 1:1 AC-SE 800	1102	92.4	0.38	18
$ZnCl_2$ 1:1 AC 600	1217	49.5	0.27	40
$ZnCl_2$ 1:1 AC 800	1018	51.8	0.24	36
$ZnCl_2$ 1:1 AC-SE 600	1308	50.7	0.30	36
$ZnCl_2$ 1:1 AC-SE 800	1217	56.0	0.29	29

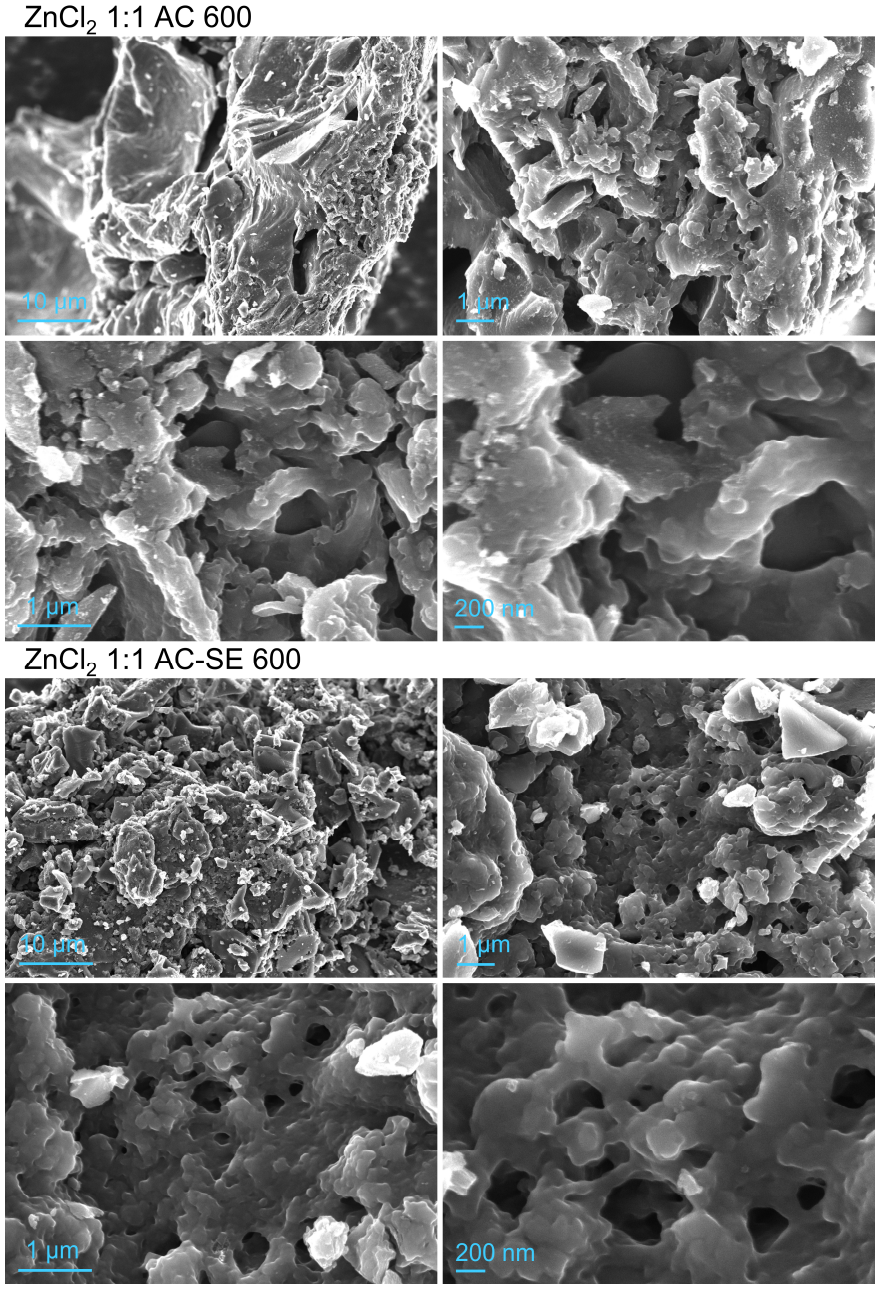

Figure 10. SEM images of the carbon materials at different magnifications. AC: activated carbon, reference without steam explosion. AC-SE: steam-exploded activated carbon. 600: pyrolysis temperature (ºC).

A more efficient impregnation of the steam-exploded materials likely explains the improved surface area, higher degree of graphitization, and lower yield for the material activated with $ZnCl_2$. The steam explosion opens the biomass structure and facilitates better impregnation (Jedvert et al. 2012). With $ZnCl_2$ homogeneously positioned in the biomass, volatile release, and pore formation could be promoted instead of char-forming reactions. KOH, on the other hand, acts on the carbonized material where steam-exploded materials have a higher relative elemental carbon content (Erixon & Björklund 2018), thus a potential for reaching higher carbonization yields.

The surfaces of the carbon materials were further examined with SEM-EDX. Figure 10 exemplifies the magnified porous structure of carbons activated with 1:1 $ZnCl_2$ and pyrolyzed at 600 ºC, and Table 10 displays the average elemental composition of surfaces mapped with SEM-EDX. Interestingly, traces of sulfur were found in the steam-exploded carbons. The sulfur is a remnant of the H_2SO_4 added to the steam explosion. However, the covalent inclusion of sulfur in the carbon matrix likely occurred during the pyrolysis step since steam explosion with H_2SO_4 only leads to minor traces of sulfur in washed pretreated solids (Wang *et al.* 2018). Paper III discusses thiophene, sulfoxide, sulfone, and sulfate functionalities detected in steam-exploded carbons by XPS.

Table 10. Surface mapping of elemental composition through SEM-EDX. AC: activated carbon, reference without steam explosion. AC-SE: steam-exploded activated carbon. 600/800: pyrolysis temperature (ºC).

	C (%)	**O (%)**	**S (%)**	**Cl (%)**
$ZnCl_2$ 1:1 AC 600	93.6	5.4	0.0	1.0
$ZnCl_2$ 1:1 AC 800	95.7	3.6	0.0	0.7
$ZnCl_2$ 1:1 AC-SE 600	90.2	6.8	2.3	0.7
$ZnCl_2$ 1:1 AC-SE 800	95.1	3.4	0.9	0.6

4.2.2 Dye adsorption

Reactive orange 16 (RO16) is an azo dye frequently used in the textile industry. In Paper III, the potential of the carbon materials as RO16 adsorbents was investigated. The steam-exploded carbons performed better than the corresponding reference in terms of dye removal ratio (Figure 11), maximum adsorption, and rate of adsorption. For carbons activated with $ZnCl_2$ 1:1 at 600 ºC, Langmuir maximum adsorption reached 218 mg/g for the steam-exploded carbon and 92 mg/g for the reference carbon. The higher

surface area, additional functionalities, and the graphitic structure may explain the elevated performance of the steam-exploded carbon.

The adsorption rate was initially controlled by boundary layer diffusion to the external surfaces (t <1 min) followed by intraparticle diffusion (t = 1-120 min) into the porous materials. The adsorption was of an intermediate initial adsorption type, meaning that adsorption onto external surfaces and intraparticle diffusion were both significant (Wu *et al.* 2009). Further discussions regarding potential adsorption mechanisms and characteristics of the adsorption process are found in Paper III.

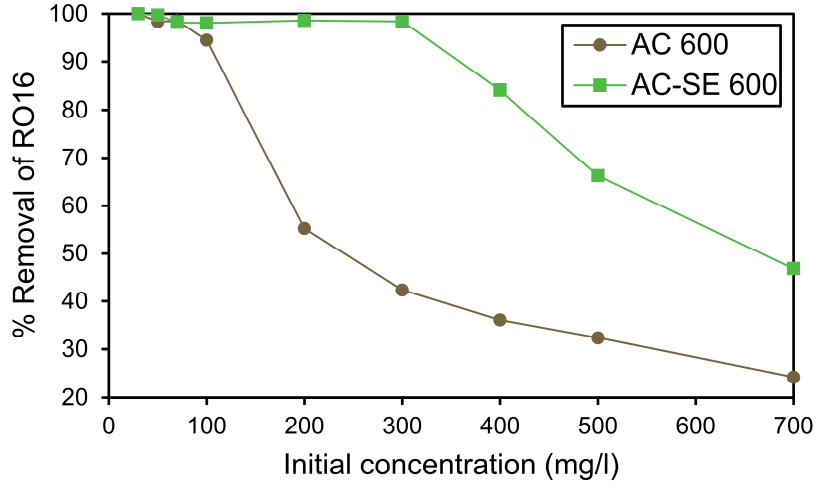

Figure 11. RO16 dye removal for carbons from bark activated with 1:1 $ZnCl_2$. AC: activated carbon, reference without steam explosion. AC-SE: steam-exploded activated carbon. 600: pyrolysis temperature (ºC).

4.3 Monitoring and quantification of degradation products

Trials were undertaken to investigate the possible recovery of degradation products from softwood bark. Furthermore, Raman spectroscopy was tested to monitor and quantify the degradation products in the blow steam (Paper IV). The utilized bark had a composition of 5.4% arabinan, 2.9% galactan, 29.1% glucan, 4.2% mannan, 0.7% rhamnan, and 3.6% xylan.

4.3.1 Degradation products in blow steam

Blow steam condensates were frequently sampled from the steam explosion process (74 samples collected at 5-minute intervals), and different degradation products, chiefly from the polysaccharide degradation pathway, were selected for HPLC quantification (Paper IV). The degradation products were furfural, acetic acid, methanol, formic acid, 5-methylfurfural (5-MF), 5-HMF, and levulinic acid. Furfural was the predominant degradation product in condensates from dilute acid (H_2SO_4) treatments >5100 mg/l, while 5-MF, 5-HMF, and levulinic acid occurred in lower concentrations <390 mg/l.

Furfural originates from the degradation of pentoses (Jönsson *et al.* 2013), present as arabinan in pectin sidechains and mainly as xylan in the hemicellulose. Especially arabinan was shown to degrade rapidly during steam explosion (Paper I) and is thus an easily accessible source of furfural. 5-HMF and levulinic acid, on the other hand, are derived from the degradation of hexoses (Jönsson *et al.* 2013). Although these are present in the hemicellulose, they degraded less rapidly than the arabinan and thus occurred in much lower concentrations in the blow steam. Furthermore, 5-HMF is highly hygroscopic, which may retain it in the water phase during a steam explosion discharge rather than flashing to the blow steam. 5-MF is derived from the degradation of 6-deoxyaldohexoses such as rhamnan (Moe *et al.* 2022), found only in low concentrations in the feedstock and was thus only present in low concentrations in the steam.

The furfural concentration in the blow steam from the continuous process was sampled during startup, stoppage, and three changes of process conditions (Figure 12). The figure shows a gradual shift in furfural concentration upon every instant change in process condition that eventually plateaus approaching steady-state conditions. At around 200 min, there was a disturbance related to the steam supply to the reactor. This disturbance was visual as a momentary decrease in furfural concentration. Altogether, this shows that measuring furfural concentration in the blow steam is an accessible way of monitoring process fluctuations and steady-state conditions.

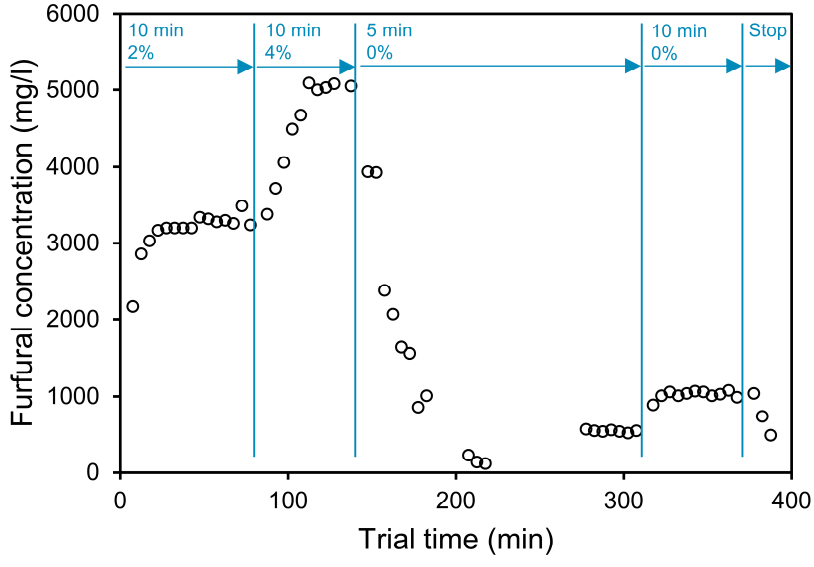

Figure 12. The furfural concentration measured by HPLC in blow steam condensates from steam explosion versus progressed time. At t = 0, the first biomass material exited the reactor. Prevailing process conditions are noted in blue (residence time 5 or 10 min and H_2SO_4 concentration 0-4% w/w on biomass total solids). Temperature was constant at 200 ºC.

4.3.2 Raman spectroscopy for analysis of blow steam

Raman spectra obtained from the blow steam were used in Paper IV to model the concentration of furfural, acetic acid, methanol, formic acid, 5-MF, 5-HMF, and levulinic acid. Through multivariate calibration (OPLS), good models could be established for the concentration of furans from Raman spectra.

Raman spectra of the pure components diluted to different concentrations revealed clear Raman signatures for the furans, containing several peaks linear to the concentration already from 100 mg/l and upwards. Apart from the excellent Raman fingerprint, furfural also occurred in high concentrations in the blow steam relative to all other compounds and well over the concentration threshold for peak linearity, which explains the excellent predictability. Figure 13 shows that even when estimated from a single point in the Raman spectra, a decent approximation of the furfural concentration can be made.

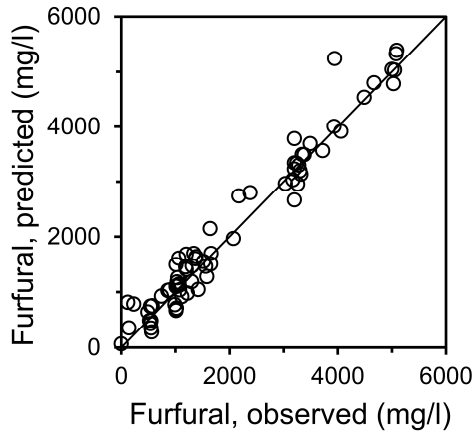

Figure 13. Furfural concentrations determined from the peak at 1078 cm^{-1} in the Raman spectra (predicted) versus furfural concentration measured with HPLC (observed).

Some covariation with furfural explains the good models for the other furans (5-HMF and 5-MF). Models for the other compound are further discussed in Paper IV.

The continuous pilot process was operated at a steam-to-biomass infeed ratio of approximately 5 kg/kg. However, scaling up the process overcomes some physical restrictions related to the discharging of the biomass, and a commercial facility can operate at a much lower steam-to-biomass ratio. In this case, chemicals in the blow steam would be more concentrated.

Figure 14 displays the Raman spectra of reference chemicals at concentrations 2-5 times higher than discovered in blow steam condensates during the pilot tests. Interestingly, the peaks appearing for acetic acid at 2946 cm^{-1} and methanol at 2846 and 2954 cm^{-1} are now in their linear range, which occurred somewhere above 1500 mg/l. Thus, better quantitative information regarding acetic acid and methanol concentration can likely be derived from Raman spectroscopy of blow steam condensates from commercial-scale steam explosion units. On the other hand, both formic and levulinic acid have very weak Raman signatures, and no helpful information regarding their concentration can be expected at concentrations below 5000 mg/l with the tested methodology.

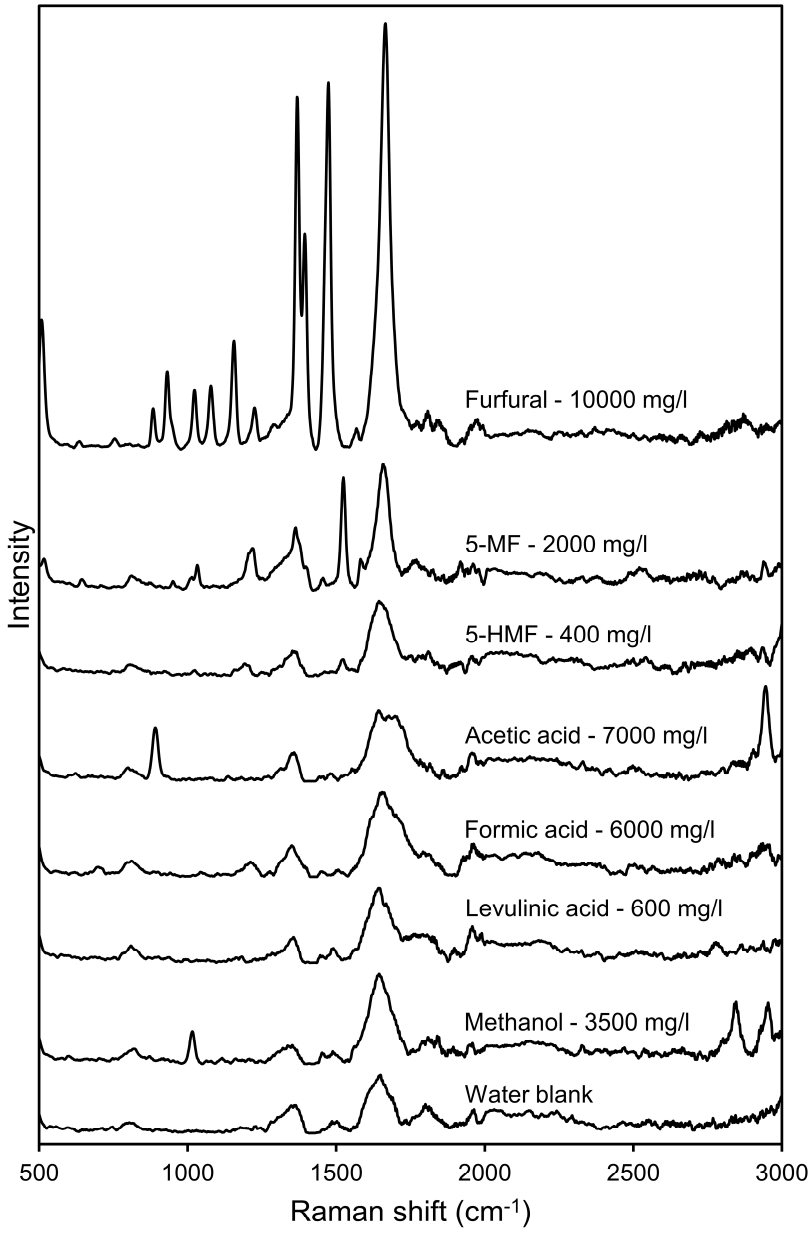

Figure 14. Raman reference spectra of seven degradation products in the steam explosion blow steam. The concentrations have been set to approximately match their interrelation in the blow steam condensate.

5. Conclusion

This work has given better insight into the potential utilization of softwood bark to produce sugars, ethanol, or activated carbon via a steam explosion processing step. In addition, novel strategies to produce activated carbon and quantify degradation products in the steam explosion blow steam were investigated. The main conclusions are summarized below.

Softwood bark was pretreated in a continuous and scalable steam explosion system, and the substrates were evaluated for sugar and ethanol recovery after enzymatic hydrolysis and fermentation at 12% total solids. Results showed that softwood bark is a problematic material to pretreat and hydrolyze with enzymes, and only with a CTec3 HS dosage of 10% w/w glucose conversions up to 0.57 was obtained. Acceptable ethanol yields from hexose (0.45 g/g) could be achieved after 24 h fermentation with *S. cerevisiae*. However, concentrations were not adequately high to consider ethanol from bark feasible with the tested process setup.

The carbocation scavenger 2-naphthol was investigated as an additive to steam explosion to overcome bark recalcitrance. The scavenger was provided as a ground powder at a dosage of 5% w/w before entering the steam explosion reactor. The data showed that the desired reactions between 2-naphthol and lignin had occurred. However, no positive effects on enzymatic digestibility were observed for pretreatments with sulfuric acid or autocatalytic pretreatments at severity factor 3.94 or below. On the upside, only a minor inhibition of the yeast was detected, highlighting that cross-inhibition between 2-naphthol and lignocellulosic degradation products can be overcome with mild pretreatments and continuous steam explosion. Furthermore, an ethanol-washing sequence before enzymatic hydrolysis could detoxify the substrates and boost the fermentation yield.

Instead, the bark's potential application as an activated carbon material for adsorbent usage was investigated. In addition, steam explosion with sulfuric acid catalyst was evaluated as a pretreatment step before activation and pyrolysis. Results revealed that steam explosion before activation improved the carbon surface area, and additional sulfur functionalities were introduced compared to reference materials without steam explosion. Consequently, the steam-exploded materials outperformed the reference materials as adsorbents of reactive orange 16. The highest BET specific surface area attained was 1308 m^2/g, and the maximum Langmuir adsorption of reactive orange 16 was 218 mg/g. The novel application of a steam explosion preconditioning step lays a foundation for future research in biobased carbon materials for environmental and energy applications.

Finally, to monitor and quantify the degradation products present in the steam explosion blow steam, several condensates from the continuous steam explosion process were collected and analyzed with Raman spectroscopy. OPLS modeling showed that models could be obtained to predict the furfural concentration from Raman spectra accurately. Furfural is not only a potentially valuable byproduct, but its quantity can also reveal fluctuations in a continuous process. These findings open the possibility of rapidly measuring furfural concentrations in blow steam condensates applicable to laboratory and online analysis.

6. Outlook and future work

Bioethanol from softwood bark via steam explosion pretreatment, enzymatic hydrolysis, and fermentation is by now a well-explored topic. There seems to be a general understanding that water-soluble extractives are one major issue when pretreatments are conducted under acidic conditions. Any further research targeting biochemicals from bark should preferably focus on the removal, but more importantly, the valorization of this process stream. Still, even with the removal of extractives, a game-changer is needed to valorize the carbohydrates in the bark fully.

The carbocation scavenger 2-naphthol was not revealed as a game-changer in this work. However, there is still room for investigation of carbocation effects in a steam explosion process at high severity (<4.5) or with an acid catalyst when a preceding removal of water-extractable compounds has been included. Since the composition of bark differs significantly from wood, no information regarding the usefulness of 2-naphthol as an additive in an industrially scalable, continuous steam explosion step can be derived from this work. Further trials on woody materials would also be required, including newly revealed methods for 2-naphthol provision at high liquid-to-solids ratios. How the detoxification with ethanol could be appropriately integrated into the process, for example, by looping it back to the 2-naphthol impregnation, could also be further studied.

Steam explosion as a preconditioning step for producing activated carbon showed promising results. However, it would be useful to more thoroughly explore and map the optimum process conditions for a wide range of activation chemicals. Further exploration should be combined with evaluating the potential of resulting carbons for various applications, such as adsorbents of different types of pollutants and possibly as part of energy

storage systems, e.g., electrode materials in batteries. Another exciting aspect is the small particle size of the steam-exploded materials and the potential adverse effects of drying, e.g., hornification. Studies should be initiated to investigate whether drying and grinding before activation can be omitted if steam explosion preconditioning is included in the process.

Finally, Raman spectroscopy is a promising technique for quantifying furfural and potentially other degradation products in blow steam condensates. However, the finding calls for very little additional research, and industrial development efforts could proceed from here.

References

Aarum, I., Solli, A., Gunnarsson, H., Kalyani, D., Devle, H., Ekeberg, D. & Stenstrøm, Y. (2019). Comparison of pyrolyzed lignin before and after milled wood lignin purification of Norway spruce with increasing steam explosion. Wood Science and Technology, 53(3), 601-618. https://doi.org/10.1007/s00226-019-01088-x

Aldea, J., Ruiz-Peinado, R., del Río, M., Pretzsch, H., Heym, M., Brazaitis, G., Jansons, A., Metslaid, M., Barbeito, I., Bielak, K., Granhus, A., Holm, S.-O., Nothdurft, A., Sitko, R. & Löf, M. (2021). Species stratification and weather conditions drive tree growth in Scots pine and Norway spruce mixed stands along Europe. Forest Ecology and Management, 481, 118697. https://doi.org/10.1016/j.foreco.2020.118697

Andersson, S., Serimaa, R., Paakkari, T., Saranpää, P. & Pesonen, E. (2003). Crystallinity of wood and the size of cellulose crystallites in Norway spruce (*Picea abies*). Journal of Wood Science, 49(6), 531-537. https://doi.org/10.1007/s10086-003-0518-x

Anerud, E., Routa, J., Bergström, D. & Eliasson, L. (2020). Fuel quality of stored spruce bark – Influence of semi-permeable covering material. Fuel, 279, 118467. https://doi.org/10.1016/j.fuel.2020.118467

Borrega, M., Pihlajaniemi, V., Liitiä, T., Wikström, L. & Tamminen, T. (2021). Evaluation of chemical additives in hydrothermal pre-treatment of wood for the integrated production of monosugars and hydrolysis lignins for PLA-based biocomposites. Biomass Conversion and Biorefinery. https://doi.org/10.1007/s13399-021-01365-5

Boussaid, A., Cai, Y., Robinson, J., Gregg, D.J., Nguyen, Q. & Saddler, J.N. (2001). Sugar recovery and fermentability of hemicellulose hydrolysates from steam-exploded softwoods containing bark. Biotechnol Prog, 17(5), 887-92. https://doi.org/10.1021/bp010092b

Chamberlin, C., Carter, D. & Jacobson, A. (2018). Measuring residence time distributions of wood chips in a screw conveyor reactor. Fuel Processing Technology, 178, 271-282. https://doi.org/10.1016/j.fuproc.2018.06.005

Chandel, A.K., Garlapati, V.K., Singh, A.K., Antunes, F.A.F. & da Silva, S.S. (2018). The path forward for lignocellulose biorefineries: Bottlenecks, solutions, and perspective on commercialization. Bioresource Technology, 264, 370-381. https://doi.org/10.1016/j.biortech.2018.06.004

Chu, Q., Song, K., Wang, J., Hu, J. & Chen, X. (2019). Improving enzymatic saccharification of hardwood through lignin modification by carbocation scavengers and the underlying mechanisms. Bioresource Technology, 294, 122216. https://doi.org/10.1016/j.biortech.2019.122216

Chu, Q., Tong, W., Wu, S., Jin, Y., Hu, J. & Song, K. (2021). Modification of lignin by various additives to mitigate lignin inhibition for improved enzymatic digestibility of dilute acid pretreated hardwood. Renewable Energy, 177, 992-1000. https://doi.org/10.1016/j.renene.2021.06.048

Correa, C.R. & Kruse, A. (2018). Biobased Functional Carbon Materials: Production, Characterization, and Applications—A Review. Materials, 11(9), 1568. https://doi.org/10.3390/ma11091568

da Silva, A.S.A., Espinheira, R.P., Teixeira, R.S.S., de Souza, M.F., Ferreira-Leitão, V. & Bon, E.P.S. (2020). Constraints and advances in high-solids enzymatic hydrolysis of lignocellulosic biomass: a critical review. Biotechnology for Biofuels, 13(1), 58. https://doi.org/10.1186/s13068-020-01697-w

Dąbrowski, A. (2001). Adsorption — from theory to practice. Advances in Colloid and Interface Science, 93(1), 135-224. https://doi.org/10.1016/S0001-8686(00)00082-8

dos Reis, G.S., Guy, M., Mathieu, M., Jebrane, M., Lima, E.C., Thyrel, M., Dotto, G.L. & Larsson, S.H. (2022). A comparative study of chemical treatment by MgCl2, ZnSO4, ZnCl2, and KOH on physicochemical properties and acetaminophen adsorption performance of biobased porous materials from tree bark residues. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 642, 128626. https://doi.org/10.1016/j.colsurfa.2022.128626

dos Reis, G.S., Larsson, S.H., Mathieu, M., Thyrel, M. & Pham, T.N. (2021). Application of design of experiments (DoE) for optimized production of micro- and mesoporous Norway spruce bark-activated carbons. Biomass Conversion and Biorefinery. https://doi.org/10.1007/s13399-021-01917-9

Eilers, P.H.C. (2004). Parametric Time Warping. Analytical Chemistry, 76(2), 404-411. https://doi.org/10.1021/ac034800e

Erixon, M. & Björklund, P. (2018). Steam exploded pellets made from bark and residues - A new possible value stream for the pulp and paper industry. Appita Journal, 71, 236-241.

European Commission Directorate-General for Research and Innovation (2018). A sustainable bioeconomy for Europe – Strengthening the connection between economy, society and the environment – Updated bioeconomy strategy. Publications Office. https://data.europa.eu/doi/10.2777/792130

Ferraz, D. & Pyka, A. (2023). Circular economy, bioeconomy, and sustainable development goals: a systematic literature review. Environmental Science and Pollution Research. https://doi.org/10.1007/s11356-023-29632-0

Frankó, B. (2018). Implications of feedstock diversity on forest-based ethanol production. Diss. Lund University. https://lup.lub.lu.se/search/ws/files/42803966/Spikfil_Balazs_F.pdf

Frankó, B., Carlqvist, K., Galbe, M., Lidén, G. & Wallberg, O. (2018). Removal of Water-Soluble Extractives Improves the Enzymatic Digestibility of Steam-Pretreated Softwood Barks. Applied Biochemistry and Biotechnology, 184(2), 599-615. https://doi.org/10.1007/s12010-017-2577-2

Freundlich, H. (1906). Over the adsorption in solution. J. Phys. chem, 57, 385-471.

Galbe, M. & Wallberg, O. (2019). Pretreatment for biorefineries: a review of common methods for efficient utilisation of lignocellulosic materials. Biotechnology for Biofuels, 12(1), 26. https://doi.org/10.1186/s13068-019-1634-1

Galbe, M. & Zacchi, G. (2012). Pretreatment: The key to efficient utilization of lignocellulosic materials. Biomass and Bioenergy, 46, 70-78. https://doi.org/10.1016/j.biombioe.2012.03.026

Garrote, G., Domínguez, H. & Parajó, J.C. (1999). Hydrothermal processing of lignocellulosic materials. Holz als Roh- und Werkstoff, 57(3), 191-202. https://doi.org/10.1007/s001070050039

Gorzsás, A (2018). MCR-ALS GUI (v4c) [application]. Umeå University. https://www.umu.se/en/research/infrastructure/visp/downloads/ [24 April 2023]

Hames, B., Ruiz, R., Scarlata, C., Sluiter, A., Sluiter, J. & Templeton, D. (2008). Preparation of Samples for Compositional Analysis. (Laboratory Analytical Procedure NREL/TP-510-42620). NREL. https://www.nrel.gov/docs/gen/fy08/42620.pdf

Hannerz, M. & Ekström, H. (2023). Nordic Forest Statistics 2023, Resources, industry, trade, prices, environment and climate. Nordic Forest Research. https://nordicforestresearch.org/wp-content/uploads/2023/06/Nordisk-skogsstatistik-2023-mindre.pdf

Hansen, L.D., Østensen, M., Arstad, B., Tschentscher, R., Eijsink, V.G.H., Horn, S.J. & Várnai, A. (2022). 2-Naphthol Impregnation Prior to Steam Explosion Promotes LPMO-Assisted Enzymatic Saccharification of Spruce and Yields High-Purity Lignin. ACS Sustainable Chemistry & Engineering, 10(16), 5233-5242. https://doi.org/10.1021/acssuschemeng.2c00286

Hawkins, J.G., Ward, E.R. & Whiffen, D.H. (1957). Characteristic infra-red absorption frequencies of substituted naphthalenes. Spectrochimica Acta, 10(1), 105-109. https://doi.org/10.1016/0371-1951(57)80168-4

Heidarinejad, Z., Dehghani, M.H., Heidari, M., Javedan, G., Ali, I. & Sillanpää, M. (2020). Methods for preparation and activation of activated carbon: a review. Environmental Chemistry Letters, 18(2), 393-415. https://doi.org/10.1007/s10311-019-00955-0

Ho, Y.S. & McKay, G. (1999). Pseudo-second order model for sorption processes. Process Biochemistry, 34(5), 451-465. https://doi.org/10.1016/S0032-9592(98)00112-5

Hoang, A.T., Nguyen, X.P., Duong, X.Q., Ağbulut, Ü., Len, C., Nguyen, P.Q.P., Kchaou, M. & Chen, W.-H. (2023). Steam explosion as sustainable biomass pretreatment technique for biofuel production: Characteristics and challenges. Bioresource Technology, 385, 129398. https://doi.org/10.1016/j.biortech.2023.129398

Hu, J., Arantes, V., Pribowo, A. & Saddler, J.N. (2013). The synergistic action of accessory enzymes enhances the hydrolytic potential of a "cellulase mixture" but is highly substrate specific. Biotechnology for Biofuels, 6(1), 112. https://doi.org/10.1186/1754-6834-6-112

IEA Bioenergy (2022). Global biorefinery status report 2022. IEA Bioenergy. https://www.ieabioenergy.com/wp-content/uploads/2022/09/IEA-Bioenergy-Task-42-Global-biorefinery-status-report-2022-220712.pdf

ISO (2015a) ISO 18122:2015 Solid biofuels — Determination of ash content (ISO 18122:2015). ISO

ISO (2015b) ISO 17828:2015 Solid biofuels — Determination of bulk density (ISO 17828:2015). ISO

ISO (2016) ISO 17827-1:2016 Solid biofuels — Determination of particle size distribution for uncompressed fuels — Part 1: Oscillating screen method using sieves with apertures of 3.15 mm and above (ISO 17827-1:2016). ISO

ISO (2020) ISO 21436:2020 Pulps — Determination of lignin content — Acid hydrolysis method (ISO 21436:2020). ISO

Jablonsky, M., Nosalova, J., Sladkova, A., Haz, A., Kreps, F., Valka, J., Miertus, S., Frecer, V., Ondrejovic, M., Sima, J. & Surina, I. (2017). Valorisation of softwood bark through extraction of utilizable chemicals. A review. Biotechnology Advances, 35(6), 726-750. https://doi.org/10.1016/j.biotechadv.2017.07.007

Jedvert, K., Saltberg, A., Theliander, H., Wang, Y., Henriksson, G. & Lindström, M.E. (2012). Mild steam explosion: A way to activate wood for enzymatic treatment, chemical pulping and biorefinery processes. Nordic Pulp & Paper Research Journal, 27(5), 828-835. https://doi.org/10.3183/npprj-2012-27-05-p828-835

Jönsson, L.J., Alriksson, B. & Nilvebrant, N.-O. (2013). Bioconversion of lignocellulose: inhibitors and detoxification. Biotechnology for Biofuels, 6(1), 16. https://doi.org/10.1186/1754-6834-6-16

Jönsson, L.J. & Martín, C. (2016). Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. Bioresource Technology, 199, 103-112. https://doi.org/10.1016/j.biortech.2015.10.009

Kärkkäinen, M. (1976). Density and moisture content of wood and bark, and bark percentage in the branches of birch, Norway spruce and Scots pine. https://doi.org/10.14214/sf.a14794

Katahira, R., Elder, T.J. & Beckham, G.T. (2018). A Brief Introduction to Lignin Structure. In: Beckham, G.T. (ed.) Lignin Valorization: Emerging Approaches. The Royal Society of Chemistry. Ch. 1. https://doi.org/10.1039/9781788010351-00001

Kemppainen, K., Inkinen, J., Uusitalo, J., Nakari-Setälä, T. & Siika-aho, M. (2012). Hot water extraction and steam explosion as pretreatments for ethanol production from spruce bark. Bioresource Technology, 117, 131-139. https://doi.org/10.1016/j.biortech.2012.04.080

Kemppainen, K., Siika-aho, M., Pattathil, S., Giovando, S. & Kruus, K. (2014). Spruce bark as an industrial source of condensed tannins and non-cellulosic sugars. Industrial Crops and Products, 52, 158-168. https://doi.org/10.1016/j.indcrop.2013.10.009

Klemm, D., Heublein, B., Fink, H.-P. & Bohn, A. (2005). Cellulose: Fascinating Biopolymer and Sustainable Raw Material. Angewandte Chemie International Edition, 44(22), 3358-3393. https://doi.org/10.1002/anie.200460587

Korpinen, R., Kallioinen, M., Hemming, J., Pranovich, A., Mänttäri, M. & Willför, S. (2014). Comparative evaluation of various lignin determination methods on hemicelluloserich fractions of spruce and birch obtained by pressurized hot-water extraction (PHWE) and subsequent ultrafiltration (UF). Holzforschung, 68(8), 971-979. https://doi.org/10.1515/hf-2013-0233

Krogell, J., Holmbom, B., Pranovich, A., Hemming, J. & Willfor, S. (2012). Extraction and chemical characterization of Norway spruce inner and outer bark. Nordic Pulp & Paper Research Journal, 27(1), 6-17. https://doi.org/10.3183/npprj-2012-27-01-p006-017

Lagergren, S. (1898). About the theory of so-called adsorption of soluble substances. Kungl. Svenska vetenskapsakademiens handlingar, 24, 1-39.

Langmuir, I. (1918). The adsorption of gases on plane surfaces of glass, mica and platinum. Journal of the American Chemical Society, 40(9), 1361-1403. https://doi.org/10.1021/ja02242a004

Le Normand, M., Mélida, H., Holmbom, B.R., Michaelsen, T.E., Inngjerdingen, M., Bulone, V., Paulsen, B.S. & Ek, M. (2014). Hot-water extracts from the inner bark of Norway spruce with immunomodulating activities. Carbohydrate Polymers, 101, 699-704. https://doi.org/10.1016/j.carbpol.2013.09.067

Le Normand, M., Rietzler, B., Vilaplana, F. & Ek, M. (2021). Macromolecular Model of the Pectic Polysaccharides Isolated from the Bark of Norway Spruce (*Picea abies*). Polymers, 13(7), 1106. https://doi.org/10.3390/polym13071106

Levenberg, K. (1944). A method for the solution of certain non-linear problems in least squares. Quarterly of Applied Mathematics, 2(2), 164-168. https://doi.org/10.1090/qam/10666

Li, J., Henriksson, G. & Gellerstedt, G. (2007). Lignin depolymerization/repolymerization and its critical role for delignification of aspen wood by steam explosion. Bioresource Technology, 98(16), 3061-3068. https://doi.org/10.1016/j.biortech.2006.10.018

Liu, L., Wang, J., Rosenberg, D., Zhao, H., Lengyel, G. & Nadel, D. (2018). Fermented beverage and food storage in 13,000 y-old stone mortars at Raqefet Cave, Israel: Investigating Natufian ritual feasting. Journal of Archaeological Science: Reports, 21, 783-793. https://doi.org/10.1016/j.jasrep.2018.08.008

Liu, T., Wang, P., Tian, J., Guo, J., Zhu, W., Bushra, R., Huang, C., Jin, Y., Xiao, H. & Song, J. (2024). Emerging role of additives in lignocellulose enzymatic saccharification: A review. Renewable and Sustainable Energy Reviews, 197, 114395. https://doi.org/10.1016/j.rser.2024.114395

Lynd, L.R., Weimer, P.J., Zyl, W.H.v. & Pretorius, I.S. (2002). Microbial Cellulose Utilization: Fundamentals and Biotechnology. Microbiology and Molecular Biology Reviews, 66(3), 506-577. https://doi.org/10.1128/mmbr.66.3.506-577.2002

Mason, W.H. (1926). Process and apparatus for disintegration of wood and the like. US1578609, 30 March 1926. https://worldwide.espacenet.com/patent/search/family/024973620/publication/US1578609A?q=pn%3DUS1578609A

Miranda, I., Gominho, J., Mirra, I. & Pereira, H. (2012). Chemical characterization of barks from *Picea abies* and *Pinus sylvestris* after fractioning into different particle sizes. Industrial Crops and Products, 36(1), 395-400. https://doi.org/10.1016/j.indcrop.2011.10.035

Moe, S.T., Marcotullio, G., Opedal, M.T. & Brusletto, R. (2022). Formation of 5-methylfurfural and 2-acetylfuran from lignocellulosic biomass and by Cr3+-catalyzed dehydration of 6-deoxyhexoses. Carbohydrate Research, 522, 108672. https://doi.org/10.1016/j.carres.2022.108672

Muzamal, M., Jedvert, K., Theliander, H. & Rasmuson, A. (2015). Structural changes in spruce wood during different steps of steam explosion pretreatment. Holzforschung, 69(1), 61-66. https://doi.org/10.1515/hf-2013-0234

Neiva, D.M., Araújo, S., Gominho, J., Carneiro, A.d.C. & Pereira, H. (2018). An integrated characterization of Picea abies industrial bark regarding chemical composition, thermal properties and polar extracts activity. PLOS ONE, 13(11), e0208270. https://doi.org/10.1371/journal.pone.0208270

Ngueho Yemele, M.C., Koubaa, A., Cloutier, A., Souloungangа, P., Stevanovic, T. & Wolcott, M.P. (2013). Effects of hot water treatment of raw bark, coupling agent, and lubricants on properties of bark/HDPE composites. Industrial Crops and Products, 42, 50-56. https://doi.org/10.1016/j.indcrop.2012.05.012

Normark, M., Winestrand, S., Lestander, T. & Jönsson, L. (2014). Analysis, pretreatment and enzymatic saccharification of different fractions of Scots pine. BMC Biotechnology, 14, 20. https://doi.org/10.1186/1472-6750-14-20

Olsson, L. & Hahn-Hägerdal, B. (1996). Fermentation of lignocellulosic hydrolysates for ethanol production. Enzyme and Microbial Technology, 18(5), 312-331. https://doi.org/10.1016/0141-0229(95)00157-3

Overend, R.P. & Chornet, E. (1987). Fractionation of lignocellulosics by steam-aqueous pretreatments. Philosophical Transactions of the Royal Society of London. Series A, Mathematical and Physical Sciences, 321(1561), 523-536. https://doi.org/10.1098/rsta.1987.0029

Pielhop, T. (2015). Hydrothermal/steam explosion pretreatment with carbocation scavengers for biofuels and -chemicals production. Diss. ETH Zürich. https://doi.org/10.3929/ethz-a-010675808

Pielhop, T., Larrazábal, G.O., Studer, M.H., Brethauer, S., Seidel, C.-M. & Rudolf von Rohr, P. (2015). Lignin repolymerisation in spruce autohydrolysis pretreatment increases cellulase deactivation. Green Chemistry, 17(6), 3521-3532. https://doi.org/10.1039/C4GC02381A

Pielhop, T., Reinhard, C., Hecht, C., Del Bene, L., Studer, M.H. & Rudolf von Rohr, P. (2017). Application potential of a carbocation scavenger in autohydrolysis and dilute acid pretreatment to overcome high softwood recalcitrance. Biomass and Bioenergy, 105, 164-173. https://doi.org/10.1016/j.biombioe.2017.07.005

Poletto, M., Pistor, V. & Zattera, A.J. (2013). Structural Characteristics and Thermal Properties of Native Cellulose. In: van de Ven, T. & Godbout, L. (eds) Cellulose – Fundamental Aspects. Intech. Ch. 2. https://doi.org/10.5772/50452

Rahikainen, J.L., Martin-Sampedro, R., Heikkinen, H., Rovio, S., Marjamaa, K., Tamminen, T., Rojas, O.J. & Kruus, K. (2013). Inhibitory effect of lignin during cellulose bioconversion: The effect of lignin chemistry on non-productive enzyme adsorption. Bioresource Technology, 133, 270-278. https://doi.org/10.1016/j.biortech.2013.01.075

Ralph, J., Lundquist, K., Brunow, G., Lu, F., Kim, H., Schatz, P.F., Marita, J.M., Hatfield, R.D., Ralph, S.A., Christensen, J.H. & Boerjan, W. (2004). Lignins: Natural polymers from oxidative coupling of 4-hydroxyphenyl-propanoids. Phytochemistry Reviews, 3(1), 29-60. https://doi.org/10.1023/B:PHYT.0000047809.65444.a4

Rasmussen, H., Sørensen, H.R. & Meyer, A.S. (2014). Formation of degradation compounds from lignocellulosic biomass in the biorefinery: sugar reaction mechanisms. Carbohydrate Research, 385, 45-57. https://doi.org/10.1016/j.carres.2013.08.029

Ritchie, A.G. (1977). Alternative to the Elovich equation for the kinetics of adsorption of gases on solids. Journal of the Chemical Society, Faraday Transactions 1: Physical Chemistry in Condensed Phases, 73(0), 1650-1653. https://doi.org/10.1039/F19777301650

Robak, K. & Balcerek, M. (2018). Review of Second Generation Bioethanol Production from Residual Biomass. Food Technology and Biotechnology, 56(2), 174-187. https://doi.org/10.17113/ftb.56.02.18.5428

Robert, D., Bardet, M., Lapierre, C. & Gellerstedt, G. (1988). Structural changes in aspen lignin during steam explosion treatment. Cellulose Chemistry and Technology, 22(2), 221-230.

Rosales-Calderon, O. & Arantes, V. (2019). A review on commercial-scale high-value products that can be produced alongside cellulosic ethanol. Biotechnology for Biofuels, 12(1), 240. https://doi.org/10.1186/s13068-019-1529-1

Sadezky, A., Muckenhuber, H., Grothe, H., Niessner, R. & Pöschl, U. (2005). Raman microspectroscopy of soot and related carbonaceous materials: Spectral analysis and structural information. Carbon, 43(8), 1731-1742. https://doi.org/10.1016/j.carbon.2005.02.018

Sannigrahi, P., Kim, D.H., Jung, S. & Ragauskas, A. (2011). Pseudo-lignin and pretreatment chemistry. Energy & Environmental Science, 4(4), 1306-1310. https://doi.org/10.1039/C0EE00378F

Santos, R., Hart, P., Jameel, H. & Chang, H.-M. (2013). Wood Based Lignin Reactions Important to the Biorefinery and Pulp and Paper Industries. BioResources, 8, 1456. https://doi.org/10.15376/biores.8.1.1456-1477

Savitzky, A. & Golay, M.J.E. (1964). Smoothing and Differentiation of Data by Simplified Least Squares Procedures. Analytical Chemistry, 36(8), 1627-1639. https://doi.org/10.1021/ac60214a047

SCAN (2003). SCAN-CM 49:03 Content of acetone-soluble matter (SCAN-CM 49:03). Swedish Institute for Standards

Seidel, C.-M., Brethauer, S., Gyenge, L., Rudolf von Rohr, P. & Studer, M.H. (2019). Two-stage steam explosion pretreatment of softwood with 2-naphthol as carbocation scavenger. Biotechnology for Biofuels, 12, 37. https://doi.org/10.1186/s13068-019-1373-3

Seidel, C.-M., Brethauer, S., Studer, M.H., von Rohr, P.R. & Pielhop, T. (2024). Overcoming Softwood Recalcitrance by Improved 2-Naphthol Addition to Steam Explosion Pretreatment. ACS Sustainable Chemistry & Engineering, 12(19), 7519-7531. https://doi.org/10.1021/acssuschemeng.4c01267

Sheehan, J. & Himmel, M. (1999). Enzymes, Energy, and the Environment: A Strategic Perspective on the U.S. Department of Energy's Research and Development Activities for Bioethanol. Biotechnology Progress, 15(5), 817-827. https://doi.org/10.1021/bp990110d

Siipola, V., Pflugmacher, S., Romar, H., Wendling, L. & Koukkari, P. (2020). Low-Cost Biochar Adsorbents for Water Purification Including Microplastics Removal. Applied Sciences, 10(3), 788. https://doi.org/10.3390/app10030788

Sips, R. (2004). On the Structure of a Catalyst Surface. The Journal of Chemical Physics, 16(5), 490-495. https://doi.org/10.1063/1.1746922

Sjöström, E. (1993). Wood chemistry: fundamentals and applications. 2 edition, Academic Press.

Skogsstyrelsen (2024). Avverkning – 01. Brutto- och nettoavverkad volym (milj. m3sk och m3fub) efter sortiment av stamved. År 1942–2023. (Updated 4 June 2024). Skogsstyrelsen. https://pxweb.skogsstyrelsen.se/pxweb/sv/Skogsstyrelsens%20statistikdatabas/Skogsstyrelsens%20statistikdatabas__Avverkning/JO0312_01.px/table/tableViewLayout2/ [14 July 2024]

Sluiter, A., Hames, B., Hyman, D., Payne, C., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D. & Wolfe, J. (2008a). Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples. (Laboratory Analytical Procedure (LAP) NREL/TP-510-42621). NREL. https://www.nrel.gov/docs/gen/fy08/42621.pdf

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D. & Crocker, D. (2008b). Determination of Structural Carbohydrates and Lignin in Biomass. (Laboratory Analytical Procedure (LAP) NREL/TP-510-42618). NREL. https://www.nrel.gov/docs/gen/fy13/42618.pdf

Sluiter, A., Hyman, D., Payne, C. & Wolfe, J. (2008c). Determination of Insoluble Solids in Pretreated Biomass Material. (Laboratory Analytical Procedure (LAP) NREL/TP-510-42627). NREL. https://www.nrel.gov/docs/gen/fy08/42627.pdf

Sun, Y. & Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresource Technology, 83(1), 1-11. https://doi.org/10.1016/S0960-8524(01)00212-7

Teugjas, H. & Väljamäe, P. (2013). Product inhibition of cellulases studied with 14C-labeled cellulose substrates. Biotechnology for Biofuels, 6(1), 104. https://doi.org/10.1186/1754-6834-6-104

Toor, M., Kumar, S.S., Malyan, S.K., Bishnoi, N.R., Mathimani, T., Rajendran, K. & Pugazhendhi, A. (2020). An overview on bioethanol production from lignocellulosic feedstocks. Chemosphere, 242, 125080. https://doi.org/10.1016/j.chemosphere.2019.125080

Torget, R., Himmel, M.E. & Grohmann, K. (1991). Dilute sulfuric acid pretreatment of hardwood bark. Bioresource Technology, 35(3), 239-246. https://doi.org/10.1016/0960-8524(91)90120-9

United Nations (2015). Transforming our World: the 2030 Agenda for Sustainable Development. United Nations. https://sdgs.un.org/sites/default/files/publications/21252030%20Agenda%20for%20Sustainable%20Development%20web.pdf

Vaaje-Kolstad, G., Westereng, B., Horn, S.J., Liu, Z., Zhai, H., Sørlie, M. & Eijsink, V.G.H. (2010). An Oxidative Enzyme Boosting the Enzymatic Conversion of Recalcitrant Polysaccharides. Science, 330, 219-222. https://doi.org/10.1126/science.1192231

Valentín, L., Kluczek-Turpeinen, B., Willför, S., Hemming, J., Hatakka, A., Steffen, K. & Tuomela, M. (2010). Scots pine (Pinus sylvestris) bark composition and degradation by fungi: Potential substrate for bioremediation. Bioresource Technology, 101(7), 2203-2209. https://doi.org/10.1016/j.biortech.2009.11.052

Vievard, J., Alem, A., Pantet, A., Ahfir, N.-D., Arellano-Sánchez, M.G., Devouge-Boyer, C. & Mignot, M. (2023). Bio-Based Adsorption as Ecofriendly Method for Wastewater Decontamination: A Review. Toxics, 11(5), 404. https://doi.org/10.3390/toxics11050404

Wang, Z., Wu, G. & Jönsson, L.J. (2018). Effects of impregnation of softwood with sulfuric acid and sulfur dioxide on chemical and physical characteristics, enzymatic digestibility, and fermentability. Bioresource Technology, 247, 200-208. https://doi.org/10.1016/j.biortech.2017.09.081

Wayman, M. & Lora, J.H. (1978). Aspen autohydrolysis: the effects of 2 naphthol and other aromatic compounds. Tappi, 61(6), 55-57.

Wu, F.-C., Tseng, R.-L. & Juang, R.-S. (2009). Initial behavior of intraparticle diffusion model used in the description of adsorption kinetics. Chemical Engineering Journal, 153(1), 1-8. https://doi.org/10.1016/j.cej.2009.04.042

Yamamoto, M., Niskanen, T., Iakovlev, M., Ojamo, H. & van Heiningen, A. (2014). The effect of bark on sulfur dioxide–ethanol–water fractionation and enzymatic hydrolysis of forest biomass. Bioresource Technology, 167, 390-397. https://doi.org/10.1016/j.biortech.2014.06.019

Ziegler-Devin, I., Chrusciel, L. & Brosse, N. (2021). Steam Explosion Pretreatment of Lignocellulosic Biomass: A Mini-Review of Theorical and Experimental Approaches. Frontiers in Chemistry, 9, 705358. https://doi.org/10.3389/fchem.2021.705358

Zimbardi, F., Viggiano, D., Nanna, F., Demichele, M., Cuna, D. & Cardinale, G. (1999). Steam explosion of straw in batch and continuous systems. Applied Biochemistry and Biotechnology, 77(1), 117-125. https://doi.org/10.1385/ABAB:77:1-3:117

Popular science summary

Softwood bark is a by-product of the pulp, paper, and sawmill industries. Today, it is mainly used for heat and power generation and can be considered an underused resource. This thesis investigated the potential conversion of softwood bark into marketable products.

Bioethanol is a biochemical that could be used as a liquid transportation fuel or a green platform chemical for transformation into other products that society demands. Softwood bark possesses a recalcitrance that makes it hard to convert into ethanol via traditional pathways, such as steam explosion, enzymatic hydrolysis, and fermentation. This work investigated a special additive, a so-called carbocation scavenger, that would potentially delimit some negative side reactions during the pretreatment. Unfortunately, no positive effects of the additive addition could be confirmed for bark treatments. The current outlook is that bioethanol from softwood bark is not feasible without further game-changing revelations.

Instead, the bark was turned into a biobased adsorbent, an activated carbon, via a novel process route including steam explosion. The steam explosion process was invented some 100 years ago and is used to produce engineered wood panels. Firstly, the material was steam exploded. The steam explosion opened the bark structure and made it available for the activation chemical zinc chloride. The activated biomass was then carbonized in a pyrolysis process.

The steam explosion improved the final carbon qualities regarding physical and chemical structure. Compared to reference carbons, the carbons were also better adsorbents of reactive orange 16, a dye frequently used by the textile industry but also a pollutant. The bark was revealed as a potential precursor for the manufacture of biobased carbon materials. In addition, including a steam explosion step was highlighted as an interesting option to

produce carbons with high surface area. Such carbons could have applications as adsorbents or in energy storage systems.

When discharging material from the steam explosion step, a blow steam transports the biomass. The steam is then separated from the solid material. This stream contains furfural, a potential by-product the chemical industry uses. Furthermore, its quantity reveals how well the process runs, making its quantity attractive to monitor. As a final step of this project, a model was developed based on Raman spectroscopy and statistical tools to apply to blow steam condensates. With this technology, a laser instrument and detector can be used on condensed blow steam to acquire precise information on the furfural concentration. The finding facilitates rapid and non-destructive measurement of furfural concentration and can potentially be used in online control systems for improved monitoring and process operation.

Populärvetenskaplig sammanfattning

Barrvedsbark är en biprodukt från massa-, pappers- och sågverksindustrin. Idag används den främst för värme- och elproduktion och kan betraktas som en underutnyttjad resurs. Denna avhandling undersökte den potentiella omvandlingen av barrvedsbark till säljbara produkter.

Bioetanol är en biokemikalie som kan användas som ett flytande transportbränsle eller en grön plattformskemikalie för omvandling till andra produkter som samhället efterfrågar. Barrvedsbark har en motståndskraft som gör den svår att omvandla till etanol via traditionella vägar, såsom ångexplosion, enzymatisk hydrolys och jäsning. I detta arbete undersöktes ett speciellt tillsatsmedel, en så kallad karbokatjonfångare, som potentiellt skulle kunna begränsa vissa negativa sidoreaktioner under förbehandlingen. Tyvärr kunde inga positiva effekter av tillsatsmedlet bekräftas för barkbehandlingar. Den nuvarande utsikten är att bioetanol från barrvedsbark inte är genomförbart utan ytterligare banbrytande upptäckter.

I stället omvandlades barken i detta arbete till en biobaserad adsorbent, ett aktivt kol, via en ny processväg som inkluderar ångexplosion. Ångexplosionsprocessen uppfanns för cirka 100 år sedan och används för att producera träpaneler. Först ångexploderades materialet. Ångexplosionen öppnade barkstrukturen och gjorde den tillgänglig för aktiveringskemikalien zinkklorid. Den aktiverade biomassan förkolades sedan i en pyrolysprocess.

Det kunde påvisas att ångexplosionssteget förbättrade de slutliga kolkvaliteterna avseende fysisk och kemisk struktur. Jämfört med icke ångexploderade matrial var kolen också bättre adsorbenter av reactive orange 16, ett färgämne som ofta används av textilindustrin men också en möjlig förorening. Barken visade sig vara en potentiell råvara för tillverkning av biobaserade kolmaterial. Dessutom framhölls inkluderingen av ett ångexplosionssteg som ett intressant alternativ för att producera kol med hög

ytarea. Sådana kol kan ha tillämpningar som adsorbenter eller i energilagringssystem.

Vid utmatning av material från en ångexplosionsbehandling används en blåsånga för att transportera biomassan. Ångan separeras sedan från det fasta materialet. Denna delström innehåller furfural, en potentiell biprodukt som kemisk industri använder. Dessutom avslöjar dess koncentration hur väl processen fungerar, vilket gör dess förekomst attraktiv att övervaka. Som ett sista steg i detta projekt utvecklades en modell baserad på Ramanspektroskopi och statistiska verktyg för att mäta på kondensat från blåsångan. Med denna teknik kan ett laserinstrument och en detektor användas på kondenserad blåsånga för att få exakt information om furfuralhalten. Metoden underlättar snabb och icke-förstörande mätning av furfuralhalten och kan potentiellt användas i uppkopplade kontrollsystem för förbättrad övervakning och processdrift.

Acknowledgments

Several people and organizations have helped make this project possible. First and foremost, I would like to thank my principal supervisor at SLU, Mikael Thyrel, who followed the project from start to finish and provided solid advice and a positive spirit whenever needed. Occasional talks about fly fishing may not have brought the project directly forward but provided a good pause and energy boost.

I thank Sylvia Larsson, previously at SLU, for acting as principal supervisor in the first years and injecting the project with a healthy can-do spirit. I am also grateful to Olof Melander, supervising at Valmet, for keeping the project closely connected to the industry and always leaving time for a discussion if requested.

Some fruitful collaborations must be mentioned. Stefan Stagge and Prof. Leif J. Jönsson at Umeå University provided much-valued help and expertise during the production of Paper II. It was a joy visiting your labs and sharing discussions with you! Prof. Ulla Lassi and Anne Heponiemi, University of Oulu, shared appreciated feedback and gave access to infrastructure and personnel essential for the finalization of Paper III. Also, the discussions with András Gorzás, Umeå University, must be mentioned, which was the true inspiration for the conceptualization of Paper IV. Thank you for all your shared expertise, support, and chats about the latest Arsenal game!

My coworkers at Valmet were also important. Jan Detlefsen operated the pilot equipment skillfully. Ann-Sofi Näsholm, my closest manager, made my time available to conduct this project, which was not always easy. Peter Björklund supported with never-ending ideas. Anna Borgström and Anne Christoffersen, Valmet Karlstad, provided solid support for compositional analyses and chromatography. Thank you for having me visit your lab week after week!

Several people completed important tasks for the project. Special thanks to Gunnar Kalén (SLU) for preprocessing the bark, Magnus Wiman (Novozymes) for enzyme sampling, Stefan Stagge (Umeå University) for assorted analyses in Paper II, Mattias Hedenström (Umeå University) for NMR spectroscopy, András Gorzás (Umeå University) for FTIR in Paper II, Glaydson Simões dos Reis (previously SLU) for guidance during investigation and production of Paper III, Alejandro Grimm (SLU) for help answering reviewers, Davide Bergna (University of Oulu) for BET analyses, Tao Hu (University of Oulu) for XPS, Rafal Sliz (University of Oulu) for FTIR analyses in Paper III and Cheng Choo Lee (Umeå University) for SEM-EDX support.

The utilized platforms are also acknowledged. Thanks to the Biomass Technology Centre (SLU), Vibrational Spectroscopy Core Facility (Umeå University), Swedish NMR Centre (Umeå University) and Centre for Electron Microscopy (Umeå University)

Vinnova, the Swedish Governmental Agency for Innovation Systems; Bio4Energy, the Swedish Energy Agency; and Valmet AB are thanked for providing essential financial support.

Finally, a big thanks to my family! Without your support and understanding, this project would never have been possible!

I

Bioresource Technology Reports 20 (2022) 101292

Contents lists available at ScienceDirect

Bioresource Technology Reports

journal homepage: www.sciencedirect.com/journal/bioresource-technology-reports

Carbocation scavenger pretreatment to mitigate lignin self-condensation in a semi-industrial steam explosion process

Andreas Averheim [a,*], Sylvia H. Larsson [b], Mikael Thyrel [b]

[a] Valmet AB, Fiber Technology Center, SE-851 94 Sundsvall, Sweden
[b] Swedish University of Agricultural Sciences, Department of Forest Biomaterials and Technology, SE-901 83 Umeå, Sweden

ARTICLE INFO

Keywords:
Continuous process
Design of experiments
Enzymatic hydrolysis
Pilot-scale
Softwood bark

ABSTRACT

Softwood bark is a potential resource for biorefineries, abundantly available in the Nordic countries. Meanwhile, carbocation scavenger pretreatment has been suggested as a biorefinery technology beneficial to the enzymatic conversion of softwood. This study aimed to evaluate the effect of 2-naphthol addition for monosaccharide production during enzymatic hydrolysis at high solid loadings of pilot-scale steam-exploded bark mimicking industrial conditions. Multiple linear regression analysis showed that 2-naphthol added as a dry powder with a 5 % dose reduced the hexose hydrolysis recoveries by 0.7 and 0.2 % for glucose and mannose, respectively, while increasing the acid-insoluble and acid-soluble lignin content. The main factors affecting saccharide conversions were reactor temperature and enzyme loading, while acetic acid addition and bark pre-drying had no or little effect. In general, carbocation scavenger addition in the form of 2-naphthol was detrimental to sugar hydrolysis yields, contradicting earlier literature findings.

1. Introduction

Today, softwood bark is mainly combusted for electricity and heat production in the Nordic countries, mainly at the pulp, paper, and sawmill sites where logs are debarked. However, increased pulp mill energy effectivity (Lipiäinen et al., 2022) and the abundant availability of bark on these mill sites have turned softwood bark into an emerging, potential resource for conversion into higher value, renewable end-products. Meanwhile, unit processes such as steam explosion and enzymatic hydrolysis have been researched intensively during the past decades to convert biomass into value-added end-products, such as liquid biofuels (Galbe and Zacchi, 2012), biochemicals, platform chemicals, and polymers (Cheng et al., 2020; Rachamontree et al., 2020), with a beneficial carbon footprint compared to the fossil-based solutions of today.

Patented almost 100 years ago (Mason, 1926), steam explosion was initially a process for producing fibreboard as a physio-chemical method to disintegrate lignocellulosic materials. The treatment is typically conducted in a steam environment at 160–240 °C for 1–20 min (Galbe and Zacchi, 2012), followed by a rapid depressurization. Due to the liberation of acetyl groups from the hemicellulose, the process is slightly acidic, causing autohydrolysis of the hemicellulose. Cellulose is more resistant to mildly acidic conditions during the treatment but will eventually depolymerize if the reaction conditions intensify (Overend and Chornet, 1987). At the same time, the oligo- and monosaccharides created in the degradation process will further dehydrate into hydroxymethylfurfural (HMF) and furfural (Ghoreishi et al., 2022; Li et al., 2005; Rachamontree et al., 2020). The furans contribute to the formation of pseudo-lignin, a by-product that can be detected as an increase in acid-insoluble lignin mass. Although the pseudo-lignin behaves like Klason lignin during quantification, it originates from the degradation of components other than lignin, such as hemicelluloses (Aarum et al., 2019). The native lignin itself is also affected by the steam explosion process, where temperatures typically exceed its glass-transition temperature. A redistribution occurs where lignin has shown evidence of melting and agglomerating during the high-temperature treatment (Donaldson et al., 1988). Lignin, a macromolecule mainly built from three generic units, *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S), also undergoes reactions during steam explosion. The generic units in lignin are connected through a variety of linkages such as β-O-4, β-5, β-β, 5-5, and 5-O-4 (Ralph et al., 2004), where cleavage of β-O-4 linkages in parallel with acid-catalyzed condensation reactions occur during steam explosion (Aarum et al., 2019; Li et al., 2007). By studying aspen wood, it has been suggested that the lignin is modified through reacting benzyl

* Corresponding author.
E-mail addresses: andreas.averheim@valmet.com (A. Averheim), sylvia.larsson@slu.se (S.H. Larsson), mikael.thyrel@slu.se (M. Thyrel).

https://doi.org/10.1016/j.biteb.2022.101292
Received 3 October 2022; Received in revised form 12 November 2022; Accepted 13 November 2022
Available online 17 November 2022
2589-014X/

alcohol structures via a carbonium ion intermediate (Wayman and Lora, 1978) to form stabilized carbon-carbon linkages in the macromolecular structure during acidic heat treatments (Li et al., 2007; Robert et al., 1988). Since the fractionation and condensation reactions occur almost simultaneously, sharing a common intermediate in the carbonium ion (Li et al., 2007), the mechanisms can be denoted as lignin self-condensation.

Softwood bark is a heterogenous resource in terms of its chemical composition and structure, with saccharides present as structural carbohydrates such as cellulose, hemicellulose, and pectin, and non-structural carbohydrates such as starch and monosaccharides (Frankó et al., 2018; Jablonsky et al., 2017; Le Normand et al., 2012). 20–29 % glucan, 12–15 % other polysaccharides, and 28–41 % apparent lignin are some composition ranges for pine and spruce bark reported in the literature (Frankó et al., 2018; Kemppainen et al., 2012; Miranda et al., 2012). Ash contents are typically higher than for stemwood (Miranda et al., 2012). The generous amount of extractives, >20 % reported by some authors (Frankó et al., 2018; Miranda et al., 2012), also distinguishes bark from stemwood, although extracted amounts vary depending on extraction procedure and solvent choice (Jablonsky et al., 2017; Miranda et al., 2012). Characterization of extractives from Norway spruce and Scots pine bark reveals significant fractions of lipophilic extractives, such as fatty and resin acids, and hydrophilic extractives, such as tannins, pectin, and starch (Bianchi et al., 2015; Jablonsky et al., 2017; Kemppainen et al., 2014; Le Normand et al., 2012).

In a study of hot water extraction and steam explosion as pretreatment processes for ethanol production from spruce bark (Kemppainen et al., 2012), hydrolysis yields as high as 84 % at a steam explosion temperature of 190 °C and 5 min residence time was achieved. Meanwhile, a harsher treatment at 205 °C with the addition of sulfuric acid led to lower hydrolysis yields. At the same time, a hot water extraction alone could be sufficient pretreatment if high doses of pectinase were added to the enzyme mix, according to Kemppainen et al. (2012). More recent studies (Frankó et al., 2018; Frankó et al., 2015) utilizing modest enzyme loadings and substrates with and without sulfur dioxide saturation prior to pretreatment received the best glucose yield after enzymatic hydrolysis at one of the harsher process conditions tested (210 °C and 10 min, with 2.5 % SO_2). By studying mixtures of spruce roundwood and spruce bark, it could be concluded that enzymatic conversion of bark was challenging, although fermentability was comparable to the corresponding roundwood.

Softwood is known for its recalcitrance compared with many other lignocellulosic raw materials (Galbe and Zacchi, 2012). Still, the resistance to enzymatic degradation can be overcome by adding highly nucleophilic phenolics, such as 2-naphthol, to the pretreatment (Pielhop et al., 2016). The beneficial effect of the 2-naphthol addition has been attributed to a prohibited lignin condensation (Pielhop et al., 2016), where some aromatics act as scavengers of carbonium ions (carbocation scavengers) under acidic conditions (Wayman and Lora, 1978). The pathway for lignin self-condensation could thus be broken by forming a stabilized, soluble cation by reaction between the carbocation scavenger and the charged lignin fragment. A 2-naphthol addition before pretreatment improved the enzymatic digestibility from 60 % to 86 % for a dilute acid pretreatment of spruce (Pielhop et al., 2017b), while an autohydrolysis of pine reported an increased sugar recovery from 7.6 to 16.2 % based on % initial dry wood (Borrega et al., 2021).

Pre-drying of raw materials could either be intentional, e.g., for preservation purposes to avoid microbial degradation, or unintentional, e.g., during storage at wood yards or chip treatment areas. Shrinkage and collapse of woody materials upon drying are well-known phenomena (Pang, 2002). The collapse of cell walls and pores potentially hinders the diffusion of reaction chemicals such as acids or enzymes. Several authors have investigated the drying of substrates after pretreatment prior to enzymatic hydrolysis (Esteghlalian et al., 2001; Kumar et al., 2012; Luo et al., 2011) and found it obstructive to the enzymatic conversion of biomass. On the other hand, Liu et al., 2017, studied the effect of pre-drying and resoaking of sweet potato vine before steam explosion and found the pre-drying inhibitory to the enzymatic hydrolysis yields, which was attributed to a blockage of steam and hot liquor in the pretreatment due to irreversible collapse of the wood cells and shrinkage of pores (hornification), however sparse information on the effects of pre-drying before pretreatment can be found in the literature.

In this study, softwood bark was processed under industrially relevant process conditions (60 kg/h continuous pilot-scale steam explosion, a feasible mixing technique for large-scale applications, and hydrolyzed at 12 % solids loading without preceding washing of the substrates). The aims were to determine i) how the addition of a carbocation scavenger, 2-naphthol, in the steam explosion reactor affects the recalcitrance of softwood bark to enzymatic degradation, and ii) how pre-drying of softwood bark before the steam explosion affects the composition of the pretreated substrate and the enzymatic hydrolysis.

2. Material and methods

2.1. Raw material

Softwood bark originating from trees harvested in Västerbotten county was collected in February 2019 from a pulp mill in the north-eastern part of Sweden. The average volumetric mix was: 47 % Scots pine (*Pinus sylvestris*), 50 % Norway spruce (*Picea abies*), 1 % Contorta pine (*Pinus contorta*), and 2 % reject wood. The mill's debarking process consisted of defrosting with hot water and tumbling in a debarking drum. The reject fraction, bark with an approximate stemwood content of 15 %, was shredded and pressed to remove excess liquid.

Approximately 40–50 m^3 of shredded and dewatered bark (41 % solids content) was sampled and transported to the Biomass Technology Centre, BTC, in Umeå, Sweden, and shredded in a single shaft shredder (Lindner Micromat 2000, Lindner-Recyclingtech GmBh, Spittal, Austria) with a 30 mm diameter screen size.

To avoid blockages in the continuous steam explosion pilot's discharge system and ensure a smooth operation, oversized particles were removed by screening the shredded material with a 14 mm mesh screen (Mogensen G-Sizer, Mogensen, Hjo, Sweden). The dried bark assortment (82 % solids content) was prepared by blowing warm air (40 °C) through shredded and screened bark in a flatbed dryer.

As the last step before the steam explosion, high-density particles such as sand and gravel were removed from the bark by pneumatically transporting the material through an in-house constructed stone trap at Valmet AB, Sundsvall, Sweden.

2.2. Chemicals

Before the steam explosion experiments, process chemicals were prepared by diluting 98–99 % glacial acetic acid (Swed Handling AB, Norrköping, Sweden) to 25 g/l. The 2-naphthol was prepared by hammer-milling 2-naphthol flakes, 98 % (Thermo Fisher Scientific), to a particle size <2 mm.

2.3. Steam explosion equipment

The steam explosion experiments were conducted in a continuous (60 kg/h) pilot system (Valmet BioTrac, Valmet AB, Sundsvall, Sweden) (Fig. 1). The pilot set-up consisted of a feeding system, a horizontal tube reactor, and a discharge system. A discharge screw that controls a constant volumetric flow from the feedstock buffer bin continuously fed a belt conveyor, followed by two screw conveyors into which 2-naphthol powder, diluted acetic acid, and water were accurately dosed. After that, the material entered a plug screw that sealed and maintained the pressure in the reactor. The steam treatment's residence time was controlled by the rotational speed of an auger conveyor inside the pressurized reactor. Saturated steam was used for reactor heating, and the

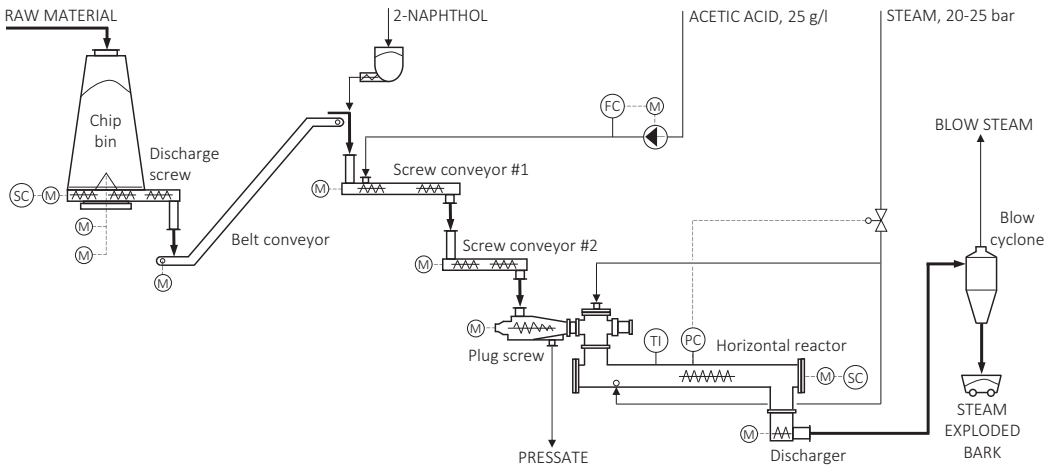

Fig. 1. Simplified process flow diagram of the continuous steam explosion system.

temperature was adjusted by controlling the pressure. Temperature, as well as pressure, was measured at the reactor top. The treated biomass was ejected through a pressure-reducing orifice at the reactor outlet. The dramatic volume expansion of the steam accelerated the pretreated material through a blow line to an atmospheric discharge cyclone.

2.4. Experimental designs

Design of experiments (DoE) was used to set up the steam explosion and enzymatic hydrolysis trial. The steam explosion experiments were performed in two experimental screening designs: A) and B).

In A), the bark's solids content was kept at 41 % (i.e., as received). The experiments were performed according to a full-factorial design with three factors. The reactor temperature was set at three levels (180, 190, and 200 °C), 2-naphthol addition at two levels (0 and 5 %), and acetic acid dosage at two levels (0 and 0.5 %). In addition, three center point experiments were performed (temperature: 190 °C, 2-naphthol: 2.5 %, and acetic acid: 0.25 %). Fifteen steam explosion experiments were conducted in design A) (Table 1).

In B), a (2^4) full-factorial design with two factors: reactor temperature (180, 190, 200, and 205 °C), and total solids of the raw material (41 and 82 %) was employed, adding up to 8 steam explosion in design B) (Table 1).

The residence time in the reactor was kept constant at 10 min for all experiments ($\log R_0$: 3.4, 3.6, 3.9, and 4.1), where $\log R_0$ is the severity factor as defined by Overend and Chornet (1987). The overlapping of the A) and B) designs resulted in 20 different steam explosion settings (Table 1).

For the enzymatic hydrolysis, enzyme loadings were 2, 4, and 10 % in design A) and 2 and 10 % in design B).

2.5. Steam explosion experiments

The 20 steam explosion experiments were performed over 3 days. The A) design's center point experiments were performed at the test series' beginning, middle, and end. At the start of each experimental day, the pilot system was run continuously for at least 1 h to assure steady-state process conditions before starting the tests. When changing the settings, the pilot system was run for 40 mins before sampling.

Before each experiment, approximately 1 l of the bark feedstock was sampled for moisture content analysis. For each experimental day, the dried samples were pooled and stored for compositional analysis (3 samples in total). For each steam explosion experiment, one general sample (9–10 kg total solids) was collected during a 10 min period at the outlet from the atmospheric discharge cyclone. Approximately 0.1 kg of each sample was gently dried for compositional analysis, and 2–3 kg was frozen for enzymatic hydrolysis.

Table 1
Experimental design for steam explosion. Chemical additions are expressed as a percentage of dry biomass feed (% on total solids).

Experiment	Reactor temperature	2-naphthol addition	Acetic acid addition	Total solids, raw material
	(°C)	(%)	(%)	(%)
A1/B1	180	0	0	41
A2/B2	190	0	0	41
A3/B3	200	0	0	41
B4	205	0	0	41
A4	180	0	0.5	41
A5	190	0	0.5	41
A6	200	0	0.5	41
A7	180	5	0	41
A8	190	5	0	41
A9	200	5	0	41
A10	180	5	0.5	41
A11	190	5	0.5	41
A12	200	5	0.5	41
A13	190	2.5	0.25	41
A14	190	2.5	0.25	41
A15	190	2.5	0.25	41
B5	180	0	0	82
B6	190	0	0	82
B7	200	0	0	82
B8	205	0	0	82

2.6. Compositional analyses of raw material and steam-exploded biomass

The total solids (Sluiter et al., 2008a) and water-insoluble solids (Sluiter et al., 2008c) of the raw material and steam-exploded material were analyzed according to laboratory analytical procedures issued by the National Renewable Energy Laboratory (NREL), as well as the determination of structural carbohydrates and lignin (Hames et al., 2008) (Sluiter et al., 2008b). Acid-soluble lignin (ASL) was quantified at 205 nm (Agilent Cary UV-100) with an extinction coefficient of 110 l/g

cm. Ash content was analyzed following the International Organization for Standardization (2015), ISO.

Acetone extraction of the raw material was conducted on the milled raw materials before determining structural carbohydrates and lignin. The extraction was performed in a Soxtec Avanti 2050 extraction unit with analytical grade acetone, 30 min of boiling, and a 120-min cycle.

Sugars were finally measured using ion chromatography (Dionex ICS-3000) utilizing pulsed amperometric detection for quantification. A stationary phase, CarboPac PA-1 precolumn and analysis column, and a mobile phase, 200 mM NaOH, separated the individual carbohydrates before detection.

2.7. Enzymatic hydrolysis

Enzymatic hydrolysis of the pretreated biomass was performed in an incubation oven (Incucell, MMM Group) at 50 °C ± 0.2 °C using 50 ml test tubes (Sarstedt) rotating at 25 rpm (Intelli-Mixer RM-2, ELMI) with a slurry weight of 20 g and 12 % total solids load. The enzyme, Cellic CTec3 HS (Novozymes, Bagsværd, Denmark), was dosed to the substrates that had been pH-adjusted to pH 5.2–5.4 with 2 M KOH. Hydrolysis was conducted with a 0.05 M citric acid/citrate buffer solution and three cylindrical stainless-steel weights (SS 2343, 3–4 g each) per tube to ensure proper mixing. The hydrolysis experiments proceeded for 96 h. The samples from the hydrolysis liquid were diluted 8–10 times with deionized water and separated in a centrifuge (Hemle Z306) with a swing-out rotor at 4500 rpm, 3350 × *g*, for 15 min. The supernatant was passed through 0.45 µm filters (Whatman Spartan 30/0.45 RC) and stored at −20 °C before being analyzed for carbohydrate composition. The monomeric sugars were measured using ion chromatography, as described in Section 2.6, but the autoclavation step was omitted. The hydrolysis experiments were conducted in triplicate, and one of the samples was repeated as an internal standard within each processed sample set. In total, 207 hydrolysis samples were processed and analyzed for monomeric carbohydrate composition.

Glucose and the summarised total monomeric carbohydrates after enzymatic hydrolysis at low (2 %), medium (4 %), and high (10 %) enzyme loadings were studied as responses to the controlled factors. These were calculated according to:

$$F_{X/TS} = 100 \times ((m_X - m_{Xb})/m_{TS}) \quad (1)$$

with

$$m_X = c_X \times D \times 1000/\rho_L \quad (2)$$

where $F_{X/TS}$ (%) denotes the fraction of monomeric sugar after hydrolysis as a fraction of the total solids fed to hydrolysis (with X as either arabinose, galactose, glucose, mannose, rhamnose, or xylose). The measured concentration of individual carbohydrates in the diluted supernatant, c_X (g/l), dilution factor D (g/g), and density of the diluted liquid, ρ_L (kg/m^3), is used for calculation, assuming that the volume of the removed insoluble solids is negligible. In Eq. (1), m_{tot} (g) corresponds to the total hydrolysis weight, m_{TS} (g) the total solids used in hydrolysis, and m_{Xb} (g), the amount of individual carbohydrate in a blank sample with corresponding enzyme addition.

2.8. Data modeling

The designs were evaluated by multiple linear regression (MLR) using the MODDE Pro 13 software package. Models for the steam explosion and enzymatic hydrolysis experimental responses, including factors, interactions, and quadratic terms, were derived from the general MLR model equation:

$$\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{e} \quad (3)$$

where $\boldsymbol{y}$ denotes the vector of observed responses, $\boldsymbol{X}$ the experimental design matrix in coded variables, $\boldsymbol{b}$ the coefficient vector, and $\boldsymbol{e}$ the residual vector. The Equation was solved by minimizing the difference between measured and predicted values through a least-squares estimate of $\boldsymbol{b}$:

$$\boldsymbol{b} = (\acute{\boldsymbol{X}}\boldsymbol{X})^{-1}\acute{\boldsymbol{X}}\boldsymbol{y} \quad (4)$$

and refined by excluding insignificant coefficients identified by the Student's *t*-test on a 95 % significance level ($p < 0.05$). Furthermore, the regression models were tested for model residuals:

$$\boldsymbol{e} = \boldsymbol{y} - \hat{\boldsymbol{y}} \quad (5)$$

where $\hat{\boldsymbol{y}}$ denotes the modeled response vector:

$$\hat{\boldsymbol{y}} = \boldsymbol{Xb} \quad (6)$$

The residuals were compared to the normal probability of the distribution to determine whether the residuals contained any vital information missing from the model and check for potential outliers. Outlier identification was performed in an observed vs. predicted plot to investigate the magnitude of the deviation for the significant outliers.

Finally, an analysis of variance was conducted. The respective model errors were tested against pure replicate error with the F-test ($p < 0.05$). The data variation explained by the models ($R2$) and their predictive ability ($Q2$) was calculated according to:

$$R2 = 1 - SS_{res}/SS_{tot} \quad (7)$$

and

$$Q2 = 1 - SS_{pre}/SS_{tot} \quad (8)$$

where SS_{res} is the sum of squares of the model residuals, SS_{tot} *is* the total sum of squares corrected for the mean, and SS_{pre} is the prediction sum of squares by the leave-one-out cross-validation method (Eriksson et al., 2008). Models with an $R2$ higher than 0.7, $Q2$ higher than 0.5, and an $R2$-$Q2$ difference smaller than 0.2 were considered good. Furthermore, reproducibility of at least 0.5 was considered acceptable when analyzing the established models.

3. Results and discussion

3.1. Chemical composition of raw material and steam-exploded substrates

The compositional data of the bark feedstock and the steam-exploded substrates are summarised in Table 2. Data for individual substrates are found in the *E*-supplementary data. The raw material contained 32.0 % lignin and 50.3 % structural carbohydrates (31.6 % glucan, 5.6 % mannan, 5.5 % arabinan, and 4.2 % xylan). About 92 % of the bark composition was characterized, whereas the remaining 8 % could consist of, for example, galacturonan (Le Normand et al., 2021), suberin (Miranda et al., 2012), acetyl, and other small fraction compounds not picked up by the acetone extraction or lignin analysis. A two-stage sequential extraction of pine bark with acetone and water (Valentín et al., 2010) showed that a substantial amount (13.7 %) of the bark could be extracted with water in the second extraction stage. Uncharacterized water extractives could also explain the missing parts in the composition specification presented in Table 2.

The saccharide contents in steam-exploded substrates are reported based on their anhydro-monomeric mass and summarize the total mono- and polysaccharide content for each saccharide (Table 2). Steam explosion increased the acid-insoluble lignin (AIL) fraction to 40.3–46.7 % compared to the feedstock material, while the summarised fraction of glucan, gluco-oligomers, and glucose remained similar to the glucan in the raw material (30.3–33.2 %). The content of other saccharides was generally reduced, and the rhamnose levels approached their lower detection limit. Rhamnose was thus not considered for modeling or further evaluations.

The individual hemicellulosic saccharide compositions plotted

Table 2
Composition of raw material and steam explosion pretreated materials as a percentage of total solids (% of total solids). Saccharides include summarised poly- and monosaccharides reported based on their anhydro-monomeric mass.

Compounds	Softwood bark	Pretreated material
Carbohydrates		
Arabinose	5.5 ± 0.2	0.7–4.8
Galactose	3.0 ± 0.1	1.8–3.1
Glucose	31.6 ± 0.7	30.3–33.2
Mannose	5.6 ± 0.2	4.2–5.6
Rhamnose	0.6 ± 0.1	0.1–0.6
Xylose	4.2 ± 0.0	2.5–4.3
Lignin		
AIL	30.2 ± 0.2	40.3–46.7
ASL	1.8 ± 0.2	1.3–2.4
Other		
Acetone extractives	7.1 ± 0.0	
Ash content	2.7 ± 0.1	2.4–2.8

against reactor temperature (Fig. 2) indicates the rate of their degradation into by-products other than monosaccharides. Arabinose stands out as the structural carbohydrate with the highest decomposition rate, approaching an almost complete removal at 205 °C. Research on Norway spruce inner bark (Le Normand et al., 2021) has shown that arabinan, together with galacturonic acid, are the main constituents in bark pectin, and the pectin amount in an industrial bark collected from a paper mill after the debarking process was found to be as high as 10.5 % (Le Normand et al., 2012). It is reasonable to believe that a large portion of the arabinan characterized in the raw material is structurally bound as pectin rather than hemicellulose. Pectin can be extracted by relatively mild hot-water extraction procedures (100–160 °C) (Le Normand et al., 2014; Le Normand et al., 2021), which explains the difference in degradation kinetics between the individual polysaccharides and the rapid degradation of arabinan observed.

3.2. Sugar recovery from enzymatic hydrolysis

An enzyme loading of 2 and 4 % on total solids was utilized in this study to investigate the process under potentially feasible conditions. In comparison, the 10 % loading was implemented from a more theoretical point of view to explore the maximum potential of the substrates. The enzyme load of 4 % on total solids was in the same range as the enzyme dosage applied by Frankó et al., 2018 (5 % on water-insoluble solids, WIS) for treatments of softwood bark. On the other hand, the lower enzyme addition of 2 % was utilized in this study to investigate if the application of carbocation scavenger could decrease enzyme consumption with maintained sugar recoveries.

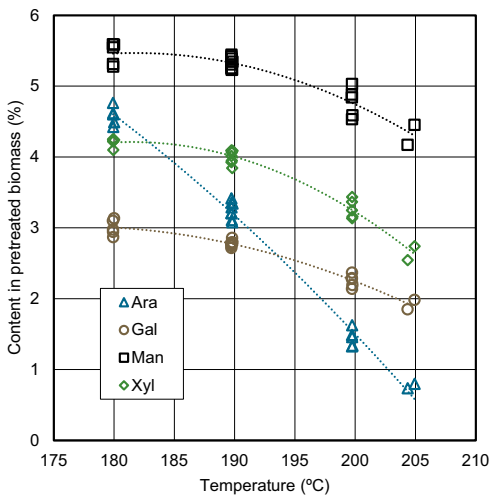

Fig. 2. Total hemicellulosic saccharide contents in steam-exploded bark vs. steam explosion temperature (dotted lines as a guide for the eye). Saccharides include summarised poly- and monosaccharides reported based on their anhydro-monomeric mass. Ara: arabinose, Gal: galactose, Glu: glucose, Man: mannose, Xyl: xylose.

The monomeric carbohydrate contents after enzymatic hydrolysis are summarised in Table 3. The glucose production ranged from 7.0 to 19.4 % based on total solids fed to enzymatic hydrolysis, corresponding to 22 to 57 % theoretical yield. Only at 10 % enzyme addition, and pretreatment temperatures above 200 °C, did the theoretical yields exceed 50 %. At 4 % enzyme loading, the theoretical glucose yield reached up to 42 % for the substrates processed at 200 °C steam explosion temperature and 0.5 % acetic acid dosage, corresponding to a production of 14.7 % monomeric glucose based on total solids fed to hydrolysis. This production level corresponds to 12.2 % glucose, 1.6 % xylose, and 1.2 % mannose based on the mass of dry bark fed to steam explosion, assuming a total solids yield of 83 %. Obtaining good data from gravimetrical mass balances utilizing this continuous pilot system would require a much longer duration of sampling at each process condition. However, extending the trial to obtain this data was not a prioritized option.

The theoretical glucose yield from softwood bark achieved in this study, ranging from 32 to 42 % at a 4 % enzyme addition, was in line with the findings by Frankó et al. (2018) but far from the yields reported by Kemppainen et al. (2012). However, Kemppainen et al. (2012) used substrates washed after pretreatment and a low solids loading (1 % WIS concentration) in enzymatic hydrolysis. This configuration would reduce inhibition of the enzymes from the substrate solid and liquid phase (Jönsson and Martín, 2016) but at the same time drastically reduce the potential feasibility of the process. In addition, the method for yield determination, DNS reducing sugars, was recognized as uncertain by the authors and was later shown less reliable when working with pretreated slurries (Deshavath et al., 2020).

In this study, monomeric arabinose, galactose, mannose, and xylose recovery from enzymatic hydrolysis ranged from 0.2 to 3.0 % (Table 3). Potentially the hydrolysis yields presented here could be improved somewhat by adding a hot-water extraction step before the steam explosion, as shown beneficial by other researchers (Frankó et al., 2018; Kemppainen et al., 2012).

3.3. Modeling of substrate compositions after steam explosion and sugar recoveries from enzymatic hydrolysis

A principal component analysis (PCA) of the steam explosion experimental settings (Table 1) showed that the trial plan had been accurately fulfilled. However, the PCA revealed that experiment A9 (200 °C, 5 % 2-naphthol, 0 % acetic acid) proved an outlier due to deviating feed settings, thus removed from further evaluations.

In total, 26 models were considered, eight explaining the composition of steam-exploded substrates and five predicting the sugar recoveries from enzymatic hydrolysis for each experimental design. Model diagnostics (Table 4) revealed that good, or excellent models for arabinose, galactose, mannose, xylose, and lignin contents in the substrate after steam explosion could be obtained ($R2$: 0.84–0.99, $Q2$: 0.71–0.98). The models predicting glucose and ash content were poor, mainly due to similarities between the samples, regardless of treatment condition.

The monosaccharide production at a 2 % enzyme dosage was modest, and a significant model for glucose could not be obtained. Thus, this dosage level was excluded from further modeling, instead focusing on the 4 and 10 % enzyme loadings. Design A) with temperature, 2-naphthol, and acetic acid as factors could be modeled with enzyme loading as an additional model factor (4 and 10 %), while design B) with

Table 3
Monomeric carbohydrates, after enzymatic hydrolysis, are reported based on their monomeric mass as a fraction of total solids fed to enzymatic hydrolysis (% of total solids). Average values lacking standard deviation are based on duplicates due to the removal of outliers. Ara: arabinose, Gal: galactose, Glu: glucose, Man: mannose, Xyl: xylose.

	2 % enzyme addition					4 % enzyme addition					10 % enzyme addition				
	Ara	Gal	Glu	Man	Xyl	Ara	Gal	Glu	Man	Xyl	Ara	Gal	Glu	Man	Xyl
A1/	1.8 ±	0.4 ±	10.1 ±	0.5 ±	1.3 ±	2.1 ±	0.4 ±	12.1 ±	0.9 ±	1.5 ±	2.5 ±	0.7 ±	15.4 ±	1.7 ±	1.8 ±
B1	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.3	0.0	0.0
A2/	1.9 ±	0.5 ±	10.7 ±	0.7 ±	1.8 ±	2.0 ±	0.6 ±	13.2 ±	1.2 ±	2.0 ±	2.3 ±	0.9 ±	15.7 ±	2.1 ±	2.2 ±
B2	0.0	0.0	0.3	0.0	0.0	0.1	0.0	0.5	0.1	0.1	0.0	0.0	0.4	0.1	0.1
A3/	1.1 ±	0.5 ±	9.3 ±	0.8 ±	1.4 ±	1.2 ±	0.7 ±	13.2 ±	1.4 ±	1.8 ±	1.2 ±	1.1 ±	18.3 ±	2.6 ±	2.1 ±
B3	0.0	0.0	0.4	0.1	0.1	0.0	0.0	0.3	0.1	0.0	0.0	0.0	0.3	0.0	0.0
B4	0.8 ±	0.6 ±	10.5 ±	0.8 ±	1.3 ±						0.7 ±	1.0 ±	18.1 ±	2.3 ±	1.7 ±
	0.1	0.1	0.4	0.1	0.1						0.0	0.0	0.1	0.0	0.0
A4	1.9 ±	0.4 ±	9.8 ±	0.5 ±	1.3 ±	2.1	0.5	12.5	0.9	1.5	2.4 ±	0.7 ±	14.6 ±	1.7 ±	1.7 ±
	0.0	0.0	0.1	0.0	0.0						0.0	0.0	0.1	0.0	0.0
A5	2.1	0.5	11.0	0.7	1.8	2.2 ±	0.6 ±	13.4 ±	1.2 ±	2.0 ±	2.5 ±	0.9 ±	16.9 ±	2.4 ±	2.5 ±
						0.1	0.1	0.8	0.1	0.1	0.1	0.1	0.5	0.2	0.3
A6	1.4 ±	0.7 ±	11.3 ±	0.9 ±	1.8 ±	1.4 ±	0.8 ±	14.7 ±	1.4 ±	1.9 ±	1.5 ±	1.1 ±	18.6 ±	2.4 ±	2.2 ±
	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.1	0.0	0.0
A7	1.8 ±	0.4 ±	9.3 ±	0.4 ±	1.3 ±	2.0 ±	0.5 ±	11.5 ±	0.7 ±	1.5 ±	2.3 ±	0.7 ±	13.8 ±	1.6 ±	1.8 ±
	0.0	0.0	0.2	0.0	0.1	0.1	0.0	0.6	0.0	0.1	0.0	0.0	0.2	0.0	0.0
A8	1.9 ±	0.4 ±	9.9 ±	0.6 ±	1.8 ±	2.1 ±	0.6 ±	13.0 ±	1.2 ±	2.0 ±	2.3 ±	0.9 ±	15.7 ±	2.1 ±	2.3 ±
	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.4	0.0	0.1
A10	1.8 ±	0.2 ±	8.7 ±	0.3 ±	1.2 ±	2.0 ±	0.3 ±	11.4 ±	0.8 ±	1.4 ±	2.3 ±	0.6 ±	13.7 ±	1.5 ±	1.7 ±
	0.1	0.0	0.3	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.1	0.1	0.2	0.2	0.2
A11	2.0	0.5	9.2	0.5	1.7	2.2 ±	0.6 ±	13.3 ±	1.0 ±	2.0 ±	2.3 ±	0.8 ±	15.2 ±	1.8 ±	2.2 ±
						0.0	0.0	0.4	0.0	0.0	0.1	0.0	0.1	0.2	0.0
A12	1.3 ±	0.5 ±	10.4 ±	0.7 ±	1.6 ±	1.4 ±	0.6 ±	13.4 ±	1.2 ±	1.7 ±	1.4 ±	0.9 ±	17.3 ±	2.1 ±	1.9 ±
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.2	0.0	0.0
A13	2.0 ±	0.4 ±	9.4 ±	0.4 ±	1.6 ±	2.1	0.5	12.5	0.9	1.8	2.3 ±	0.7 ±	15.2 ±	1.7 ±	2.0 ±
	0.1	0.0	0.3	0.0	0.1						0.1	0.0	0.7	0.1	0.1
A14	1.8 ±	0.4 ±	8.8 ±	0.4 ±	1.6 ±	2.1 ±	0.6 ±	13.1 ±	1.0 ±	1.9 ±	2.3 ±	0.8 ±	15.6 ±	1.8 ±	2.1 ±
	0.1	0.0	0.6	0.1	0.1	0.0	0.0	0.2	0.0	0.0	0.1	0.0	0.5	0.1	0.0
A15	2.0 ±	0.5 ±	10.2 ±	0.6 ±	1.8 ±	2.2 ±	0.7 ±	13.6 ±	1.1 ±	2.0 ±	2.4 ±	0.9 ±	15.9 ±	2.1 ±	2.2 ±
	0.1	0.0	0.5	0.1	0.1	0.1	0.0	0.6	0.0	0.1	0.1	0.0	0.3	0.1	0.1
B5	2.5 ±	0.5 ±	10.4 ±	0.6 ±	1.6 ±						3.0 ±	0.9 ±	15.1 ±	1.8 ±	2.0 ±
	0.0	0.0	0.4	0.0	0.0						0.0	0.0	0.3	0.0	0.0
B6	2.3 ±	0.5 ±	10.5 ±	0.6 ±	1.7 ±						2.5 ±	0.9 ±	16.1 ±	2.0 ±	2.2 ±
	0.0	0.0	0.6	0.1	0.1						0.1	0.0	0.5	0.0	0.1
B7	1.3 ±	0.7 ±	11.0 ±	0.9 ±	1.7 ±						1.3 ±	1.1 ±	17.6 ±	2.3 ±	2.0 ±
	0.0	0.0	0.2	0.0	0.0						0.0	0.0	0.1	0.0	0.0
B8	0.8 ±	0.8 ±	11.5 ±	1.0 ±	1.4 ±						0.7 ±	1.1 ±	19.4 ±	2.3 ±	1.7 ±
	0.0	0.0	0.2	0.0	0.0						0.0	0.0	0.2	0.0	0.0

Table 4
Model diagnostics for the modeled responses; substrate composition as summarised poly- and monosaccharides after steam explosion (SE) and monosaccharide recovery from enzymatic hydrolysis (EH).

Design:		A)		B)	
Modeled response	Process	*R2*	*Q2*	*R2*	*Q2*
Arabinose	SE	0.99	0.98	0.99	0.98
	EH	0.99	0.98	0.92	0.85
Galactose	SE	0.95	0.92	0.93	0.88
	EH	0.94	0.91	0.79	0.57
Glucose	SE	0.19	−0.06	0.06	−0.61
	EH	0.95	0.92	0.89	0.79
Mannose	SE	0.88	0.77	0.87	0.75
	EH	0.94	0.93	0.79	0.67
Xylose	SE	0.96	0.93	0.98	0.95
	EH	0.86	0.81	0.91	0.73
ASL	SE	0.92	0.86	0.94	0.85
AIL	SE	0.84	0.71	0.98	0.94
Ash	SE	0.35	0.16	0.85	0.61

temperature and raw material total solids as factors was modeled strictly at the 10 % enzyme addition level. Good to excellent models were produced (*R2*: 0.86–0.99 and *Q2*: 0.81–0.98) for experimental design A) with enzyme loading as a model factor (Table 4). Experimental design B) yielded good models for arabinose, glucose, mannose, and xylose production from enzymatic hydrolysis (*R2*: 0.92–0.79 and *Q2* 0.79–0.67). However, the galactose model was weaker, with a slightly too high difference between *R2* and *Q2*. Galactose was, apart from rhamnose, the saccharide with the lowest concentration in the hydrolysis liquid, making it more exposed to statistical noise, which might explain the model's weakness.

The model coefficients for design A) are found in the *E*-supplementary material and for design B) in Table 5. Coefficients are only presented for models considered good (defined in Section 2.8).

Table 5
Model coefficients for the experimental design B). RTS: raw material total solids, T: steam explosion temperature.

Modeled response		β_0	β_1 (RTS)	β_2 (T)	β_{22} (T × T)
Arabinose	SE	2.6		−2.0	
	EH	1.9		−1.1	
Galactose	SE	2.6		−0.6	
Glucose	EH	16.8		1.9	
Xylose	SE	3.8		−0.8	−0.4
	EH	2.2		−0.1	−0.4
Mannose	SE	5.0		−0.6	
	EH	2.1		0.3	
ASL	SE	1.8	0.2	0.3	
AIL	SE	42.5		3.5	1.5

3.4. Influence of temperature, 2-naphthol, and acetic acid on lignin content and sugar recovery

The modeled relationships between lignin contents in pretreated substrates versus temperature and 2-naphthol dosage are displayed in Fig. 3. Although the temperature was the predominating factor in the studied design range, both acid-insoluble and acid-soluble lignin content increased with increasing 2-naphthol addition (Fig. 3). A study of lignin self-condensation during autohydrolysis of softwood (Pielhop et al., 2017b) noted that ASL increased during treatments with carbocation scavenger compared to control samples; however, AIL was more or less considered unchanged. The increased AIL content observed in this study when adding 2-naphthol to pretreatment has not been investigated further. However, one possible cause might be unwanted 2-naphthol reactions yielding acid-insoluble condensation products, for example, reaction with tannins found in softwood bark, or possibly, the increased apparent AIL is due to unreacted 2-naphthol adding to the acid-insoluble fraction. Pielhop et al. (2017b) applied extensive washing to the substrates before AIL measurement, whereas this study focused on whole-slurry treatments, which might leave more traces of 2-naphthol in the solid phase.

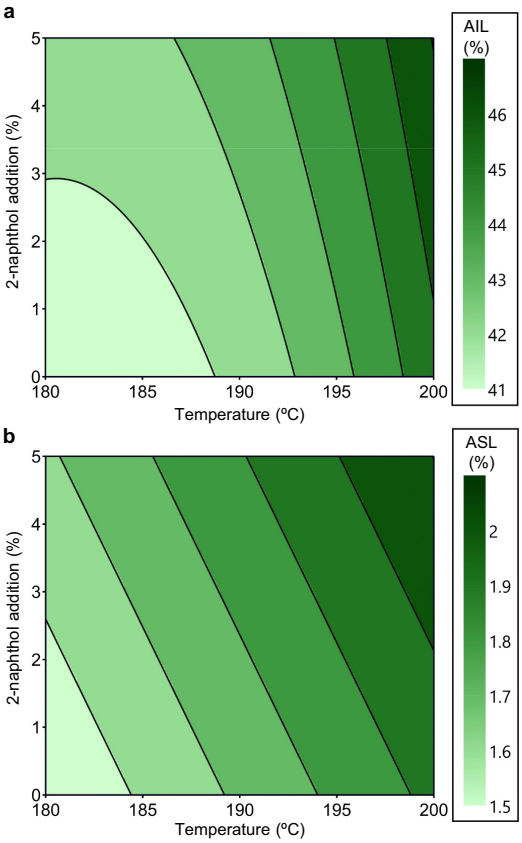

Fig. 3. Contour plots showing modeled values for a) AIL and b) ASL content in steam-exploded bark at different 2-naphthol addition levels (% on raw material total solids) and steam explosion temperatures and an acetic acid load of 0.25 % on raw material total solids.

On the other hand, the increase of ASL upon scavenger addition was attributed to an increased UV-absorption due to the integration of 2-naphthol into the lignin structure and potentially an increase in lignin solubility (Pielhop et al., 2017b). However, ASL measurements are also sensitive to other system changes, for example, interferences in the UV spectra from other compounds (Hatfield and Fukushima, 2005). Extensive conclusions will not be drawn based on the small changes observed (Fig. 3).

For sugar recoveries from enzymatic hydrolysis in general, steam explosion temperature had a significant impact and was, apart from the enzyme loading, the predominant factor explaining the production of monosaccharides. The modeled level of glucose after enzymatic hydrolysis, shown as a contour plot at a fixed acetic acid addition of 0.25 % (Fig. 4), displays a positive impact from increased temperature and enzyme loading. Additionally, there is a beneficial impact from the interaction between steam explosion temperature and the enzyme loading, which could be explained by an increased enzymatic availability for the cellulose with increased temperature within the tested range. The contours (Fig. 4) also show a slightly negative effect on glucose recovery (0.7 % at $\log R_0$ 3.4–3.9 and enzyme loadings 4–10 %) with an increasing 2-naphthol addition which contradicts previous findings reported in the literature on softwoods (Borrega et al., 2021; Hansen et al., 2022; Pielhop et al., 2017a; Pielhop et al., 2016; Pielhop et al., 2017b; Seidel et al., 2019). To investigate an industrially feasible approach, this study has focused on a simplified 2-naphthol dosage, a steam explosion severity that aims to prevent carbohydrate degradation, omission of washing steps after the steam explosion, and reasonable solids loading during enzymatic hydrolysis (12 % total solids). In part, this has also been investigated by others.

During the early pioneering efforts to utilize carbocation scavengers in pretreatment (Pielhop et al., 2017a; Pielhop et al., 2016; Pielhop et al., 2017b), enzymatic hydrolysis was conducted at 1 % (*w*/w) cellulose concentration on washed substrates. More recent work by other authors (Borrega et al., 2021; Hansen et al., 2022; Seidel et al., 2019) was conducted at higher solids loading in the enzymatic hydrolysis stage. Borrega et al. (2021) applied 5 % WIS concentration on washed substrates from pine and showed an apparent positive effect of the scavenger addition at $\log R_0$ above 4.4 with an increase in sugar recovery from 7.6 to 16.2 %. Hansen et al. (2022) performed whole-slurry enzymatic treatments of pretreated spruce at 10 % total solids and noted a positive effect of scavenger addition at severities above 4.0. However, the improvement in hydrolysis yield was slight at severity 4.2 but drastic at severity 4.5. By application of 2-naphthol as a carbocation scavenger, the authors reported a complete enzymatic glucan conversion compared to <70 % for the control ($\log R_0$: 4.5, enzyme loading: 16 mg protein/g total solids). Seidel et al. (2019), on the other hand, conducted a co-fermentation with simultaneous saccharification and fermentation (SSF) set-up on softwood pretreated with 2-naphthol and detected a lower ethanol yield when the treatment was performed at 5 % cellulose concentration compared to 1 %, attributed to sensitivity to inhibitor concentration when 2-naphthol was present in the slurries. Although the saccharification yields benefitted from the carbocation scavenger treatment, the fermentability was very poor when 2-naphthol was present in the more concentrated slurries.

A pilot-scale study (Pielhop et al., 2017a) investigated different ways of adding 2-naphthol to the biomass before pretreatment. 2-naphthol was either dissolved in a solvent or directly mixed as a powder with the biomass. Both strategies improved the glucose yields from enzymatic hydrolysis, although dissolving in solvent was more efficient for increasing the saccharification yields. The authors reported a cellulose digestibility for spruce of 52.6 % (2-naphthol mixed directly with biomass), 83.7 % (2-naphthol in ethanol), and 33.9 % for the control ($\log R_0$: 5.3, enzyme loading: 30 FPU/g cellulose). The applied mixing strategy (adding 2-naphthol directly to biomass) in combination with a high liquid-to-wood ratio in the pretreatment reactor has similarities to this present study. However, Pielhop et al. (2017a) described that the

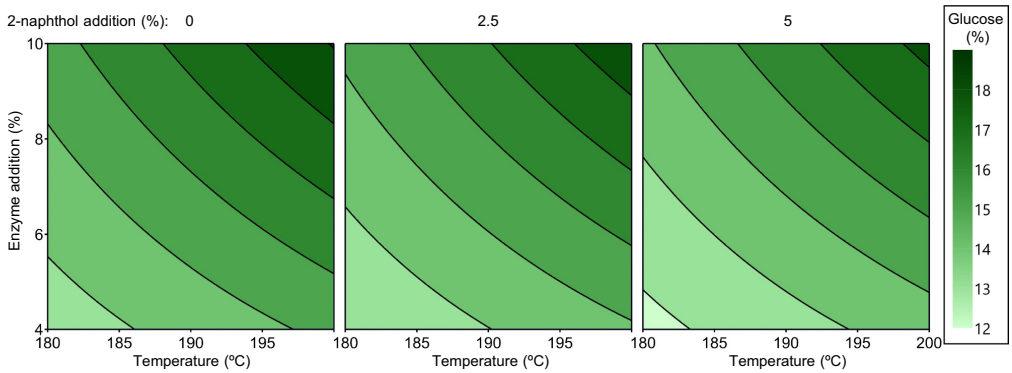

Fig. 4. Contour plots showing modeled levels of glucose (% of substrate solids) vs. steam explosion temperature and enzyme dosage (% on substrate solids) at three different 2-naphthol dosages (% on raw material solids) and an acetic acid load of 0.25 % on raw material total solids.

pretreated slurries needed filtering, which suggests a higher pretreatment liquid-to-solids ratio than applied within this study (~1.5:1).

The effect on hemicellulosic saccharide yields from 2-naphthol addition, on the other hand, was insignificant or minimal (Table 3). Mannose was the hemisaccharide affected the most by the 2-naphthol addition with 0.2 % lower recovery based on substrate total solids. Fig. 5 shows contour plots of the modeled levels of arabinose, galactose, mannose, and xylose after enzymatic hydrolysis at a fixed 2-naphthol and acetic acid addition (2.5 and 0.25 %, respectively). Increasing the temperature from 180 to 200 °C positively impacted galactose and mannose recovery and negatively affected the arabinose content. Xylose levels reached a maximum between 190 and 195 °C. The lower recovery of arabinose at 200 °C was due to degradation in the steam explosion stage (Fig. 2).

It can be deduced from the available information that high severities are required to benefit from the carbocation scavenger treatment.

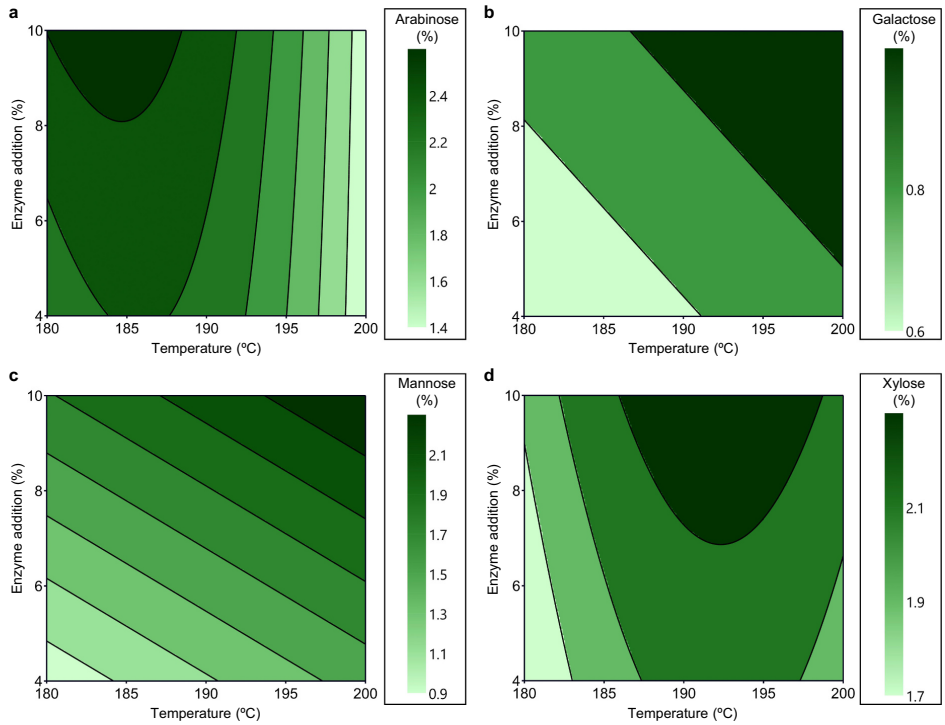

Fig. 5. Contour plots showing modeled levels of arabinose, galactose, mannose, and xylose (% of substrate total solids) vs. steam explosion temperature and enzyme dosage (% on substrate total solids).

However, it has been concluded (Pielhop et al., 2017b) that the beneficial effect of 2-naphthol addition strongly depends on the type of biomass feedstock utilized. In addition, lignin content and composition, preferably a high amount of G units, most likely play a vital role in increased effectivity. Other researchers have indicated that the apparent Klason lignin (acid-insoluble lignin) might be elevated due to the condensation of extractives (Burkhardt et al., 2013; Torget et al., 1991), for example, phenolic compounds such as tannins. Consequently, an overestimated lignin content could explain the lacking effect of 2-naphthol addition on enzymatic hydrolysis yields in this study.

Acetic acid addition to pretreatment had no, or very little, impact on the lignin content in substrates or hydrolysis yields (Table 3). Potentially it helped kick-start the pre-hydrolysis reactions during steam explosion pretreatment. Still, the effect was entirely overpowered by the other treatment conditions contributing to the severity of the process.

3.5. Effect of pre-drying on substrate composition and sugar recovery

The coefficients for models derived from the pre-drying trials, experimental design B), are presented in Table 5. Pre-drying raw materials before the steam explosion had a minor effect on the acid-soluble lignin, which increased with the pre-drying treatment. Still, as discussed in Section 3.4, no further conclusions can be drawn from this slight shift in ASL content due to the nature of the analysis procedure (Hatfield and Fukushima, 2005). Apart from ASL, no good models were obtained that revealed any significant effect of pre-drying on the composition of steam-exploded substrates or sugar recoveries from enzymatic hydrolysis (Table 5). The substrates performed equally during enzymatic hydrolysis regardless of initial bark moisture and acid-soluble lignin content after steam explosion, indicating no impact from hornification effects for softwood bark dried to 18 % moisture content.

4. Conclusions

Softwood bark was steam explosion pretreated at logR_0 3.4–4.1, and the pectic saccharide arabinose degraded rapidly during the treatment. Enzyme loading and pretreatment temperature were the predominant factors influencing the recovery of monosaccharides during enzymatic hydrolysis of pretreated substrates, and the highest yields were achieved at 205 °C, logR_0 4.1.

Meanwhile, 5 % 2-naphthol added as a powder to the bark before the pretreatment negatively impacted the hexose recovery, 0.7 and 0.2 % based on substrate total solids for glucose and mannose, respectively, during enzymatic hydrolysis. The use of 2-naphthol probably requires higher severity factors, which decreases its potential for treatments of softwood bark due to the risk of condensing the tannins and impairing the enzymatic hydrolysis.

Pre-drying and resoaking the bark before pretreatment had no impact on the production of monosaccharides during enzymatic hydrolysis.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biteb.2022.101292.

Funding

This work was supported by Vinnova (Swedish Governmental Agency for Innovation Systems), grant number 2017-05408, and Valmet AB. Also, we thank Bio4Energy, a strategic research environment appointed by the Swedish government, for supporting this work.

CRediT authorship contribution statement

Andreas Averheim: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Visualization. **Sylvia H. Larsson:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – review & editing, Funding acquisition. **Mikael Thyrel:** Conceptualization, Methodology, Validation, Formal analysis, Writing – review & editing, Project administration.

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.

Acknowledgments

We thank Magnus Wiman at Novozymes for kindly facilitating the sampling of the enzyme. Jan Detlefsen at Fiber Technology Center, Valmet AB, Sundsvall, Sweden, is acknowledged for operational work with steam explosion runs, and Anna Borgström and Anne Christoffersen at Valmet AB, Karlstad, for support and data extraction during ion chromatography analyses. The authors also thank Gunnar Kalén at the Biomass Technology Centre, Umeå, Sweden, for technical support during pre-processing of the biomass.

References

Aarum, I., Solli, A., Gunnarsson, H., Kalyani, D., Devle, H., Ekeberg, D., Stenstrøm, Y., 2019. Comparison of pyrolyzed lignin before and after milled wood lignin purification of Norway spruce with increasing steam explosion. Wood Sci. Technol. 53 (3), 601–618.

Bianchi, S., Kroslakova, I., Janzon, R., Mayer, I., Saake, B., Pichelin, F., 2015. Characterization of condensed tannins and carbohydrates in hot water bark extracts of european softwood species. Phytochemistry 120, 53–61.

Borrega, M., Pihlajaniemi, V., Liitiä, T., Wikström, L., Tamminen, T., 2021. Evaluation of chemical additives in hydrothermal pre-treatment of wood for the integrated production of monosugars and hydrolysis lignins for PLA-based biocomposites. Biomass Conversion Biorefinery. https://doi.org/10.1007/s13399-021-01365-5.

Burkhardt, S., Kumar, L., Chandra, R., Saddler, J., 2013. How effective are traditional methods of compositional analysis in providing an accurate material balance for a range of softwood derived residues? Biotechnol. Biofuels 6 (1), 90.

Cheng, Y.-S., Mutrakulcharoen, P., Chuetor, S., Cheenkachorn, K., Tantayotai, P., Jayex Panakkal, E., Sriariyanun, M., 2020. Recent situation and progress in biorefining process of lignocellulosic biomass: toward green economy. Appl. Sci. Eng. Prog. 13.

Deshavath, N.N., Mukherjee, G., Goud, V.V., Veeranki, V.D., Sastri, C.V., 2020. Pitfalls in the 3, 5-dinitrosalicylic acid (DNS) assay for the reducing sugars: interference of furfural and 5-hydroxymethylfurfural. Int. J. Biol. Macromol. 156, 180–185.

Donaldson, L.A., Wong, K.K.Y., Mackie, K.L., 1988. Ultrastructure of steam-exploded wood. Wood Sci. Technol. 22 (2), 103–114.

Eriksson, L., Johansson, E., Kettaneh-Wold, N., Wikström, C., Wold, S., 2008. Design of Experiments: Principles and Applications, 3rd ed. Umetrics AB, Umeå.

Esteghlalian, A.R., Bilodeau, M., Mansfield, S.D., Saddler, J.N., 2001. Do enzymatic hydrolyzability and simons' stain reflect the changes in the accessibility of lignocellulosic substrates to cellulase enzymes? Biotechnol. Prog. 17 (6), 1049–1054.

Frankó, B., Galbe, M., Wallberg, O., 2015. Influence of bark on fuel ethanol production from steam-pretreated spruce. Biotechnol. Biofuels 8.

Frankó, B., Carlqvist, K., Galbe, M., Lidén, G., Wallberg, O., 2018. Removal of water-soluble extractives improves the enzymatic digestibility of steam-pretreated softwood barks. Appl. Biochem. Biotechnol. 184 (2), 599–615.

Galbe, M., Zacchi, G., 2012. Pretreatment: the key to efficient utilization of lignocellulosic materials. Biomass Bioenergy 46, 70–78.

Ghoreishi, S., Løhre, C., Hermundsgård, D.H., Molnes, J.L., Tanase-Opedal, M., Brusletto, R., Barth, T., 2022. Identification and quantification of valuable platform chemicals in aqueous product streams from a preliminary study of a large pilot-scale steam explosion of woody biomass using quantitative nuclear magnetic resonance spectroscopy. Biomass Conversion Biorefinery. https://doi.org/10.1007/s13399-022-02712-w.

Hames, B., Ruiz, R., Scarlata, C., Sluiter, A., Sluiter, J., Templeton, D., 2008. Preparation of Samples for Compositional Analysis. Natianal Renewable Energy Laboratory. NREL/TP-510-42620.

Hansen, L.D., Østensen, M., Arstad, B., Tschentscher, R., Eijsink, V.G.H., Horn, S.J., Várnai, A., 2022. 2-Naphthol impregnation prior to steam explosion promotes LPMO-assisted enzymatic saccharification of spruce and yields high-purity lignin. ACS Sustain. Chem. Eng. 10 (16), 5233–5242.

Hatfield, R., Fukushima, R.S., 2005. Can lignin be accurately measured? Crop Sci. 45 (3), 832–839.

International Organization for Standardization, 2015. Solid Biofuels — Determination of Ash Content, ISO 18122:2015. Geneva, CH.

Jablonsky, M., Nosalova, J., Sladkova, A., Haz, A., Kreps, F., Valka, J., Miertus, S., Frecer, V., Ondrejovic, M., Sima, J., Surina, I., 2017. Valorisation of softwood bark through extraction of utilizable chemicals. A review. Biotechnol. Adv. 35 (6), 726–750.
Jönsson, L.J., Martín, C., 2016. Pretreatment of lignocellulose: formation of inhibitory by-products and strategies for minimizing their effects. Bioresour. Technol. 199, 103–112.
Kemppainen, K., Inkinen, J., Uusitalo, J., Nakari-Setälä, T., Siika-aho, M., 2012. Hot water extraction and steam explosion as pretreatments for ethanol production from spruce bark. Bioresour. Technol. 117, 131–139.
Kemppainen, K., Siika-aho, M., Pattathil, S., Giovando, S., Kruus, K., 2014. Spruce bark as an industrial source of condensed tannins and non-cellulosic sugars. Ind. Crop. Prod. 52, 158–168.
Kumar, L., Tooyserkani, Z., Sokhansanj, S., Saddler, J.N., 2012. Does densification influence the steam pretreatment and enzymatic hydrolysis of softwoods to sugars? Bioresour. Technol. 121, 190–198.
Le Normand, M., Edlund, U., Holmbom, B.R., Ek, M., 2012. Hot-water extraction and characterization of spruce bark non-cellulosic polysaccharides. Nord. Pulp Pap. Res. J. 27 (1), 18–23.
Le Normand, M., Mélida, H., Holmbom, B.R., Michaelsen, T.E., Inngjerdingen, M., Bulone, V., Paulsen, B.S., Ek, M., 2014. Hot-water extracts from the inner bark of Norway spruce with immunomodulating activities. Carbohydr. Polym. 101, 699–704.
Le Normand, M., Rietzler, B., Vilaplana, F., Ek, M., 2021. Macromolecular model of the pectic polysaccharides isolated from the barkof Norway Spruce (Picea abies). Polymers 13 (7), 1106.
Li, J.B., Henriksson, G., Gellerstedt, G., 2005. Carbohydrate reactions during high-temperature steam treatment of aspen wood. Appl. Biochem. Biotechnol. 125 (3), 175–188.
Li, J., Henriksson, G., Gellerstedt, G., 2007. Lignin depolymerization/repolymerization and its critical role for delignification of aspen wood by steam explosion. Bioresour. Technol. 98 (16), 3061–3068.
Lipiäinen, S., Kuparinen, K., Sermyagina, E., Vakkilainen, E., 2022. Pulp and paper industry in energy transition: towards energy-efficient and low carbon operation in Finland and Sweden. Sustain. Prod. Consum. 29, 421–431.
Liu, L.-Y., Qin, J.-C., Li, K., Mehmood, M.A., Liu, C.-G., 2017. Impact of moisture content on instant catapult steam explosion pretreatment of sweet potato vine. Bioresour. Bioprocess. 4 (1), 49.
Luo, X., Zhu, J.Y., Gleisner, R., Zhan, H., 2011. Effects of drying-induced fiber hornification on enzymatic saccharification of lignocelluloses. Cellulose 18, 1055–1062.
Mason, W.H., 1926. Process and Apparatus for Disintegration of Wood and the Like. Vol. Patent No. US 1,578,609A. United States.
Miranda, I., Gominho, J., Mirra, I., Pereira, H., 2012. Chemical characterization of barks from Picea abies and Pinus sylvestris after fractioning into different particle sizes. Ind. Crop. Prod. 36 (1), 395–400.
Overend, R.P., Chornet, E., 1987. Fractionation of lignocellulosics by steam-aqueous pretreatments. Philos. Trans. R. Soc. Lond. Ser. A Math. Phys. Sci. 321 (1561), 523–536.
Pang, S., 2002. Predicting anisotropic shringkage of softwood part 1: theories. Wood Sci. Technol. 36 (1), 75–91.
Pielhop, T., Larrazábal, G., Rudolf von Rohr, P., 2016. Autohydrolysis pretreatment of softwood – enhancement by phenolic additives and the effects of other compounds. Green Chem. 18 (19), 5239–5247.
Pielhop, T., Amgarten, J., Studer, M.H., von Rohr, P.R., 2017a. Pilot-scale steam explosion pretreatment with 2-naphthol to overcome high softwood recalcitrance. Biotechnol. Biofuels 10 (1), 130.
Pielhop, T., Reinhard, C., Hecht, C., Del Bene, L., Studer, M.H., Rudolf von Rohr, P., 2017b. Application potential of a carbocation scavenger in autohydrolysis and dilute acid pretreatment to overcome high softwood recalcitrance. Biomass Bioenergy 105, 164–173.
Rachamontree, P., Douzou, T., Cheenkachorn, K., Sriariyanun, M., Rattanaporn, K., 2020. Furfural: a sustainable platform chemical and fuel. Appl. Sci. Eng. Prog. 13, 2020.
Ralph, J., Lundquist, K., Brunow, G., Lu, F., Kim, H., Schatz, P.F., Marita, J.M., Hatfield, R.D., Ralph, S.A., Christensen, J.H., Boerjan, W., 2004. Lignins: natural polymers from oxidative coupling of 4-hydroxyphenyl- propanoids. Phytochem. Rev. 3 (1), 29–60.
Robert, D., Bardet, M., Lapierre, C., Gellerstedt, G., 1988. Structural changes in aspen lignin during steam explosion treatment. Cellul. Chem. Technol. 22 (2), 221–230.
Seidel, C.-M., Brethauer, S., Gyenge, L., Rudolf von Rohr, P., Studer, M.H., 2019. Two-stage steam explosion pretreatment of softwood with 2-naphthol as carbocation scavenger. Biotechnol. Biofuels 12, 37-37.
Sluiter, A., Hames, B., Hyman, D., Payne, C., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Wolfe, J., 2008. Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples. National Renewable Energy Laboratory. NREL/TP-510-42621.
Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2008. Determinataion of Structural Carbohydrates and Lignin in Biomass. National Renewable Energy Laboratory. NREL/TP-510-42618.
Sluiter, A., Hyman, D., Payne, C., Wolfe, J., 2008. Determination of Insoluble Solids in Pretreated Biomass Material. National Renewable Energy Laboratory. NREL/TP-510-42627.
Torget, R., Himmel, M.E., Grohmann, K., 1991. Dilute sulfuric acid pretreatment of hardwood bark. Bioresour. Technol. 35 (3), 239–246.
Valentín, L., Kluczek-Turpeinen, B., Willför, S., Hemming, J., Hatakka, A., Steffen, K., Tuomela, M., 2010. Scots pine (Pinus sylvestris) bark composition and degradation by fungi: potential substrate for bioremediation. Bioresour. Technol. 101 (7), 2203–2209.
Wayman, M., Lora, J.H., 1978. Aspen autohydrolysis: the effects of 2 naphthol and other aromatic compounds. Tappi 61 (6), 55–57.

II

Averheim *et al.*
Biotechnology for Biofuels and Bioproducts (2024) 17:102
https://doi.org/10.1186/s13068-024-02552-y

Biotechnology for Biofuels and Bioproducts

RESEARCH

Open Access

Separate hydrolysis and fermentation of softwood bark pretreated with 2-naphthol by steam explosion

Andreas Averheim[1,3*], Stefan Stagge[2], Leif J. Jönsson[2], Sylvia H. Larsson[3] and Mikael Thyrel[3]

Abstract

Background 2-Naphthol, a carbocation scavenger, is known to mitigate lignin condensation during the acidic processing of lignocellulosic biomass, which may benefit downstream processing of the resulting materials. Consequently, various raw materials have demonstrated improved enzymatic saccharification yields for substrates pretreated through autohydrolysis and dilute acid hydrolysis in the presence of 2-naphthol. However, 2-naphthol is toxic to ethanol-producing organisms, which may hinder its potential application. Little is known about the implications of 2-naphthol in combination with the pretreatment of softwood bark during continuous steam explosion in an industrially scalable system.

Results The 2-naphthol-pretreated softwood bark was examined through spectroscopic techniques and subjected to separate hydrolysis and fermentation along with a reference excluding the scavenger and a detoxified sample washed with ethanol. The extractions of the pretreated materials with water resulted in a lower aromatic content in the extracts and stronger FTIR signals, possibly related to guaiacyl lignin, in the nonextractable residue when 2-naphthol was used during pretreatment. In addition, cyclohexane/acetone (9:1) extraction revealed the presence of pristine 2-naphthol in the extracts and increased aromatic content of the nonextractable residue detectable by NMR for the scavenger-pretreated materials. Whole-slurry enzymatic saccharification at 12% solids loading revealed that elevated saccharification recoveries after 48 h could not be achieved with the help of the scavenger. Glucose concentrations of 16.9 (reference) and 15.8 g/l (2-naphthol) could be obtained after 48 h of hydrolysis. However, increased inhibition during fermentation of the scavenger-pretreated hydrolysate, indicated by yeast cell growth, was slight and could be entirely overcome by the detoxification stage. The ethanol yields from fermentable sugars after 24 h were 0.45 (reference), 0.45 (2-naphthol), and 0.49 g/g (2-naphthol, detoxified).

Conclusion The carbocation scavenger 2-naphthol did not increase the saccharification yield of softwood bark pretreated in an industrially scalable system for continuous steam explosion. On the other hand, it was shown that the scavenger's inhibitory effects on fermenting microorganisms can be overcome by controlling the pretreatment conditions to avoid cross-inhibition or detoxifying the substrates through ethanol washing. This study underlines the need to jointly optimize all the main processing steps.

Keywords Steam explosion, Softwood bark, 2-Naphthol, Enzymatic hydrolysis, Fermentation, Inhibition

*Correspondence:
Andreas Averheim
andreas.averheim@valmet.com
Full list of author information is available at the end of the article

Background

Since the introduction of the carbocation scavenger 2-naphthol in a pretreatment process to enhance subsequent enzymatic hydrolysis in 2015 [1], several authors have investigated scavenger pretreatments. Feedstocks such as poplar [1–4], spruce [1, 4–6], pine [4, 7, 8], larch [9], bamboo [10], birch [7], and beech [4] have been studied, confirming the enhancing potential of adding 2-naphthol to the pretreatment. On the other hand, the toxicity of 2-naphthol to fermentation organisms [6] must be adequately investigated and addressed if feasible process concepts are to be presented.

The inclusion of 2-naphthol presumably counteracts the condensation of lignin during the acidic processing of lignocellulosic biomasses, such as hydrothermal, steam explosion, or dilute acid pretreatments. These pretreatments have been widely researched for biomass conversion into biofuels, e.g., second-generation bioethanol via enzymatic hydrolysis and fermentation, renewable platform chemicals, or biomaterials [11]. Apart from depolymerizing hemicellulose [11], the processing of lignocellulosic materials under these acidic conditions affects acid-labile structures in lignin, especially by the cleavage of β-O-4 linkages followed by instant repolymerization reactions to form condensed C–C structures [12, 13]. The reactions proceed via a carbocation intermediate. Thus, a nucleophilic reagent, such as 2-naphthol, acts as a carbocation scavenger and, in that way, prohibits condensation reactions [1, 12, 14].

The repolymerization of lignin is considered an obstacle to the enzymatic conversion of pretreated biomass and one of the main factors behind softwood recalcitrance [4]. Condensed lignin may act as a physical barrier [2], but more importantly, it promotes nonproductive binding and deactivation of the enzyme [2, 3, 8, 9]. Mitigated lignin condensation by the addition of 2-naphthol during pretreatment can increase biomass porosity and decrease lignin surface coverage, thus diminishing steric hindrance [2]. It has been proposed that the nonproductive binding of enzymes can be minimized due to reduced inhibitory phenolics [3, 8] and decreased lignin surface area [1]. Interestingly, it has also been discovered that pretreatment with lignins derived from 2-naphthol treatments may promote the activity of lytic polysaccharide monooxygenases present in modern enzyme cocktails, consequently promoting the oxidative depolymerization of cellulose [5].

Phenolic compounds may inhibit the fermentation of saccharides into ethanol with baker's yeast *Saccharomyces cerevisiae* [15]. Although pretreatments with carbocation scavengers have been extensively studied in combination with enzymatic treatments, information regarding the fermentability of broths in the presence of the phenolic compound 2-naphthol is scarce. Seidel et al. [6] conducted fermentations using two-stage pretreated softwood as a substrate, including washed and unwashed substrates, at various solid loadings. The study showed that whole-slurry fermentation at 10% solids loading, which could be considered relevant for industrial applications [16, 17], was severely inhibited by the presence of 2-naphthol. The threshold concentration for inhibition was lower than that in more dilute systems, indicating cross-inhibition between 2-naphthol and degradation products from hemicelluloses, such as acetic acid, furfural, and 5-hydroxymethylfurfural (5-HMF) [6].

This investigation aimed to study the effect of 2-naphthol addition on softwood bark pretreated via continuous steam explosion prior to enzymatic saccharification followed by fermentation using *Saccharomyces cerevisiae*. The enzymatic saccharification and fermentation steps were kept apart in a separate hydrolysis and fermentation (SHF) setup, to facilitate stepwise analysis of the 2-naphthol effects. This approach differs from previous studies regarding the substrate, pretreatment approach, and analyses of the effects of the inclusion of 2-naphthol. In addition, a simple detoxification procedure was tested to study whether any potential inhibitory effects could be easily overcome.

Methods

Raw material

The raw material was softwood bark collected at a pulp mill along the northern coast of Sweden. The material was downsized by coarsely shredding it over a 30-mm sieve followed by screening through a 14-mm mesh screen. High-density particles such as sand and gravel were removed before the steam explosion pretreatment to ensure stable operation. The bark consisted of a mixture of Norway spruce (*Picea abies* Karst. L.) and Scots pine (*Pinus sylvestris* L.) with a total solids content of 41%. Further details regarding the sampling and preprocessing procedures are reported elsewhere [18].

Steam explosion pretreatment

The bark was pretreated in a pilot system for continuous steam explosion (Valmet BioTrac, Valmet AB, Sundsvall, Sweden), as detailed in the literature [18]. A feed rate of approximately 60 kg h^{-1} on a dry basis, a reactor temperature of 200 °C (corresponding to 14.5 barg steam pressure), and a residence time of 10 min were applied. The chemicals, 98–99% glacial acetic acid (Swed Handling AB, Sweden) diluted to 2.5% (w/v) and 98% 2-naphthol (Thermo Fisher Scientific) milled in a knife-mill (Retsch SM 200) to a particle size < 2 mm were added to the biomass in the atmospheric part of the pretreatment system according to the schematic illustration in

Fig. 1. An even and continuous dosage of chemicals and biomass, together with tumbling of the mixture in two conveyor screws before entering the plug screw, ensured the mixing of the chemicals with the biomass. Two steam explosion conditions were applied using 0 and 5% (w/w) 2-naphthol addition, each with 0.5% (w/w) acetic acid dosage based on the total solid content of the biomass feed.

Samples from tests with 2-naphthol (2N) and without (Ref) were taken directly from the steam explosion system outlet and stored frozen before further analysis. In addition, a third sample was produced by detoxifying the sample treated with 2-naphthol (2N EW). The sample was mixed with ethanol at ambient temperature at a ratio of 1:3.2 (w/w) for 15 min, after which the ethanol was decanted. This procedure was repeated three times. The ethanol was then removed through filtering, after which the final undissolved residue was evaporated at ambient temperature under a fume hood.

Substrate characterization

Determination of furans, total aromatics, and total phenolic content in hydrolysates

To obtain the hydrolysates, the substrates were first diluted from ~ 40% to 20% total solids with boiling water. The slurries were kneaded in a kitchen mixer (Bosch MUM8) at the low-speed setting for 15 min, and hydrolysates were then extracted by mechanical compression of the slurries through a wire cloth. The total aromatic content (TAC) of the liquids was measured by UV–Vis absorption at 280 nm [19] in 96-well microplates using an Epoch™ 2 spectrophotometer and Gen 5™ ver. 1.10 software (BioTek Instruments, Inc., USA, as described in Wang et al. 2018). TAC values will cover heteroaromatics, such as furans, and aromatics, such as phenolic substances. The main furans, furfural, and 5-HMF were analyzed separately using a Dionex UltiMate 3000 HPLC system (Thermo Fisher Scientific, Waltham, MA, USA) as described previously [20, 21]. The total aromatic content, excluding the furans, could then be calculated by accounting for their absorbance at 280 nm by running a blank sample with corresponding 5-HMF and furfural concentrations.

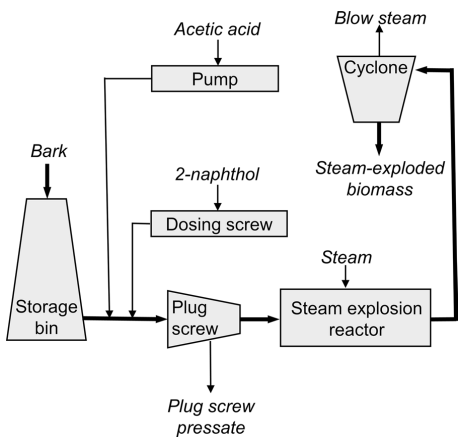

Fig. 1 Schematic illustration of the steam explosion system

The Folin–Ciocalteu assay was used to determine the total phenolic content [22]. Twenty µL of each sample and a series of vanillin standards at concentrations ranging from 0–300 mg/L were incubated with tenfold dilutions of Folin–Ciocalteu reagent (Merck) for 5 min. Eighty µL of 1 M sodium carbonate solution was added to all the samples. The samples were mixed thoroughly and kept at ambient temperature for 45 min. Afterwards, the UV–Vis absorption at 760 nm was determined using 96-well microplates, and the total phenolic content was calculated based on vanillin standards.

Solvent extraction of pretreated materials

The extracts were removed from the pretreated materials by extraction with cyclohexane/acetone (9:1 v/v) for nonpolar extractives and then with ethanol for polar extractives in a Dionex ASE 350 system (Dionex, Sunnyvale, CA). The nonextractable solids were dried in a fume hood between extractions to allow complete evaporation of the solvents. The extract yield was determined for each extraction by evaporating the solvent and drying at 105 °C overnight.

Cyclohexane/acetone extraction was carried out at 140 °C and 100 bar pressure for four cycles, while the second extraction with ethanol was conducted at 80 °C for eight cycles. The cycle time was eight minutes per cycle, and the rinse volume was 150%. The nonextractable solids were dried in a fume hood and transferred to fresh cells for ethanol extraction. The second extraction was conducted at 80 °C for eight cycles with a residence time of 8 min each and a rinse volume of 150%.

After the two-stage extraction, the nonextractable solids were subjected to acid hydrolysis to determine the acid-insoluble lignin (Klason lignin) content [23].

Nuclear magnetic resonance spectroscopy analysis

Samples for ^{1}H nuclear magnetic resonance (NMR) spectroscopy were prepared from the dried cyclohexane/acetone extracts by dissolving approximately 20 mg of each sample in 600 µL of acetone-d_6. The spectra were recorded on a Bruker 600 MHz Avance III HD spectrometer equipped with a BBO cryo-probe. Eight scans were

recorded with a relaxation delay of 1 s and a sweep width of 20 ppm.

^{13}C cross-polarization magic angle spinning (CP-MAS) NMR spectroscopy was performed on samples from the solid nonextractable fractions after cyclohexane/acetone extraction. A Bruker 500 MHz Avance III spectrometer operating at a ^{13}C frequency of 125.75 MHz and equipped with a 4 mm MAS probe was used. Approximately 80 mg of each sample was transferred, as a dry powder, into a 4 mm ZrO_2 rotor. A 1 ms contact time was used, with a ramped ^{1}H pulse amplitude (50–100%). Spinal64 ^{1}H decoupling was applied during the acquisition time, and 8192 scans were accumulated for each spectrum at a spin rate of 10 kHz. Adamantane was used as an external chemical shift reference.

CP-MAS spectra were recorded at ambient temperature, and ^{1}H spectra were recorded at 298 K. All spectra were processed in Topspin 3.6 (Bruker Biospin, Germany).

Fourier transform infrared spectroscopy analysis

Samples for Fourier transform infrared spectroscopy (FTIR) were prepared by washing the steam-exploded materials extensively with 20 °C tap water, drying them without forced convection at 45 °C, and milling them with a knife-mill (IKA A11 basic, IKA-Werke GmbH & Co) to < 0.5 mm. The samples were mixed with IR spectroscopy grade KBr and manually ground in an agate mortar and pestle, and their spectra were measured [24]. Spectra in the range of 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1} were recorded on a Bruker IFS 66v/S spectrometer (Bruker Corporation). 128 scans were coadded for background (pure KBr) and sample and collected in OPUS version 5 software (Bruker Corporation). The data were then processed using MATLAB R2021b (MathWorks) with the open-source graphical user interface available from the Vibrational Spectroscopy Core Facility at Umeå University [25]. The spectral range was cut to the fingerprint region 500–1850 cm^{-1}, and baseline correction was performed using asymmetrical least squares [26] with $\lambda = 100\ 000$ and $p = 0.001$. Finally, total area normalization without curve smoothing was conducted in the cut spectral range.

Carbohydrate composition

The carbohydrate compositions of the pretreated materials were measured according to the laboratory analytical procedures available from National Renewable Energy Laboratory. Sugar concentrations were determined with an HPAEC system, Dionex ICS-6000, with CarboPac SA-10 guard and analytical columns. The content of monomeric sugars in the hydrolyzate was subtracted from the total amount of saccharides in the pretreated materials to obtain the total amount of poly- and oligosaccharides.

Enzymatic saccharification

The steam-exploded materials were subjected to SHF treatments. Enzymatic hydrolysis was performed by shaking flask experiments in an incubation shaker (Infors Ecotron, Infors, Switzerland) with a batch size of 75 g, a total solids loading of 12% (w/w), and a temperature of 50 °C. The pH of the samples was adjusted with KOH to 5.2, after which 58 mM citric acid/citrate buffer and enzyme were added. The enzyme used was Cellic CTec3 HS (Novozymes, Bagsværd, Denmark), and the dosage of enzyme was 4% (w/w) on substrate total solids. Shaking was set at 250 rpm for the first four h and then at 150 rpm for the following 72 h.

The batch was sampled at 0, 4, 24, and 48 h, after which the samples were diluted ~ 10 times, and the solid phase was separated through centrifugation. The monosaccharide concentration in the supernatant, filtered through a 0.2-µm nylon membrane (Millipore), was then determined through high-performance anion-exchange chromatography (HPAEC). A Dionex ICS-6000 system (Sunnyvale, CA, USA) equipped with a 4 × 250 mm separation column and a 4 × 50 mm guard column (both CarboPac PA1, Dionex) and pulsed amperometric detection were used for this analysis.

The final sample from the hydrolysis (72 h) was centrifuged without prior dilution, and the hydrolysis liquid was stored frozen for further fermentation testing.

Fermentation

To assess the inhibitory effect of pretreatment liquids on yeast, fermentation tests were performed with the industrial *Saccharomyces cerevisiae* strain Ethanol Red (Fermentis, Marcq en Baroeul, France) using fermentation of a synthetic glucose solution as a reference. All hydrolysis liquids were supplemented with 0.5 mL of a nutrient solution containing 150 g/L yeast extract, 75 g/L $(NH_4)_2HPO_4$, 3.75 g/L $MgSO_4 \cdot 7\ H_2O$, and 238.2 g/L $NaH_2PO_4 \cdot H_2O$, and the initial pH was adjusted to 5.5 before inoculation. Freeze-dried Ethanol Red was rehydrated by suspending it in sterile water five times its weight for 30 min at 35 °C, and 1 mL of the suspension was then added to the medium to an initial cell concentration of 2 g/L. The fermentations were run in 30-mL glass flasks containing 25 mL of yeast culture and were agitated with a magnetic stirrer. The flasks were sealed with rubber plugs pierced with cannulas to remove carbon dioxide and were incubated for 48 h in a heating chamber at 180 rpm and 30 °C.

Sampling was conducted after 0, 4, 24, and 48 h, and the ethanol concentration was measured with an Agilent

1260 Infinity high-performance liquid chromatography (HPLC) system (Santa Clara, CA, USA) equipped with a refractive index detector, an autoinjector, and a column oven. An Aminex HPX-87H column and a 125-0131 Standard Cartridge Holder guard column, supplied by Bio-Rad Laboratories AB (Solna, Sweden), were used for separation at 55 °C. The temperature of the detector was set to 55 °C. The eluent was 0.005 M sulfuric acid, supplied at a 0.6 mL/min flow rate.

Initial and residual sugar concentrations were determined with the HPAEC system, Dionex ICS-6000, with CarboPac SA-10 guard and analytical columns.

Results and discussion

Extraction with water

The steam-exploded materials were extracted with water to investigate the effects of the addition of 2-naphthol. The phenolic content in water extracts is mainly derived from lignin, whereas carbohydrate degradation gives rise to substances such as furans (including furfural and 5-HMF) and aliphatic carboxylic acids, which, in addition to lignin, affect the aromatic content. The extract potentially contains inhibitory compounds that are detrimental to the growth of microorganisms and to the efficiency of enzymes and consequently diminish ethanol yields [15].

The total phenolic content in biomass samples pretreated with or without 2-naphthol was similar, indicating that scavenger addition did not affect lignin (Fig. 2). However, the total aromatic content, determined from the UV–Vis absorption at 280 nm, revealed a small but significant difference between the two samples. The furan content was analyzed by reversed-phase HPLC to exclude furans derived from the hemicelluloses from the aromatic content. Furans are often found in high concentrations as sugar degradation products after hydrothermal pretreatment [11]. The 5-HMF and furfural concentrations were similar but significantly ($p < 0.05$) different (Fig. 2). Sample 2N had a slightly lower concentration of furans, which could indicate a slower dissolution of hemicellulose when 2-naphthol was used in pretreatment. Possibly, this is explained by slight mass and heat transfer issues when the scavenger was provided as a milled powder to the process. However, this was not supported by analyses of pH and carbohydrate content. Treatment at a range of severity conditions would be required to be able to draw further conclusions regarding furan formation related to the presence of 2-naphthol.

Although the furan concentration could partially explain the difference in total aromatic content, it was still significantly ($p < 0.05$) different even after the absorbance stemming from 5-HMF and furfural was deducted from the total absorbance at 280 nm (Fig. 2), which shows that more aromatics derived from sources other than carbohydrates were extractable with water when the treatment was performed without the carbocation scavenger. If the scavenger had reacted as expected, an increase in

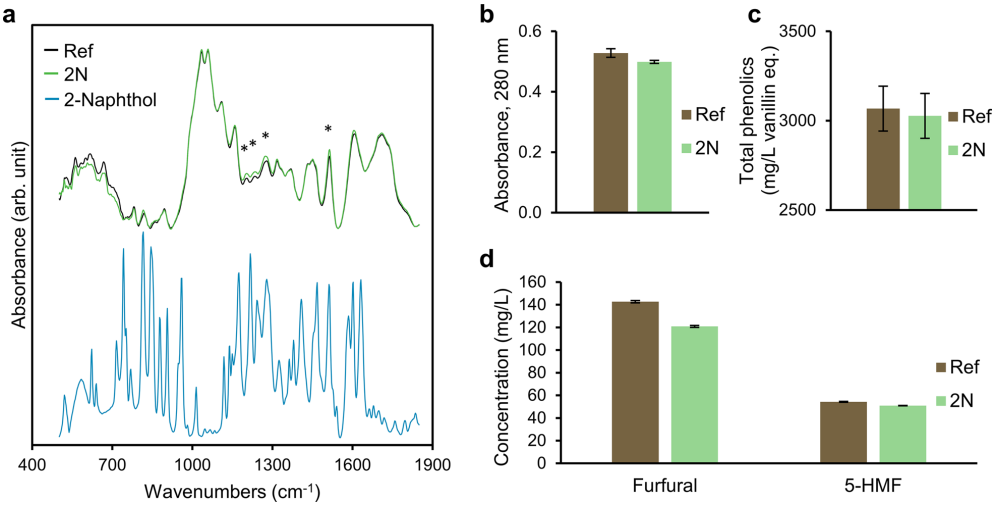

Fig. 2 Cut and area-normalized FTIR spectra of steam-exploded biomass samples washed with water (**a**), as well as total aromatics indicated by UV—Vis absorbance at 280 nm (**b**), and total phenolic (**c**), furfural and 5-HMF (**d**) concentrations in the water extracts. The contribution of the furans to the total aromatic content was subtracted from the UV—Vis absorbance at 280 nm

water-extractable aromatics would be expected. However, a third source of aromatics, tannins, is commonly present in softwood bark and may play a part in the aromatic content. Tannins may also participate in reactions with 2-naphthol, increasing the complexity of the system. Therefore, in this case, the slight difference in aromatic content cannot be used to draw further conclusions regarding 2-naphthol reactions.

The FTIR spectra (Fig. 2) reveal an intensification of the peaks at approximately 1514, 1273, 1232, and 1203 cm^{-1} for the scavenger-pretreated material, which may indicate more G (guaiacyl) lignin units [27–30]. More G-lignin units could imply that the carbocation scavenger retained the lignin structure to a greater degree. However, no indication of 1,2-disubstituted naphthalenes, indicated by strengthened IR signals at 750 and 815 cm^{-1} [1, 30], could be detected, which contradicts earlier findings on pretreated spruce wood [1]. Thus, drawing conclusions regarding 2-naphthol reactions based on FTIR data is impossible in this case. On the other hand, no distinctive features of pristine 2-naphthol were found in the steam-exploded material after washing with water, implying that its concentration, if present, was below the detection limit.

Extraction with cyclohexane/acetone and ethanol

Ground and sieved bark samples Ref and 2N were sequentially extracted with a cyclohexane/acetone mixture followed by ethanol as a polar solvent. The extraction of the reference sample with cyclohexane showed high variability, with extraction yields ranging from 5.8 to 13.0% (Fig. 3). Industrial bark is a heterogeneous material, but as the bark was milled and mixed well before the experiments, the variation is surprising. Approximately 6.0% of the extractives were removed from sample 2N with the nonpolar solvent mixture. The extractions with ethanol did not show the same variability for the two samples. Ethanol removed 8.5–8.7% of the polar compounds from the bark samples, with a slightly greater fraction extracted from the reference sample. Overall, this study did not reveal a higher extraction yield for the material pretreated with 2-naphthol, as previously shown

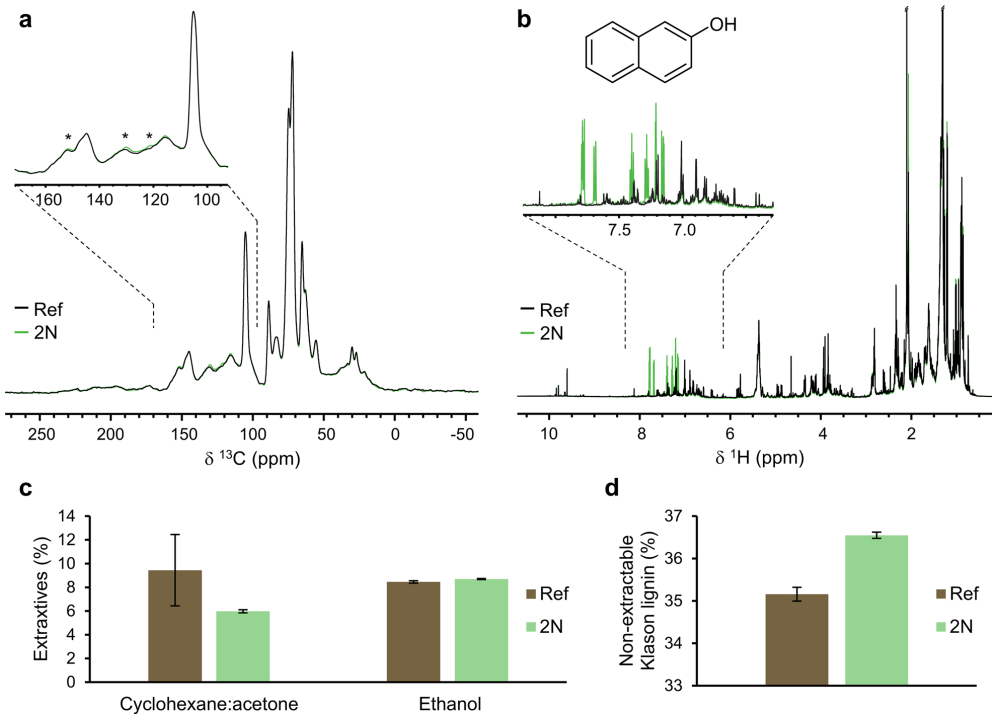

Fig. 3 ^{13}C CP-MAS NMR spectra of steam-exploded biomass, nonextractable with cyclohexane/acetone (**a**) and ^{1}H NMR of cyclohexane/acetone extracts (**b**), as well as the extracted mass fractions (**c**) and nonextractable Klason lignin after 2-stage extraction (**d**)

for autocatalyzed spruce [1]. This may be explained by differences in solvent choices and extraction procedures.

The fraction of Klason lignin in the nonextractable residue is displayed in Fig. 3. The sample treated with 2-naphthol had a higher content of acid-insoluble lignin, which could be due to the inclusion of 2-naphthol in the lignin structure by integration of naphthalene rings, as previously shown in the literature [1, 7].

Figure 3 also shows ^{13}C CP-MAS and ^{1}H NMR spectra of the nonextractable residue and dissolved extracts from cyclohexane/acetone extraction. The ^{13}C spectra revealed a slight increase in the intensity of the peak in the aromatic region at 115–156 ppm [32] for sample 2N. Whether this is caused by pristine 2-naphthol remaining in the solid structure or lignin substituted with 2-naphthol cannot be concluded with the current resolution. In contrast, the ^{1}H spectra of the extracts revealed unsubstituted 2-naphthol (7.15–7.8 ppm) in the 2N sample (green in Fig. 3b), indicating that some of the added scavenger remained unreacted in the steam-exploded materials.

Carbohydrate composition

The carbohydrate composition of the pretreated materials, Table 1, revealed a higher glucan content and lower content of the hemisaccharides (mannan and galactan) in the reference sample compared to the sample treated with 2-naphthol. The content of monosaccharides, however, was identical in the two samples. The slightly higher amount of hemisaccharides in the sample pretreated with 2-naphthol coincides with the lower concentration of degradation products (furfural and 5-HMF).

Enzymatic saccharification

The conversion of polysaccharides from bark solids obtained after pretreatment with or without 2-naphthol addition was examined by enzymatic saccharification in a whole-slurry treatment at 12% solids loading. Glucose was the predominant monosaccharide in the hydrolysates, and the concentration was between 15.7 and 17 g/l (Fig. 4a), corresponding to a glucose conversion of 0.39 and 0.40, respectively. The recalcitrance of softwood bark is known from the literature, and its

Table 1 Carbohydrate composition of the pretreated materials. The polymeric and oligomeric contents (glucan, mannan, and galactan) are reported based on anhydro monomeric mass, while the monomeric contents (glucose, mannose, and galactose) are reported based on the actual monomeric mass

	Ref	2N
Glucan (%)	31.4	28.8
Glucose (%)	0.5	0.5
Mannan (%)	4.2	4.5
Mannose (%)	0.2	0.2
Galactan (%)	1.6	1.8
Galactose (%)	0.6	0.6

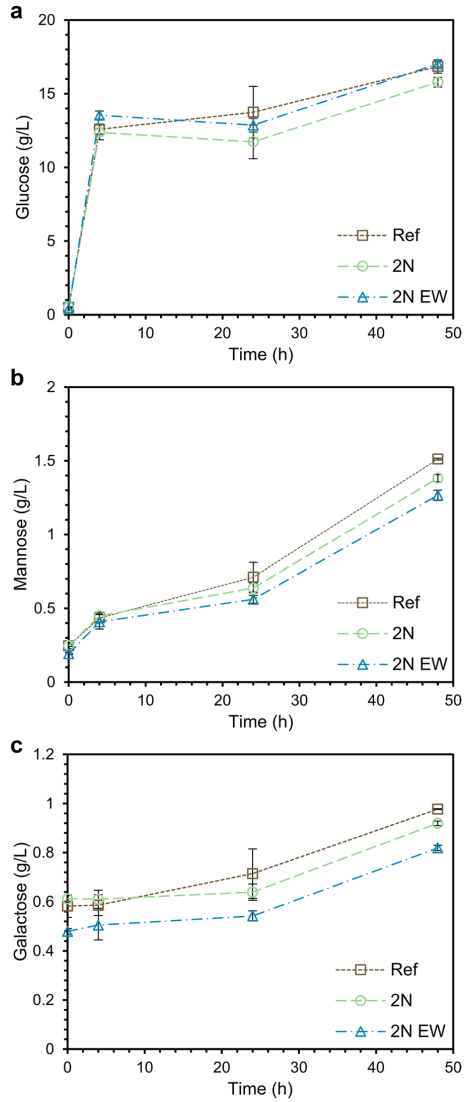

Fig. 4 Concentrations of the fermentable sugars glucose (**a**), mannose (**b**), and galactose (**c**) as a function of residence time during enzymatic hydrolysis

glucose recovery is comparable to other findings [33]. The concentrations of other monosaccharides were much lower than those of glucose and decreased in the following order: xylose, arabinose, mannose, galactose, fructose, and rhamnose. Part of the saccharide content, especially arabinose, stems from pectin fractions typically found in softwood bark [34].

The general trend for the summarized hexose concentrations in the hydrolysates (Fig. 4) was that the reference (Ref) contained the highest concentrations, whereas the hydrolysate from the scavenger-pretreated bark (2N), had a 6.5% lower concentration of hexoses compared to Ref. The total hexose concentrations in the hydrolysate of the 2N EW sample (2N washed with ethanol) were similar to those of the Ref sample. The significantly lower concentrations in the hydrolysate of sample 2N compared to sample Ref is most likely caused by the lower initial glucan concentration in the pretreated substrate (Table 1). In contrast, more glucose was released from glucan in sample N2 EW, which indicates the removal of possibly inhibitory reaction products. Ethanol can remove polar compounds and some nonpolar compounds that may have been attached to the surface of the biomass. The removal of such compounds may, in turn, lead to better accessibility of attachment sites for cellulose-degrading enzymes and possibly less deactivation. In addition, ethanol treatment may have removed water-soluble substances derived as byproducts of the pretreatment process and improved the efficiency of carbohydrate-degrading enzymes [15, 35]. On the other hand, some of the hemicellulose was depolymerized and dissolved during the steam explosion and was inevitably removed during ethanol washing, which explains the lower concentrations of pectic and hemicellulosic saccharides after enzymatic hydrolysis for the detoxified sample (2N EW).

It is evident that 2-naphthol, as a carbocation scavenger, could not be efficiently used for softwood bark in a steam explosion process with the tested setup to improve the enzymatic conversion of resulting substrates at these relatively low severities (severity factor 3.94). This study, together with a preceding study investigating a range of autohydrolysis conditions [18], trials with sulfuric acid as a pretreatment catalyst (data not shown) and enzymatic saccharification conducted on washed substrates (data not shown) unanimously revealed that enzymatic saccharification yields and recoveries did not improve from the addition of a scavenger. However, autocatalytic conditions at higher severities remain to be investigated. Recent research on improved 2-naphthol addition could also unlock yet undiscovered potential in terms of scavenger efficiency for softwood bark treatments [36].

As indicated by the analysis of extracts and nonextractable residues, unreacted 2-naphthol was left to some degree in the steam-exploded material. However, no desired reaction between lignin and 2-naphthol was observed. Although generally considered a useful additive for softwood pretreatment [1, 5, 7, 8], 2-naphthol efficiency could not be proven at severity 3.94 for the treatment of softwood bark. The bark composition, which includes, for example, tannins and pectin [34] at a much higher fraction than in stemwood may have contributed to the challenges of pretreating this material.

Fermentation

Cultivations of ethanol red yeast using hydrolysates from bark pretreated with or without the addition of 2-naphthol were undertaken to investigate the possible inhibitory effects. Table 2 shows the change in yeast cell mass indicated by the optical density of the fermented broths measured at 600 nm (OD_{600}). All the Ref, 2N, and 2N EW cultures remained in the lag phase during the first 6 h of cultivation. The growth phase occurred between the 6 and 24 h samples. Therefore, the exact growth rate in the exponential growth phase could not be calculated due to a lack of observations between 6 and 24 h. However, it appears that cultures from the Ref hydrolysate grew faster than cultures grown on hydrolysate from 2-naphthol-pretreated biomass, judging from the accumulated growth at 24 h. Hampered growth indicates an inhibitory effect that may be attributed to the addition of 2-naphthol, especially due to the presence of pristine 2-naphthol in the fermentation broths. 2-Naphthol is structurally similar to well-known inhibitors, such as phenols, 5-HMF, and furfural [15], and potentially possesses related inhibitory effects.

Ethanol release from glucose conversion by the yeast cells was first observed after 24 h (Fig. 5). At the same time, glucose was not detectable in the culture broth. Further yeast growth was based on the consumption of other monosaccharides, such as mannose and galactose, that were present in the enzymatic hydrolysate. Analysis of the culture broth by HPAEC revealed that the yeast consumed mannose, galactose, and glucose. The galactose concentration also decreased from approximately

Table 2 Optical density measured at 600 nm (OD_{600}) for the fermentation broths and yield of ethanol from the fermentable sugars (glucose, mannose, and galactose) at different fermentation times

	OD_{600} (-)			Yield (g/g)	
	6 h	24 h	48 h	24 h	48 h
Ref	0.062	0.963	1.160	0.45	0.45
2N	0.072	0.796	1.011	0.45	0.46
2N EW	0.056	0.829	1.230	0.49	0.49

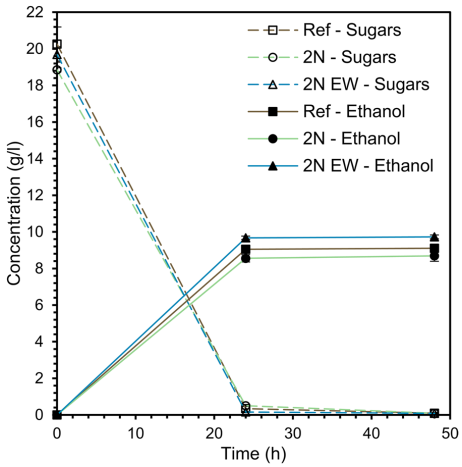

Fig. 5 Concentrations of ethanol and fermentable sugars (glucose, mannose, and galactose) as a function of fermentation time

0.75–0.9 g/L to zero after 48 h and was almost completely consumed after 24 h. Ethanol red yeast has been shown to consume galactose in nutrient-rich media, especially those containing nitrogen [37].

The yield of fermentable sugars (glucose, mannose, and galactose) to ethanol (Table 2) revealed that the fermentation yield was unaffected by the 2-naphthol dosage, although the yeast cell growth was slightly lower. On the other hand, the detoxified sample, 2N EW, performed somewhat but significantly better than the other samples in terms of ethanol yield, reaching close to the theoretical limit of 0.51 g/g. The improved performance confirmed that the ethanol washing procedure could also remove other inhibitors. Previous studies on softwood [6] have indicated a strong cross-inhibitory effect between 2-naphthol and furans and possibly other compounds such as organic acids. This study showed that inhibition can be overcome by selecting pretreatment conditions, such as mild pretreatment conditions and a continuous process where furfural is continuously stripped from the substrate. However, under mild pretreatment conditions, the potentially positive effect of scavenger addition may not be adequate. Considering the growing number of published works utilizing 2-naphthol pretreatments for the enzymatic conversion of biomass [1–10, 38], surprisingly few studies have investigated fermentation to target an actual market product. This study highlights the necessity for jointly optimizing all the main process steps since 2-naphthol may have a beneficial effect on hydrolysis yields depending on the raw material, pretreatment severity, and dosage, which in turn, due to cross-inhibition, has a contradictory impact on fermentation recovery if bioethanol is the intended product.

Conclusions

This study showed that the carbocation scavenger 2-naphthol is not a useful pretreatment additive for the production of sugars or bioethanol from softwood bark via a continuous and industrially scalable steam explosion process at severity factor 3.94. A lower initial growth rate indicated the yeast growth was slightly inhibited upon scavenger addition. Moreover, no beneficial effects on sugar recovery from enzymatic hydrolysis were detected. However, ethanol yields after 24 h were not negatively affected by scavenger addition, showing that cross-inhibitory effects can be overcome if the correct parameters for the pretreatment process are selected. Furthermore, a simple detoxification procedure was demonstrated, showing that ethanol yields can be improved further by washing the substrates with ethanol before enzymatic hydrolysis and fermentation, which likely removes 2-naphthol together with other inhibitors. Overall, this information may prove helpful when investigating carbocation scavenger treatments for different raw materials and highlights the importance of jointly investigating and optimizing all the main process steps.

Acknowledgements

We gratefully acknowledge Magnus Wiman at Novozymes, Bagsværd, for enzyme sampling. Gunnar Kalén at Biomass Technology Center, Umeå, is thanked for his valuable support during the feedstock preprocessing. At the same time, Jan Detlefsen at the Valmet Fiber Technology Center, Sundsvall, is recognized for the skillful operation of the steam explosion pilot. We would also like to acknowledge Mattias Hedenström at the Swedish NMR Centre at Umeå University for NMR support and András Gorzás at the Vibrational Spectroscopy Platform at Umeå University for conducting the FTIR measurements.

Author contributions

All authors participated in the study's conceptualization. AA supervised the steam explosion trials and conducted enzymatic saccharifications, while SS performed the fermentations, extractions, compositional analyses, and HPLC analyses. AA and SS both contributed to HPEAC measurements. AA and SS prepared the original draft, while MT and LJJ performed the critical review. LJJ, MT, and SHJ conducted the funding acquisition.

Funding

Open access funding provided by Swedish University of Agricultural Sciences. This work was funded by Vinnova, the Swedish Governmental Agency for Innovation Systems (grant number 2017–05408), Bio4Energy (www.bio4energy.se), the Swedish Energy Agency (P2022-00569), and Valmet AB.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Fiber Technology Center, Valmet AB, 851 94 Sundsvall, Sweden. [2]Department of Chemistry, Umeå University, 901 87 Umeå, Sweden. [3]Department of Forest Biomaterials and Technology, Swedish University of Agricultural Sciences, 901 83 Umeå, Sweden.

Received: 8 May 2024 Accepted: 8 July 2024
Published online: 17 July 2024

References

1. Pielhop T, Larrazábal GO, Studer MH, Brethauer S, Seidel C-M. Rudolf von Rohr P: Lignin repolymerisation in spruce autohydrolysis pretreatment increases cellulase deactivation. Green Chem. 2015;17(6):3521–32.
2. Chu Q, Song K, Wang J, Hu J, Chen X. Improving enzymatic saccharification of hardwood through lignin modification by carbocation scavengers and the underlying mechanisms. Bioresour Technol. 2019;294: 122216.
3. Chu Q, Tong W, Wu S, Jin Y, Hu J, Song K. Modification of lignin by various additives to mitigate lignin inhibition for improved enzymatic digestibility of dilute acid pretreated hardwood. Renewable Energy. 2021;177:992–1000.
4. Pielhop T, Reinhard C, Hecht C, Del Bene L, Studer MH. Rudolf von Rohr P: Application potential of a carbocation scavenger in autohydrolysis and dilute acid pretreatment to overcome high softwood recalcitrance. Biomass Bioenerg. 2017;105:164–73.
5. Hansen LD, Østensen M, Arstad B, Tschentscher R, Eijsink VGH, Horn SJ, Várnai A. 2-Naphthol Impregnation Prior to Steam Explosion Promotes LPMO-assisted enzymatic saccharification of spruce and yields high-purity lignin. ACS Sustain Chem Eng. 2022;10(16):5233–42.
6. Seidel C-M, Brethauer S, Gyenge L. Rudolf von Rohr P, Studer MH: Two-stage steam explosion pretreatment of softwood with 2-naphthol as carbocation scavenger. Biotechnol Biofuels. 2019;12:37–37.
7. Borrega M, Pihlajaniemi V, Liitiä T, Wikström L, Tamminen T: Evaluation of chemical additives in hydrothermal pre-treatment of wood for the integrated production of monosugars and hydrolysis lignins for PLA-based biocomposites. *Biomass Convers Biorefinery* 2021.
8. Madadi M, Bakr MMA, Abdulkhani A. Alleviating lignin repolymerization by carbocation scavenger for effective production of fermentable sugars from combined liquid hot water and green-liquor pretreated softwood biomass. Energy Convers Manage. 2022;251: 114956.
9. Lai C, Jia Y, Zhou C, Yang C, Shen B, Zhang D, Yong Q. Facilitating enzymatic digestibility of larch by in-situ lignin modification during combined acid and alkali pretreatment. Bioresour Technol. 2020;311: 123517.
10. He J, Huang C, Lai C, Wang Z, Yuan L, Ragauskas A, Yan Y, Yong Q. Revealing the mechanism of lignin re-polymerization inhibitor in acidic pretreatment and its impact on enzymatic hydrolysis. Ind Crops Prod. 2022;179: 114631.
11. Galbe M, Wallberg O. Pretreatment for biorefineries: a review of common methods for efficient utilisation of lignocellulosic materials. Biotechnol Biofuels. 2019;12(1):26.
12. Li J, Henriksson G, Gellerstedt G. Lignin depolymerization/repolymerization and its critical role for delignification of aspen wood by steam explosion. Bioresour Technol. 2007;98(16):3061–8.
13. Robert D, Bardet M, Lapierre C, Gellerstedt G. Structural changes in aspen lignin during steam explosion treatment. Cellul Chem Technol. 1988;22(2):221–30.
14. Wayman M, Lora JH. Aspen autohydrolysis: the effects of 2 naphthol and other aromatic compounds. Tappi. 1978;61(6):55–7.
15. Jönsson LJ, Martín C. Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. Bioresour Technol. 2016;199:103–12.
16. Robak K, Balcerek M. Current state-of-the-art in ethanol production from lignocellulosic feedstocks. Microbiol Res. 2020;240: 126534.
17. da Silva ASA, Espinheira RP, Teixeira RSS, de Souza MF, Ferreira-Leitão V, Bon EPS. Constraints and advances in high-solids enzymatic hydrolysis of lignocellulosic biomass: a critical review. Biotechnol Biofuels. 2020;13(1):58.
18. Averheim A, Larsson SH, Thyrel M. Carbocation scavenger pretreatment to mitigate lignin self-condensation in a semi-industrial steam explosion process. Bioresour Technol Rep. 2022;20: 101292.
19. Wang Z, Wu G, Jönsson LJ. Effects of impregnation of softwood with sulfuric acid and sulfur dioxide on chemical and physical characteristics, enzymatic digestibility, and fermentability. Bioresour Technol. 2018;247:200–8.
20. Wang Z, Gräsvik J, Jönsson LJ, Winestrand S. Comparison of [HSO4]−, [Cl]− and [MeCO2]− as anions in pretreatment of aspen and spruce with imidazolium-based ionic liquids. BMC Biotechnol. 2017;17(1):82.
21. Martín C, Peinemann JC, Wei M, Stagge S, Xiong S, Jönsson LJ. Dilute-sulfuric acid pretreatment of de-starched cassava stems for enhancing the enzymatic convertibility and total glucan recovery. Ind Crops Prod. 2019;132:301–10.
22. Singleton VL, Orthofer R, Lamuela-Raventós RM: [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. In: *Methods in Enzymology.* vol. 299: Academic Press; 1999: 152–178.
23. Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D: Determination of Structural Carbohydrates and Lignin in Biomass. In: *Laboratory Analytical Procedure (LAP).* Golden, CO: National Renewable Energy Laboratory; 2008.
24. Gorzsás A, Sundberg B: Chemical Fingerprinting of Arabidopsis Using Fourier Transform Infrared (FT-IR) Spectroscopic Approaches. In: *Arabidopsis Protocols.* Sanchez-Serrano JJ, Salinas J. Totowa, NJ, eds.: Humana Press; 2014: 317–352.
25. Downloads at Vibrational Spectroscopy Core Facility, Umeå University. https://www.umu.se/en/research/infrastructure/visp/downloads/. Accessed 24 April 2023.
26. Eilers PHC. Parametric Time Warping. Anal Chem. 2004;76(2):404–11.
27. Pandey K, Pitman A. FTIR studies of the changes in wood chemistry following decay by brown-rot and white-rot fungi. Int Biodeterior Biodegradation. 2003;52:151–60.
28. Pandey KK. A study of chemical structure of soft and hardwood and wood polymers by FTIR spectroscopy. J Appl Polym Sci. 1999;71(12):1969–75.
29. Ganne-Chédeville C, Jääskeläinen A-S, Froidevaux J, Hughes M, Navi P. Natural and artificial ageing of spruce wood as observed by FTIR-ATR and UVRR spectroscopy. Holzforschung. 2012;66(2):163–70.
30. Vanderghem C, Richel A, Jacquet N, Blecker C, Paquot M. Impact of formic/acetic acid and ammonia pre-treatments on chemical structure and physico-chemical properties of Miscanthus x giganteus lignins. Polym Degrad Stab. 2011;96(10):1761–70.
31. Hawkins JG, Ward ER, Whiffen DH. Characteristic infra-red absorption frequencies of substituted naphthalenes. Spectrochim Acta. 1957;10(1):105–9.
32. Love GD, Snape CE, Jarvis MC. Comparison of leaf and stem cell-wall components in barley straw by solid-state 13C NMR. Phytochemistry. 1998;49(5):1191–4.
33. Frankó B, Carlqvist K, Galbe M, Lidén G, Wallberg O. Removal of water-soluble extractives improves the enzymatic digestibility of steam-pretreated softwood barks. Appl Biochem Biotechnol. 2018;184(2):599–615.
34. Krogell J, Holmbom B, Pranovich A, Hemming J, Willfor S. Extraction and chemical characterization of Norway spruce inner and outer bark. Nord Pulp Pap Res J. 2012;27(1):6–17.
35. Ko JK, Um Y, Park Y-C, Seo J-H, Kim KH. Compounds inhibiting the bioconversion of hydrothermally pretreated lignocellulose. Appl Microbiol Biotechnol. 2015;99(10):4201–12.
36. Seidel C-M, Brethauer S, Studer MH, von Rohr PR, Pielhop T. Overcoming softwood recalcitrance by improved 2-naphthol addition to steam explosion pretreatment. ACS Sustain Chem Eng. 2024;12(19):7519–31.
37. Díez-Antolínez R, Hijosa-Valsero M, Paniagua-García AI, Garita-Cambronero J, Gómez X. Yeast screening and cell immobilization on inert supports for ethanol production from cheese whey permeate with high lactose loads. PLoS ONE. 2019;13(12): e0210002.
38. Pielhop T: Hydrothermal/steam explosion pretreatment with carbocation scavengers for biofuels and -chemicals production. ETH Zürich; 2015.

Publisher's Note

III

Bioresource Technology 400 (2024) 130698

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Enhanced biobased carbon materials made from softwood bark via a steam explosion preprocessing step for reactive orange 16 dye adsorption

Andreas Averheim [a], Glaydson Simões dos Reis [b], Alejandro Grimm [b], Davide Bergna [c], Anne Heponiemi [c], Ulla Lassi [c], Mikael Thyrel [b,*]

[a] Valmet AB, Fiber Technology Center, SE-851 94 Sundsvall, Sweden
[b] Swedish University of Agricultural Sciences, Department of Forest Biomaterials and Technology, SE-901 83 Umeå, Sweden
[c] University of Oulu, Research Unit of Sustainable Chemistry, FI-90570 Oulu, Finland

HIGHLIGHTS

- A novel process for active carbon using steam explosion preconditioning is presented.
- Steam explosion prior to activation improves carbon surface area and functionality.
- Maximum adsorption (218 mg g^{-1}) was obtained using steam-exploded material.
- Steam exploded materials showed higher efficiencies in synthetic effluent dye removal.
- The large surface area of carbons supports pore-filling as main adsorption mechanism.

ARTICLE INFO

Keywords:
Biobased carbon materials
Steam explosion
Softwood bark
Reactive orange 16: Adsorption
Activated carbon

ABSTRACT

The growing textile industry produces large volumes of hazardous wastewater containing dyes, which stresses the need for cheap, efficient adsorbing technologies. This study investigates a novel preprocessing method for producing activated carbons from abundantly available softwood bark. The preprocessing involved a continuous steam explosion preconditioning step, chemical activation with $ZnCl_2$, pyrolysis at 600 and 800 °C, and washing. The activated carbons were subsequently characterized by SEM, XPS, Raman and FTIR prior to evaluation for their effectiveness in adsorbing reactive orange 16 and two synthetic dyehouse effluents. Results showed that the steam-exploded carbon, pyrolyzed at 600 °C, obtained the highest BET specific surface area (1308 m^2/g), the best Langmuir maximum adsorption of reactive orange 16 (218 mg g^{-1}) and synthetic dyehouse effluents (>70 % removal) of the tested carbons. Finally, steam explosion preconditioning could open up new and potentially more sustainable process routes for producing functionalized active carbons.

1. Introduction

Biobased carbon materials have been researched intensively in recent years for the potential use in, for example, supercapacitors (Correa & Kruse, 2018; Wang et al., 2020), batteries (Correa & Kruse, 2018; Zhang et al., 2015), adsorbents (Bilal et al., 2022; Praveen et al., 2022; Qasem et al., 2021), and catalysts (Correa & Kruse, 2018). Biomass has the potential to replace fossil-based precursors such as coal or graphite for these applications and, in addition, provide improved properties or new functionalities that may increase the final product's performance.

Biobased adsorbents have been used by humanity for thousands of years and cover a wide range of materials that can be used to purify gases or liquids (Dąbrowski, 2001). Biomass sources such as low-cost byproducts from agriculture or industry are of particular interest as precursors as these may provide cost-efficient and sustainable materials. A wide range of agricultural residues has been investigated in literature as raw materials for adsorbent production (Ioannidou & Zabaniotou, 2007), for example, corn straw, rice straw, wheat straw, bagasse, corn stover, and cotton residues together with industrial residues such as

* Corresponding author.
E-mail addresses: andreas.averheim@valmet.com (A. Averheim), glaydson.simoes.dos.reis@slu.se (G. Simões dos Reis), alejandro.grimm@slu.se (A. Grimm), anne.heponiemi@oulu.fi (A. Heponiemi), ulla.lassi@oulu.fi (U. Lassi), mikael.thyrel@slu.se (M. Thyrel).

https://doi.org/10.1016/j.biortech.2024.130698
Received 15 January 2024; Received in revised form 11 April 2024; Accepted 11 April 2024
Available online 12 April 2024

spruce bark (dos Reis et al., 2021b), and sludges (Won et al., 2006) to mention a few. The wide range of potential raw materials and choices for processing also gives rise to a wide selection of adsorbent materials that could be produced for tailor-made purposes.

A large surface area is a typical characteristic of biochars used as adsorbents due to their meso- and microporous structure (Dąbrowski, 2001), which can be achieved through one or several physical, thermal and chemical treatment steps (Bilal et al., 2022; Correa & Kruse, 2018; Praveen et al., 2022). Thermal conversion processes, such as pyrolysis, hydrothermal carbonization (HTC), or gasification, are often involved in producing biochar (Praveen et al., 2022), while activation steps could be incorporated in the process such as physical activation by steam or CO_2, or chemical activation with chemicals such as H_3PO_4, K_2CO_3, KOH or $ZnCl_2$ to obtain an activated carbon (AC) (Bilal et al., 2022; Correa & Kruse, 2018). The materials may also be subjected to post-treatments to incorporate additional functionalities that improve the adsorption capacity (Qasem et al., 2021).

Apart from the traditionally used thermal conversion processes, steam explosion (STEX) has attracted some attention during the last couple of years as a process of interest for adsorbent production. Whereas steam activation is a process for developing carbon materials porosity and functionalities, conducted at temperatures above 700 °C in an environment partially containing steam at low or moderate pressure, steam explosion is performed at lower temperatures (160–260 °C) at the corresponding steam saturation pressure. The process is terminated by rapidly reducing the pressure, leading to the explosive decompression of the lignocellulosic material. Together with the thermal treatment, which facilitates the degradation of hemicellulose and softening of lignin, the pressure release causes a structural breakdown of the biomass (Hoang et al., 2023). A study of coconut husks showed that STEX could be utilized in a single stage to produce an adsorbent superior to the raw coconut husk powder for the adsorption of Cu^{2+} and Cd^{2+}, which was attributed to an increased crystallinity and porosity due to the thermal degradation of hemicellulose (Pinheiro Nascimento & Barros Neto, 2021). In another study, the authors used a similar process denoted hydrothermal-pressure preconditioning eruption in a multistage treatment. Pine sawdust was preconditioned and then steam-activated by pyrolysis at 900 °C. The resulting carbons could be used for perfluorooctanoic acid and methylene blue adsorption, where they outperformed a commercial activated carbon in adsorption capacity (Yang & Cannon, 2021). In contrast, the adsorption of Ni^{2+} from simulated wastewater onto steam-exploded poplar was used in another study utilizing multistage treatments. By pyrolysis of the biomass-nickel residue, a Ni-doped carbon fiber with a surface area of 1480 m^2/g could be produced (Yang et al., 2021). However, the few studies present in the literature using STEX as a pretreatment have all employed lab-scale batch process conditions while the industrial standard uses continous operations.

Adsorption is widely used for water purification as it is versatile, cost-efficient, and relatively easy to set up and operate (Bilal et al., 2022; Praveen et al., 2022; Qasem et al., 2021). At the same time, an ever-growing textile industry (Textile Exchange, 2022) and increased environmental awareness have put focus on the vast amounts of dyehouse effluents generated by this industry (Kant, 2012). The dye-containing wastewater threatens aquatic environments and, ultimately, human health. Azo dyes, for example, are commonly employed for dyeing textiles and are suspected to be carcinogenic due to the formation of aromatic amines during their degradation (Al-Tohamy et al., 2022). Reactive orange 16 (RO16) is a frequently used azo dye, and several authors have investigated its adsorption onto a variety of adsorbents (Calvete et al., 2010; dos Reis et al., 2021b; dos Santos et al., 2014; Malakootian & Heidari, 2018; Marrakchi et al., 2017; Rosa et al., 2008; Shah et al., 2020; Won et al., 2006). For example, dos Reis et al., 2021b, worked with biobased activated carbons from softwood bark (*Picea abies*) produced via activation with KOH and $ZnCl_2$ and could reach a Langmuir maximum adsorption capacity of 355 and 90 mg g^{-1} respectively. Softwood bark is considered an underused resource mainly utilized for low-value applications (dos Reis et al., 2021b) and was thus selected as raw material for this work.

This investigation aimed to study the effect of novel semi-industrial STEX preconditioning of softwood bark for producing functionalized ACs for adsorption. The impact of STEX treatment before $ZnCl_2$-activation at two carbonization temperatures on produced AC characteristics such as morphology, specific surface area and porosity, surface chemistry, AC composition, and adsorption of RO16 and two synthetic dyehouse effluents were evaluated.

2. Materials and methods

2.1. Raw material and preprocessing

The raw material used in the study was a mixed industrial bark of Norway spruce (*Picea abies*) and Scots pine (*Pinus Sylvestris*) from a pulp mill in northern Sweden. The bark was shredded, and the screened fraction <14 mm was selected for the steam treatments. A junk trap removed sand and gravel from the bark before the steam explosion (STEX) treatments. Additional details regarding the sampling and preparations are reported elsewhere (Averheim et al., 2022).

2.1.1. Steam explosion

A pilot system for continuous STEX (Valmet BioTrac, Valmet AB, Sundsvall, Sweden) was used to precondition the already shredded bark. The system is described in detail in the literature (Averheim et al., 2022), and the schematic illustration is available in the e-supplementary material. The bark was processed at 200 °C reactor temperature at a residence time of 10 min. The feed rate was approximately 52 kg h^{-1} on a dry basis (127 kg h^{-1} on an as-received basis at 41 % total solids content).

Sulphuric acid, 37 % (Swed Handling AB, Sweden), diluted to 8.1 % (w/v), was added to the biomass in the atmospheric part of the pretreatment system to reach 3.8 % (w/w) sulphuric acid dose on raw material total solids. The sulphuric acid going into the reactor was 2.2 % (w/w), accounting for the acidity of the liquid pressed out from the plug screw.

Sampling was conducted from the discharge cyclone during steady-state operation (>60 min of operation at a stable temperature and feed rate). Steam-exploded material was then collected for 5 min for further evaluation. Ten reference samples of the unprocessed bark were also collected from the outlet of the raw material buffer bin and were mixed into a composite sample. The steam-exploded and reference barks were dried at 45 °C to constant weight and stored in sealed plastic bags at 4 °C before further treatment or characterization.

2.1.2. Characterization of raw and steam-exploded bark

The carbohydrate composition of the raw reference bark and steam-exploded bark was determined following National Renewable Energy Laboratory (NREL) laboratory analytical procedures (Hames et al., 2008; Sluiter et al., 2008) and ash content according to ISO 18122:2015. A spectrophotometer (Agilent Cary UV-100) was used for the determination of acid-soluble lignin at 205 nm using 110 L g^{-1} cm^{-1} as extinction coefficient, and the neutral carbohydrates were quantified through ion chromatography (Dionex ICS-3000 with CarboPac PA-1 precolumn and analysis column).

2.2. Preparation of carbon materials

The reference bark was ground in a Retsch SM 200 knife mill (Retsch GmbH) with a 5 mm screen sieve, followed by a Fritsch Pulversisette 14 mill (Fritsch GmbH) equipped with a 0.5 mm sieve. The SE material was ground directly in the Fritsch Pulverisette using the 0.5 mm sieve. By visual inspection, this material already had a small particle size but was still ground for fair comparison with the reference material.

Activation and pyrolysis were then conducted according to principles described in the literature (dos Reis et al., 2021a). The reference bark and the steam-exploded bark were mixed with the activation chemical $ZnCl_2$ at a mass ratio 1:1 on a dry basis (20 g biomass per test). Milli-Q water was added under continuous stirring until a thick, homogenous paste was formed. The paste was allowed to soak for two hours, whereafter, the paste was left in an oven to dry at 60 °C overnight. Samples were then pyrolyzed at 600 or 800 °C in a reactor heated by a furnace. Nitrogen gas was used to keep an inert atmosphere during the process. The heating rate was 10 °C min^{-1} and the desired temperature was kept for one hour. The oven was then cooled to <100 °C before the nitrogen purge was turned off, and the samples were removed and left to cool to ambient temperature.

The pyrolyzed samples were weighed and ground using a Fritsch Pulversisette 14 mill (Fritsch GmbH) equipped with a 0.2 mm sieve. Acid washing was conducted to remove residues of the activation chemical. The materials were put in a flask connected to a re-condenser with 6 M HCl and kept at 70 °C under agitation for two hours. The slurry was dewatered over a glass microfiber filter (Whatman GF/A grade), and the solid was resuspended in hot Milli-Q water and washed repeatedly to a constant pH of the filtrate. The washed carbons were dried at 105 °C and then weighed and stored in sealed plastic tubes.

Four carbon materials were produced, two from raw bark (AC 600 and AC 800) and two from steam-exploded bark (AC-SE 600 and AC-SE 800), where 600 and 800 denote the pyrolysis temperature.

2.3. Characterization of carbon materials

N_2 isotherms were measured with a sorptometer (Tristar, Micrometrics Instrument Corporation) to obtain the specific surface area (S_{BET}), pore volume, meso- (S_{MESO}), and micropore area (S_{MICRO}) for the carbon materials.

XPS was conducted for C1s (280–298 eV), O1s (525–545 eV), and S2p (157–175 ev) using a Thermo Scientific ESCALAB 250Xi XPS system equipped with an Al Kα X-ray source. The measurements were performed using standard protocols (dos Reis et al., 2021a). No S2p signals were detected in samples AC 600 and 800 during the XPS spectral survey, and consequently, these samples were not scanned in detail for S2p.

Raman spectra were recorded using a Bruker BRAVO handheld unit with its vial accessory to determine the degree of graphitization (I_D/I_G). A full spectral range recording (300–3200 cm^{-1}) at a resolution of 2 cm^{-1} was conducted, with integration time set on automatic mode. Single scans were recorded for every sample and collected in the OPUS version 5 software (Bruker Corporation). Data processing was conducted in MATLAB R2021b (MathWorks) with the open-source graphical user interface available from the Vibrational Spectroscopy Core Facility at Umeå University (https://www.umu.se/en/research/infrastructure/visp/downloads/). The spectral range was trimmed to 1000–1900 cm^{-1}, and a total area normalization with Savitzy-Golay filtering polynomial order = 1 and frame rate = 7 was performed. No baseline correction was needed. Peak fitting was finally conducted in Origin Pro 2020b to separate the D and G peaks from other peaks relating to disorder (Sadezky et al., 2005), and I_D/I_G was calculated from their integrated areas.

Fourier transform infrared (FTIR) spectra were recorded in attenuated total reflection (ATR) mode using a Thermo Scientific Nicolet iS5 FTIR spectrometer equipped with a Ge crystal designed for measuring highly colored samples. Spectra were recorded in 600–4000 cm^{-1} wavelength range with a 0.5 cm^{-1} step size. The surface morphology was analyzed using scanning electron microscopy (SEM) (Carl Zeiss Evo SEM) with an acceleration voltage of 5 kV and magnification ranging from 5 k to 25 k.

2.4. Dye adsorption testing and regeneration

Adsorption testing was performed on synthetic effluents with reactive orange 16 dye (RO16) and mixed effluents A and B (see section 2.4.1). A batch adsorption procedure was used, where the carbon materials were contacted with the effluent at an adsorbent dosage of 1.5 g/L under continuous mixing (IKA KS250 laboratory shaker) at ambient temperature for a predetermined time (t). After that, the adsorption process was stopped by centrifugation in a Sorvall ST16R centrifuge and decanting the supernatant. For tests with RO16, the initial concentration of the effluent (C_i) and the final concentration of the supernatant (C_f) was determined with a Shimadzu UV-1800 UV/visible scanning spectrophotometer by measuring absorbance at 494 nm. For the mixed effluents (A and B), a spectral scan in the range 180–800 nm was performed to determine the total chromophore removal.

Testing was conducted to determine RO16 adsorption kinetics (C_i = 200 mg/L, and t = 0.1 to 4 h), and equilibrium (C_i = 30–1000 mg/L, and t = 19 h). Total chromophore removal from effluents A and B was performed at t = 19 h. All testing was conducted at pH 6. The formulation and calculation of adsorption isotherms and kinetics are described in the e-supplementary material.

For the regeneration tests, the active carbons that showed the best performance were loaded using RO16 solution (400 mg/L). Loaded carbons underwent water rinse to eliminate any residual unadsorbed dye. Subsequently, they were dried overnight at 50 °C in an oven. The dried loaded carbons were exposed to a solution containing 0.2 M NaOH and 20 % EtOH, followed by agitation for 12 h to facilitate the desorption of the dye. The separated dye was then isolated from the carbon, which underwent multiple rinses with water to eliminate any remaining eluent. Afterwards, it was dried overnight at 50 °C. The dye removal capacity of the regenerated adsorbent was then reassessed. This process was repeated for four consecutive cycles of adsorption and desorption.

2.4.1. Preparation of mixed effluents

The mixed effluents A and B each consisted of eight dyes, mainly of Azo type, in the presence of sodium dodecyl, sodium acetate, sodium sulfate, and ammonium chloride. The pH of the effluents ranged between 5.2 and 5.4. The specified composition of the effluents is found elsewhere (dos Reis et al., 2023a).

3. Results and discussion

3.1. Composition of preprocessed materials

The composition of the raw material before activation and pyrolysis, Table 1, may impact the yield and properties of the AC and was thus analyzed. The raw bark consisted mainly of cellulose, hemicellulose, and lignin, but also a significant fraction of uncharacterized substances (14.8 %). Based on the literature, this uncharacterized fraction chiefly constitutes a variety of extractives, such as tannin and acidic sugars found in pectin (Le Normand et al., 2014). STEX is known to hydrolyze and degrade the hemicelluloses, a mechanism especially pronounced with an acid catalyst present (Hoang et al., 2023), which was confirmed in this work. The STEX treatment altered the composition significantly, with a decrease in neutral sugars due to the degradation of hemicelluloses, Table 1. Concomitantly, the lignin content increased drastically due to the removal of other components, but potentially also due to

Table 1
Composition of the raw and steam-exploded bark as a percentage of its dry weight. Neutral carbohydrates summarise the cellulose and hemicellulose content and are reported based on the anhydro-monomeric mass, while total lignin summarises the acid-soluble and acid-insoluble lignin.

Compounds	Raw bark	Steam-exploded bark
Neutral carbohydrates	50.5	44.5
Total lignin	32.0	49.9
Ash content	2.7	2.5
Other	14.8	3.1

pseudo-lignin formation. Pseudo-lignin is an acid-insoluble degradation product formed from the degradation of hemicellulosic sugars into diols and furans (Aarum et al., 2019). In addition, the phenolic tannins found in the bark can also condense to form acid-insoluble residues, contributing to the Klason lignin (Torget et al., 1991). Altogether, this may explain the elevated lignin content in the steam-exploded bark and the lowering of the non-qualitative fraction named "other" in Table 1.

3.2. Properties and yield of activated carbon materials

The degree of graphitization (I_D/I_G) determined from Raman spectra is widely employed for evaluating the microstructure of carbon materials (Merlen et al., 2017). I_D/I_G is the ratio between the G band, found around 1582 cm^{-1} indicating sp^2 hybridized carbon (graphitic structure), and the D band, around 1350 cm^{-1} related to a more defective sp^3 hybridized carbon structure (Pimenta et al., 2007; Sadezky et al., 2005).

The spectra from the ACs prepared from bark are shown in Fig. 1. Apart from the D and G peaks, the spectra clearly revealed a third peak around 1186–1196 cm^{-1} (Sadezky et al., 2005) which was separated from the D band through peak fitting before calculating I_D/I_G (Table 2). Steam-exploded bark carbons (AC-SE 600 and 800) expressed an apparent intensification of the G peak in relation to the D peak compared to those prepared from raw bark (AC 600 and 800) and the lowest I_D/I_G (1.08) was achieved at 600 °C with the steam-exploded bark. The steam-exploded precursor had a higher degree of aromaticity due to the elevated lignin content (Deng et al., 2016) compared to the raw bark, which may explain the more pronounced graphitization for these materials. Furthermore, carbons produced at 800 °C had a higher I_D/I_G than carbons pyrolyzed at 600 °C, indicating a more disordered structure. Moreover, FTIR spectra from the ACs (Fig. 1e) provided limited information regarding the influence of steam-explosion on AC properties.

One of the essential characteristics of any carbonaceous material is its porosity, e.g., the S_{BET}, S_{MESO}, and S_{MICRO}, as well as pore volume. These porous features, shown by SEM in the e-supplementary material, significantly influence the materials' ability to adsorb pollutants by adsorption. Table 2 shows the biobased materials' porosity data, i.e., S_{BET}, S_{MESO}, and S_{MICRO}, pore volume, and pyrolysis mass yield. The results show that the samples subjected to STEX exhibited higher S_{BET} values. A possible explanation is that when a sample is subjected to STEX, its structure opens up (Muzamal et al., 2015), increasing the contact area available for the chemical activator, which, in turn, provokes an increase in the S_{BET} value.

Table 2 also shows that STEX treatment did shift the micro-mesoporosity interrelational pore structure slightly towards a more microporous structure. In addition, it was responsible for an 11 % and 21 % pore volume increase for the samples pyrolyzed at 600 and 800, respectively. These results suggest that STEX influenced the textural properties of bio-based carbons. Adsorbents with higher S_{BET} and pore volume are promising candidates for adsorption applications (Dąbrowski, 2001).

X-ray photoelectron spectroscopy (XPS) was used to investigate the surface composition and functionality of the carbons further. The XPS survey spectra revealed the self-evident presence of carbon (C) and oxygen (O) in all carbons, whereas the STEX-pretreated carbons also contained weak signals of sulfur (S) (Fig. 1). Spectral deconvolution of S2p correlated to thiophene (C – S – C, 164.1–164.2 eV), sulfoxide (C – SO – C, 165.3–165.4 eV), sulfone (167.7–167.9 eV), and sulfate (169.1–169.3 eV). These sulfur states imply an abundance of functional groups that have the potential to enhance the adsorptive capacity of the STEX-pretreated carbons (Vigneshwaran et al., 2021). Analysis of the FTIR data (Fig. 1e) concerning sulfur functionalities was inconclusive, which may be explained by a low sulfur concentration. Apart from sulfur

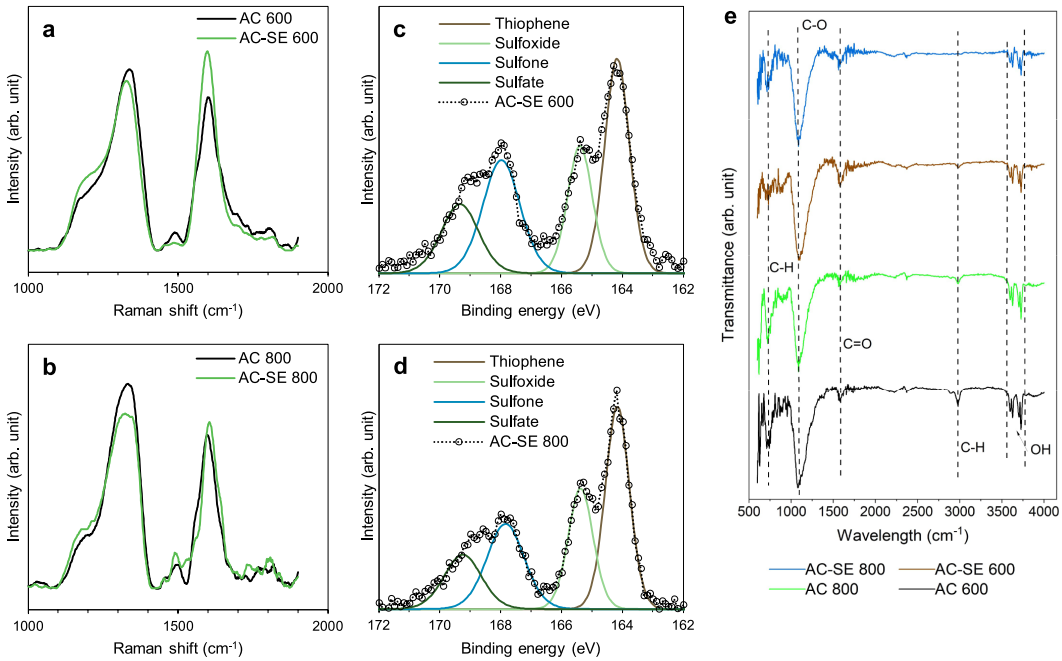

Fig. 1. Raman spectra from AC:s pyrolyzed at 600 (a) and 800 °C (b) and S2p XPS spectra for steam-exploded AC:s pyrolyzed at 600 (c) and 800 °C (d) and FTIR spectra of pyrolyzed AC:s (e).

Table 2
Textural properties and pyrolysis yield for the bark AC:s. The degree of graphatization I_D/I_G is expressed as a ratio of the integrated areas of the D and G peaks.

Sample	S_{BET}	S_{MESO}	S_{MICRO}	$S_{MESO\%}$	$S_{MICRO\%}$	Pore volume	I_D/I_G	Mass yield
	($m^2\ g^{-1}$)	($m^2\ g^{-1}$)	($m^2\ g^{-1}$)	(%)	(%)	($cm^3\ g^{-1}$)	(–)	(%)
AC 600	1217	615	602	50.5	49.5	0.27	1.41	40
AC 800	1018	490	528	48.2	51.8	0.24	2.03	36
AC-SE 600	1308	645	662	49.3	50.7	0.30	1.08	36
AC-SE 800	1217	535	682	44.0	56.0	0.29	1.81	29

functionalities, the XPS peak table (found in the e-supplementary material) revealed a higher fraction of C-C bonds for AC-SE 600 than the other carbon materials. The elevation of C-C bonds is likely caused by graphitic structures being especially pronounced in AC-SE 600, indicated by its degree of graphitization (Table 2).

High lignin content positively impacts biochar yields from pyrolysis without activation chemicals (Cagnon et al., 2009). However, the mass yield (Table 2) was somewhat lower for AC-SE than the AC at the corresponding temperature, although it had a higher lignin content. The lower yield may indicate a more efficient impregnation, promoting reactivity and pore formation. Consequently, yield decreases, but quality parameters improve due to a more homogenous treatment and efficient use of the activation agent.

3.3. Adsorption kinetics

Fig. 2 shows the profiles of the RO16 adsorption kinetics for the carbon materials. The curves display similar trends regardless of temperature and STEX treatment. However, the samples subjected to STEX adsorbed more RO16 molecules than the AC samples pyrolyzed at the corresponding temperature. The better adsorption performances of the SE–AC samples could be related to their higher specific surface areas and larger pore volumes. For instance, high S_{BET} means more accessible, active sites for the adsorption of RO16 molecules, promoting the adsorptive properties.

The kinetic behavior was further evaluated by assessing the models' suitability, considering R^2_{adj} and *SD* values (dos Reis et al., 2023a). The kinetic model parameters and statistical information are presented in Table 3. General order best described the RO16 uptake, based on R^2_{adj} and *SD*, within the studied timeframe (0–360 min). The general order equation determines the apparent reaction order based on the experimental data (dos Santos et al., 2014), making it a versatile function that can be fitted to many different cases (Guo & Wang, 2019). However, the maximum uptake (Table 3) estimated by this model reaches higher than the theoretical maximum (200 mg g^{-1}) for the carbons pyrolyzed at 800 °C and values much higher than those obtained after 19 h for the 600 °C carbons. This overprediction may be due to the complexity of the adsorption onto microporous materials where the adsorption rate is governed by four major phenomena, specifically external and internal mass transfer, surface diffusion, and the adsorption/desorption elementary process (Dąbrowski, 2001). An intraparticle diffusion (IPD) model (Weber & Morris, 1963) was applied to describe the adsorption process by investigating the linear regions of q_t as a function $t^{0.5}$. The modeling is further described in the e-supplementary material, and the

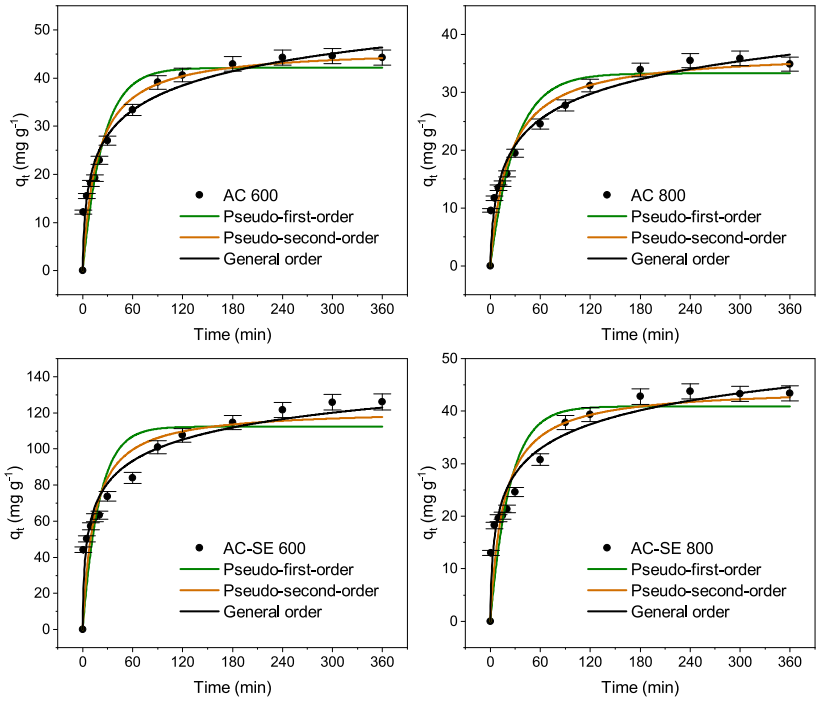

Fig. 2. Kinetic models and experimental data for RO16 adsorption.

Table 3
Kinetic model parameters for RO16 adsorption.

	AC 600	AC 800	AC-SE 600	AC-SE 800
Pseudo-first-order				
q_1 (mg g^{-1})	42.1	33.3	112	40.9
k_1 (min^{-1})	4.14×10^{-2}	3.33×10^{-2}	5.04×10^{-2}	4.24×10^{-2}
R^2	0.912	0.892	0.798	0.848
R^2_{adj}	0.904	0.883	0.781	0.835
SD (mg g^{-1})	4.47	3.94	17.4	5.59
Pseudo-second-order				
q_2 (mg g^{-1})	46.2	37.1	122	44.5
k_2 (g mg^{-1} min^{-1})	1.24×10^{-3}	1.21×10^{-3}	6.01×10^{-3}	1.40×10^{-3}
R^2	0.947	0.934	0.875	0.904
R^2_{adj}	0.943	0.929	0.864	0.896
SD (mg g^{-1})	3.46	3.08	13.7	4.43
General order				
q_n (mg g^{-1})	236	169	352	177
k_n (g mg^{-1})$^{n-1}$ (min^{-1})	1.84×10^{-65}	7.89×10^{-51}	3.66×10^{-39}	6.65×10^{-49}
n (–)	27.7	22.8	15.7	21.9
R^2	0.969	0.963	0.950	0.955
R^2_{adj}	0.964	0.956	0.941	0.947
SD (mg g^{-1})	2.75	2.42	9.05	3.17
IPD (0–120 min)				
C (mg g^{-1} min$^{-0.5}$)	8.90	6.58	36.3	10.6
k_p (mg g^{-1})	3.05	2.24	6.53	2.68
R^2	0.989	0.994	0.993	0.985
R_i	0.78	0.79	0.66	0.73

model constants are shown in Table 3. The investigation revealed two initial zones, the first one <1 min related to boundary layer diffusion to the external surface of the adsorbents, quantified by the constant C, and the second one 1–120 min related to intraparticle diffusion described by the IPD rate constant k_p (Bhat et al., 2023). The constant, C, was positive for all tested carbon materials. Thus, reaction kinetics for RO16 removal were governed by the combined boundary layer and intraparticle diffusion effect.

In literature, the adsorption of RO16 onto a variety of biobased carbon materials is modeled as general order (dos Reis et al., 2021b; dos Reis et al., 2023a; dos Santos et al., 2014) as well as pseudo-second-order (Malakootian & Heidari, 2018; Marrakchi et al., 2017; Rosa et al., 2008; Shah et al., 2020). In this study, the pseudo-second-order was the second-best model, followed by the pseudo-first-order, which was poorly adapted to the experimental data, judging from R^2_{adj} and SD. The fact that pseudo-second-order could be reasonably adapted to the data implies that chemisorption was rate-limiting. However, different potential adsorbent-adsorbate chemical interactions and pore diffusion add to the complexity (Lin et al., 2023; Sutthasupa et al., 2023).

3.4. Adsorption equilibria

Adsorption isotherms are helpful tools for describing and understanding how adsorbates interact with adsorbents. Numerous adsorption isotherm models have been proposed over the years (Al-Ghouti & Da'ana, 2020; Foo & Hameed, 2010). Langmuir, Freundlich and Sips models were employed in this work to study the equilibria.

The isotherms and their parameters are displayed in Fig. 3 and Table 4. The figure shows that the samples subjected to STEX (AC-SE 600 and AC-SE 800) reached a plateau at a lower initial effluent concentration than those without STEX, especially pronounced at 600 °C pyrolysis temperature. Meanwhile, the % Removal was higher for the STEX-treated carbons compared to the reference at the corresponding temperature, which could be explained by the higher BET surface area and, potentially, additional functionalities.

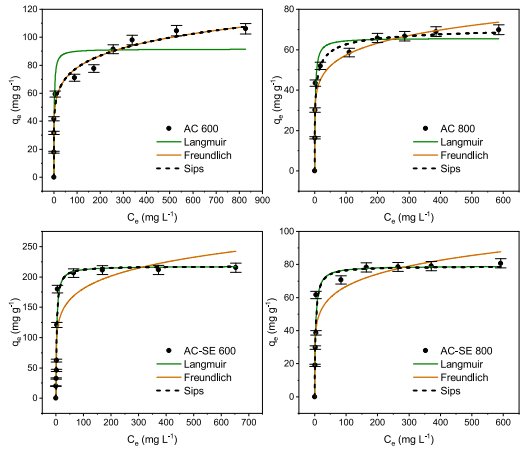

Fig. 3. Isotherm models based and experimental data for RO16 adsorption.

Table 4
Equilibrium isotherm model parameters for RO16 adsorption.

	AC 600	AC 800	AC-SE 600	AC-SE 800
Freundlich				
k_F (mg g^{-1})(L mg^{-1})$^{1/nF}$	39.0	29.8	88.1	32.9
n_F (–)	6.61	7.05	6.41	6.51
R^2	0.958	0.924	0.844	0.897
R^2_{adj}	0.953	0.915	0.827	0.884
SD (mg g^{-1})	7.92	7.11	36.7	10.0
Langmuir				
Q_{max} (mg g^{-1})	91.6	65.7	218	79.2
K_L (L mg^{-1})	0.573	0.467	0.372	0.312
R^2	0.892	0.935	0.949	0.960
R^2_{adj}	0.879	0.927	0.943	0.955
SD (mg g^{-1})	12.7	6.57	21.0	6.26
Sips				
Q_{ms} (mg g^{-1})	6.43×10^3	72.8	218	79.0
K_S (L mg^{-1})	6.09×10^{-3}	0.516	0.372	0.304
n_S (–)	0.153	0.537	1.00	1.00
R^2	0.958	0.943	0.949	0.960
R^2_{adj}	0.947	0.927	0.936	0.948
SD (mg g^{-1})	8.40	6.58	22.3	6.71

Analogous to the kinetic evaluation, the suitability of the models was determined based on R^2_{adj} and SD. Generally, Sips was the model that best could explain the experimental data for the four different carbons. The Sips isotherm model combines the Langmuir and Freundlich isotherm model (Al-Ghouti & Da'ana, 2020; Foo & Hameed, 2010), which explains its adaptability in this case. However, studying the individual carbons, Langmuir was an equally good fit for the experimental data for the SE-AC samples, indicating homogenous adsorption (Foo & Hameed, 2010). On the contrary, the RO16 uptake on AC samples did not plateau in the same way with increasing concentration (Fig. 3), and AC 600 could be well fitted with the Freundlich isotherm. Adaptation to the Freundlich isotherm could indicate a more heterogeneous surface and multilayer adsorption (Foo & Hameed, 2010). The difference in adsorption equilibria between SE-AC and AC suggests that SE-AC have equally energized adsorption sites (Bhat et al., 2023), whereas this is less pronounced for the AC samples. Although the uptake increased with increasing concentration for AC 600 long after AC-SE 600 was saturated with adsorbate, its % Removal was always inferior to AC-SE 600 due to

the high amount of residual dye left in solution at higher adsorbate concentrations. AC-SE 600 was thus the best AC for RO16 removal in this study, with a Langmuir maximum uptake of 218 mg g^{-1}.

The performance, in terms of Langmuir maximum RO16 uptake (Q_{max}), of the carbons AC 600, AC 800, and AC-SE 800 is similar to other carbons made from softwood bark with $ZnCl_2$ as an activator (dos Reis et al., 2021b), while AC-SE 600 on the other hand stands out as a more than twice as promising adsorbent. A compilation of Q_{max} for some other biobased adsorbents in literature is presented in the e-supplementary material. A few of the carbons display Q_{max} values higher than AC-SE, such as AC from spruce bark activated with KOH (dos Reis et al., 2021b), Se-doped biochar from spruce bark (dos Reis et al., 2023a), carbonized Brazilian-pine fruit shell (Calvete et al., 2010) and cross-linked quaternary chitosan salt (Rosa et al., 2008). The latter stands out with an uptake as high as 1060 mg g^{-1}.

However, the magnitude of the textile industry and its potential need for wastewater treatment (Al-Tohamy et al., 2022) calls for cheap bulk adsorbents with a good enough performance. One of the exciting features of AC-SE 600 is the high mass yield (36 %) compared with, for example, KOH-activated spruce bark (14 %) (dos Reis et al., 2021b). Furthermore, its processing is based on a novel STEX technology, an abundant raw material, and relatively cheap chemicals (H_2SO_4 and $ZnCl_2$), which makes steam-exploded activated carbons an interesting prospect for the future.

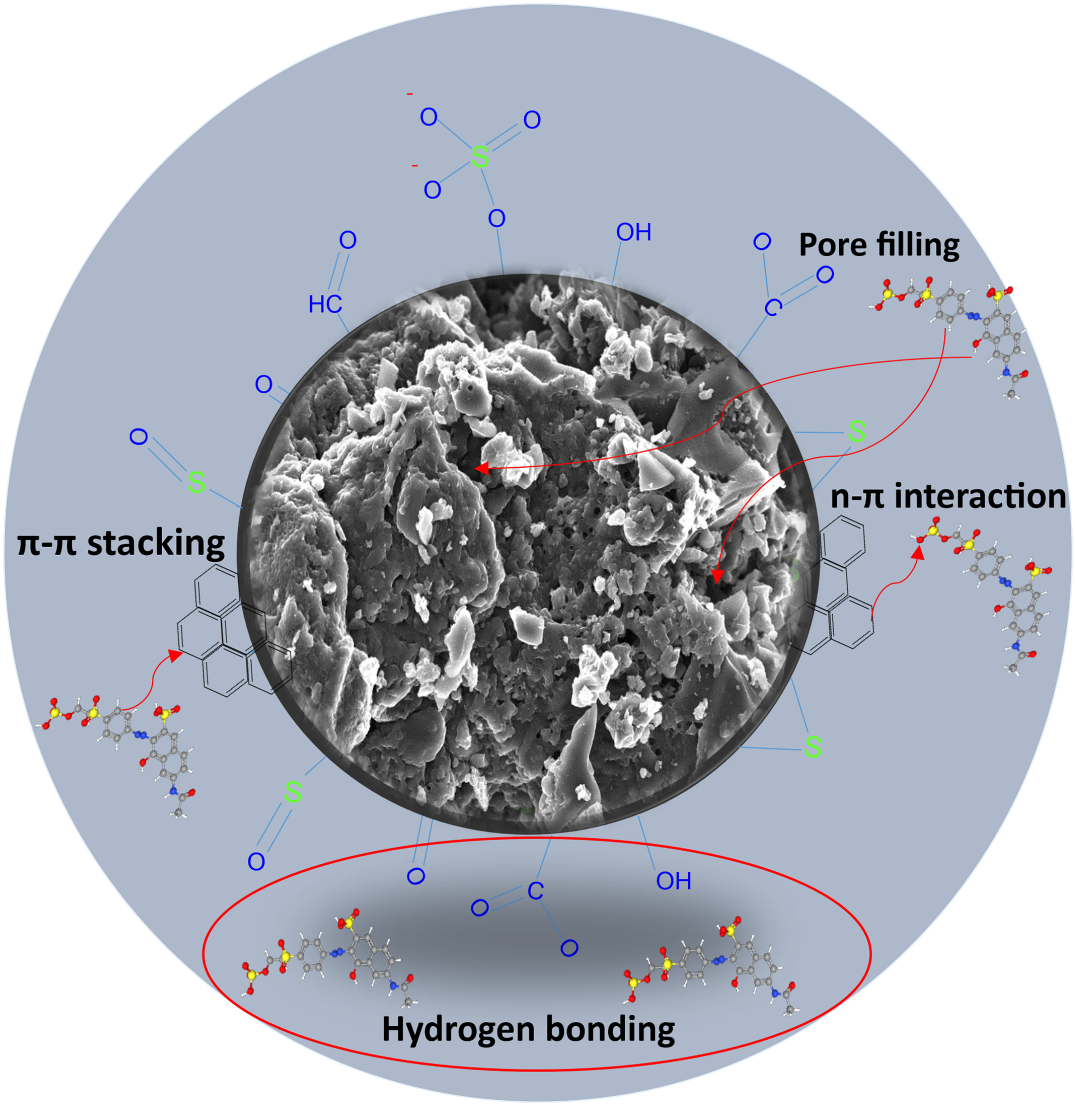

Fig. 4. Possible mechanisms for adsorption of RO16 onto carbon adsorbent.

3.5. Adsorbent regeneration

The AC-SE 600 °C and the AC 600 °C were used to evaluate the regeneration studies. The tests were performed using an adsorbent dosage of 2.5 g/L available in the e-supplementary material. The AC-SE 600 carbon performed better compared to the AC 600, and results showed that it can be reused at least three times, losing approximately 40 % of its initial removal performance. This loss of performance could be due to pore blockage by adsorbate molecules getting sized inside small pores, as well as mechanical damage to the carbon matrix during cycling. To sum up, the AC-SE 600 biochar exhibited good reusability for a second cycle. However, further experiments on testing different eluents could help the biochar to reach even higher adsorption performances after two or more cycles.

3.6. Possible adsorption mechanisms of RO16 on carbon adsorbents

XPS performed on a dye-loaded sample (AC-SE 600) showed higher amounts of S, N, O, and Na (all constituents of RO16), validating the dye adsorption on the carbon surface (e-supplementary material). Spectral deconvolution revealed amide (O = C-NH, 531.8 eV) and sulfonate (SO_3^-, 167,4 eV) functionalities, only present in the dye-loaded sample. Fig. 4 shows a proposal of the interaction mechanism of RO16 with carbon-based materials. Because the carbon adsorbents displayed very high surface areas, the dominant mechanism should be the pore filling. Furthermore, due to the RO16 molecule dimensions (1.68 nm (length), 1.42 nm (width)) (Calvete et al., 2010), it would be expected that RO16 molecule can easily fit into the big micropores (1.7 – 2.0 nm) and mesopores (>2.0 nm) present in the carbon adsorbents. Besides pore filling, hydrogen bonding, π- π stacking, and n- π interactions are also involved in the overall adsorption process. Hydrogen bonding occurs between H of the RO16 molecules and the carbon materials' O, H, and S atoms (dos Reis et al., 2023b). For the *π-π* interactions, RO16 has functionalities that can create a strong electron-withdrawing effect on the aromatic ring from the carbon, which allows the aromatic ring to bind to the electron donor carbon adsorbent surfaces that establish *π-π* interactions (Lima et al., 2024). Donor-acceptor interactions (n–π interaction) between the oxygen and sulfur (electron-donating) on the adsorbent surface and the π-system in the aromatic rings of the dye molecules (electron acceptor) could also contribute to the adsorption mechanism (Sayed et al., 2024).

3.7. Treatment of two synthetic dyehouse effluents

A real dyehouse effluent may contain various dyes, salts, and trace metals (Yaseen & Scholz, 2019). Therefore, the carbon materials potential for chromophore removal was tested on two synthetic effluents prepared from several dyes and salts (dos Reis et al., 2023b). UV–Vis spectra for the effluents before and after treatment and the % Removal of chromophores for the individual carbons are presented in Fig. 5. Analogous to the RO16 adsorption, AC-SE 600 was the best carbon for chromophore removal, achieving 71 and 73 % chromophore removal for effluent A and B, respectively. In contrast, the second-best carbon, AC 600, removed 48 and 54 %. These results show that the carbons are suited for the removal of a range of different dyes in a salt-containing environment. In addition, it highlights the benefit of subjecting the materials to STEX prior to a chemical activation step. This could be investigated further by optimizing the process for maximum yield and performance at minimum energy and chemical expenditure, investigating other activation chemicals and adsorbates, and even looking towards other carbon applications such as supercapacitors and batteries. Likewise, the importance of handling wasted adsorbents and desorbed pollutants sustainably, should be further studied and developed.

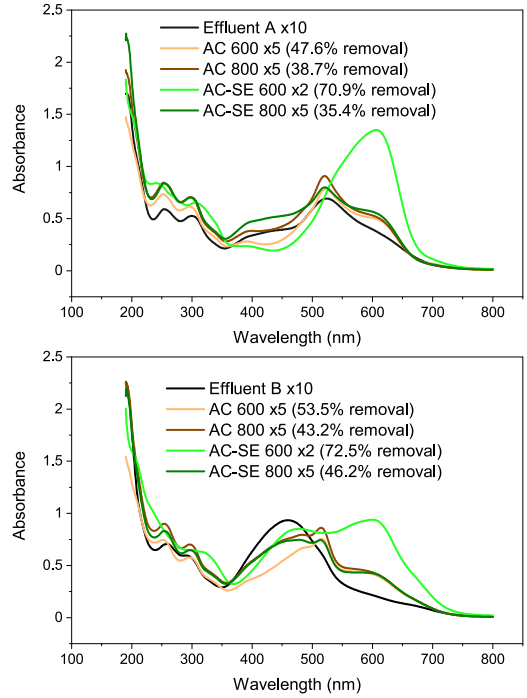

Fig. 5. UV–Vis spectra for the two synthetic dyehouse effluents A and B (ten times dilution) and residual effluent (two or five times dilution) after treatment with each of the four carbon materials. The % removal is calculated as a difference between the integrated curves before and after treatment.

4. Conclusions

This study demonstrated the feasibility of STEX preconditioning for softwood bark for producing functionalized ACs with high adsorption capacities. The STEX-treated carbons showed favourable characteristics with increased specific surface area, pore volume, and aromaticity, leading to enhanced adsorption compared to conventionally produced ACs. The adsorption kinetics and equilibrium studies revealed the effectiveness of the STEX-treated carbons in dye removal (Q_{max} 218 mg g^{-1}). Moreover, the regeneration studies showed the potential for reusability of STEX-treated ACs. This research shows that innovative methods like STEX for producing carbonaceous adsorbents for wastewater treatment lay a foundation for future advancements in the field.

Funding

This work was supported by Vinnova (Swedish Governmental Agency for Innovation Systems), grant number 2017–05408, Bio4Energy, a strategic research environment appointed by the Swedish government, and Valmet AB.

CRediT authorship contribution statement

Andreas Averheim: Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Glaydson Simões dos Reis:** Writing – review & editing, Validation, Methodology, Investigation, Conceptualization. **Alejandro Grimm:** Writing – review & editing, Investigation. **Davide Bergna:** Investigation. **Anne Heponiemi:**

Writing – review & editing. **Ulla Lassi:** Writing – review & editing, Resources. **Mikael Thyrel:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization.

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.

Acknowledgment

We thank Gunnar Kalén at the Biomass Technology Center, Umeå, Sweden, and Jan Detlefsen at Valmet Fiber Technology Center, Sundsvall, Sweden, for operational work during the preprocessing of biomass and steam explosion runs, respectively. Furthermore, Tao Hu and Rafal Sliz at Oulu University, Research Unit of Sustainable Chemistry, are acknowledged for their skilful performance of FTIR and XPS measurements. In addition, Umeå University Vibrational Spectroscopy Core Facility, Umeå, Sweden, is acknowledged for granting access to a suitable Raman instrument.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2024.130698.

References

Aarum, I., Solli, A., Gunnarsson, H., Kalyani, D., Devle, H., Ekeberg, D., Stenstrøm, Y., 2019. Comparison of pyrolyzed lignin before and after milled wood lignin purification of Norway spruce with increasing steam explosion. Wood Sci. Technol. 53 (3), 601–618.

Al-Ghouti, M.A., Da'ana, D.A., 2020. Guidelines for the use and interpretation of adsorption isotherm models: A review. J. Hazard. Mater. 393, 122383.

Al-Tohamy, R., Ali, S.S., Li, F., Okasha, K.M., Mahmoud, Y.A.G., Elsamahy, T., Jiao, H., Fu, Y., Sun, J., 2022. A critical review on the treatment of dye-containing wastewater: Ecotoxicological and health concerns of textile dyes and possible remediation approaches for environmental safety. Ecotoxicol. Environ. Saf. 231, 113160.

Averheim, A., Larsson, S.H., Thyrel, M., 2022. Carbocation scavenger pretreatment to mitigate lignin self-condensation in a semi-industrial steam explosion process. Bioresource Technology Reports 20, 101292.

Bhat, S., Uthappa, U.T., Sadhasivam, T., Altalhi, T., Soo Han, S., Kurkuri, M.D., 2023. Abundant cilantro derived high surface area activated carbon (AC) for superior adsorption performances of cationic/anionic dyes and supercapacitor application. Chem. Eng. J. 459, 141577.

Bilal, M., Ihsanullah, I., Hassan Shah, M.U., Bhaskar Reddy, A.V., Aminabhavi, T.M., 2022. Recent advances in the removal of dyes from wastewater using low-cost adsorbents. J. Environ. Manage. 321, 115981.

Cagnon, B., Py, X., Guillot, A., Stoeckli, F., Chambat, G., 2009. Contributions of hemicellulose, cellulose and lignin to the mass and the porous properties of chars and steam activated carbons from various lignocellulosic precursors. Bioresour. Technol. 100 (1), 292–298.

Calvete, T., Lima, E.C., Cardoso, N.F., Vaghetti, J.C.P., Dias, S.L.P., Pavan, F.A., 2010. Application of carbon adsorbents prepared from Brazilian-pine fruit shell for the removal of reactive orange 16 from aqueous solution: Kinetic, equilibrium, and thermodynamic studies. J. Environ. Manage. 91 (8), 1695–1706.

Correa, C.R., Kruse, A., 2018. Biobased Functional Carbon Materials: Production, Characterisation, and Applications—A Review. Materials 11 (9), 1568.

Dąbrowski, A., 2001. Adsorption — from theory to practice. Adv. Colloid Interface Sci. 93 (1), 135–224.

Deng, J., Li, M., Wang, Y., 2016. Biomass-derived carbon: synthesis and applications in energy storage and conversion. Green Chem. 18 (18), 4824–4854.

dos Reis, G.S., Larsson, S.H., Mathieu, M., Thyrel, M., Pham, T.N., 2021a. Application of design of experiments (DoE) for optimized production of micro- and mesoporous Norway spruce bark activated carbons. Biomass Convers. Biorefin.

dos Reis, G.S., Larsson, S.H., Thyrel, M., Pham, T.N., Claudio Lima, E., de Oliveira, H.P., Dotto, G.L., 2021b. Preparation and application of efficient biobased carbon adsorbents prepared from spruce bark residues for efficient removal of reactive dyes and colors from synthetic effluents. Coatings 11 (7), 772.

dos Reis, G.S., Thivet, J., Laisné, E., Srivastava, V., Grimm, A., Lima, E.C., Bergna, D., Hu, T., Naushad, M., Lassi, U., 2023a. Synthesis of novel mesoporous selenium-doped biochar with high-performance sodium diclofenac and reactive orange 16 dye removals. Chem. Eng. Sci. 281, 119129.

dos Reis, G.S., Bergna, D., Grimm, A., Lima, E.C., Hu, T., Naushad, M., Lassi, U., 2023b. Preparation of highly porous nitrogen-doped biochar derived from birch tree wastes with superior dye removal performance. Physicochemical and Engineering Aspects, Colloids and Surfaces A, p. 669.

dos Santos, D.C., Adebayo, M.A., de Fátima Pinheiro Pereira, S., Prola, L.D.T., Cataluña, R., Lima, E.C., Saucier, C., Gally, C.R., Machado, F.M., 2014. New carbon composite adsorbents for the removal of textile dyes from aqueous solutions: Kinetic, equilibrium, and thermodynamic studies. Korean J. Chem. Eng. 31 (8), 1470–1479.

Foo, K.Y., Hameed, B.H., 2010. Insights into the modeling of adsorption isotherm systems. Chem. Eng. J. 156 (1), 2–10.

Guo, X., Wang, J., 2019. A general kinetic model for adsorption: Theoretical analysis and modeling. J. Mol. Liq. 288, 111100.

Hames, B., Ruiz, R., Scarlata, C., Sluiter, A., Sluiter, J., Templeton, D., 2008. Preparation of samples for compositional analysis. Natianal Renewable Energy Laboratory. NREL/TP-510-42620.

Hoang, A.T., Nguyen, X.P., Duong, X.Q., Ağbulut, Ü., Len, C., Nguyen, P.Q.P., Kchaou, M., Chen, W.-H., 2023. Steam explosion as sustainable biomass pretreatment technique for biofuel production: Characteristics and challenges. Bioresour. Technol. 385, 129398.

Ioannidou, O., Zabaniotou, A., 2007. Agricultural residues as precursors for activated carbon production—A review. Renew. Sustain. Energy Rev. 11 (9), 1966–2005.

Kant, R., 2012. Textile dyeing industry an environmental hazard. Nat. Sci. 4, 22–26.

Le Normand, M., Mélida, H., Holmbom, B.R., Michaelsen, T.E., Inngjerdingen, M., Bulone, V., Paulsen, B.S., Ek, M., 2014. Hot-water extracts from the inner bark of Norway spruce with immunomodulating activities. Carbohydr. Polym. 101, 699–704.

Lima, E.C., Ponce-Vargas, M., Naushad, M., Thue, P.S., dos Reis, G.S., Mello, B.L., Rabiee, N., Abatal, M., Korany Seliem, M., Badawi, M., 2024. Removal of methylparaben from aqueous effluents using biobased carbon material. Experimental and DFT calculations. J. Mol. Liq. 397, 124194.

Lin, S., Yu, J., Yao, A., Tian, S., Liao, H., Zhan, Y., Xiao, H., Lan, J., 2023. Mussel-inspired fabrication of pH-responsive pomelo peels as "smart" bio-based adsorbents for controllable removal of both cationic and anionic dyes. Sep. Purif. Technol. 326, 124744.

Malakootian, M., Heidari, M.R., 2018. Reactive orange 16 dye adsorption from aqueous solutions by psyllium seed powder as a low-cost biosorbent: kinetic and equilibrium studies. Appl Water Sci 8 (7), 212.

Marrakchi, F., Ahmed, M.J., Khanday, W.A., Asif, M., Hameed, B.H., 2017. Mesoporous carbonaceous material from fish scales as low-cost adsorbent for reactive orange 16 adsorption. J. Taiwan Inst. Chem. Eng. 71, 47–54.

Merlen, A., Buijnsters, J.G., Pardanaud, C., 2017. A guide to and review of the use of multiwavelength raman spectroscopy for characterizing defective aromatic carbon solids: from graphene to amorphous carbons. Coatings 7 (10), 153.

Muzamal, M., Jedvert, K., Theliander, H., Rasmuson, A., 2015. Structural changes in spruce wood during different steps of steam explosion pretreatment. Holzforschung 69.

Pimenta, M.A., Dresselhaus, G., Dresselhaus, M.S., Cançado, L.G., Jorio, A., Saito, R., 2007. Studying disorder in graphite-based systems by Raman spectroscopy. PCCP 9 (11), 1276–1290.

Pinheiro Nascimento, P.F., Barros Neto, E.L., 2021. Steam Explosion: Hydrothermal Pretreatment in the Production of an Adsorbent Material Using Coconut Husk. Bioenergy Res. 14 (1), 153–162.

Praveen, S., Jegan, J., Bhagavathi Pushpa, T., Gokulan, R., Bulgariu, L., 2022. Biochar for removal of dyes in contaminated water: an overview. Biochar 4 (1), 10.

Qasem, N.A.A., Mohammed, R.H., Lawal, D.U., 2021. Removal of heavy metal ions from wastewater: a comprehensive and critical review. *npj Clean.* Water 4 (1), 36.

Rosa, S., Laranjeira, M.C.M., Riela, H.G., Fávere, V.T., 2008. Cross-linked quaternary chitosan as an adsorbent for the removal of the reactive dye from aqueous solutions. J. Hazard. Mater. 155 (1), 253–260.

Sadezky, A., Muckenhuber, H., Grothe, H., Niessner, R., Pöschl, U., 2005. Raman microspectroscopy of soot and related carbonaceous materials: Spectral analysis and structural information. Carbon 43 (8), 1731–1742.

Sayed, N.S.M., Ahmed, A.S.A., Abdallah, M.H., Gouda, G.A., 2024. ZnO@ activated carbon derived from wood sawdust as adsorbent for removal of methyl red and methyl orange from aqueous solutions. Sci. Rep. 14 (1), 5384.

Shah, J.A., Butt, T.A., Mirza, C.R., Shaikh, A.J., Khan, M.S., Arshad, M., Riaz, N., Haroon, H., Gardazi, S.M.H., Yaqoob, K., Bilal, M., 2020. Phosphoric acid activated carbon from melia azedarach waste sawdust for adsorptive removal of reactive orange 16: equilibrium modelling and thermodynamic analysis. Molecules 25 (9), 2118.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2008. Determinataion of Structural Carbohydrates and Lignin in Biomass. National Renewable Energy Laboratory. NREL/TP-510-42618.

Sutthasupa, S., Koo-amornpattana, W., Worasuwannarak, N., Prachakittikul, P., Teachawachirasiri, P., Wanthong, W., Thungthong, T., Inthapat, P., Chanamarn, W., Thawonbundit, C., Srifa, A., Ratchahat, S., Chaiwat, W., 2023. Sugarcane bagasse-derived granular activated carbon hybridized with ash in bio-based alginate/gelatin polymer matrix for methylene blue adsorption. Int. J. Biol. Macromol. 253, 127464.

Textile Exchange. 2022. Preferred Fiber & Materials Market Report.

Torget, R., Himmel, M.E., Grohmann, K., 1991. Dilute sulfuric acid pretreatment of hardwood bark. Bioresour. Technol. 35 (3), 239–246.

Vigneshwaran, S., Sirajudheen, P., Karthikeyan, P., Meenakshi, S., 2021. Fabrication of sulfur-doped biochar derived from tapioca peel waste with superior adsorption performance for the removal of Malachite green and Rhodamine B dyes. Surf. Interfaces 23, 100920.
Wang, J., Zhang, X., Li, Z., Ma, Y., Ma, L., 2020. Recent progress of biomass-derived carbon materials for supercapacitors. J. Power Sources 451, 227794.
Weber, W.J., Morris, J.C., 1963. Kinetics of adsorption on carbon from solution. J. Sanit. Eng. Div. 89 (2), 31–59.
Won, S.W., Choi, S.B., Yun, Y.-S., 2006. Performance and mechanism in binding of Reactive Orange 16 to various types of sludge. Biochem. Eng. J. 28 (2), 208–214.
Yang, Y., Cannon, F.S., 2021. Preparation of activated carbon from pine sawdust with hydrothermal-pressure preconditioning. J. Environ. Chem. Eng. 9 (6), 106391.
Yang, G., Pan, C., Yang, H., Feng, N., 2021. Carbon-supported nickel catalyst prepared from steam-exploded poplar by recovering Ni(II). BioResources 16, 5481–5493.
Yaseen, D.A., Scholz, M., 2019. Textile dye wastewater characteristics and constituents of synthetic effluents: a critical review. Int. J. Environ. Sci. Technol. 16 (2), 1193–1226.
Zhang, L., Liu, Z., Cui, G., Chen, L., 2015. Biomass-derived materials for electrochemical energy storages. Prog. Polym. Sci. 43, 136–164.

Acta Universitatis Agriculturae Sueciae
Doctoral Thesis No. 2024:61

Softwood bark is an underutilized bioeconomy resource. This thesis focuses on the valorization of softwood bark through a steam explosion processing step. Producing second-generation bioethanol from the bark via enzymatic hydrolysis and fermentation proved challenging, even with enhancing additives. Instead, innovative techniques were developed for producing biobased carbon materials through steam explosion, activation, and pyrolysis, as well as monitoring value-added byproducts from steam explosion using Raman spectroscopy. These novel methods also have potential applications for other feedstocks.

Andreas Av**erhei**m received his doctoral education at the Department of Forest Biomaterials and Technology, SLU, Umeå, and his Master of Science in Chemical Engineering at Luleå University of Technology.

Acta Universitatis Agriculturae Sueciae presents doctoral theses from the Swedish University of Agricultural Sciences (SLU).

SLU generates knowledge for the sustainable use of biological natural resources. Research, education, extension, as well as environmental monitoring and assessment are used to achieve this goal.

ISSN 1652-6880
ISBN (print version) 978-91-8046-352-2
ISBN (electronic version) 978-91-8046-388-1