



LUND UNIVERSITY

Process development and environmental assessment within softwood based biorefineries

Carlqvist, Karin

2022

Document Version:

Publisher's PDF, also known as Version of record

[Link to publication](#)

Citation for published version (APA):

Carlqvist, K. (2022). *Process development and environmental assessment within softwood based biorefineries*. [Doctoral Thesis (compilation), Division of Chemical Engineering]. Chemical Engineering, Lund University.

Total number of authors:

1

Creative Commons License:

Unspecified

General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Process development and environmental assessment within softwood based biorefineries

KARIN CARLQVIST

CHEMICAL ENGINEERING | FACULTY OF ENGINEERING | LUND UNIVERSITY



Process development and environmental assessment within softwood based biorefineries

Karin Carlqvist

Department of Chemical Engineering

Lund University, Sweden

2022



LUND
UNIVERSITY

DOCTORAL DISSERTATION

by due permission of the Faculty of Engineering, Lund University, Sweden.
To be defended in lecture hall KC:A at Kemicentrum, Naturvetarvägen 14, Lund.
March 4, 2022 at 9:00 a.m.

Faculty opponent

Prof. Magdalena Svanström, Department of Technology Management and
Economics, Chalmers, Sweden.

Organization LUND UNIVERSITY Department of Chemical Engineering Box 124, SE-22100 Lund, Sweden		Document name DOCTORAL DISSERTATION	
		Date of issue 2022-03-04	
Author(s) Karin Carlqvist		Sponsoring organization Bo Rydin Foundation (Paper I)	
Title and subtitle Process development and environmental assessment within softwood based biorefineries			
Abstract We urgently need to move from a fossil-based to a bio-based society. From a Swedish perspective, a promising way forward are biorefineries with an integrated production of materials, chemicals, and energy from the softwood species Scots pine (<i>Pinus sylvestris</i>) and Norway spruce (<i>Picea abies</i>). A diverse set of process routes will be necessary to produce all intermediates and commercial products. In this thesis, the focus is the production of phenolic compounds through extraction, and production of fermentable sugars through steam explosion pretreatment with subsequent enzymatic hydrolysis. Although renewable, the annual biomass production is limited, and efficient biorefinery processes are essential. By understanding the structural or chemical differences within the feedstock, processes can be optimized further. In this thesis, it is shown how more juvenile tissue, or sapwood, of Scots pine required lower severity in steam explosion pretreatment than older tissue, heartwood, or knots for the production of fermentable sugars. Pretreating different wood tissues of Scots pine at different severity can thus increase the overall yield of fermentable sugars. Bark is chemically significantly different from wood and the production of fermentable sugars from bark is not feasible using the same processes as for wood. In this thesis, it is shown how removing water-soluble extractives prior to steam explosion pretreatment, and enzymatic hydrolysis can increase the yield of glucose by 30 % and 11 % in the enzymatic hydrolysis of bark from Norway spruce and from Scots pine, respectively. To develop new process technologies with favorable environmental profiles, it is essential to quantitatively assess the potential environmental impact of different process choices through life cycle assessment (LCA), early during process development. In this thesis, the potential environmental impact of the production of cationized tannins from high-pressure hot water extract of Norway spruce bark was evaluated. The potential environmental impacts when using the three different extraction technologies: hot water extraction, ultrasound extraction, and supercritical extraction, for the production of phenolic compounds, were also assessed and compared. For the production of 1 kg cationized tannins the overall contribution to climate change was estimated to be between 1.2-4.7 kg CO ₂ eq. The potential impact on climate change for the three extraction technologies were estimated to 0.48-0.68, 5.9-11, and 5.8-6.3 kg CO ₂ -eq. per kg of produced phenolic compounds for hot water extraction, ultrasound assisted extraction, and supercritical fluid extraction, respectively. The need for chemical solvents and reagents were identified as primary hotspots, i.e. critical aspects to be prioritized for action, in all studied systems and for most of the studied impact categories. Simple, water-based systems, therefore, have substantial advantages. In addition, it will be necessary with sustainably produced solvents and reagents to reach a sustainable biobased economy.			
Key words sugar platform, extractives, steam explosion pretreatment, bark, enzymatic hydrolysis, phenolic compounds, bioeconomy, LCA, <i>Pinus sylvestris</i> , <i>Picea abies</i> , pine, spruce			
Classification system and/or index terms (if any)			
Supplementary bibliographical information		Language English	
ISSN and key title		ISBN 978-91-7422-858-8 (print) 978-91-7422-859-5 (pdf)	
Recipient's notes	Number of pages 80		Price
	Security classification		

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature

Date 2022-01-24

Process development and environmental assessment within softwood based biorefineries

Karin Carlqvist

Department of Chemical Engineering

Lund University, Sweden

2022



LUND
UNIVERSITY

Cover illustration by Vertyr

Copyright pp 1-80 Karin Carlqvist 2022

Paper 1 © 2015 Springer Nature

Paper 2 © 2017 Springer

Paper 3 © 2020 Society of Chemical Industry and John Wiley & Sons

Paper 4 © 2021 Elsevier

Faculty of Engineering at Lund University

Department of Chemical Engineering

ISBN 978-91-7422-858-8 (print)

ISBN 978-91-7422-859-5 (pdf)

Printed in Sweden by Media-Tryck, Lund University

Lund 2022



Media-Tryck is a Nordic Swan Ecolabel certified provider of printed material. Read more about our environmental work at www.mediatryck.lu.se

MADE IN SWEDEN 

*“We don’t inherit the earth from our ancestors,
we borrow it from our children.”*

-Native American proverb

Contents

Abstract	ix
Populärvetenskaplig sammanfattning.....	x
List of publications	xii
Author contributions.....	xiii
Acknowledgements.....	xiv
Abbreviations and symbols	xv
1 Introduction	1
1.1 Scope and outline	2
2 Understanding the feedstock.....	5
2.1 Structure and function of the tree stem	5
2.2 Chemistry of wood and bark.....	6
2.2.1 Extractives	7
2.2.2 Carbohydrates.....	8
2.2.3 Lignin.....	11
2.2.4 Inorganics	13
2.2.5 Suberin	13
2.3 Chemical characteristics of the used feedstocks	13
2.3.1 Methods used for characterization of biomass	14
2.3.2 The heterogeneity of tree stems.....	15
2.3.3 Bark from Scots pine and Norway spruce.....	17
2.3.4 Important outcomes	18
3 Softwood based biorefineries	19
3.1 Overview of production routes and products	21
3.2 Production of phenolic compounds	23
3.3 Production of fermentable sugars	24
3.3.1 Steam explosion pretreatment	25
3.3.2 Enzymatic hydrolysis	28

3.4	Process development within softwood biorefineries	31
3.4.1	Methods for pretreatment and enzymatic hydrolysis	32
3.4.2	Influence of tissue type on processability	34
3.4.3	Removal of extractives to enhance hydrolysis	38
3.4.4	Energy and mass flows in extraction	41
3.4.5	Important outcomes	44
4	Environmental assessment	45
4.1	Towards an LCA framework	46
4.1.1	Goal and scope definition	48
4.1.2	Inventory analysis	49
4.1.3	Impact assessment	51
4.1.4	Interpretation	54
4.2	LCA of bark-derived phenolic compounds	55
4.2.1	Methodological considerations	56
4.2.2	Chemicals and material	58
4.2.3	Electricity	61
4.2.4	Heat	62
4.2.5	Important outcomes	63
5	Conclusion and outlook	65
6	References	69

Abstract

We urgently need to move from a fossil-based to a bio-based society. From a Swedish perspective, a promising way forward are biorefineries with an integrated production of materials, chemicals, and energy from the softwood species Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*). A diverse set of process routes will be necessary to produce all intermediates and commercial products. In this thesis, the focus is the production of phenolic compounds through extraction, and production of fermentable sugars through steam explosion pretreatment with subsequent enzymatic hydrolysis.

Although renewable, the annual biomass production is limited, and efficient biorefinery processes are essential. By understanding the structural or chemical differences within the feedstock, processes can be optimized further. In this thesis, it is shown how more juvenile tissue, or sapwood, of Scots pine required lower severity in steam explosion pretreatment than older tissue, heartwood, or knots for the production of fermentable sugars. Pretreating different wood tissues of Scots pine at different severity can thus increase the overall yield of fermentable sugars. Bark is chemically significantly different from wood and the production of fermentable sugars from bark is not feasible using the same processes as for wood. In this thesis, it is shown how removing water-soluble extractives prior to steam explosion pretreatment, and enzymatic hydrolysis can increase the yield of glucose by 30 % and 11 % in the enzymatic hydrolysis of bark from Norway spruce and from Scots pine, respectively.

To develop new process technologies with favorable environmental profiles, it is essential to quantitatively assess the potential environmental impact of different process choices through life cycle assessment (LCA), early during process development. In this thesis, the potential environmental impact of the production of cationized tannins from high-pressure hot water extract of Norway spruce bark was evaluated. The potential environmental impacts when using the three different extraction technologies: hot water extraction, ultrasound extraction, and supercritical extraction, for the production of phenolic compounds, were also assessed and compared. For the production of 1 kg cationized tannins the overall contribution to climate change was estimated to be between 1.2-4.7 kg CO₂ eq. The potential impact on climate change for the three extraction technologies were estimated to 0.48-0.68, 5.9-11, and 5.8-6.3 kg CO₂-eq. per kg of produced phenolic compounds for hot water extraction, ultrasound assisted extraction, and supercritical fluid extraction, respectively. The need for chemical solvents and reagents were identified as primary hotspots, i.e. critical aspects to be prioritized for action, in all studied systems and for most of the studied impact categories. Simple, water-based systems, therefore, have substantial advantages. In addition, it will be necessary with sustainably produced solvents and reagents to reach a sustainable biobased economy.

Populärvetenskaplig sammanfattning

Den svenska skogen är högtintressant som källa för att ersätta behovet av fossila råvaror. I den här avhandlingen beskrivs hur gran och tall kan användas som råvaror för att tillverka material och kemikalier, tillsammans med el- och värmeproduktion. Avhandlingen handlar om hur processerna kan förbättras och hur den miljömässiga påverkan minimeras.

Vi rör oss från ett samhälle baserat på fossila till ett baserat på förnyelsebara råvaror. Bioraffinaderier (biorefineries), där biomassa (från skogs- och jordbruk, alger mm) som råvara för energi, material och kemikalier förväntas få en ökad betydelse i denna omställning. I Sverige där två tredjedelar av ytan är täckt av skogsmark, främst gran och tall, är barrträd (softwood) en högtintressant råvara. Trä består till största delen av de tre strukturgivande delarna cellulosa, hemicellulosa och lignin. Både cellulosa och hemicellulosa utgörs av långa sockerkedjor. Den tredje strukturgivande komponenten lignin är en komplex struktur av olika ringformade kolväten. Dessutom innehåller trä en mängd lösliga ämnen, ofta kallade extraktivämnen, som olika fenoler, fetter och socker. Det finns ett stort antal möjliga användningsområden för de olika kemiska komponenterna i trä. Med genomtänkta processer för hur råvaran används kan material, kemikalier, el och värme produceras utöver dagens huvudsakliga produkter papper, pappersmassa och sågade trävaror.

Ett sätt att tillverka olika kommersiellt intressanta kemikalier från trä är att ta hjälp av mikroorganismer, som bakterier eller jäst, som kan omvandla socker till olika värdefulla kemikalier. Problemet är att lång evolution har gjort trä till ett material som är oerhört hållbart och socker i trä, i form av cellulosa och hemicellulosa, är en del av en komplex struktur. Ett träd som fälls och blir liggande i skogen kommer att ligga kvar i åtminstone årtionden. Därför används processer med både värme, kemikalier och enzymer för att frigöra sockret. Processerna har tidigare optimerats för olika träslag. Men en trädstam är inte en homogen struktur. Förutom bark som har markant särskiljande utseende består veden av kärnved, splintved och kvistar, som kan särskiljas genom olika färgskiftningar. Att olika delar av veden har olika kemisk sammansättning och även olika egenskaper är sedan tidigare känt inom skogsindustrin. Trots detta finns det mycket få vetenskapliga studier som behandlat vad olikheterna inom veden har för betydelse i en process för att framställa socker. I den här avhandlingen visas hur processen att tillverka socker påverkas av vilken del av tallstammen som används. Att producera socker från bark har visat sig vara extra svårt. En trolig orsak kan vara att det finns kemiska ämnen i just bark som stör processen. I den här avhandlingen beskrivs hur man genom att först extrahera barken från gran och tall med varmt vatten kan producera mer socker som mikroorganismer kan använda.

Extraktion av bark innebär inte bara att efterföljande processteg för att producera socker kan förbättras. Lösningen, extraktet, som fås innehåller även många intressanta ämnen till exempel olika ringformade kolföreningar kallade fenoler. Fenoler kan användas till flera olika produkter. I den här avhandlingen analyseras de miljömässiga konsekvenserna av en process där en sorts fenoler, kallade tanniner, modifieras kemiskt för att sedan kunna användas vid vattenrening. Studien visade på vikten av att noggrant välja de kemikalier som används i processen. Dessutom måste en utvärdering göras om alla processteg verkligen är nödvändiga, eftersom vissa steg kan ha stor miljöpåverkan.

Fenoler kan utvinnas ur bark genom extraktion med flera olika tekniker. De flesta av dem är i ett tidigt utvecklingsskede och används ännu inte i kommersiell skala. För att utforma miljömässigt hållbara processer är det dock viktigt att kvantifiera och utvärdera miljöpåverkan (environmental assesment) av olika processval redan i ett tidigt utvecklingsskede, även då tillgången till data är bristfällig. Det är dock viktigt att beräkningarna görs för en uppskalad process med till exempel industriellt relevanta metoder för värmeförsel. I den här avhandlingen visar beräkningar för framtida industriella processer att det krävs betydligt högre utbyten för de mer komplexa och resurskrävande teknikerna jämfört med enklare tekniker för att kompensera för den ökade miljöpåverkan komplexa processer medför.

Förhoppningen är att den här avhandlingen kan bidra med en ökad för hur processer i bioraffinaderier behöver anpassas efter vilka delar av barrträds stam som används som råvara. Dessutom är förhoppningen att den belyser vikten av att i ett tidigt skede utvärdera miljöpåverkan och därigenom kunna fatta avgörande beslut för att designa mer miljömässigt hållbara processer.

List of publications

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals. The papers are appended at the end of the thesis

- I. Johansson, S., **Carlqvist, K.**, Kataria, R., Ulvcrona, T., Bergsten, U., Arshadi, M., Galbe, M., Lidén, G., 2015. Implications of differences in macromolecular composition of stem fractions for processing of Scots pine. *Wood Science and Technology* 49, 1037–1054. <https://doi.org/10.1007/s00226-015-0739-3>
- II. 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, 599–615. <https://doi.org/10.1007/s12010-017-2577-2>
- III. **Carlqvist, K.**, Arshadi, M., Mossing, T., Östman, U., Brännström, H., Halmemies, E., Nurmi, J., Lidén, G., Börjesson, P., 2020. Life-cycle assessment of the production of cationized tannins from Norway spruce bark as flocculants in wastewater treatment. *Biofuels, Bioproducts and Biorefining* 14, 1270–1285. <https://doi.org/10.1002/bbb.2139>
- IV. **Carlqvist, K.**, Wallberg, O., Lidén, G., Börjesson, P., 2022. Life cycle assessment for identification of critical aspects in emerging technologies for the extraction of phenolic compounds from spruce bark. *Journal of Cleaner Production* 333, 130093. <https://doi.org/10.1016/j.jclepro.2021.130093>

I have also contributed to the following peer-reviewed publication, which is not included in this thesis.

Carrasco, C., Cuno, D., **Carlqvist, K.**, Galbe, M., Lidén, G., 2015. SO₂ -catalysed steam pretreatment of quinoa stalks. *Journal of Chemical Technology & Biotechnology* 90, 64–71. <https://doi.org/10.1002/jctb.4286>

All papers are reproduced with the permission of their respective publishers.

Author contributions

- I. I did all the experimental work for the digestibility study and some of the analyses of pretreated material. I controlled the calculations for raw material and pretreatment and did all the calculations for the digestibility study. I did half of the writing and most of the formatting of the manuscript, including figures. All authors read and approved the manuscript.
- II. I did preparatory experiments with bark. I participated in the design and coordination of the study and in the preparation of the manuscript. All authors read and approved the manuscript.
- III. I planned the life cycle assessment, performed the data collection, made all calculations, analyzed the results, prepared the figures, and wrote the manuscript. All authors read and approved the manuscript.
- IV. I planned the life cycle assessment, performed the data collection, did most of the simulation work in Aspen plus, made all calculations not included in Aspen plus, analyzed the results, prepared the figures, and wrote the manuscript. All authors read and approved the manuscript.

Acknowledgements

First, I would like to express my deepest gratitude to my main supervisor, Prof. Gunnar Lidén. Thank you for your relentless optimism and unfailing support over the years. You are everything one could look for in a doctoral supervisor.

I would also like to show my gratitude to my co-supervisors, Prof. Henrik Stålblbrand and Prof. Pål Börjesson, and their research groups. Thank you for all your support and guidance in the “world of enzymes” and for letting me get to know the “art of life cycle assessment.”

Apart from my supervisors, I won't forget to express my gratitude to the rest of my co-authors. A special thanks to all collaborating researchers at the Swedish University of Agricultural Sciences (SLU) and Natural Resources Institute Finland (Luke) taking part in the projects Tallraff and TanWat.

I would also like to thank *all* my colleagues at the Department of Chemical Engineering at Lund University for the supportive atmosphere at the department. Whether it concerned troubleshooting laboratory equipment, mastering new computer software, or discussing the research, help was never far away. You have been like a second family, and we have shared both the greatest joys and the deepest sorrows of life.

I likewise very much appreciate my mentor Dr. Anneli Petersson. Your mentorship has been an invaluable gift over the past couple of years.

Finally, I would like to thank my family. Without your support, I would never have been able to finish. I love you all.

Karin Carlqvist

Lund, January 2022

Abbreviations and symbols

AIL	Acid insoluble lignin
ASL	Acid soluble lignin
CML	Centrum voor Milieukunde Leiden
DP	Degree of polymerization
DW	Dry weight
EJ	Exajoule
Gton	Gigaton
HPHWE	High pressure hot water extraction
HPLC	High performance liquid chromatography
HWE	Hot water extraction
ISO	International Organization for Standardization
LCA	Life cycle assessment or life cycle analysis
LCI	Life cycle inventory analysis
LCIA	Life cycle impact assessment
PM ₁₀	Fine particulate matter with a diameter of less than 10 µm
RI	Refractive index
SFE	Supercritical fluid extraction
STEX	Steam explosion
UAE	Ultrasound assisted extraction
WIS	Water insoluble solids

1 Introduction

During the past centuries, we have developed a society dependent on fossil resources for energy, fuels, and chemicals (Figure 1.1). However, there are many reasons, such as limited reserves, self-sufficiency, and not least climate change, calling for a transition from fossil to renewable sources. The biorefinery concept has been introduced for integrated production of food, feed, materials, chemicals, fuels, and energy from biomass (Kamm and Kamm, 2004). Biorefineries will most likely grow in importance in the coming decades to enable a less fossil-dependent society and local self-sufficiency in the supply of energy carriers and chemicals.

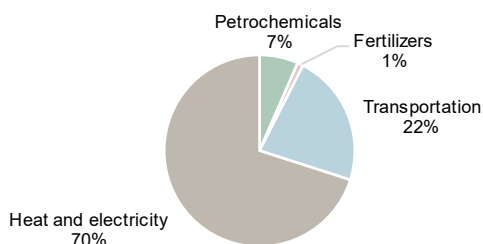


Figure 1.1. Proportions of the current use of fossil resources based on energy content (Bos and Broeze, 2020).

Although biomass is in principle a renewable resource, its production is inherently restricted by available land and sustainability constraints (Popp et al., 2014). Today, the annual use of fossil resources is about 15 Gton (corresponding to 440 EJ). The total annual harvest of wood, crop, and grass is 12 Gton (or 110 EJ), which is in the same range in mass as the fossil resource use, but only has a fourth of the energy content. Unharvested residues amounts to an additional 15 Gton (or 190 EJ) (Bos and Broeze, 2020). This means that efficiency improvements, utilization of side streams, and recycling within biorefineries will be crucial for reaching a biobased economy.

Two-thirds of the land in Sweden is forest land, most of which is producing or, is capable of producing commercial forest products. The two dominant tree species, constituting approximately 80 % of the standing tree volume, are the softwood species Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*) (Swedish National Forest

Inventory, 2021). These two species are also the most important ones for the forest industry. In 2020 the total roundwood consumption in Swedish sawmills and pulp mills was estimated to be 75 million m³ under bark. Approximately 90 % were pine and spruce, primarily produced in Sweden (Biometria, 2021).

Given the abundance of these two species and the already existing infrastructure for processing in sawmills and in pulp & paper industry, Norway spruce and Scots pine, are given choices for the development of Swedish biorefineries. With an advanced cascade use of the trees, energy, chemicals, and materials can be produced, in addition to today's major products of sawn wood and wood pulp. One example is the fermentative production of ethanol from hemicellulose sugars released in the sulfite process, which has a historically important role for the chemical industry in Sweden (Glessinger, 1952). One current example of a commercial softwood biorefinery is Borregaard in Norway. Their primary raw material is Norway spruce, which is converted to a diverse set of products within the product groups lignin-based biopolymers, specialty cellulose, vanillin, and bioethanol (Rødsrud et al., 2012). These products are based on the structural components lignin, hemicellulose, and cellulose. In addition, wood and bark, contain soluble components with a large spectrum of possible applications, both in specialized applications in the pharma-field (Jablonsky et al., 2017) and in bulk applications, for instance, as adhesives and binders for production of boards (Roffael, 2016), or as coagulants for water clarification (Bello et al., 2020).

For truly sustainable development, it is crucial to consider the entire production system and all the relevant environmental impacts through life cycle assessment (LCA). This allows hotspots, i.e. critical aspects with a large impact on the environmental performance, to be identified. Most of the environmental effects are locked already during the design phase. Therefore, it is essential to consider the environmental effects early in process development, even if this implicates a high degree of uncertainty of the LCA.

1.1 Scope and outline

This thesis concerns the use of wood and bark of Scots pine and Norway spruce as feedstocks in biorefineries, from both an experimental and systems analysis perspective. It primarily examines the upstream processes extraction, pretreatment, and enzymatic hydrolysis for the production of phenolic compounds and sugars.

The specific research questions in focus of this work were:

- How are pretreatment and enzymatic hydrolysis affected by the different tissue types of the wood log?
- Can upstream removal of extractives enhance the enzymatic hydrolysis of bark?
- What are the most significant environmental issues in the production of cationized tannins from the bark of Norway spruce, and how can the process be improved?
- What are the critical environmental aspects of different emerging technologies for extracting phenolic compounds from bark of Norway spruce?

This thesis summarizes the work described in four papers and is divided into five chapters. The second chapter describes the structure and chemistry of Scots pine and Norway spruce logs, including the feedstock analysis presented in **Papers I and II**. The third chapter covers processes and products within softwood biorefineries. Here the work on pretreatment and enzymatic hydrolysis reported in **Papers I and II** as well as mass and energy flows in extraction in **Papers III and IV**, is discussed. The fourth chapter covers the theory of life cycle assessment methodology as well as the environmental assessment of extraction of Norway spruce bark in **Papers III and IV**. Finally, chapter 5 provides a conclusion and outlook of this thesis.

2 Understanding the feedstock

This thesis, as explained in the previous chapter, concerns the use of wood and bark from Norway spruce and Scots pine as feedstocks in biorefineries. These materials are not homogeneous, as is apparent already from looking at the cross-section of a tree (Figure 2.1). Most noticeable are the concentric circles, with the central circles being darker colored (heartwood) surrounded by lighter colored circles (sapwood), and an outermost coarse layer (bark). In addition, the wood is scattered with darker patches (knots). Also on a molecular level, wood can be described as a composite material of the three main biopolymers cellulose, hemicelluloses, and lignin, together with minor amounts of extractives and inorganics. Therefore, this type of biomass is commonly referred to as lignocellulose (Bermek and Eriksson, 2009).

This chapter will start with an overview of the function, structure, and chemical composition of the tree stem of softwoods. This is followed by a description of the main methods used to evaluate, as well as the results and discussion, of the difference in chemical composition within and between two Scots pine trees (**Paper I**), and the difference between bark from Scots pine and Norway spruce (**Paper II**).

2.1 Structure and function of the tree stem

The tree stem has three main functions. Firstly, it supports the crown. Secondly, it is a storage of water, carbohydrates, and minerals. Thirdly, it is used for the transportation of water, nutrients, energy, and hormones within the tree (Pallardy, 2007a). Figure 2.1 shows the cross-section of a Scots pine stem. The wood, or xylem, consists of several layers of annual increments of vertically ordered elongated cells (Ek et al., 2009), resembling cones stacked on top of each other (Pallardy, 2007a). The older central part, called heartwood, contains dark-colored dead cells and mainly function as mechanical support. (Pallardy, 2007a). The outer and more juvenile part of the wood is called sapwood. It conducts sap, strengthens the stem, and stores and synthesizes biochemicals (Wiedenhoft and Miller, 2005). Due to a more closed structure, native heartwood is less susceptible to biological degradation than sapwood (Gjerdrum, 2003). This makes

it a useful construction material where durability is essential, for instance, for window frames (Ek et al., 2009).



Figure 2.1. To the left, the cross-section of a Scots pine stem with the darker heartwood surrounded by lighter sapwood and outermost the bark. To the right, boards of Scots pine (left) and Norway spruce (right) with knots visible as darker patches.

Modification of the photo "Pinus silvestris cross beentree" by Beentree available at https://commons.wikimedia.org/w/index.php?title=File:Pinus_silvestris_cross_beentree.jpg&oldid=477154119#file used under CC BY-SA 3.0 (<https://creativecommons.org/licenses/by-sa/3.0/deed.en>)

Where the branches are attached to the tree stem, knots are formed. This part of the wood, particularly in coniferous species which do not lose their needles in the winter, needs to tolerate high mechanical loads from wind and snow. In contrast to the vertical fibers of sapwood and heartwood, the fibers of the knots are horizontal. They also have a higher density, darker color, and a higher extractive content than the rest of the wood since this part of the tree can be prone to attacks from pathogens. Knots are considered a defect of the stem wood in pulp and sawmills (Ek et al., 2009) due to higher recalcitrance to the pulping process as well as giving poor mechanical properties of sawn timber (Kebbi-Benkeder et al., 2015).

The outermost layer of the tree stem, the bark, also has two distinct parts. The inner bark, or phloem, consists of living cells transporting the nutrients between the leaves/needles and the root (Ek et al., 2009). The outer bark consists of dead cells that reduce water loss and protect the stem from mechanical injury. Actively dividing cells responsible for the growth of the trunk, called the cambium, is only a single cell layer between the xylem and the phloem (Pallardy, 2007a).

2.2 Chemistry of wood and bark

Except for water, the major chemical component of a living tree, two-thirds of wood cells are typically made of carbohydrate-based polymers and one-third of lignin, on a

dry weight basis. In fact, wood almost solely consists of the elements carbon, oxygen, and hydrogen. Softwoods generally have a higher cellulose content, higher lignin, and lower pentosan content than hardwoods (Rowell et al., 2005). However, the exact chemical composition varies between individual trees and depends on several factors such as species, growth conditions, and age.

Structurally the cell wall is a composite of load-bearing microfibrils, semicrystalline aggregates of cellulose, and a middle lamella of hemicellulose, pectin, and lignin, binding the individual cells together (Hänninen et al., 2011). In addition to the structural components, the tree stem holds a vast set of soluble compounds often referred to as extractives and a minor amount of inorganic components or ash (Rowell et al., 2005). The chemical composition of bark is more complex than that of stem wood and, contains, for instance, suberin (hydroxy acid complexes) as well as a higher and more diverse content of extractives (Browning, 1967).

2.2.1 Extractives

“Extractives” is an umbrella term used for many different soluble compounds such as waxes, tannins, terpenes, and non-structural sugars. The extractives may be a chemical defense against pathogens as well as a physical barrier to protect the rest of the wood (Kirker et al., 2013; Wiedenhoef and Miller, 2005). The extractives content of Norway spruce wood has been reported to be 2.2 to 3.3 % (Frankó et al., 2015; Kačíková et al., 2013) and for Scots pine wood 2.6 to 4.5 % (Normark et al., 2014; Rowell et al., 2005). Knots and heartwood generally have a higher extractive content than sapwood (Willför et al., 2005). The extractive content in bark is usually even higher and more diverse than for wood of the same species (Browning, 1967). For Scots pine bark, the extractives content have been reported to be around 19 % (Miranda et al., 2012; Valentín et al., 2010) and for Norway spruce bark in the interval 22 to 32 % (Frankó et al., 2015; Halmemies et al., 2021; Miranda et al., 2012; Picea et al., 2021).

In addition to the difference between different tissue types, many other factors influence the determined content of extractives and make it problematic to compare reported values from bark analysis (Jensen et al., 1975). This can be factors such as age, growing conditions of the tree, the height of the tree stem of sampling, sampling method, storage time between sampling and analysis, and extraction method. Figure 2.2. intends to give an idea of the extractives present in Norway spruce bark based on a study by Halmemies et al.(2021) where lipophilic components first were extracted using hexane, and a water extraction was subsequently made. Lipophilic extractives constituted 4.8 % and water-soluble extractives constituted 30 % of the dry bark.

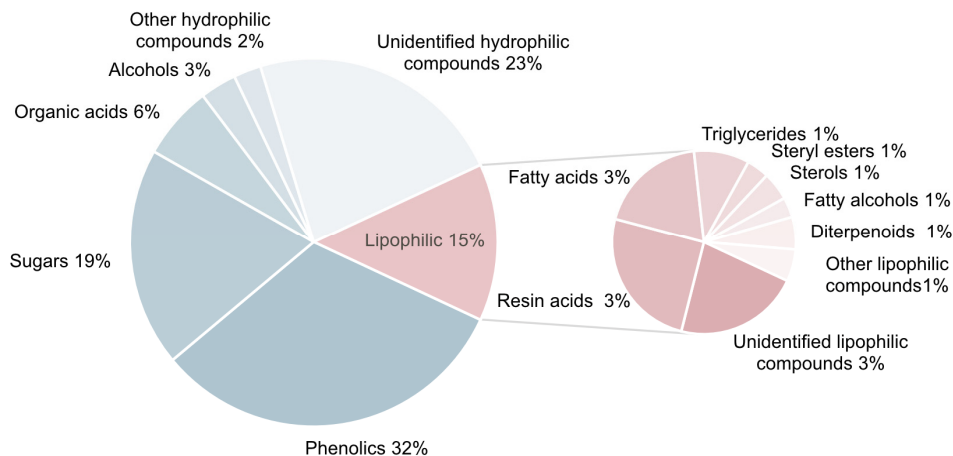


Figure 2.2. Extractives in bark from Norway spruce, sampled during winter. Blue represents water extract, and pink represents lipophilic extractives extracted with hexane. The percentage of different lipophilic extractives in the smaller circle represents the share of total extractives (Halmemies et al., 2021).

As can be seen in Figure 2.2 most of the extractives of Norway spruce bark are water soluble, whereof the majority are different sugars and phenolic compounds. In bark, the main water soluble monosaccharides are glucose and fructose (Laks, 1991). Sucrose, a disaccharide of glucose and fructose, plays an important role in plant metabolism. For most plant species this soluble disaccharide is the main form for carbohydrate transport within the plant. The phenolic compounds are, for instance stilbenes, flavonoids, and tannins (Beltrán-Heredia et al., 2011; Halmemies et al., 2021). Tannins are complex polyphenolic compounds with a size in the range between 500 and a few thousand daltons (Be(Sturm and Tang, 1999). ltrán-Heredia et al., 2011). The type of tannins is species dependent (Laks, 1991).

2.2.2 Carbohydrates

Most of the carbohydrate content of the tree stem is present as polymers, mainly as cellulose and hemicellulose, but minor amounts of starch and pectin are also found (Rowell et al., 2005). In addition, there are water-soluble monosaccharides, mainly glucose and fructose, predominantly in the bark (Laks, 1991).

Cellulose

Cellulose is often stated to be the most abundant organic polymer on earth (Bermek and Eriksson, 2009). It is a polymer of glucose (D-glucopyranose) units linked together by β -(1 \rightarrow 4)-glucosidic bonds (Figure 2.3). The degree of polymerization (DP), the number of glucose units, is suggested to be at least 9,000–10,000 on average (Rowell et al., 2005). However, the repeating unit in the polymer is the disaccharide cellobiose, since adjacent sugars are rotated by 180 degrees (Horn et al., 2012). This is the reason for the long, straight, non-helical shape of the cellulose chain.

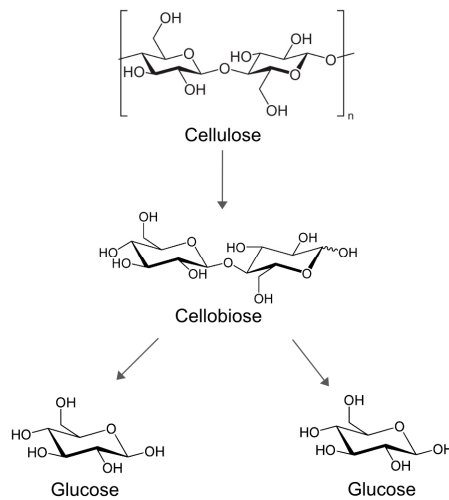


Figure 2.3. Partial structure of the cellulose polymer. The repeating unit, cellobiose, consists of two perpendicular D-glucopyranose units.

The cellulose chains can form intra- and intermolecular hydrogen bonds, organizing the chains parallel within a layer. In turn, the layers are held together by weak van der Waals forces (Rowell et al., 2005). In this way, wood cellulose chains can be organized as microfibrils, a linear structure of approximately 24 to 36 aggregated cellulose chains (Endler and Persson, 2011; Fernandes et al., 2011). The cellulose can be densely packed as crystalline cellulose or have a lower packing degree as amorphous cellulose (De Carvalho et al., 2016). Most of the native wood cellulose is highly crystalline (Rowell et al., 2005). The structure and degree of polymerization are similar for bark and sapwood cellulose (Laks, 1991), but the degree of crystallinity is lower in the bark (Rowell et al., 2005). The cellulose microfibrils are not a fully detached structure but are probably associated with, for instance, hemicellulose (Åkerholm and Salmén, 2001), which form a complex network around the cellulosic fibers (Várnai et al., 2011).

Hemicellulose

While cellulose is made of only the hexose glucose, hemicellulose is a group of heteropolymers made from a mixture of different hexose and pentose sugars. Hemicellulose polymers are also considerably smaller than cellulose, with an average DP of 100–200. One of the main hemicelluloses in softwood, galactoglucomannan (Figure 2.4), is based on a backbone of D-glucose and D-mannose with branches of single units of D-galactose. In addition, the backbone polymer also has acetyl groups substituted on an average of every three to four hexose units (Rowell et al., 2005).

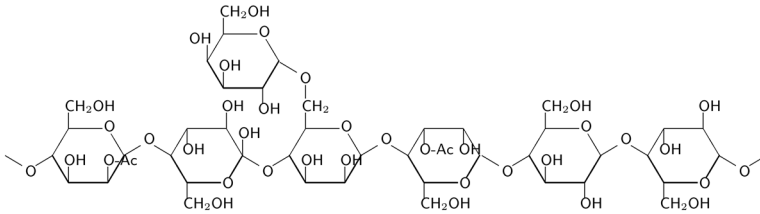


Figure 2.4. Partial structure of the hemicellulose galactoglucomannan.

The second most common hemicellulose of softwoods is arabinoglucuronoxylan, based on units of xylopyranose, D-glucopyranosyluronic acid, and L-arabinofuranose. Furthermore, there are minor hemicelluloses in softwoods based on L-arabinofuranose, D-galactopyranose, D-glucopyranouronic acid, and D-galactopyroanuronic acid (Rowell et al., 2005). The hemicelluloses present in wood are also present in the bark of the same species. In addition, there are polysaccharides in bark that are not found in wood (Browning, 1967). The hemicellulose composition of hardwoods is rather different from that in softwoods, with predominantly glucuronoxylans that has a xylan backbone of D-xylopyranose units (Rowell et al., 2005).

Other carbohydrates

Two minor polysaccharides in wood are starch and pectin. Starch is, like cellulose, made up of glucose (D-glucopyranose). However, in contrast to cellulose, the units are linked with α -(1 \rightarrow 4) glucosidic bonds resulting in a helix shape of the polymer chain. In addition, there are α -(1 \rightarrow 6) glucosidic bonds creating a branched structure (Rowell et al., 2005). In the stem, the storage of starch is low in more mature cells resulting in a radial difference of starch concentration with the lowest amount of carbohydrate stored in the heartwood. In the bark, the concentration of stored carbohydrates is usually high (Pallardy, 2007b). Pectin is a polymer of mainly α -(1 \rightarrow 4) linked D-galacturonic acid, but it often also contains L-arabinofuranose and D-galactopyranose.

Pectin is present in small amounts, except in the inner bark where it is found in high concentration and acts as a binder (Rowell et al., 2005).

Polysaccharides in Norway spruce and Scots pine

The polysaccharides in wood and bark are complicated to analyze, and the polysaccharide composition is often reported simply as the constituting carbohydrate monomers (see section 2.3.1). The presence of glucose, arabinose, galactose, mannose, and xylose units, the main constituents of polysaccharides in softwoods, are thus often reported as the corresponding polymers glucan, arabinan, galactan, mannan, and xylan, respectively. Norway spruce and Scots pine have been reported to have a similar composition of carbohydrate constituents on a dry biomass basis between species, whereas there are considerable differences between the wood and bark of the same species. Glucan, the main fraction, has been reported to constitute 39-43 % of the wood for both species (Frankó et al., 2015; Kempainen et al., 2014; Normark et al., 2014). In contrast, a lower glucan content of 23-31 % and 29-42 % has been reported for the bark of Norway spruce (Frankó et al., 2015; Kempainen et al., 2014) and Scots pine (Normark et al., 2014; Valentín et al., 2010), respectively. The most abundant hemicelluloses of softwoods, are galactoglucoman and arabinoglucuronoxylan. The mannan content has been reported to be 9.9-14 %, the xylan content 3.8-6.6 %, galactan 1.3-3.1 %, and arabinan 0.7-2.0 % of the wood of Scots pine (Normark et al., 2014) and Norway spruce (Frankó et al., 2015; Kempainen et al., 2014). In contrast has the bark of Scots pine (Normark et al., 2014; Valentín et al., 2010) and Norway spruce (Frankó et al., 2015; Kempainen et al., 2014) been reported to have a more even distribution between the main sugar monomers of 2.2-12 % mannan, 2.8-5.4 % xylan, 0.8-3.2 % galactan, and 2.4-4.5 % arabinan.

2.2.3 Lignin

Lignin is often referred to as a phenolic polymer, although it strictly speaking is not a polymer but a macromolecule since there are no clear repeating units. The function of lignin in wood is to act as an adhesive between individual cells, which reinforces the load-bearing structures. Lignin also protects against pathogens. The hydrophobic nature furthermore controls the water content inside the cell wall and enables transport of water (Hänninen et al., 2011). Lignin forms covalent bonds with polysaccharides, mainly hemicelluloses, resulting in what is referred to as lignin carbohydrate complexes, which may impair pulping processes (Deshpande et al., 2018). The lignin content of softwoods is usually higher than for hardwoods (Rowell et al., 2005).

From a chemical perspective, lignin is a three-dimensional, amorphous, and highly complex macromolecule that consists of substituted (propylene-)phenolics. There are three main precursors: p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, differing in the number of methoxy groups on the benzene ring (Figure 2.5). In softwood, the primary lignin precursor is coniferyl alcohol, which has one methoxy group on the benzene ring (Rowell et al., 2005).

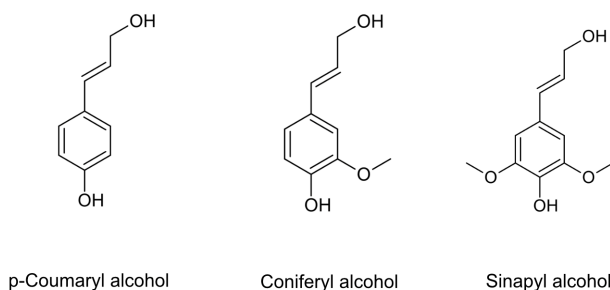


Figure 2.5. The three main lignin precursors p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol.

Although new analytical methods, particularly 2D NMR as well as insights of the metabolic pathways of lignin through gene technology, have provided more information on the lignin composition, the structural details of lignin are still under exploration (Ralph et al., 2019). Lignin has previously been described as having a “highly condensed 3D structure,” but studies indicate it may be less branched and more linear than commonly assumed (Yue et al., 2016). The relative proportions of the precursors in the lignifying zone are controlled by both genetic and environmental factors (Boerjan et al., 2003). However, the polymerization of the complex structure is believed to take place by purely chemical radical coupling and is not by enzymatically controlled reactions (Ralph et al., 2019).

The lignin content is often determined after acid hydrolysis of the biomass, where the solid residue is referred to as acid-insoluble lignin or Klason lignin and is measured gravimetrically. The solubilized lignin is referred to as acid soluble lignin and is measured spectrophotometrically (Rowell et al., 2005; Sluiter et al., 2012). For Norway spruce, a lignin content of 29-34 % and 28-37 % have been reported for wood and bark, respectively (Frankó et al., 2015; Kemppainen et al., 2014; Miranda et al., 2012). Similarly, Scots pine has been reported to have a lignin content of 26-30 % and 28-45 % of wood and bark, respectively (Miranda et al., 2012; Normark et al., 2014; Rowell et al., 2005; Rusanen et al., 2019; Valentín et al., 2010).

Carbohydrate degradation under acidic conditions (Shinde et al., 2018) can result in an increased apparent Klason lignin content, referred to as “pseudo-lignin.” A high bias in lignin values may also be obtained due to the presence of extractives such as, polyflavonoids and other high molecular weight condensed tannins or suberin (Rowell et al., 2005).

2.2.4 Inorganics

The amount of inorganic components, appearing as “ash” in analyses, varies depending on both tree species and the environmental growth conditions. The main elements, about 80 %, are typically Ca, Mg, and K, and the remaining 20 % consist of many different elements (Rowell et al., 2005). The ash content of bark is generally higher than for wood (Laks, 1991). For instance, has the ash content of wood and bark been reported to be 0.2-0.4 % and 3.1-4.6 %, respectively, for Norway spruce (Kemppainen et al., 2014; Miranda et al., 2012) and Scots pine (Miranda et al., 2012; Normark et al., 2014; Rowell et al., 2005).

2.2.5 Suberin

Suberin are hydroxy acid complexes (with both polyaliphatic and polyaromatic domains) that functions as a transport barrier for water and solutes as well as a protective barrier (Franke and Schreiber, 2007). Suberin is present in bark, but not in wood and can be determined by analyzing suberin-derived products after cleavage by alkaline hydrolysis (Krogell et al., 2012). Most species have a content of 5 % suberin in the outer bark, but cork oak and some birch species may have 35-45 % (Browning, 1967). A suberin content of less than 2 % has been reported for bark from Norway spruce and Scots pine (Miranda et al., 2012).

2.3 Chemical characteristics of the used feedstocks

In this section, the chemical differences between bark from Scots pine and Norway spruce, as well as the difference within tissue types of Scots pine wood, is discussed (**Papers I-II**). The analyses of the biomass feedstocks deserve attention since it is the foundation for the use of the feedstock and strongly influences both the methods used and the results of any further processing.

2.3.1 Methods used for characterization of biomass

Knowing the feedstock composition accurately is necessary to assess the potential of the feedstock, determine process design and evaluate the yields in relation to the maximum theoretical yields. As discussed earlier, many factors affect the actual chemical composition of woody biomass, and it is therefore essential to always have a recent analysis of the actual feedstock to be able to evaluate a process. It is important to remember that the results are highly dependent on the method and that it therefore might be difficult to compare the results from different studies when different analytical methods have been used.

Characterizing complex solid materials such as lignocellulosic biomass chemically is intrinsically difficult. The laboratory procedures provided by the National Renewable Energy Laboratory (NREL)¹ to characterize biomass have become standards within the biorefinery research community to characterize the macromolecular composition of biomass. These procedures were also the basis of the analysis of the biomass in **Paper I** and **Paper II**.

The biomass characterization follows the flow scheme in Figure 2.6. First, the particle size is adjusted by milling (Hames et al., 2008). Subsequently, water-soluble components are extracted and then more hydrophobic components are extracted using 95 % ethanol (Sluiter et al., 2008b). The polymeric carbohydrates in the extractive free biomass are then hydrolyzed by a two-step acid hydrolysis (Sluiter et al., 2012). Firstly using 72 % sulfuric acid at 30°C and secondly diluting to 4 % and continuing the hydrolysis at 121°C. The resulting carbohydrate monomers are quantified using high-performance liquid chromatography (HPLC). Acid soluble lignin (ASL) is determined spectrophotometrically (only **Paper II**). The solid residue after acid hydrolysis is measured gravimetrically and referred to as acid insoluble lignin (AIL). Dry matter and ash content are determined by drying/ashing the samples at 105 °C and 575 °C, respectively (Sluiter et al., 2008a).

The strong acid hydrolysis may result in further degradation of carbohydrate monomers (see 3.3.1). In order not to underestimate the carbohydrate content in the sample, a “sugar recovery standard” with the studied carbohydrate monomers is treated together with the biomass samples. In this way, the degradation during the acid hydrolysis can be compensated for in the calculations. These analytical procedures do not give any precise structural information of the biomass. For instance, are all the glucose monomers from the acid hydrolysis simply reported as glucan.

¹ <https://www.nrel.gov/bioenergy/biomass-compositional-analysis.html>

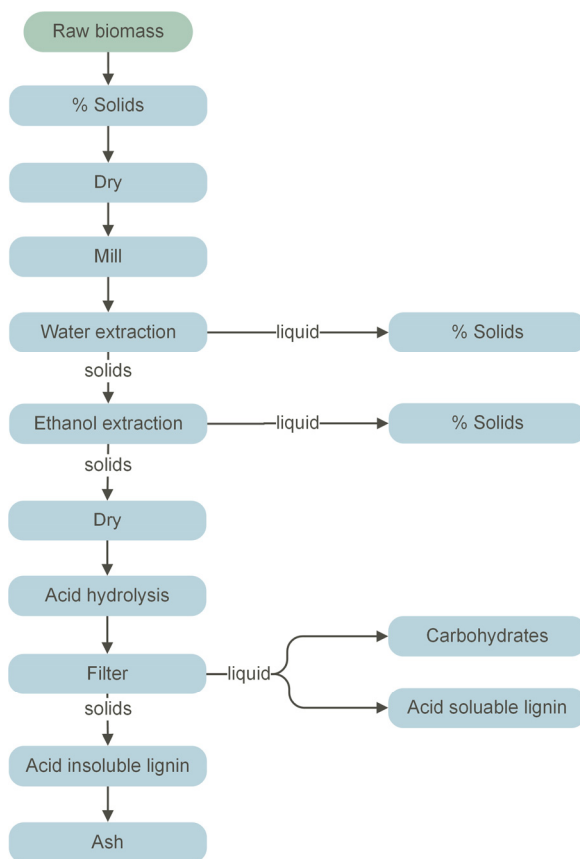


Figure 2.6. Flow scheme for the analysis of raw biomass.

2.3.2 The heterogeneity of tree stems

As described in previous sections, it is well known that different parts of the tree stem have different structures and chemical compositions. In the study reported in **Paper I**, the aim was to see how these differences would affect the processability in a biorefinery. The chemical composition of different parts of two contrasting trees of Scots pine (*Pinus sylvestris*) were analyzed (Figure 2.7). In addition to the data published in **Paper I**, the same analysis was made for bark from one of the trees (unpublished).

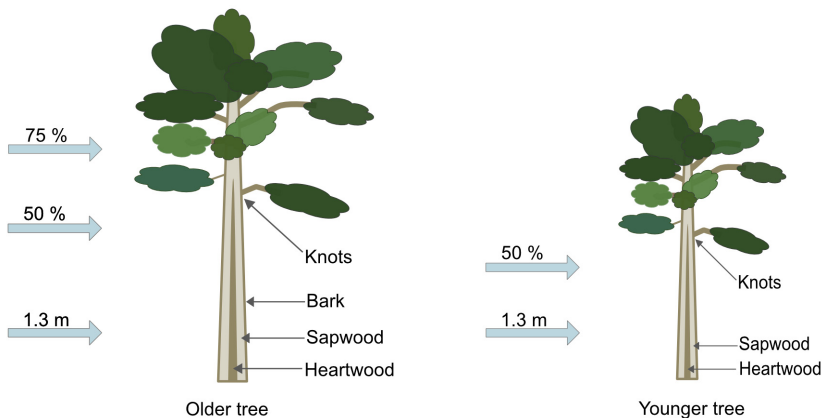


Figure 2.7. To study the difference in chemical composition and processability within as well as between two Scots pine trees (**Paper I**) samples of sapwood and heartwood were collected at 1.3 m, 50 %, and 75 % of the tree height from one older tree and one younger tree. Knots (**Paper I**) and bark (unpublished) was collected from the whole stem.

As expected, the largest differences in chemical composition were seen between the wood and the bark (Figure 2.8). However, there was also a noticeable difference between different tissue types of the wood.

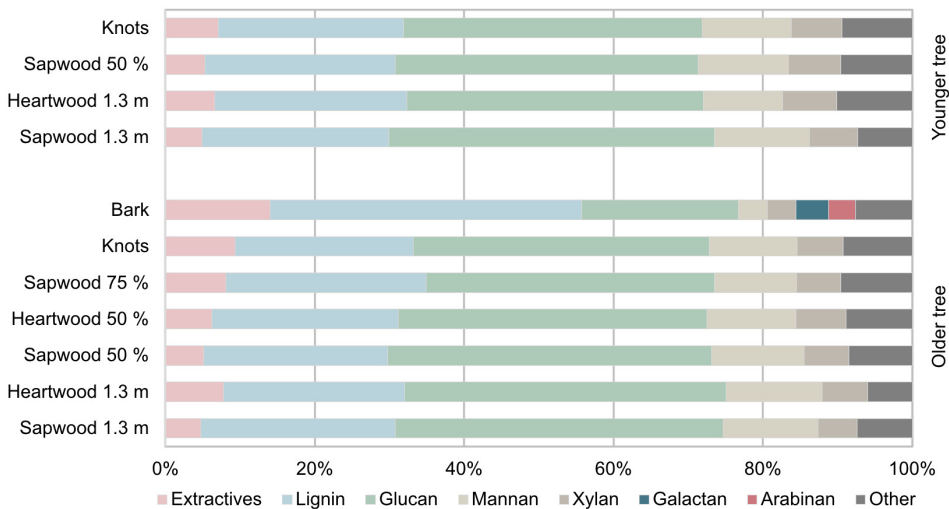


Figure 2.8. Chemical composition (as % of dry material) of different fractions from two Scots pine trees (**Paper I** and unpublished) sampled as described in Figure 2.7. Extractives are given as the sum of water and ethanol extractives.

The differences in macromolecular carbohydrate contents between the wood fractions were small. One exception was the xylan content, which was higher in heartwood than in sapwood at the same tree height. The analyzed bark had a glucan content of 20 %, which is approximately only half of that found in the wood. The total contents of hemicellulose sugars were similar, but the proportions of the constituent sugars were different. In stem wood, the hemicellulose was mainly based on xylose and mannose, while there was a more even distribution between arabinan, galactan, mannan, and xylan units in the bark hemicellulose.

As expected, significant differences in total extractive contents were seen between the wood fractions, where the heartwood had an extractive content which was 1–3 wt% higher than that in sapwood (corresponding to a relative increase of 20–60 %) for samples at the same height. The lignin content of bark (42 %) also appeared to be considerably higher than for wood (24–27 %). The values were in agreement with previously reported values (see section 2.2).

One should keep in mind that the analytical procedures used have been optimized for corn stover, although they are claimed to work well also on woody feedstocks (Sluiter and Sluiter, 2011a). There are components in bark making it problematic to use the same methods as used to analyze wood. Lignin values may be biased due to the presence of substances such as suberin and tannins (Rowell et al., 2005). However, with the prior water and ethanol extraction the interference from extractives should be limited. In addition, the interference from suberin should be minor since Scots pine and Norway spruce bark have been reported to have a suberin content of less than 2 % (section 2.2.1).

2.3.3 Bark from Scots pine and Norway spruce

The use of bark as a feedstock for sugar-based biorefineries poses extra challenges compared with wood (chapter 3). Therefore, the study reported in **Paper II** examined if processability could be improved by first extracting disturbing compounds from bark of Scots pine and Norway spruce.

Compositional analysis of the feedstock showed that the provided bark from both species had a similar overall chemical composition (Figure 2.9). The major difference was a higher content of water-soluble extractives 17 %, glucan 25 %, and ash 2.3 % in Norway spruce bark, compared with Scots pine bark which had 13 % water soluble extractives, 20 % glucan, and 0.9 % ash. The pine bark had a higher content of lignin, 41 %, compared with 30 % for Norway spruce bark. The values agreed with previously reported compositions for Scots pine and Norway spruce bark (see section 2.2).

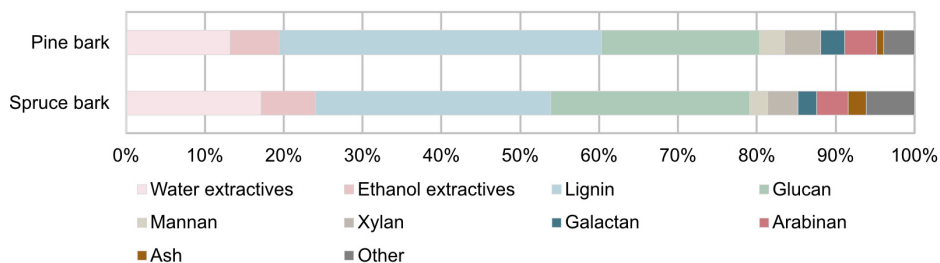


Figure 2.9. Chemical composition (as % of dry material) of bark from Norway spruce and Scots pine (**Paper II**).

2.3.4 Important outcomes

The results of the feedstock characterization in the present work underline several important facts for biorefinery operations:

- There are chemical differences within a Scots pine tree stem with, for instance, a higher content of extractives in the heartwood. However, the differences in macromolecular carbohydrate contents are mainly insignificant (**Paper I**).
- The bark of Scots pine is chemically very different from the wood, with a higher content of extractives and lignin and different types of hemicelluloses (**Papers I and II** and unpublished).
- The major chemical difference between bark from Norway spruce and Scots pine was that Norway spruce bark had a higher content of water-soluble extractives, glucan, and ash compared with Scots pine bark. Scots pine bark, on the other hand, had a higher lignin content compared with Norway spruce bark (**Paper II**).

3 Softwood based biorefineries

The biorefinery concept is analogous to that of a petroleum refinery (Galbe and Wallberg, 2019). In both types of refineries, raw materials are processed into a wide range of marketable products such as energy, materials, and chemicals (Katakojwala and Mohan, 2021). Apart from the renewable/non-renewable nature of the feedstock, the most significant difference lies in the fact that petroleum is a fairly homogeneous liquid feedstock that with, comparatively, simple processing can give a selected range of carbon and hydrogen-based molecules to be used as platform chemicals for a variety of products. In contrast, biorefineries can be based on many different feedstocks, most of which are solid and of widely varying compositions (Attard et al., 2020). Due to the chemical and structural diversity of lignocellulosic materials, it is likely not possible to design one biorefinery capable of processing all sorts of feedstocks (Galbe and Wallberg, 2019).

Softwood has for thousands of years been used as a fuel and construction material but also as a source of chemicals, for instance, to produce tar used for wood preservation. Ever since the wood pulping technologies were developed in the 19th century, softwood has also been an important feedstock for pulp and paper production (Ek et al., 2009). The use of wood for papermaking gradually led to improved knowledge of wood's chemical structure. With the development of new physicochemical and biotechnological processes over the last century, it is now possible to produce a variety of chemicals, fuels, energy, and even foods and feeds, from softwood within the biorefinery concept. To reach economic feasibility as well as to use material and energy resources efficiently, future biorefineries must make efficient use of the whole feedstock and at the same time produce several products in parallel.

This chapter starts with an overview of how wood and bark from Scots pine and Norway spruce can be converted to various products using different process options. The production of phenolic compounds and fermentable sugars are described in more detail to reflect the scope of this thesis.

The overview is followed by a description of the main methods, results, and discussions from the studies on; the influence of tissue type on pretreatment and enzymatic hydrolysis (**Paper I**); the effect of removing extractives prior to pretreatment and enzymatic hydrolysis (**Paper II**), and process design and scaleup of extraction (**Papers III and IV**).

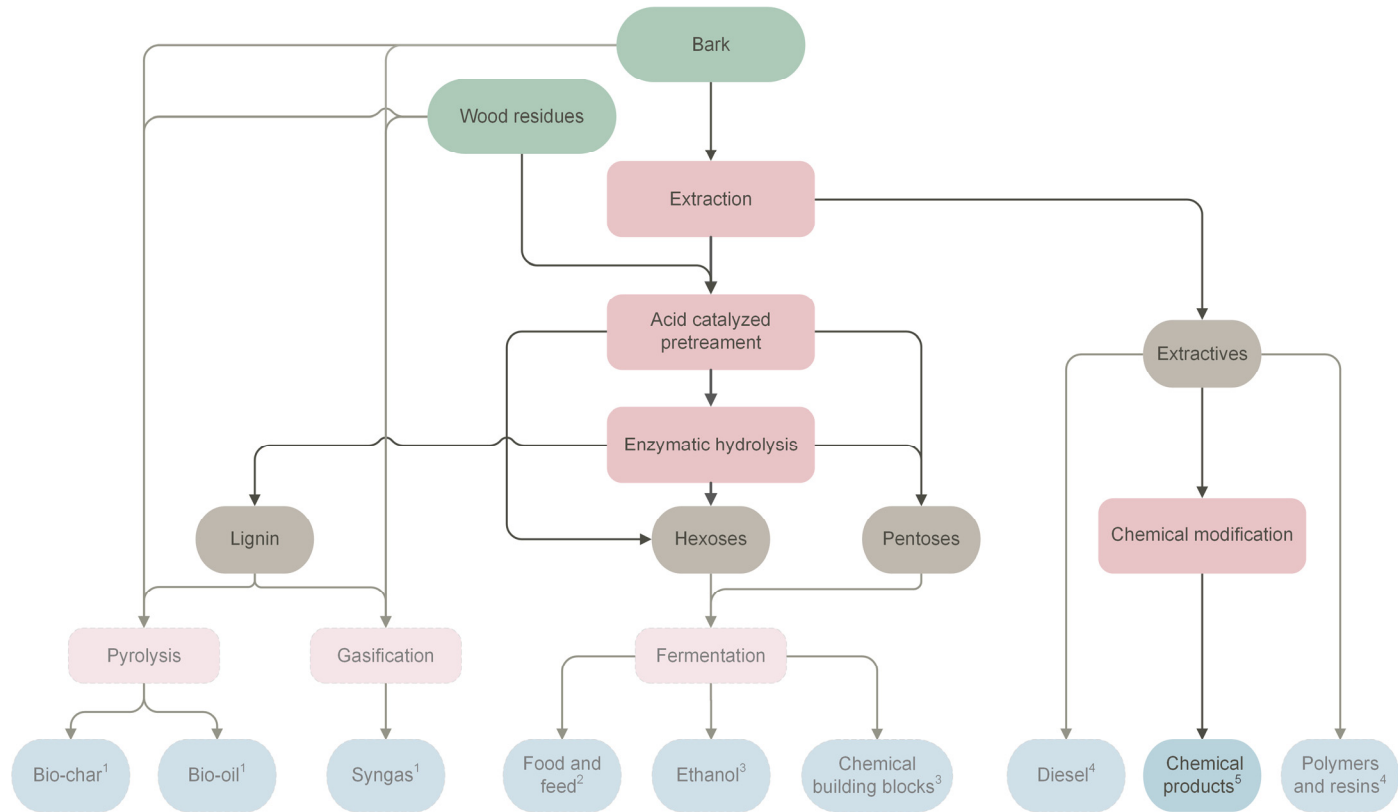


Figure 3.1. Overview of some possible process routes and products in a softwood based biorefinery based on acidic pretreatment. Bark and wood residues can come from, for instance, a pulp mill or sawmill. In addition, is heat production through the combustion of the whole or part of the biomass an alternative. Darker color indicates that processes are covered in **Papers I-IV**. ¹Seo et al. (2022) ²Bajpai (2017) ³Galbe and Wallberg (2019) ⁴Eriksson et al. (2018) and Varila (2020), ⁵Bello et al. (2020)

3.1 Overview of production routes and products

As described previously in chapter 2, wood has four principal groups of chemical components, namely cellulose, hemicellulose, lignin, and extractives, with different chemical properties, making them useful for different purposes (Figure 3.1).

There are many products based directly on the use of the *cellulose or hemicellulose* polymer, for instance, pulp, paper, textile, nano- and bacterial-celluloses, films, coating, and hydrogels (Chandel et al., 2018). Another possibility, which is the basis of many biorefineries, is the conversion of the sugars in the cellulose and hemicellulose to desired chemical products. This is usually referred to as the sugar platform type of biorefinery. Early examples were ethanol factories built adjacent to sulfite paper mills at the beginning of the 20th century, that fermented the sugars released from the sulfite process (Glessinger, 1952). These sugars mainly come from the hemicellulose, and since the hemicellulose of softwood mainly contains glucose and mannose, common baker's yeast (*Saccharomyces cerevisiae*) could be used for the fermentation (Rødsrud et al., 2012). Metabolic engineering has led to the possibility to introduce additional metabolic routes that also allow utilization of pentose sugars in e.g. *S. cerevisiae* (Jeffries and Jin, 2004; Nijland and Driessen, 2020). Utilizing the pentoses in softwood is not as crucial as for hardwoods or grasses, where the pentose content of the hemicelluloses is considerably higher. However, it has been estimated that xylose fermentation can improve the ethanol yield from Norway spruce by 8 % (Sassner et al., 2008). It is also possible to use chemical processes to produce acetate, carboxymethyl cellulose, methyl cellulose, and hydroxyethyl cellulose from the cellulose and hemicellulose (Majewicz and Podlas, 2000). Bioethanol was for a long time the main chemical product of interest from the sugar platform. However, in later decades have platform chemicals, which can be used to produce many different compounds, been given more attention. For example, three organic acids (Figure 3.2) used today as starting material for many high-value replacement products are succinic, fumaric, and malic acid (Galbe and Wallberg, 2019).

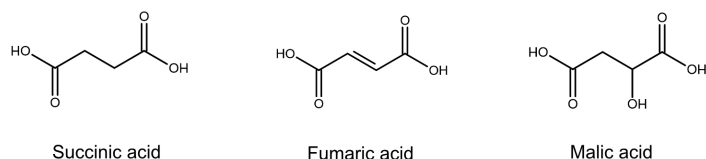


Figure 3.2. Succinic, fumaric, and malic acid acid are three important platform chemicals produced via the sugar platform (Galbe and Wallberg, 2019).

If microorganisms are used for the conversion, cell mass will be produced in addition to the targeted chemicals. Produced bacteria, fungi, or algae are rich in proteins and can be used for either biogas production or single-cell protein for food/feed production (Bajpai, 2017).

Lignin is the largest renewable source of aromatic building blocks available in Nature. To obtain low molecular weight compounds, lignin first needs to be isolated and then depolymerized via catalytic or biocatalytic methods. Unfortunately, the chemical complexity and rigidity of lignin have made it difficult to produce specific, well-defined smaller aromatic products at high yields. The most significant industrial streams of lignin are today created in the pulp and paper industry, where most of the lignin is burnt for energy recovery (Sun et al., 2018). A small fraction is used to produce different lignosulfonates, polydisperse products, mainly used as dispersants (Aro and Fatehi, 2017). Another well-established product is vanillin which is mainly used as a food additive (Rødsrud et al., 2012). The research output on lignin conversion has increased rapidly during the last decade. Lignin is seen as an attractive renewable feedstock for producing the central petroleum-derived platform chemicals phenol, benzene, toluene, and xylene (Figure 3.3) (Sun et al., 2018). Other examples of lignin products are carbon fibers and adhesives (Chandel et al., 2018).

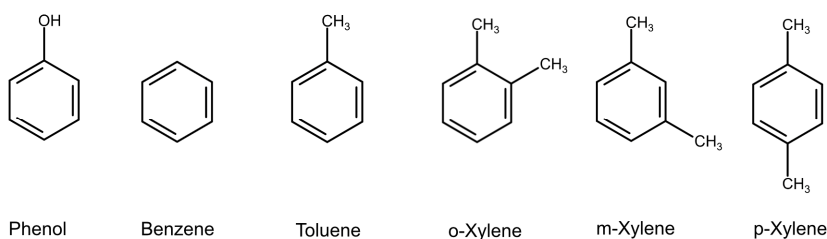


Figure 3.3. Phenol, benzene, toluene, and xylene (ortho, meta and para) are important petroleum-derived platform chemicals that can also be produced from lignin (Sun et al., 2018).

The compounds referred to as **extractives** are a heterogeneous group of chemical components including **waxes, phenolic compounds, and non-structural sugars**, predominantly present in bark. An advantage from a process point of view, of this group of compounds is the ease of isolation in which much - or all - of the native structure can be retained. Fatty and resin acids from pine wood can readily be separated during pulp production and are in great demand for use in the production of, for instance, tall oil diesel in the case of fatty acids or glues and inks in the case of resin acids (Eriksson et al., 2018). One group of industrially well explored phenolic extractives are tannins. Tannins have traditionally been used in leather manufacturing (leather tanning), using

bark or wood rich in tannin. The industrial extraction of tannins started in the middle of the 19th century and peaked around the second world war. Today tannins are mainly produced from the subtropical species Quebracho (*Schinopsis balansae*) and Black wattle (*Acacia mearnsii*) and used for leather tanning and adhesives for wood panels or other wood products (Pizzi, 2008). However, with the increased interest for renewable feedstocks, the research outputs of using tannins from conifers present in sub-arctic climate, have increased. Some examples are to use tannins as coagulants for water clarification (Bello et al., 2020), the production of foams (Varila, 2020), and as a food preservative (Raitanen et al., 2020).

In addition to the above-described valorization to specific chemical products, there are also several *unspecific methods* to use biomass. Heat, power, biochar, bio-oil, and syngas (methane) can be produced from either the whole biomass or part of the biomass by thermochemical conversion processes such as pyrolysis (torrefaction), hydrothermal treatment, gasification and combustion (Seo et al., 2022). However, in these conversions most of the native structures and their potential are lost.

A challenge for the future is finding biorefinery processes that make use of the whole biomass in the best way. Often, a process optimized to use one component may impair the use of another. This thesis focuses on upstream activities to produce phenolic compounds and/or sugars for further processing, described in the following two sections.

3.2 Production of phenolic compounds

The group of nonstructural compounds referred to as extractives, prominent in bark, can be isolated through solid-liquid extraction. The basis of the extraction is to find a suitable solvent system favoring the solubility of the target compound. The optimal solvent not only depends on the compound of interest but also on the biomass extracted (Venkatesan et al., 2019). For the extraction of phenolic compounds from bark, polar solvents, usually based on water in combination with an organic co-solvent such as acetone, ethanol, methanol, or ethyl acetate, are used (Tanase et al., 2019). An alkaline solution is needed for high molecular weight phenolic compounds to prevent precipitation (Laks, 1991). As the polar solvents also dissolve sugars and other carbohydrates, a purification step is sometimes added after the extraction (**Paper III**).

Several physical methods can be used to improve the extraction. Most common is to apply heat, as in hot water extraction, or heat in combination with increased pressure, as in high-pressure hot water extraction. Pressurized hot water extraction is the basis of

today's industrial extraction of phenolic compounds from bark (Kemppainen et al., 2014). However, high temperatures and long exposure times can lead to the degradation of sensitive components. Emerging technologies for the extraction of phenolic compounds from bark include the use of ultrasound and supercritical fluids. Ultrasonic waves, with a frequency between audible waves and microwaves, can enhance extraction by facilitating the penetration of the solvent into the bark through creating micro-channels. This allows for extraction at lower temperatures (Rodrigues and Fernandes, 2017). Supercritical fluids have properties between gases and liquids, with high diffusivity and low viscosity, providing high mass-transfer rates. The most used supercritical fluid for extraction is supercritical carbon dioxide as it is inert, non-toxic, and exists as a supercritical fluid at relatively low pressure and temperature (Cunha et al., 2018). Some advantages and disadvantages of four different extraction methods are summarized in Table 3.1.

Choosing the most suitable extraction technology is a complex task. Yield, selectivity, and productivity are always essential factors from a process economic point of view, as is the choice of solvent and other consumables. Furthermore, it is necessary to consider the environmental performance of different extraction technologies, as discussed in chapter 4.

Table 3.1. Four extraction methods and some of their advantages and disadvantages (Ameer et al., 2017; Da Porto et al., 2014; Jovanovic et al., 2017; Tiwari, 2015).

Extraction method	Advantages	Disadvantages
Hot water	Simple apparatus and method Low investment cost	Degradation of thermosensitive compounds
Pressurized hot water	Relatively simple Low investment cost	Degradation of thermosensitive compounds
Ultrasound assisted	Flexible Relative low investment cost Favorable for thermosensitive compounds	High electricity demand
Supercritical fluid	Simple separation and recovery of solvent Favorable for thermosensitive compounds	Complex High investment cost

3.3 Production of fermentable sugars

As described above in section 3.1, microorganisms can convert sugar to a wide range of valuable products, in what is described as the sugar platform biorefinery. However, sugar from cellulose and hemicellulose are not directly available for microorganisms but need to be obtained through hydrolysis. Softwood is more recalcitrant than most

agricultural or herbaceous crops and residues, and the challenges in production of fermentable sugars from softwood has been subject to much research for the last decades (Galbe and Wallberg, 2019).

There are several possible approaches to access the monomeric sugars in biomass. They are usually based on degradation and solubilization of one of the main structural components cellulose, hemicellulose or lignin. Pretreatment methods, i.e. the processes which improves accessibility of the enzymes to the carbohydrates, have traditionally been classified as physical, chemical, physicochemical or biological methods, but are often a combination two or more methods (Figure 3.4) (Galbe and Wallberg, 2019). This structure is still useful for the work in this thesis, with the focus on the production of fermentable sugars.

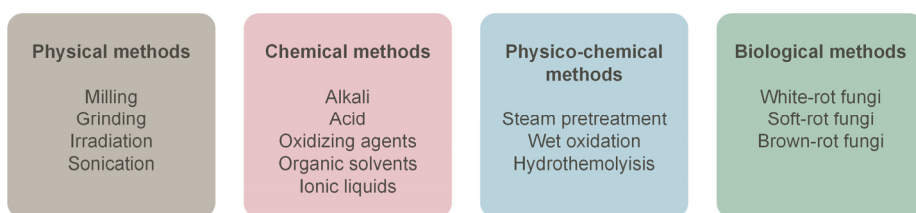


Figure 3.4. Examples of pretreatment methods for lignocellulosic biomass. Traditionally, pretreatment methods have been classified as physical, chemical, physicochemical, or biological. However, in reality, pretreatment technologies can be a combination of two or more methods (Galbe and Wallberg, 2019).

The most common approach has been to use enzymes in combination with a thermochemical pretreatment method, to produce fermentable sugars. In principle, a fully enzymatic process for biomass conversion gives the possibility of a high selectivity without unwanted degradation products as formed in thermochemical conversion (Horn et al., 2012). However, native lignocellulose is very recalcitrant to enzymatic degradation, so pretreatment of the biomass is therefore needed to reach reasonable enzymatic conversion rates to fermentable sugars. Furthermore, the cost of enzymes is a significant factor in the overall process economy, and pretreatment may release monosaccharides – typically from hemicellulose – and thereby reduce the need for costly enzymes (Pedersen and Meyer, 2010).

In the current work, steam explosion pretreatment was followed by enzymatic hydrolysis using commercial enzyme cocktails.

3.3.1 Steam explosion pretreatment

One of the most studied methods for pretreatment is steam explosion or steam pretreatment (Galbe and Wallberg, 2019). The method originates from the century old

Masonite process but became popular as a method to increase enzymatic hydrolysis of lignocellulose in the 1980s (Ruiz et al., 2021). One of its advantages is its relative simplicity. The biomass is subjected to high pressure steam at 160 to 230 °C for 3 to 20 min. This is followed by a rapid decompression, denoted by the word “explosion.” The necessity of the explosion has been debated over many years (Ruiz et al., 2021) but several studies under the last decade support need of explosion to improve the enzymatic digestibility of softwoods. Pielhop et al. (2016) found that the enzymatic digestibility of spruce wood chips improved to about 90 % with explosion compared with about 60 % without explosion.

Acidic pretreatment methods hydrolyze the polysaccharides while alkaline pretreatments alter the lignin structure and remove it from the solid biomass (Pedersen and Meyer, 2010). If no catalyst is added to the steam explosion pretreatment, organic acids in the feedstock will catalyze autohydrolysis. This is not as pronounced for softwoods since they have a low content of organic acids (Galbe and Wallberg, 2019). However, even if no acid catalyst is added before steam pretreatment, recycling the process water might lead to an acidic pretreatment due to accumulation of acetic acid (Larsen et al., 2008).

Figure 3.5 gives a schematic overview of neutral to acid catalyzed steam explosion pretreatment that is the pretreatment method used in this thesis for subsequent enzymatic hydrolysis.

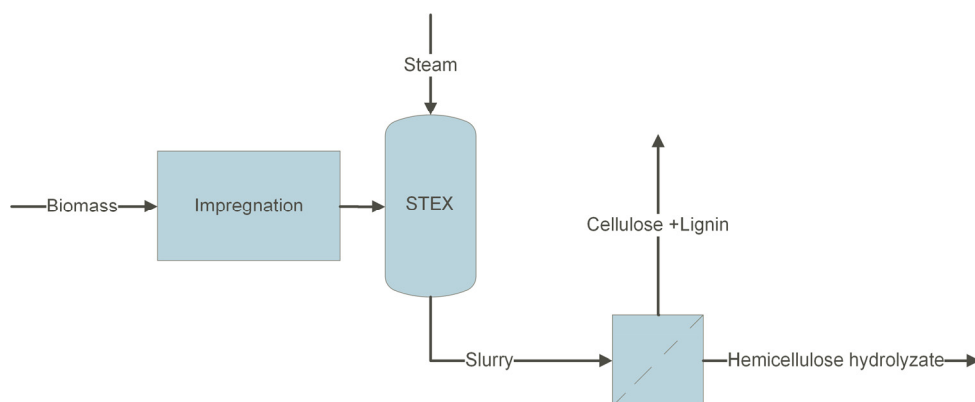


Figure 3.5. Schematic description of neutral to acid catalyzed steam explosion (STEX) pretreatment of biomass.

At acidic conditions cellulose and hemicelluloses are degraded via acid hydrolysis to oligo- and monosaccharides (Pedersen and Meyer, 2010). The branched structure of hemicelluloses reduce the chemical and thermal stability and are usually hydrolyzed and degraded at a lower severity compared with cellulose (Palmqvist and Hahn-

Hägerdal, 2000). If the severity is high enough the desired monosaccharides are further degraded to furans for instance HMF (5-hydroxymethyl hydroxymethyl-2-furaldehyde) and 2-furfuraldehyde. They are inhibitors to glycolytic enzymes as well as inhibit fermentation (Pedersen and Meyer, 2010). HMF and 2-furfuraldehyde can in turn be hydrated to formic acid and levulinic acid. In addition, can acetic acid be formed from hemicellulose acetyl groups by oxidation, which are fermentation inhibitors (Figure 3.6) (Almeida et al., 2011).

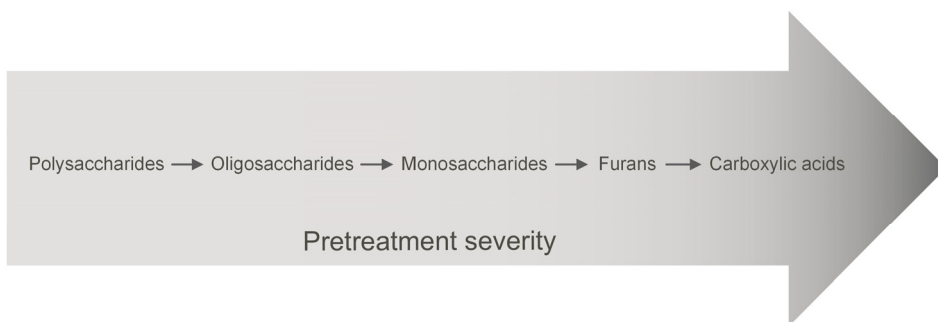


Figure 3.6. The degradation of the polysaccharides cellulose and hemicellulose depends on the pretreatment severity. For the sugar platform, monosaccharides are the desired products while furans and carboxylic acids impair subsequent enzymatic hydrolysis and fermentation (Almeida et al., 2011; Pedersen and Meyer, 2010).

The three major parameters that determine the effect of pretreatment are temperature, time and pH (Pedersen and Meyer, 2010). To be able to compare the harshness or severity of different operating conditions of steam explosion, the severity factor was developed from previous similar concepts in the pulp and paper industry. For acid catalyzed steam explosion, the pretreatment can be described using the combined severity factor (CSF) defined as:

$$CSF = \log \left[t \cdot \exp \left(\frac{T - T_{ref}}{14.75} \right) \right] - pH \quad (1)$$

where t (min) is residence time, T (K) is pretreatment temperature and T_{ref} (K) is a reference temperature set to 373 K (Pedersen and Meyer, 2010) (Chum et al., 1990). The optimal pretreatment severity is a balance between improved enzymatic accessibility and degradation of the desired product.

3.3.2 Enzymatic hydrolysis

The use of enzymes allows the design of industrial processes with high selectivity that can be performed under mild conditions, and enzymes are a central part of biorefinery research. The discovery of fungal enzymes with high capacity of degrading cellulose in the 1950s opened entirely new possibilities to produce sugars for fermentation from wood. However, it was the oil crisis in the 1970s that truly triggered the interest in using enzymes to degrade cellulose industrially (Allen et al., 2009).

Today, it is understood that natural lignocellulosic biomass degradation results from the concomitant action of many different enzymes. The minimum mixture of hydrolytic enzymes (glycoside hydrolases) needed to degrade the cellulose consists of cellobiohydrolases, endoglucanases, and β -glucosidases. The cellobiohydrolases cleave off cellobiose units from the end of the cellulose chain (there are two types, starting from either the reducing or non-reducing end). The endoglucanases hydrolyze internal glucosidic bonds and thereby create new ends for cellobiohydrolases. Finally, β -glucosidases hydrolyze liberated cellobiose into two glucose units. In the last decade, much attention has been directed to the lytic polysaccharide monooxygenases (LPMO), a different group of enzymes shown to be important for cellulose degradation. The LPMOs cleave glycosidic bonds through oxidation instead of hydrolysis (Horn et al., 2012).

Access to the cellulose in native biomass is hindered by the other carbohydrate-based structures hemicellulose and pectin. Therefore, enzymes attacking these structures will provide additional fermentable sugars and also affect the cellulose degradation (Berlin et al., 2007; Várnai et al., 2011). The degradation of the branched hemicellulose polymers is to some extent analogous to that of cellulose. Depending on the type of hemicellulose, the backbones are cleaved through hydrolysis by endomannanases, endoxylanases, or endoglucanases. In addition, many LPMOs also oxidatively cleave the hemicellulose backbones (Østby et al., 2020). Released mannan and xylose oligomers are hydrolyzed by β -1,4-mannosidase and β -1,4-xylosidase. In addition, several types of hydrolases are needed, including galactosidases and acetyl esterase, to release the substituents on the backbone (Van Den Brink and De Vries, 2011). Pectic substances are degraded by the consecutive action of pectin methyl esterase and pectate lyase or pectin lyases directly (Gilbert et al., 2008). The pectin-degrading enzymes are probably more important in softwood degradation compared with hardwood degradation (Couturier et al., 2015).

Another structural component hindering cellulose degradation is lignin. Lignin is much more heterogeneous than the carbohydrate-based components and degradation takes

place mainly through oxidation, by various laccases and peroxidases, as well as by Fenton oxidation chemistry (Bugg et al., 2011).

In addition to hydrolytic and oxidative enzymes that directly degrade the biomass, auxiliary enzymes, such as swollenin and loosenin, have a synergistic effect by increasing the access of other enzymes through disrupting inter- and intramolecular hydrogen bonds (Gourlay et al., 2013).

In nature, wood extractives hinder the degradation of wood. However, at least one fungus, *Phlebiopsis gigantea*, may produce various enzymes, including lipases, to efficiently transform or degrade conifer extractives (Iwata et al., 2021).

Factors affecting the enzymatic hydrolysis

Within biorefineries, enzyme blends, referred to as enzyme cocktails, are used for lignocellulose degradation. Since enzymes are expensive, extensive research efforts are made to improve the efficiency of the hydrolysis step (Galbe and Wallberg, 2019). Factors affecting the enzymatic degradation of lignocellulose include enzyme-related factors, substrate-related factors, process conditions, and the presence of inhibitors (Figure 3.7).

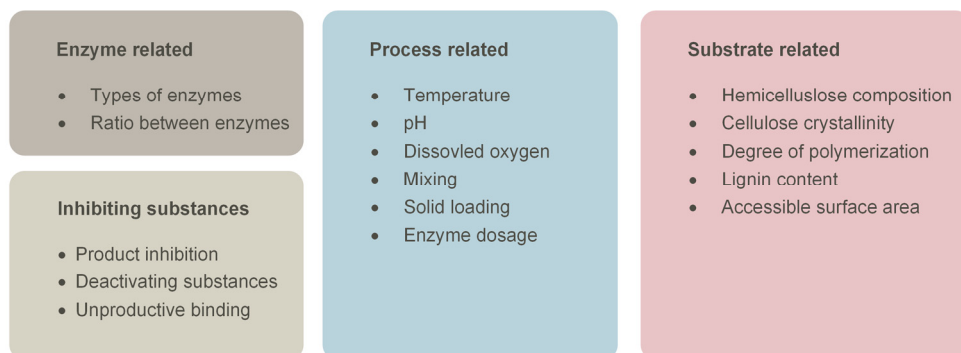


Figure 3.7. Factors affecting the enzymatic hydrolysis can be related to the enzymes used, the process conditions, the substrate to be degraded, or the presence of inhibiting substances.

Commercially available *enzyme cocktails* are usually based on fungal enzymes (Merino and Cherry, 2007). The most used fungus in the industrial production of cellulases is *Trichoderma reesei* (the asexual stage of *Hypocrea jecorina*). Wild-type variants of this fungus produce a mixture of needed basic hydrolytic enzymes, but the secreted amounts have been drastically increased through, initially, mutagenesis and selection, and more recently targeted genetic engineering (Martinez et al., 2008). In addition to improvements of the fungal hosts, growth medium, and growth conditions (Østby et

al., 2020), enzyme cocktails may be enhanced by supplementation of additional enzymes attacking e.g. remaining hemicellulose (Várnai et al., 2011) or pectin (Berlin et al., 2007) in the pretreated material. Currently, the development seems to move towards on-site production and more “tailored enzyme cocktails,” optimized for different biomasses and pretreatments (Østby et al., 2020).

Several products released during pretreatment or enzymatic hydrolysis can affect the enzyme activity negatively. Product inhibition is common among enzyme catalyzed reactions (Schmidt et al., 1983). For example, it has been observed that cellobiohydrolases and β -glucosidase are inhibited by their end-products cellobiose and glucose (Andrić et al., 2010). Therefore, the *presence and ratio of enzymes* catalyzing consecutive reactions are essential (Østby et al., 2020). End-product inhibition can also be overcome by continuously removing the end product, for instance, by simultaneous saccharification and fermentation (Kadić and Lidén, 2017).

Phenolics and furans, formed during pretreatment or present in the native biomass, can deactivate enzymes (Laks, 1991; Olsen et al., 2016; Pedersen and Meyer, 2010). The amount of inhibiting substances can be kept low by operating the pretreatment at low severity (Söderström et al., 2003), and by hot water extraction of the solid material prior to pretreatment (**Paper II**).

Unproductive binding, by adsorption of enzymes to lignin or “pseudo-lignin” formed from carbohydrates during pretreatment, increases the needed enzyme load. (Wang and Jönsson, 2018). Unproductive binding can be overcome by adding non-catalytic proteins binding to lignin in place of the enzymes (Kumar and Wyman, 2009) or by adding nonionic surfactants, such as polyethylene glycol, to disrupt the hydrophobic interaction with lignin (Eriksson et al., 2002; Rocha-Martín et al., 2017).

Substrate related factors include cellulose crystallinity, degree of polymerization, lignin content, hemicellulose content, and available surface area (Meng and Ragauskas, 2014). The accessibility of the cellulose surface to the enzyme is clearly important (Wiman et al., 2012), and this is strongly affected by activities upstream of the enzymatic hydrolysis, mainly the pretreatment, but also the type of biomass (Piccolo et al., 2010).

Process conditions during hydrolysis, such as temperature, pH, and dissolved oxygen level, affect enzyme activity and stability (Østby et al., 2020). Enzymes have different specific temperature and pH intervals where they show maximal catalytic activity. Operating outside this range may lead to a lower activity or denaturation of the enzyme (Herlet et al., 2017). The enzymatic activity is generally positively correlated with the temperature as a higher temperature gives a higher reaction velocity and lowers the viscosity of the reaction mixture. In addition, is the risk of contamination lower at elevated temperatures (Turner et al., 2007). Even though most enzymes are favored by

elevated temperatures, as long as they are stable, the oxygen solubility in the reaction mixture will decrease with temperature (Xing et al., 2014). The presence of oxygen and/or H₂O₂ is essential for the oxidative action of lytic polysaccharide monooxygenases. However, lytic polysaccharide monooxygenases can be oxidatively damaged if oxygen and/or H₂O₂ are present in the absence of a substrate (Eijsink et al., 2019). Depolymerization of pretreated biomass is usually performed at pH 4.5 - 5.0 and 40 to 50°C (Horn et al., 2012).

It is desirable to have a high *solid loading* to improve the economic feasibility and increase energy efficiency (Da Silva et al., 2020). However, a decreased yield at higher water insoluble solid loadings has often been seen (Kristensen et al., 2009; Modenbach and Nokes, 2013). The reason may be higher concentrations of inhibiting substances, either end-products or compounds from the pretreatment. In addition, highly viscous lignocellulose slurries with poor mixing may cause local end-product accumulation, leading to product inhibition (Kadić and Lidén, 2017). Also, the availability of free water has been put forward as an explanation for decreased yields at high solids loadings (Weiss et al., 2019)

In conclusion, it is essential to carefully consider the combination of the upstream pretreatment, enzyme cocktail, and the process conditions for effective industrial enzymatic hydrolysis of lignocellulosic biomass.

3.4 Process development within softwood biorefineries

In this section, the work on process development within softwood biorefineries reported in **Papers I-IV** is presented (Figure 3.8). The section starts with an overview of the methods used for pretreatment and enzymatic hydrolysis of wood and bark from Scots pine and Norway spruce (**Papers I and II**). Secondly, the difference in effects of pretreatment and enzymatic digestibility between tissue types of stems are discussed (**Paper I**). Also the effect on the enzymatic digestibility of bark by removing extractives prior to pretreatment is discussed (**Paper II**). Finally, the modeled mass and energy balances of upscaled systems for bark extraction, which were the basis of the environmental assessments described in chapter 4, are discussed (**Papers III and IV**). The results reported in **Papers I and II** were based on laboratory experiments, while modeling was essential for the results reported in **Papers III and IV**.

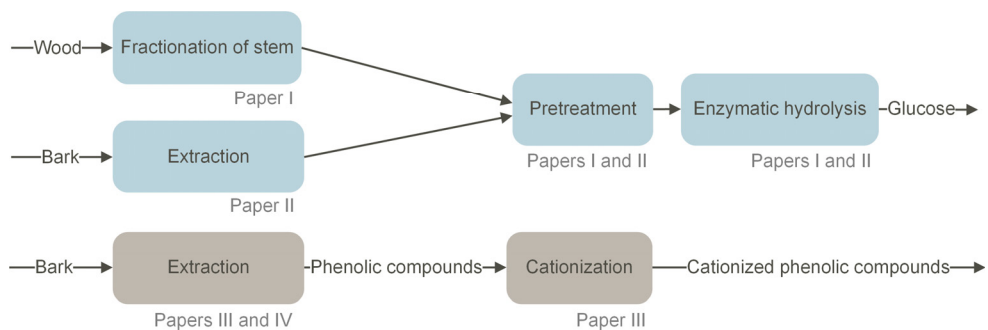


Figure 3.8. Overview of the studies reported in **Papers I-IV**.

3.4.1 Methods for pretreatment and enzymatic hydrolysis

The studies reported in **Papers I and II** both concerned possible process improvements for steam explosion pretreatment and subsequent enzymatic hydrolysis for the production of fermentable sugars. The feedstocks used have previously been discussed in section 2.3. The feedstocks were pretreated in a similar way. First, the material was chipped and milled to obtain particles less than 10 mm in size. If SO_2 catalysis was used in the pretreatment, the biomass was impregnated with gaseous SO_2 (2.5 wt %, based on the moisture content of raw material) as described by Monavari et al. (2009). The steam explosion pretreatment took place in a 10 L reactor, described by Bondesson et al. (2014) loaded with batches of typically 600-700 g of dry matter (Figure 3.9).



Figure 3.9. The biomass was pretreated by steam explosion in batches of typically 600-700 g of dry matter in a 10 L reactor described by Bondesson et al. (2014).

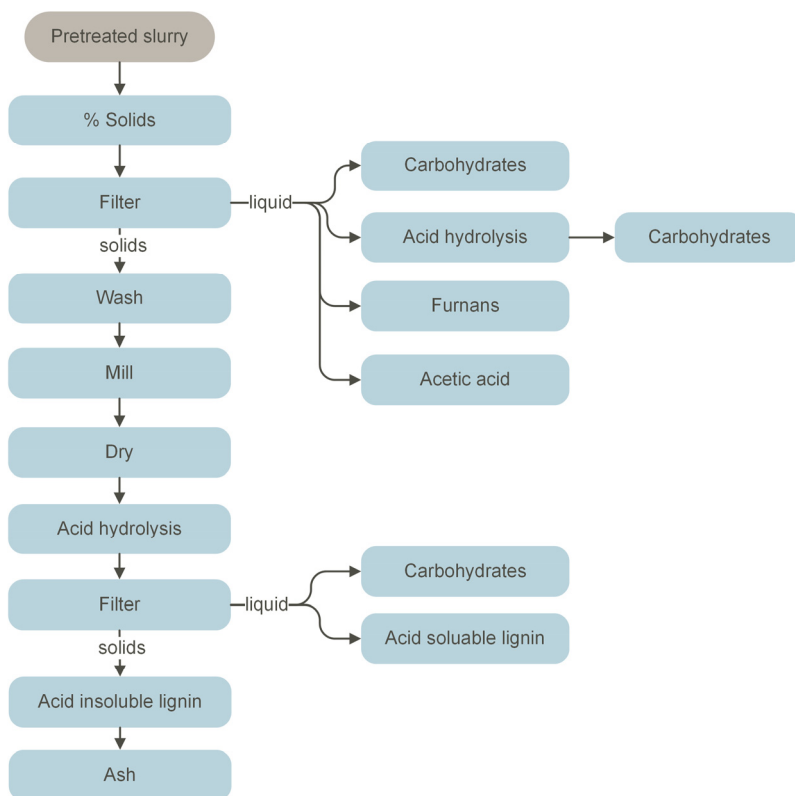


Figure 3.10. Flow scheme for analysis of chemical composition of pretreated slurries.

Table 3.1. Conditions for the enzymatic hydrolysis in the studies reported in **Papers I and II.**

Parameter	Paper I	Paper II
Reactor	250-ml laboratory flasks	2-L bioreactors
Load	150 g	1 kg
WIS	2 %	10 %
pH	4.8	5
Temperature	50 °C	45 °C
Time	72 hours	96 hours
Enzyme cocktail	Cellic CTec2	Cellic CTec3
Enzyme dosage	0.2 g/g glucan	0.05 g/g WIS

The pretreated slurries were analyzed according to laboratory procedures provided by the National Renewable Energy Laboratory (Sluiter and Sluiter, 2011b), as summarized in Figure 3.10. Due to limitation in amounts of biomass material, it was generally not possible to perform replicates of all steam explosion pretreatment experiments. Previous experiments in the same pretreatment unit has shown that the variance between pretreatment batches is within the same range as the variance of the analytical methods (Wiman et al., 2012). After steam explosion pretreatment, the biomass was enzymatically hydrolyzed according to the conditions listed Table 3.1.

3.4.2 Influence of tissue type on processability

The biorefinery concept implies working with several feedstocks, for instance, different tree species, which have different process requirements. As well as there are a chemical and structural differences between tree species, there are also differences between trees of the same species as well as differences between parts within one tree (section 2.3.2). At the time of **Paper I**, these differences were well known, but the implications for the processability in a biorefinery had not been given much attention. The focus of **Paper I** was to study steam explosion pretreatment and enzymatic hydrolysis of different fractions of pine from the perspective of the sugar platform (Figure 3.11).

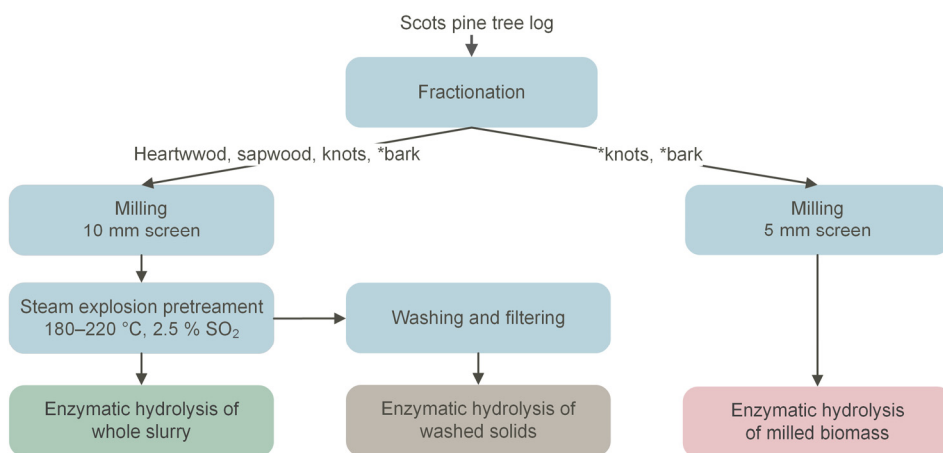


Figure 3.11. Schematic outline of the evaluation of pretreatment and enzymatic hydrolysis of different tissue types of Scots pine (**Paper I**) *Unpublished

Heartwood, sapwood, and knots from two pine trees (section 2.3.2), were subjected to steam explosion at different temperatures. Pretreated fractions from one of the trees were then enzymatically hydrolyzed, both as whole slurries and as washed solids. In

addition to the data published in **Paper I**, the enzymatic hydrolysis of pretreated bark, as well as enzymatic hydrolysis of milled knots and bark without steam explosion pretreatment, was evaluated (unpublished).

The pretreatment by acid catalyzed steam explosion mainly hydrolyzes the hemicellulose. In Figure 3.12 the total recovered glucose, mannose, and lignin, i.e. from both the water insoluble solid and liquid fraction, after pretreatment at 210 °C is presented. Glucose, that originally mainly is part of the cellulose polymer, was the best-preserved sugar with a recovery of at least 67 % for all studied tissue fractions. Approximately 1/3 of the recovered glucose was found in the liquid as monomers and oligomers after pretreatment, whereas the remainder was still part of the water insoluble polymeric fraction. The hemicellulose fraction, in contrast, depolymerized to a very high extent in the pretreatment and mannose was only recovered above the limit of detection in the liquid fraction as monosaccharides, but also some oligosaccharides, for all tree fractions. The recovery of mannose was between 44 % and 71 % for all fractions, except for bark where it was only 24 %. Sugars in the liquid fraction are susceptible to further degradation, and mannose was consequently degraded to a larger extent than glucose. The heartwood samples were generally less affected than sapwood and knots by the pretreatment, resulting in higher recovery of the hemicellulose sugar mannose. The older tree was also less susceptible to pretreatment, seen as higher recoveries of sugars, compared with the younger tree.

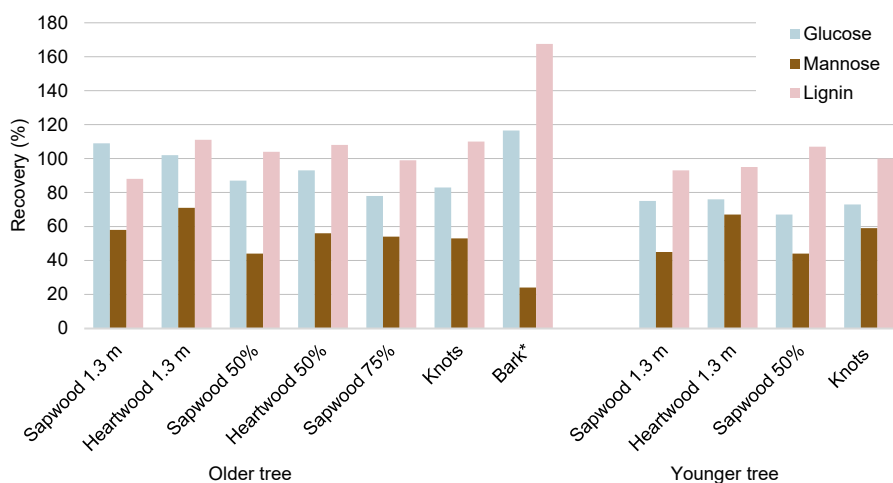


Figure 3.12. Total recovery (in the liquid and the water insoluble solid) of glucose, mannose, and lignin after steam explosion pretreatment at 210 °C, 2.5 wt %, SO₂ for 5 min of sapwood, heartwood, and knots sampled at different heights in one older and one younger tree (**Paper I**). *The data of the bark fraction of the older tree (pretreated in the same way) and calculated lignin recoveries for all fractions are previously unpublished.

The apparent low recovery of mannose together with a very high recovery of glucose for bark stands out and might be biased. In the calculation, only the sugars in the extractives free material were accounted for. However, compared with wood there are more water soluble carbohydrates in bark and for Scots pine bark approximately 25 % of the water soluble extractive are different carbohydrates (Laks, 1991). The high carbohydrate content of bark is not accounted for in the used analytical procedure. It is therefore possible that bark is more susceptible to steam explosion than indicated in Figure 3.12.

The lignin appears to be preserved in the solid fraction according to the analysis of acid insoluble lignin, although the amount of lignin in the liquid fraction was not measured. However, the lignin recovery for the bark stands out compared with the wood. A lignin recovery of 168 % for bark is clearly unreasonable and is likely caused by formation of “pseudo-lignin” from degradation products or the interface of for instance tannins or suberin (see section 2.2.3).

The highest total yield of glucose in the liquid fraction after steam explosion came from sapwood for both the younger and the older tree. The higher glucose yield coincided with a lower yield of the hemicellulose sugars mannose and xylose, resulting to a lower yield of the hemicellulose sugars mannose and xylose, resulting in that the sum of glucose, mannose, and xylose was even between the different fractions (data in **Paper I**). The amount of formed degradation products HMF, Furfural, and Acetic acid confirms that a larger degradation of sugars is linked to the higher glucose yields after pretreatment (data in **Paper I**).

When the steam explosion pretreatment was studied over a temperature interval for fractions of sapwood sampled at 1.3 m, heartwood sampled at 1.3 m, and knots, from the older tree, sapwood was again found more susceptible to pretreatment (Figure 3.13). Pretreatment is absolutely necessary to obtain enzymatic digestibility, as illustrated for knots and bark where only milling before enzymatic hydrolysis results in very low yields in the enzymatic hydrolysis (Figure 3.13). The enzymatic digestibility of pretreated sapwood, knots, and heartwood depended strongly on the severity of the pretreatment. Heartwood required higher severity of the pretreatment (Figures 3.13 and 3.14) and at the highest severity studied, at 220 °C, the glucose yield of heartwood was the best of all wood tissues studied, 31 g/100 g DW, corresponding to 65 % of the total glucose in the material. Since the glucan content in the raw heartwood, sapwood and knots were similar, the trend is the same regardless of if the yield is expressed in percent of theoretical or as g/g DW.

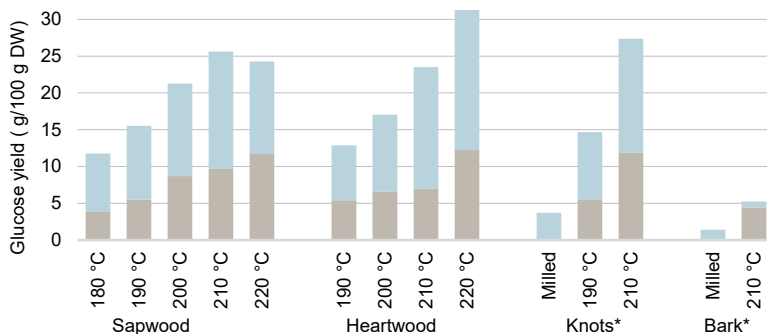


Figure 3.13. Glucose obtained per mass of material (g/ 100 g DW) after pretreatment and subsequent enzymatic hydrolysis of the older tree (**Paper I**). *Values for bark and milled knots are previously unpublished.

Comparing the enzymatic digestibility of both washed pretreated solids and whole slurry it was seen that the digestibility was increased by removing the liquid fraction. However, the initial hydrolysis rate for the whole slurries were faster than those of washed fibers. After 24 hours the glucose yield of the whole slurries had almost reached their final values and were higher compared with washed fibers in all cases except for sapwood pretreated at 220 °C. However, after 72 hours the glucose yield from most washed fibers were superior. It is likely that this behavior is due to readily available oligomeric glucose in the slurries (Table 8 **Paper I**). The enzymatic hydrolysis of bark was highly impaired and gave very low yields at the studied pretreatment conditions and less than 5 % of the glucose was accessed after 72 hours (Figure 3.14).

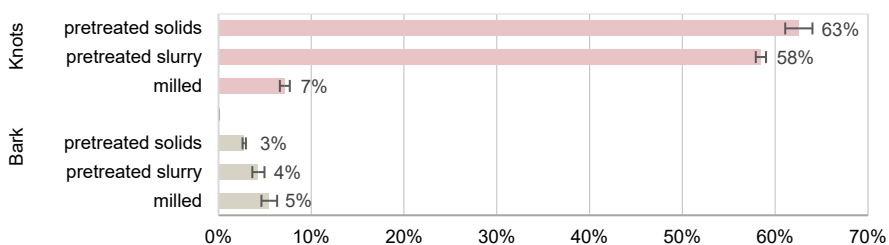


Figure 3.14. Glucose yield (%) after 72 hours enzymatic hydrolysis of the whole slurry and washed water insoluble solids of pretreated knots (**Paper I**) and bark (unpublished). In addition, is the glucose yield of the enzymatic hydrolysis after only milling knots and bark presented (unpublished).

To reach higher yields at the studied pretreatment conditions the enzyme dosage for bark needed to be increased considerable (Figure 3.15) to levels which are much higher than economically feasible.

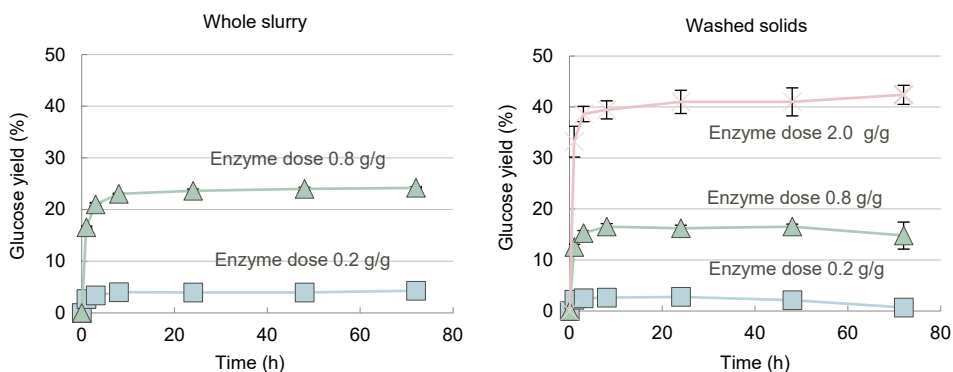


Figure 3.15. Glucose yield (%) during 72 hours enzymatic hydrolysis of the whole slurry and washed water insoluble solids of pretreated bark (unpublished).

3.4.3 Removal of extractives to enhance hydrolysis

The literature as well as initial studies of steam explosion pretreatment and enzymatic hydrolysis of bark linked to **Paper I** indicated significant challenges of using bark as a feedstock for the sugar platform. As mentioned previously, can some compounds referred to as extractives impair the enzymatic hydrolysis (section 3.3.2). The effect of extraction prior to acid catalyzed steam explosion pretreatment and enzymatic hydrolysis of bark from Norway spruce and Scots pine was therefore assessed in **Paper II** (Figure 3.16).

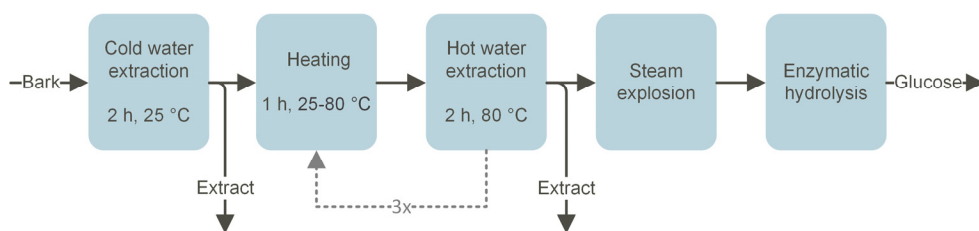


Figure 3.16. Simplified overview of the single time pre-extraction (1x HWE) of bark in **Paper II**. Some experiments also used a more thorough extraction, where the hot water extraction step was repeated three times (3x HWE).

In initial experiments using Scots pine bark (see section 3.4.2), the bark appeared to have an increased content of acid insoluble lignin after acid catalyzed steam explosion

pretreatment. The same was observed in the study reported in **Paper II**, using bark from both Scots pine and Norway spruce (Figure 3.17).

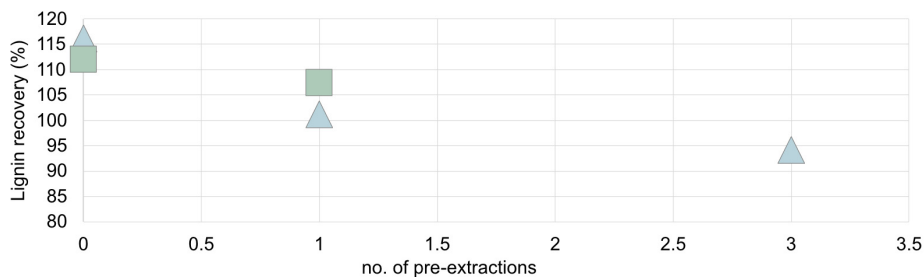


Figure 3.17. Calculated lignin recoveries (acid insoluble lignin) in the water insoluble fraction after acid catalyzed steam explosion pretreatment (210 °C, 5 min, 2.5 % SO₂) of bark from Norway spruce (▲) and Scots pine (■) plotted against the number of pre-extractions.

The unreasonable mass balance for lignin over the pretreatment step, was correlated with the extent of pre-extraction. The lowest total lignin recovery of 94 % was obtained with the thorough tree times hot water extraction of Norway spruce bark. This supports the hypothesis that water soluble extractives are deposited in the water-insoluble fraction after acid catalyzed steam explosion and are detected as acid insoluble lignin or “pseudo-lignin” in the analysis. The effect of pre-extraction on the lignin recovery was not as pronounced for steam explosion without acid catalyst (**Paper II**).

The degree of hydrolysis, based on total available glucose oligomers and polymers, after 96 hours of enzymatic hydrolysis was higher for bark from Scots pine compared with bark from Norway spruce (Figure 3.18). Pre-extraction improved the digestibility for bark from both species, even though the difference was more pronounced for bark from Norway spruce (30% compared with 11%). The difference between the effect of the single pre-extraction and the more thorough 3 times pre-extraction was small. This correlates to small differences in water soluble extractives removed with the more thorough extraction (Figure 3.18).

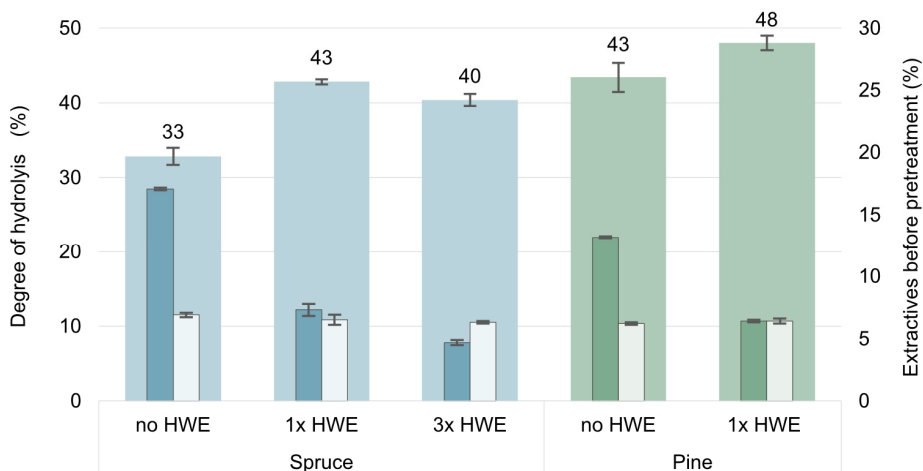


Figure 3.18. Degree of enzymatic hydrolysis of bark without pre-extraction (no HWE), single time pre-extraction (1x HWE), and tree times pre-extraction (3xHWE) from Scots pine (green) and Norway spruce (blue) before steam explosion pretreatment (**Paper II**). The darker narrow bar show the content of water extractives (%) while the lighter narrow bar represent the content of ethanol extractives prior to steam explosion pretreatment. The degree of hydrolysis is calculated after 96 hours enzymatic hydrolysis at 10 wt % WIS loading, 45 °C, pH 5 using Cellic CTec3 enzyme cocktail at a dose of 5 wt % based on WIS. The values are based on the sum of total available glucose oligomers and polymers (both in liquid as well as in the water insoluble fraction) and the error bars show the lowest and highest values of digestibility and standard deviation of material analysis. The value of three time pre-extraction is previously unpublished.

However, it is still necessary with a pretreatment that is severe enough in order to obtain a reasonable overall glucose yield. Removing the acid catalyst or removing the acid catalyst and lowering the temperature of the steam explosion, corresponding to lower pretreatment severity, resulted in considerably lower yields of steam explosion as well as lower overall yields (Figure 3.19).

It is difficult to compare the absolute values between the enzymatic hydrolysis of wood and bark described in section 3.4.2 with the data discussed here (**Paper II**). due to differences in the experiments with respect to mixing, WIS content and enzyme blends used (CTec2 vs CTec3). The enzyme cocktails Cellic CTec2 and Cellic CTec3 are both commercially available from Novozymes, but CTec3 is a further enhanced enzyme cocktail allowing a lower dosage, and has been reported to work well also at high solid loadings (Sun et al., 2015; Teter, 2012).

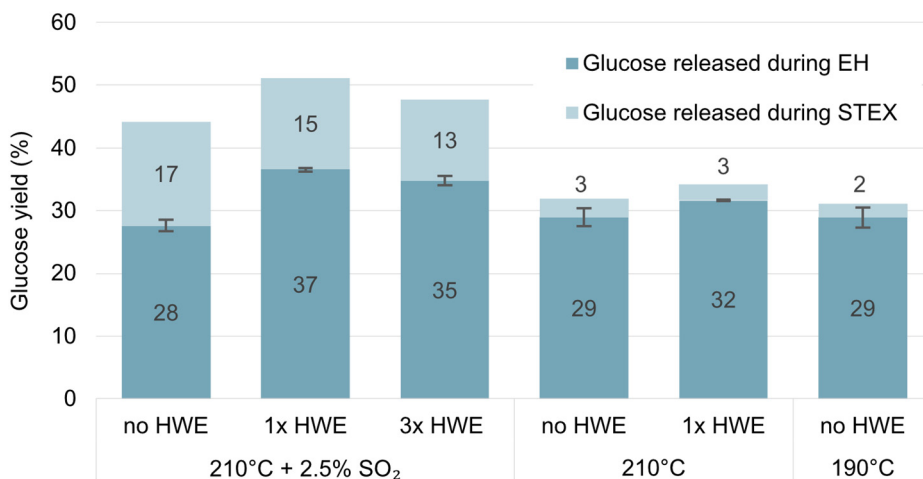


Figure 3.19. Glucose yields (as % of theoretical) after steam explosion pretreatment and enzymatic hydrolysis of bark from Norway spruce without pre-extraction (no HWE), single time hot water-extracted (1x HWE), or three times hot water extracted (3x HWE) (**Paper II**). The top bars represent glucose released during steam explosion pretreatment for 5 min at 210 °C, with 2.5 % SO₂, 210 °C without acid catalyst, and 190 °C without acid catalyst. The lower bars represent the yield of enzymatic hydrolysis at 10 % WIS loading, 45 °C, pH 5 for 96 hours using Cellic CTec3 enzyme cocktail at a dose of 5 wt % based on WIS. The error bars show the lowest and highest values.

The work reported in **Paper II** leads to the suggestion that extractives should be removed as the first step in the processing of bark or bark-rich materials in a biorefinery using the sugar platform. The obtained extractives, for instance phenolic compounds, could also be used to add value to the biorefinery. In the next section, process designs for the extraction of phenolic components from bark within a biorefinery will be analyzed in more detail with respect to the energy and mass flows.

3.4.4 Energy and mass flows in extraction

Mass and energy balances are at the very core of chemical engineering process design. Apart from the obvious engineering needs, it is essential to set-up mass and energy balances at an early phase of process development to be able to evaluate the environmental performance. To not get a skewed picture, the evaluations need to be made of the future industrial systems. This implies estimating the mass and energy balance of upscaled systems from lab-scale or pilot scale data. In this section, the modelled systems for the production of phenolic compounds from Norway spruce bark reported in **Papers III and IV** (Figure 3.20) will be discussed. This was the basis of the environmental assessment in chapter 4.

Paper III concerns the extraction of tannins from Norway spruce bark using high-pressure hot water extraction with subsequent purification and chemical modification.

In this study, the calculations were mainly made manually, and some simplifications were made calculating the heat demand for solvent recovery, and heat integration was not applied. In the study reported in **Paper IV** the focus was the extraction process per se, and the comparison of the three extraction technologies hot water extraction, ultrasound assisted extraction, and supercritical fluid extraction. The systems were in this study modelled using the software Aspen plus, which allowed using more advanced models for solvent regeneration and also included heat integration.

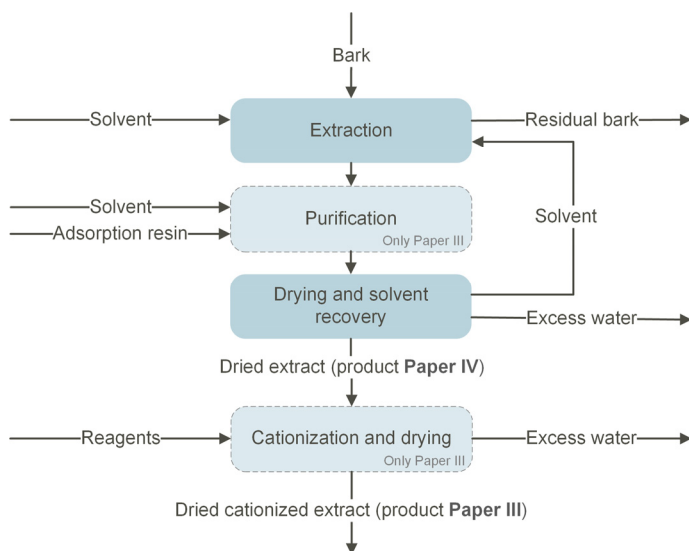


Figure 3.20. Schematic representation of the process steps and mass flows of the modeled systems for the production of phenolic compounds from Norway spruce bark reported in **Papers III and IV**. Purification and cationization were only included in the study reported in **Paper III**.

The mass and energy flow modeling allowed calculations of electricity and net heat demand per kg product (Figures 3.20 and 3.21). The end-products were defined differently in the two studies. In the study reported in **Paper III** the product was chemically modified and approximately half of the final product originated from the bark extract (see mass balance Figure 3 **Paper III**). In **Paper IV**, the product was defined as total phenolic contents measured as gallic acid equivalents. Although somewhat affected by the different end products, it is clearly seen that the electricity demand in ultrasound assisted extraction and supercritical fluid extraction is large compared with the two other systems (Figure 3.21). In the ultrasound assisted extraction the major contributor is the ultrasound generation, whereas for the supercritical fluid extraction it is the need of high-pressure pumps and compressors. Dewatering residual bark (pressing) was a minor contributor in all studied systems, as was the stirring in the high viscous cationization step. Ultrasound assisted extraction stands out as having a very

large electricity demand. However, the actual electricity need for ultrasound generation is a value with significant uncertainty, since no study evaluating the effect on bark extraction of changing ultrasound power was found.

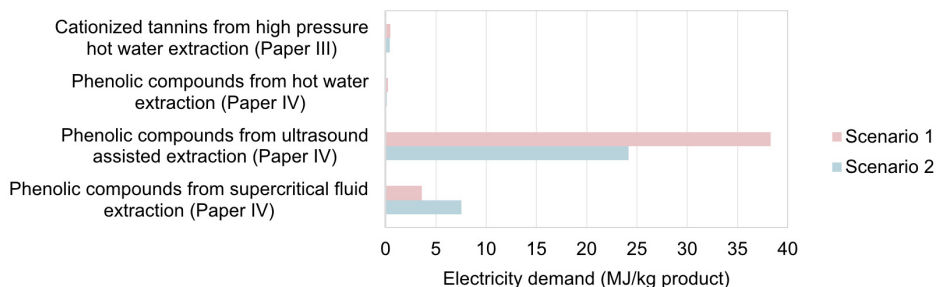


Figure 3.21. Electricity demand for the production of 1 kg product defined as 1 kg cationized tannins from high pressure hot water extraction (**Paper III**) or 1 kg total phenolic compounds measured as Gallic Acid Equivalents using hot water extraction, ultrasound assisted extraction or supercritical fluid extraction (**Paper IV**).

Compared with the electricity demands, the heat demands of the studied production processes were more similar (Figure 3.22).

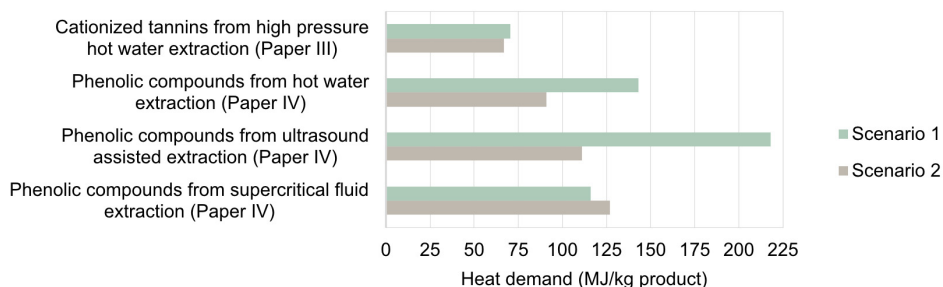


Figure 3.22. Heat demand for the production of 1 kg product defined as 1 kg cationized tannins from high pressure hot water extraction (**Paper III**) or 1 kg total phenolic compounds measured as Gallic Acid Equivalents using hot water extraction, ultrasound assisted extraction or supercritical fluid extraction (**Paper IV**).

The primary heat need stems from drying and solvent recovery. When heat integration was applied, the net heat demand was reduced by 35–60 % for the three studied extraction processes (**Paper IV**). Even though heat integration was not applied in the model for the production of cationized tannins, it still appears from Figure 3.22 as the process has a comparably low heat demand. There are two main explanations to this. Firstly, it is important to remember that cationized tannins from high pressure hot

water extraction is reported per kg cationized tannins, whereas the other extraction technologies are reported per kg total phenolic compounds produced. The energy requirement per kg extract (including drying and solvent recovery) is thus approximately a factor 4 to 6 higher for the high-pressure hot water extraction and cationization (see the mass of dry extract in mass the balances Figure 3 **Paper III** and Figure 2 **Paper IV**). Secondly, the purification step through adsorption lowered the heat demand for drying the extract. As most of the water was removed in this step and replaced by the ethanol eluent (that has a lower latent heat of vaporization compared with water), less heat was eventually required to dry the extract.

3.4.5 Important outcomes

The work on pretreatment and enzymatic hydrolysis, as well as design of extraction pinpointed several important facts for biorefinery operations.

- There is a difference in optimal pretreatment processing conditions for the fractions of Scots pine stem wood, despite mainly similar macromolecular compositions (**Paper I**). More juvenile tissue, or sapwood, of Scots pine required lower severity in steam explosion pretreatment than older tissue, heartwood, or knots.
- Scots pine bark differs substantially from other tree fractions regarding both composition and processing requirements (**Papers I and II**, and unpublished).
- Removing water-soluble extractives from Scots pine and Norway spruce bark prior to acid catalyzed steam explosion pretreatment and enzymatic hydrolysis improved the glucose yield by 11 and 30%, respectively (**Paper II**).
- Ultrasound assisted extraction or supercritical fluid extraction have a high electricity demand compared with hot water extraction or high-pressure hot water extraction (**Papers III and IV**).
- The heat demand for the studied extraction processes mainly stems from drying and solvent recovery, and the process conditions of the extraction is of minor importance for the heat demand (**Papers III and IV**).
- It was estimated that heat integration can reduce net heat demand by 35-60 % for hot water extraction, ultrasound assisted extraction, and supercritical fluid extraction. Thus, it is valuable to assess an upscaled system where heat integration can be included (**Paper IV**).
- Input data with high uncertainty and high impact on the mass and energy balances, making them essential to evaluate comprehensively early in process development, included possible solvent recovery when using organic co-solvents and ultrasound power needed for ultrasound assisted extraction (**Paper IV**).

4 Environmental assessment

When working for a sustainable development, it is necessary to take a systems perspective and consider the whole life cycle (Figure 4.1) of the solution and all the relevant impacts caused by the solution. It is also essential that these impacts are quantified. Otherwise, there is a significant risk of “problem shifting” where the intended solution to one problem leads to new and often ignored problems (Hauschild et al., 2017). In this context, life cycle assessment (LCA) is an essential tool to identify and quantify potential environmental impacts related to a product’s life cycle. This knowledge can also be a valuable support for funding decisions and new legislation (Cooper and Gutowski, 2020; Thonemann et al., 2020). In line with this, the published research on LCA has increased considerably for the last 15 years (Gaurav et al., 2021).

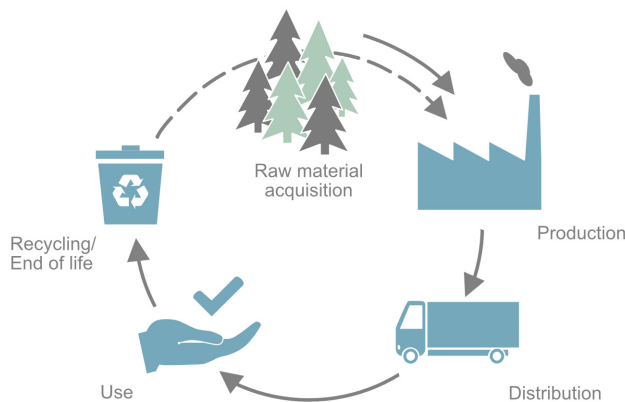


Figure 4.1. The life cycle of a product includes raw material acquisition, production, distribution, use, and recycling or end of life.

It has been estimated that roughly 80 % of all environmental effects are de facto determined during the design phase (Tischner et al., 2000). Therefore, it is pivotal to use LCA already in the early developmental phase before many design parameters are locked, as it is then possible to make technological changes that substantially improve

the environmental profile of a product or process (Moni et al., 2020). Nonetheless, the early developmental phase also implicates a high degree of uncertainty and many unknowns that complicates the LCA work.

In this chapter, the principles of LCA will be explained with an emphasis on the evaluation of emerging technologies. The outcomes of **Papers III and IV** reporting studies of the potential environmental impact of the production of phenolic compounds derived from the extraction of Norway spruce bark, will also be discussed.

4.1 Towards an LCA framework

In the late 1960s and early 1970s, nearly identical ideas of comprehensive LCA were developed in the USA and Europe (Hunt et al., 1996). In these early years, the assessments were called Resource and Environmental Profile Analysis (REPA) (Hunt et al., 1996) or Ecobalances (Hauschild et al., 2017). The focus was from the start solid waste issues, the role of recycling, and the use of reusable products, while energy was not considered as important until the energy crisis in the middle of the 1970s (Hunt et al., 1996). These early assessments were more similar to what we today would call a life cycle inventory (LCI), and the use of environmental impact factors to perform a life cycle impact assessment (LCIA) developed during subsequent decades (Klöpffer and Grahl, 2014).

It was not until the 1990s that the public, through increased media attention, became aware of the concept and the term LCA became the norm (Hunt et al., 1996). At the beginning of this century, the scope of the LCAs was broadened, and their application on macro-level questions, for instance, national energy systems and waste management systems, increased (Hauschild et al., 2017). An increased implementation in policies, especially in the European Union, could also be observed (Sonnemann et al., 2018). The need to harmonize the evolving LCA methods became apparent at the end of the last century as it was realized that two studies that aimed to answer the same question and compared nearly the same technologies could come to different conclusions (Hauschild et al., 2017). Today, the predominant standards for LCA are the 14040 and 14044 standards from the International Organization for Standardization (ISO). They are part of the ISO 14000 family regarding standards for environmental management (Klöpffer and Grahl, 2014).

LCA is, according to ISO, defined as “a tool to assess the potential environmental impacts and resources used throughout a product’s life cycle, that is from raw material acquisition, via production and use stages, to waste management” (ISO 14040, 2006).

According to ISO 1440, LCA has four mandatory phases: Goal and Scope Definition, Life Cycle Inventory Analysis (LCI), Life Cycle Impact Assessment (LCIA), and Interpretation, as seen in Figure 4.2. An iterative approach is often necessary, and the phases are hence connected with two-way arrows in the figure.

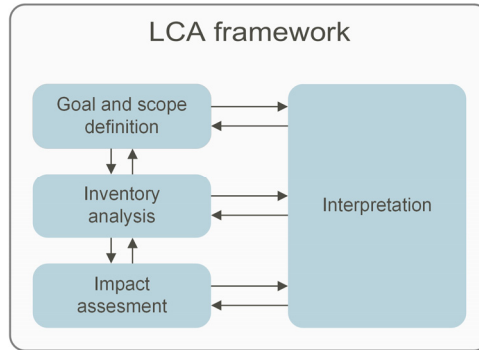


Figure 4.2. According to International Organization for Standardization (ISO) standard 1440, life cycle assessment (LCA) has four mandatory phases: goal and scope definition, life cycle inventory analysis, life cycle impact assessment, and interpretation. The two-way arrows indicate the iterative nature of LCA.

Over the last decade, the interest in applying the LCA methodology to emerging technologies has increased. The reason, as mentioned above, is the awareness that it is crucial to use LCA before important design parameters are locked, in order to make the most extensive improvements to the system's environmental impact. However, as LCA of emerging technologies implicates a high degree of uncertainty and many unknowns it complicates the assessment. According to Bergerson et al. (2020) the specific issues related to emerging production systems are not sufficiently addressed in the existing ISO standards. Neither is the nomenclature well defined and coupled to a specific methodology within the research community. Various prefixes such as prospective, early stage, ex ante, anticipatory, explorative, and scenario based, are used together with the term LCA to describe the analysis of systems in early developmental phase but might have a different meaning in different research groups. In this thesis, the term "LCA of emerging technologies" is used to describe the use of LCA to evaluate technologies in the early developmental phase.

Discussions are ongoing for a framework for LCA of emerging technologies to tackle the higher degree of uncertainty and ensure comparability. Since 2007, 17 peer-reviewed publications with methodological guidance on performing prospective LCAs on emerging technologies have been published (Thonemann et al., 2020). However, the basis is still the ISO 14040 and ISO 14044 standards. In the next section, the four

mandatory phases of LCA are discussed in more detail regarding assessing emerging technologies.

4.1.1 Goal and scope definition

The first LCA phase is to define the goal and scope. The goal includes why the LCA was performed, for whom, and how the results intend to be reported and used. There are no detailed requirements of how thorough an LCA needs to be. The ISO 14040 states that the scope should “ensure that the breadth, depth, and detail of the study are compatible and sufficient to address the stated goal” (ISO 14040, 2006). It is essential to define the studied system harmoniously so that there is not a mismatch in for instance, the LCA method, the developmental stage or time. Some of the elements defined in the scope to ensure transparency and comparability for LCA are further discussed below.

Functional unit

The functional unit (FU) is used to provide a reference to which the inputs and outputs are related to be able to compare LCA results. By using a function, systems providing very different products, but fulfill the same need, can be compared. The functional unit is coupled to an amount of needed product, the reference flow. In the chemical industry output-based definitions of functional units, for instance, the production of 1 kg of a specific chemical is the most common choice (Thonemann et al., 2020). This does not reflect the final function in an end use product but makes it practical to compare equivalent specific chemicals as ingredients in final products. To ensure comparability it is then important to also consider properties such as purity and dry weight.

System boundaries

To avoid problem shifting and ensure comparability, the whole life cycle from raw material acquisition to disposal of waste should preferably be included within the system boundaries, a so-called cradle-to-grave assessment. The main problem of setting the system boundaries of emerging technologies is that use and end-of-life stages are often unknown at an early stage. An alternative is to perform a cradle-to-gate assessment covering the environmental impact from raw material acquisition to finished product, ready to leave at the factory gate. Limiting the LCA to a cradle-to-gate assessment is not a problem if the LCA is used to find hotspots, critical aspects which have the largest impact on the environmental performance, within the production system. Neither is it a problem if the LCA is used to compare different production technologies resulting in chemically equivalent products. However, it is essential to include the whole

production system, including downstream processing, heat- and solvent recovery, to avoid a skewed result and make fair comparisons possible.

LCA method

LCA methods can generally be divided into two main groups: attributional LCA or consequential LCA. Attributional LCAs are usually based on *stoichiometric relationships* between inputs and outputs. It is useful for identifying process hotspots and possible improvements by process improvements or new technologies. However, an attributional LCA does not examine indirect effects due to changes in surrounding systems. Consequential LCA has the goal of studying the *change caused* by the studied system. It inherently has a higher degree of uncertainty since they are typically based on economic models representing relationships between demand for inputs, prices elasticities, supply, and markets effects of co-products (Brander et al., 2008). In version 3 of the Ecoinvent database a consequential system model to be used as background data for consequential LCA studies became available. This background data is a first step facilitating consequential LCA based on Ecoinvent, which is likely to be developed further for instance by the implementation of future technology development and supply of energy. Previously, consequential LCA's have mainly been performed using compiled data from various different sources. Wernet et al. (2016) recommend users to “carefully assess the datasets that are significant for their results and determine whether more detailed information on the predicted situation within their scope is available to improve the reliability of results.” Brander et al. (2008) addressed the problem that policymakers do not tend to distinguish between attributional LCA and consequential LCA. They warn that this could lead to the wrong method being applied, a combination of the two approaches within a single analysis, a misinterpretation of the results, or an unfair comparison of results derived from different methods. Thus, transparency is crucial in this perspective and to clearly describe which methodology that has been used.

4.1.2 Inventory analysis

In the second phase, the LCI, all relevant inputs and outputs of the product system are quantified. A thorough LCA depends on an accurate understanding of the mass and energy flows in both the foreground and background system. Quality data for the LCI is therefore of vital importance. In the 1990s, several LCI databases were developed by different institutes and organizations. However, the focus was to expand the coverage, and there was a problem with the consistency of the data. In 2003 the first Ecoinvent database (v 1.01) was introduced. It gave broad coverage as well as consistent data

standards and quality. The latest version of the Ecoinvent database (3.7) was released in September 2020 (Ecoinvent, n.d.).

For emerging technologies at an early stage, only experimental data from lab-scale studies may be available. In addition, there may be considerable differences between a process at lab-scale and industrial scale. Differences usually relate to reuse and recycling of raw materials; use of waste heat, and the use of continuous processes instead of energy-intensive batch processes (Moni et al., 2020). Therefore, the system, needs to be scaled to a developed stage and at a future point in time to avoid a skewed picture of the future process and ensure comparability. Thonemann et al. (2020) argue that ideally also the background systems should be modeled for the same time period. However, even though there are models available for future electricity supply, models of future heat supply are scarce (Thonemann et al., 2020). Furthermore, all these types of forecasting models are inherently uncertain as they are affected by a wide range of external factors.

Biorefineries are multifunctional processes, delivering more than one product output or providing more than one service, making a comparison with other product systems and hotspot analysis more complicated (Hauschild et al., 2017). According to the ISO 14044 standard, the systems should primarily be divided into smaller units, and the subprocesses that provide the additional functions should be excluded. Secondly, the problem should be solved by system expansion. This means that the most likely alternative way of providing the secondary function is either included in the system lacking this function or is the multifunctional process credited for avoided production by subtraction. In cases system expansion is not possible, for instance, when there is no alternative way to produce the secondary function or when this function is unknown, the last alternative is to use allocation. Inputs and outputs of the multifunctional process or system are then divided between the different products or functions. Preferably the allocation should be made using a representative physical parameter. Secondly, another parameter, for instance, economic value, can be used (ISO 14044, 2006).

There are several allocation models available in the Ecoinvent database. One is allocation at the point of substitution (APOS). It is intended for use in attributional LCA. The model makes no difference between wastes or recyclable by-products and includes all treatment processes required for any by-products by expanding the system. In this way, recycling and reuse possibilities are promoted for produced waste since part of the impact can be allocated to useful treatment products (Wernet et al., 2016).

4.1.3 Impact assessment

To be able to evaluate and quantify environmental aspects of the studied system, the life cycle inventory is aggregated and converted to environmental impact categories using various characterization factors.

The first impact assessment methods were developed during the 1990s (Hauschild et al., 2017), and continuous development is still ongoing as more knowledge of, for instance, how human activity impacts complex systems is obtained (Klöpffer and Grahl, 2014). There is also always a tradeoff between limiting the number of impact categories to simplify the interpretation or using a large set of impact categories for a more comprehensive assessment (Sala et al., 2021). The ISO standards of LCA only includes a general definition of the life cycle impact assessment phase and does not specify environmental impact categories or characterization factors to be used (ISO 14040, 2006; ISO 14044, 2006).

Due to variations in what processes are included, geographical coverages, temporal scales, and differences in the focus of the research at the institutions where assessment methods are developed, the LCIA models have fundamental differences. For instance, this can be seen as different characterization factors for the same substance. Therefore, the results and conclusions of an LCA often depend on the choice of the LCIA method (Dekker et al., 2020).

Two of the first widely accepted methodologies were the CML (developed at Centrum voor Milieukunde Leiden) and Eco-indicator 99 (developed by PRé Consultants). CML used a midpoint approach, meaning that the LCI is translated to contribution to different mechanisms for environmental issues, for instance, climate change. Whereas Eco-indicator 99 used an endpoint approach, meaning that information is aggregated to areas of protection for instance ecosystem quality. Out of a desire for a harmonized framework, with both midpoint and endpoint indicators, ReCiPe 2008 was developed in the beginning of this century. At the same time, almost all existing midpoint and endpoint characterization models were redesigned. The name is both a metaphor that the method intends to be used as a recipe and is an acronym of the initials of the main institutes and collaborators, RIVM (Rijksinstituut voor Volksgezondheid en Milieu) and Radboud University, CML, and PRé Consultants, involved in the design of the method. (Goedkoop et al., 2013).

This thesis focuses on the midpoint categories, which are more direct and therefore have a lower degree of uncertainty. The midpoint categories of ReCiPe 2008 are listed and explained in Table 4.1.

The characterization factors of ReCiPe 2008 are primarily adapted for European conditions, and some have limited validity outside well-developed temperate regions. It is available with three different perspectives: egalitarian, hierarchist, individualist. With the hierarchism perspective, the most common policy principles with regards to timeframe and other issues are followed with, for instance, the use of a 100-year time frame for the characterization factor global warming potential.

Today, the updated version ReCiPe 2016 is available. The updated version includes characterization factors that are more representative for the global scale, and work has been done to harmonize the time horizon per cultural perspective across the various impact categories (Huijbregts et al., 2016). Dekker et al. (2020) compared ReCiPe 2008 and ReCiPe 2016 in LCAs for 152 foods consumed in the Netherlands. They found that even though the LCIA results differed, they led to comparable hotspots and ranking conclusions. The most considerable discrepancy was seen for endpoint-level LCA.

Table 4.1. Impact categories at midpoint level for ReCiPe 2008 (Goedkoop et al., 2009).

Impact category	Midpoint indicator	Unit for midpoint indicator	Explanation and midpoint characterization factor
Land use	Agricultural land occupation	m ² a	The amount of agricultural area occupied in m ² and the time of occupation in years, for instance to produce trees in a forest. At midpoint level there is no differentiation to land use types.
Climate change	Climate change	kg CO ₂ -eq.	Greenhouse gasses in the atmosphere absorb infrared radiation leading to increased global temperature. The global warming potential (GWP) is quantified relative to that of CO ₂ over a defined period of time. The GWP depends on both the specific thermal radiation absorption and the lifetime of a substance
Fossil fuel depletion	Fossil depletion	kg oil-eq.	Fossil resources like natural gas, oil and coal are limited. The fossil depletion potential (FDP) is based on the cumulative energy demand of the used resource relative to the cumulative energy demand of oil.
Toxicity	Freshwater ecotoxicity	kg 1,4-DCB-eq.	Environmental persistence (fate) and accumulation in the human food chain (exposure), and toxicity (effect) of a chemical. Freshwater ecotoxicity potential (FETP) is quantitated relative to 1,4-dichlorobenzene.
Eutrophication	Freshwater eutrophication	kg P-eq	Phosphorus enrichment of seawater leads to adverse ecological effects. Eutrophication potentials (EP freshwater) is quantified relative to emission of phosphorus from sewage treatment plants to freshwater.
Toxicity	Human toxicity	kg 1,4-DCB-eq.	Environmental persistence (fate) and accumulation in the human food chain (exposure), and toxicity (effect) of a chemical. Human toxicity potential (HTP) is quantitated relative to 1,4-dichlorobenzene.
Ionizing radiation	Ionizing radiation	kg U ²³⁵ -eq.	Routine releases of radioactive material in the nuclear fuel cycle damage humane health. The ionizing

			radiation potential (IRP) is quantified relative to uranium.
Toxicity	Marine ecotoxicity	kg 1,4-DB-eq.	Environmental persistence (fate) and accumulation in the human food chain (exposure), and toxicity (effect) of a chemical. The marine ecotoxicity potential (METP) is quantitated relative to 1,4-dichlorobenzene.
Eutrophication	Marine eutrophication	kg N-eq.	Nitrogen enrichment of seawater leads to adverse ecological effects. Eutrophication potential (EP seawater) quantitated relative to emission of nitrogen from sewage treatment plants to freshwater.
Mineral resource depletion	Metal depletion	kg Fe-eq.	Many metals are not destroyed after their use, but the cost of extraction can be higher. The mineral depletion potential (MDP) is quantified relative to the burden that current resource extraction of a metal puts on future extraction relative to extracting 1 kg iron.
Land use	Natural land transformation	m ²	The land area that is transformed from one use to another. The land use types "forest", "tropical forest" and "sea and ocean" can be recognized as natural land. A negative value corresponds to transformation to natural land. The natural land transformation potential (NLTP) makes no difference to land use types at midpoint level.
Ozone depletion	Ozone depletion	kg CFC-11-eq.	Depletion of stratospheric ozone result in increased damaging UVB- radiation. The Ozone Depleting Potential (ODP) quantifies the amount of ozone a substance can deplete relative to CFC-11 (trichlorofluoromethane).
Health damage due to PM ₁₀ and ozone	Particulate matter formation	kg PM ₁₀ -eq.	Fine Particulate Matter (PM) with a diameter of less than 10 µm (PM ₁₀) causes health problems when inhaled. Emissions of substances like sulfur dioxide ammonia and nitrogen oxides can lead to the formation of PM as quantified by the Particulate Matter Formation Potential (PMFP) and expressed in PM ₁₀ -equivalents.
Health damage due to PM ₁₀ and ozone	Photochemical oxidant formation	kg NMVOC-eq.	Ozone formed at ground level from photochemical reactions of NO _x and air pollutants have a negative impact on human health and vegetation. Ozone formation potential (OFP) is quantitated as the marginal change in the 24h-average European concentration of ozone due to a marginal change in emission of the substance and is expressed in Non Methane Volatile Organic Compounds (NMVOCs) equivalents.
Acidification	Terrestrial acidification	kg SO ₂ -eq.	Emission of some inorganic substances to air increase the acidity in the soil (via rain) to harmful levels. Terrestrial acidification potentials (TAP) are expressed in SO ₂ -equivalents,
Toxicity	Terrestrial ecotoxicity	kg 1,4-DCB-eq.	Environmental persistence (fate) and accumulation in the human food chain (exposure), and toxicity (effect) of a chemical. The terrestrial ecotoxicity potential (TETP) is quantitated relative to 1,4-dichlorobenzene.
Land use	Urban land occupation	m ² a	Describes the amount in m ² of urban area, like mineral extraction sites or traffic area, occupied and the time of occupation in years. At midpoint level there is no differentiation to land use types.
Water depletion	Water depletion	m ³ water-eq.	Expresses the total amount of water used.

4.1.4 Interpretation

In the last phase, a combined interpretation of the LCI and LCIA is made to provide results according to the goal and scope. This phase also includes drawing conclusions, defining the limitations of the LCA and providing recommendations (ISO 14040, 2006).

When it comes to the interpretation of LCAs of emerging technologies, special attention must be paid to the challenges of comparability, data availability and quality, scaling, and uncertainty (Thonemann et al., 2020). Sensitivity and uncertainty analysis is important in all types of LCAs, but is particularly important in assessments of emerging technologies. The input data which has the largest influence should be identified and effects of different scenarios or changes in performance be analyzed (Arvidsson et al., 2018). Here it is essential to distinguish sensitivity from uncertainty. It is not necessarily so that the data, which is most uncertain has the largest impact on the final results of the LCA.

The uncertainties are of different kinds. Experimentally obtained data is always affected by, for instance, measurement errors. Furthermore, the goal of different experimental studies, used methods, and ways of reporting vary. Often only lab-scale data are available for emerging technologies, which means that the LCI of the large-scale process will have to rely on models and extrapolations of lab-scale data rather than direct data from large-scale operation. However, it is well known that the transition from lab-scale to an industrial scale process can imply unforeseen consequences, and one strategy to reduce such technological risks is to include a pilot and demonstration-scale step. Also, the time aspect is essential. Gradual technology development is likely to give improvements in, for instance, product yields or reduced energy requirements following the maturity of the studied process as well as technical developments in general. Many of these uncertainties may be dealt with by using predictive scenarios, for instance, applying a reasonable development curve in terms of improved energy efficiency with scale or time of development. If it is difficult to make such predictive extrapolations or functions from learning curves, different scenarios – defining outer ranges – could instead be looked at to show uncertainties in the interpretation of the results (Arvidsson et al., 2018).

A different type of uncertainty, that is more difficult to foresee, is that larger technological shifts, providing new technology alternatives, may occur in a future scenario (Ljunggren Söderman et al., 2014). This will affect the LCA in a more profound way, for instance, by using completely different reaction pathways radically changing the process chemicals needed for a reaction.

Future scenarios also give comparability issues since the background system may have changed and is no longer “in phase” with the developed large-scale productions system of the emerging technology (Moni et al., 2020). As discussed above, in connection to consequential LCA, to model background systems as for energy supply in a future point of time is challenging.

When interpreting the LCA it is also important remember that the evaluation is dependent on the LCIA models used. The development of LCIA models does not completely keep up with the fast technical development and emerging technologies for the production of chemicals, which may therefore cause impacts at a future point in time for environmental mechanisms that are not considered today (Thonemann et al., 2020).

Overall, the interpretation of an LCA of emerging technologies requires specific care and awareness. On the other hand, the potential benefits of looking at technologies at an early stage are very substantial, e.g. regarding identification of potential hotspots and/or significant environmental benefits already in the design phase.

4.2 LCA of bark-derived phenolic compounds

In the environmental assessments reported in **Papers III and IV**, the main goal was to identify critical aspects to be prioritized for action to reduce the environmental impact, here referred to as hotspots, when producing polyphenolic compounds from Norway spruce bark. In the study reported in **Paper III** the basis was high pressure hot water extraction, followed by purification and chemical modification of tannins to be used as flocculants. In the study reported in **Paper IV** the focus was to compare three different extraction technologies, hot water extraction, ultrasound assisted extraction, and supercritical fluid extraction, in the production of phenolic compounds derived from bark. The technical aspects of these processes, including mass flow and energy calculations that were the basis for the LCIs, have already been described in section 3.4.4. Therefore, this section will mainly focus on the other three phases of the LCAs conducted. It starts with an overview of some of the methodological choices for the LCAs, followed by the most central research outputs from the studies.

4.2.1 Methodological considerations

The evaluated systems covered emerging technologies with limited information on the intended use of the products. Therefore, the system boundaries were set cradle-to-gate to only include the first phases until the product is finished and ready to leave the factory, and the functional units were 1 kg of product (Figure 4.3).

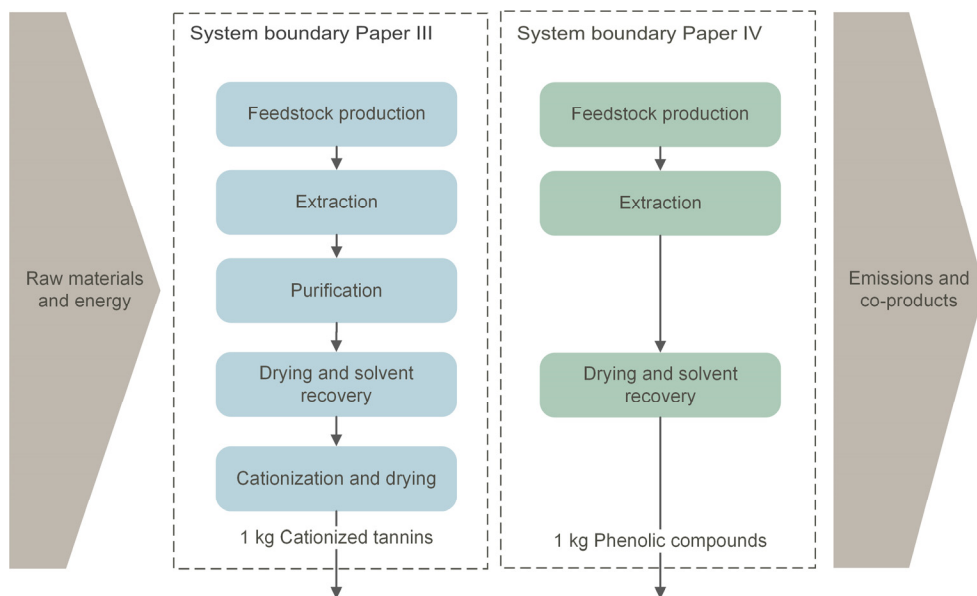


Figure 4.3. Schematic representation of the system boundaries of the LCAs reported in **Papers III and IV.**

Consequential LCA might seem like the preferable choice for LCA of emerging technologies since it is designed to describe the change caused by new processes and products. However, the inherently higher degree of uncertainty in combination with the scarcity of background data for consequential LCA led to the choice of the more robust attributional LCA as it serves the primary purpose of identifying hotspots well.

One of the biggest challenges working with LCAs of emerging technologies proved to be the LCI. The basis was pilot-scale experiments from a collaborating research group (**Paper III**) and literature data at lab-scale (**Paper IV**). However, this data only partly covered the needed process steps in a complete application. In addition, it was necessary to consider scaleup effects to evaluate the technologies in an industrial, commercial context. The work concerning process design and scaleup was covered in section 3.4.4.

The Ecoinvent 3.6 database using allocation at the point of substitution (APOS) was used for all background systems except bark production. It is reasonable to assume that

the bark could come from a sawmill. Therefore, the environmental impact of bark production was calculated based on the mass flow in a Swedish sawmill with an annual production of 370 000 m³ sawn wood (Hagberg et al., 2009). A sawmill is a multioutput system, and allocation was therefore used. The sum of the environmental burden of the timber production, transportation, and all sawmill activities was allocated to all products and co-products: sawn wood, pulp chips, sawdust, and bark. Three different allocation factors were calculated. To not underestimate the impact of the feedstock production, in accordance with ISO 14040 (2006), allocation based on volume was chosen, and 9 % of the environmental impact of upstream activities was assigned to the bark. The two other alternatives would have been allocation based on energy or economic value, corresponding to 7 % or 2 %, respectively (Figure 4.4). A sensitivity analysis performed in Paper III shows, however, that a change in allocation method will have a minor effect on the results since the impact from the production of the bark is rather limited in relation to the overall impact (see Figures 4.6 and 4.7 in section 4.2.2)

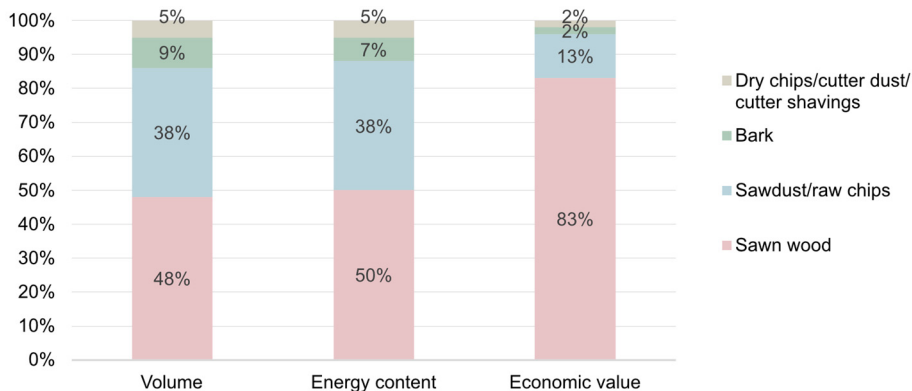


Figure 4.4. Allocation factors for sawmill activities based on volume, energy content, or economic value of all products and co-products produced (**Paper III**).

The extraction is also a multifunctional system. After extraction, residual bark can be used, for instance, as a fuel for heat production. The heating value per mass of dry material of the bark was assumed to be the same before and after extraction, which is supported in a study by Therasme et al. (2018). System expansion was therefore used to handle this additional function of the bark. After pressing the residual bark to the same dry content as before extraction, representing an equivalent energy quality, the potential environmental impact of the bark production was subtracted from the studied system (Figure 4.5).

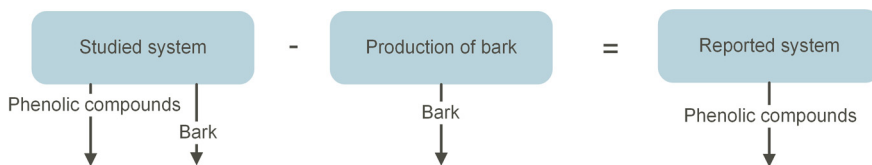


Figure 4.5. The production of phenolic compounds from bark is a multioutput system with both phenolic compounds and residual bark as outputs. System expansion was therefore used.

4.2.2 Chemicals and material

The consumption of process chemicals (other than water), such as solvents, eluents, and reagents, were major hotspots in all studied production systems in **Papers III and IV**. This underlines the necessity of closing the loops of process chemicals to reduce the potential environmental impact. It is also essential to, early during process development, evaluate if the process gains of using chemicals are worth the increased environmental impact.

In the study reported in **Paper III**, ethanol was used for elution in the purification step. The need for makeup ethanol for elution was identified as the major contributor to the potential environmental impact for the production of cationized tannins. In a first scenario, as much as 36 to 99 % of the total potential impact in the different studied impact categories could stem from the need for makeup ethanol (comparable to the adsorption step in Figure 11 **Paper III**). However, this was based on non-optimized pilot experiments where only 68 % of the water-free ethanol could be reused. The adsorption resin used in the purification could be another hotspot, accounting for 10 % of the overall contribution on climate change in the first scenario. The impact from the resin production depends on, for instance, the adsorption capacity and how many times it can be regenerated. Since there were no data on the lifetime of the resin, it was first assumed that it could be regenerated 100 times. It is probable that the first scenario led to a somewhat overestimated potential environmental impact of the studied system. Therefore, a second scenario was made where the liquid in the bark press was utilized (instead of treating it as waste), the ethanol recovery was 98 % (instead of 68 %), and adsorption resin could be regenerated 1000 times (instead of 100 times). The overall contribution to climate change shifted from 4.7 kg CO₂ eq. to 1.2 kg CO₂ eq. per kg of cationized tannins (Figure 4.6), with a similar trend for all impact categories. The lower value is within the estimated range of 0.2-2.8 kg CO₂ eq. per kg of the commercial products polyacrylamide, aluminium sulfate, and iron(III)chloride (Figure 14 **Paper III**).

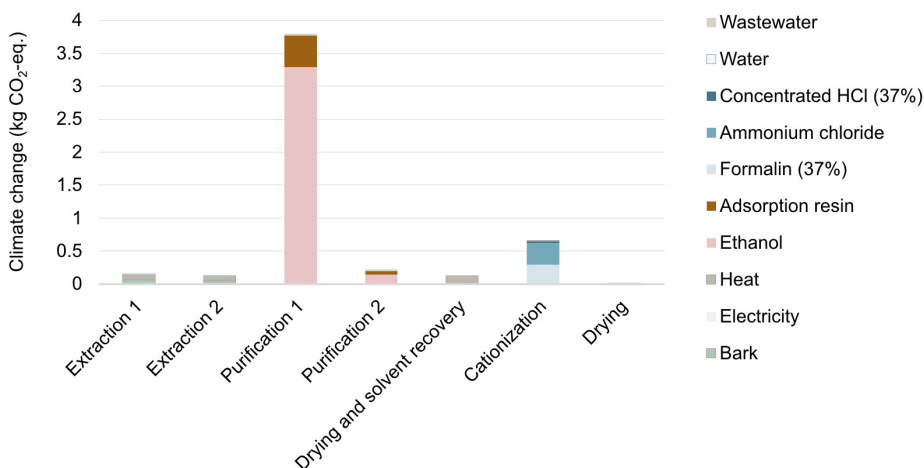


Figure 4.6. Potential impact on climate change for the production of 1 kg cationized tannins for the process steps extraction, purification through adsorption, drying of purified extract and regeneration of the solvent (ethanol for elution), cationization of the extract, and drying of the final product. The extraction category in this figure includes high-pressure hot water extraction, the production of bark (raw material), and the pressing of residual bark. Two scenarios were made for the Extraction and Purification steps. In the first scenario (denoted 1), the liquid from the bark press was treated as wastewater, the ethanol recovery was 68 %, and the adsorption resin could be regenerated 100 times. In the second scenario (denoted 2), the liquid from the bark press was utilized as the extract, the ethanol recovery was 98 %, and the adsorption resin could be regenerated 1000 times (**Paper III**).

Ethanol may also be added as a solvent to enhance extraction. However, it was estimated that the yield from ultrasound assisted extraction or supercritical fluid extraction using ethanol as a co-solvent needed to be fivefold compared with hot water extraction (Figure 4.7). From Aspen simulations it could be estimated the maximum possible ethanol recovery in ultrasound assisted extraction and supercritical fluid extraction was only 82-86 % (**Paper IV**). Even at this ethanol reuse, 70 % of the total environmental burden of most of the studied environmental impact categories of phenolic compounds produced via ultrasound assisted extraction or supercritical fluid extraction was due to the production of ethanol. As shown in the sensitivity analysis in **Paper IV** (see Figure 10), the climate performance of ethanol could be significantly improved by using lignocellulosic feedstock and resource efficient production systems. Still, the ethanol demand will account for a share of the potential impact on climate change.

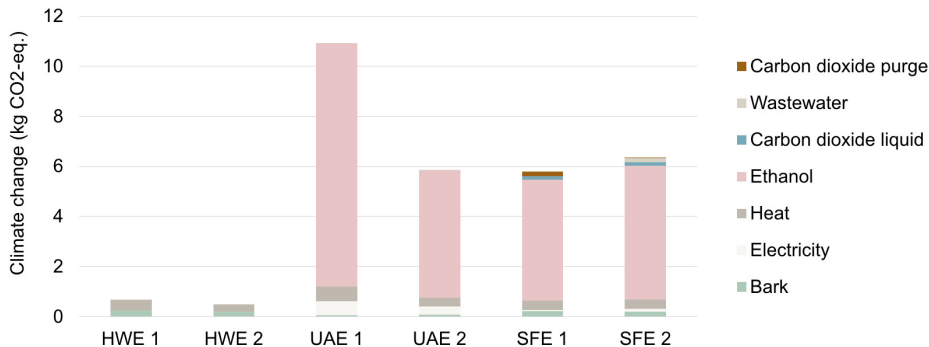


Figure 4.7. Potential impact on climate change for the production of 1 kg phenolic compounds, measured as gallic acid equivalents, using the three different extraction technologies hot water extraction (HWE), ultrasound assisted extraction (UAE), and supercritical fluid extraction (SFE). The suffixes 1 and 2 represent scenario 1 (based on yields reported in the literature) and scenario 2 (based on setting the same yields for all extraction technologies), respectively (**Paper IV**).

Most of the ethanol was calculated to be lost with the residual bark, based on the assumption of identical composition of the liquid in the bark and in the extraction bulk liquid. No experimental data on ethanol recovery from residual bark after extraction was found and this would be of interest to investigate further. The problem of low solvent recovery due to losses in the stream of residual bark is estimated to be lower when supercritical carbon dioxide is used. The solvent can then be recovered by simply decreasing the pressure. In the study of extraction with supercritical fluid extraction as much as 98 % of the carbon dioxide was estimated to be recycled (**Paper IV**), and the use of carbon dioxide was not a major hotspot.

The need for reagents always leads to a net consumption of chemicals. In the studied system the extracted tannins are cationized using formalin, ammonium chloride, and concentrated hydrochloric acid. These three chemicals are today produced using energy-intensive production processes based on fossil resources for both material and energy and therefore gives a considerable contribution to the environmental impact of the production of cationized tannins from Norway spruce bark (Figure 4.6 and **Paper III**).

As discussed above in section 4.2.1, the production of the bark is contributing less to the environmental impact than either chemicals or heat demand for all studied production systems (Figures 4.6 and 4.7). This is mainly due to the system expansion to account for the multioutput system where residual bark still can be used as a fuel.

In future LCAs, to extend the understanding of the most environmentally favorable process design to produce phenolic compounds from bark, the functional unit needs to

include the product's functionality which is critical in the final end product. Using the more simplified functional unit based on the mass of the product does not account for the complexity of the biological matrix. The extract will be a complex mixture of many different components depending on many factors, such as the choice of extraction solvent. Including functionality constraints in the functional unit might favor the use of ethanol as a co-solvent. At the same time, some process designs, such as the purification, could be simplified or omitted since the additional compounds in the extract are primarily carbohydrates and may also confer some specific properties, in this case flocculation.

4.2.3 Electricity

Choosing a relevant electricity production system is essential for a truthful environmental assessment regarding production systems requiring high electricity input. The potential impact on, for instance, climate change is well known to be strongly dependent on the choice of electricity production system depending on the share of primary fossil fuels. There is a large exchange of electricity between the countries on the Scandinavian peninsula (Gode et al., 2011), and a Nordic electricity mixture from Nordic Countries Power Association (NORDEL) was estimated to be the most relevant choice for LCAs in this geographical area. For NORDEL only 5 % stems from fossil resources. As a comparison, calculations were also made for electricity supplied by the Central Europe Power Association (CENTREL), where 70 % is generated from fossil resources (for details about electricity mixes see Table 1 **Paper IV**). In the production of cationized tannins (**Paper III**) the electricity demand was estimated to be relatively low (0.44-0.48 MJ/kg cationized tannins, see section 3.4.4) and the difference in the LCIA using electricity supplied by CENTREL or NORDEL was small. For the studied extraction processes (**Paper IV**), the electricity demands in the base case scenario were 0.27, 38, and 3.6 MJ/kg phenolic compounds for hot water, ultrasound assisted and supercritical fluid extraction, respectively. For the two later technologies with a higher electricity demand, electricity the choice of electricity production system has a significant impact on the environmental assessment. If electricity is supplied from NORDEL (mainly using hydropower and nuclear power) the electricity demand only accounts for 5 % and 0.9 % of the total contribution to climate change. In this case was the electricity demand not considered a hotspot. However, if electricity instead stems from CENTREL, with high use of coal, the contribution from the electricity need on this impact category is substantially increased, to 47 % and 12 %. The values used for calculations are based on today's production mixes. In the future, the electricity production mixes are likely to change to meet future

climate goals using an even lower share of fossil fuels and reduce the potential impact on climate change for, especially, ultrasound assisted extraction.

4.2.4 Heat

It is crucial to study a process at a relevant commercial scale to estimate the adequate heat requirements and corresponding potential environmental impacts. Most lab-scale equipment use electricity for heating. However, the use of electricity as the main heat source is unrealistic in an industrial context. Process heat will always have an impact on the environmental performance of the system, even if heat production with low carbon emissions is chosen. Heat produced from the incineration of wood chips generates 3.1 g CO₂ eq. /MJ heat (Ecoinvent, 2019a), which is an industrially relevant heat source and therefore used as base case in **Paper III** and **IV**. For comparison, electricity from NORDEL, results in the emission of 14 g CO₂ eq./MJ electricity (Ecoinvent, 2019b).

As was seen comparing the heat demand (Figure 3.21), the heat demand was within the range 67 to 218 MJ/kg product for all studied systems. Heat recovery was not included in the base case assessing the production of cationized tannins (**Paper III**), and the process energy need was therefore overestimated. In the study comparing different extraction technologies (**Paper IV**) the net heat demand could be reduced by 35-60 % by internal heat integration, according to simulations in Aspen plus.

Water is a solvent with many advantageous properties for designing environmentally sound processes. However, the high specific heat of evaporation of water results in high energy demand for water-based processes in all steps where evaporation is needed, for instance, drying and recovery of solvent. In the production of cationized tannins from Norway spruce bark, most of the energy demand stems from evaporation of the water and ethanol (**Paper III**). Using carbon dioxide at supercritical conditions as a solvent gives advantages in this respect. Firstly, changing the state of matter of carbon dioxide is less energy demanding compared with water. Secondly, the possibility to evaporate all carbon dioxide makes a high recovery and reuse possible, as discussed above concerning the chemical use (**Paper IV**).

The importance of minimizing the energy demand depends on if other hotspots in the system are present. The use of process chemicals has a much larger influence on the environmental performance. However, when the chemical demand has been minimized, reducing the heat demand should come next.

4.2.5 Important outcomes

The overall outcome from the present environmental assessments was that a simple water-based system could be favorable over other technologies, and the higher environmental impact from a more advanced process may need to be compensated with a high increase in yield. More specific conclusions made were:

- In the comparison of three different technologies for extraction of phenolic compounds from bark, the lowest environmental impact in most impact categories in absolute numbers was obtained for hot water extraction. The potential impact on climate change were estimated to 0.48-0.68, 5.9-11, and 5.8-6.3 kg CO₂-eq. per kg of produced phenolic compounds, for hot water extraction, ultrasound assisted extraction and supercritical fluid extraction, respectively. The relative difference between the different extraction technologies were similar for most studied impact categories (**Paper IV**).
- The consumption of chemicals was generally identified as the primary hotspot in extracting and modifying phenolic compounds from Norway spruce bark (**Papers III and IV**). Ethanol, when used to improve extraction (**Paper IV**) and as an eluent in downstream separation (**Paper III**), greatly contributes to the overall environmental impact of the systems if efficient recovery is not possible. This conclusion holds even if ethanol with the current most environmentally favorable profile is used. Solvents that can be recovered readily, for instance, supercritical carbon dioxide (**Paper IV**), are thus advantageous.
- The origin of chemical reagents is essential. In the studied cationization step formalin, ammonium chloride, and hydrochloric acid are used. They all have high energy requirements for production, and their precursors are generally based on fossil resources today (**Paper III**), which impair the environmental performance of the studied system.
- The use of an adsorption resin for separation can considerably contribute to the overall environmental impact. However, the data of resin need in the studied system had a high uncertainty (**Paper III**).
- For the studied processes ultrasound assisted extraction and supercritical fluid extraction, which have a substantial need of electricity, the choice of electricity production system has a significant impact on the environmental assessment (**Paper IV**). For extraction technologies with low electricity demand, like high pressure hot water extraction or hot water extraction, electricity production has little influence on the environmental performance (**Papers III and IV**).

- The limited available information on the final use of products and applications motivated the use of mass of produced intermediate product as a functional unit. However, this led to excluding the properties of the final products, which can be affected by the process design. For instance, in the case of cationized tannins, a more inclusive functional unit could have been based on the function of treating wastewater (**Paper III**).
- Limited availability of data leads to challenges in the interpretation of the LCAs. Most published lab-scale data come from proof of concept studies, where the final process design is not the main aim. In addition, data gaps lead to the need of estimations for the LCIs. Examples of LCI data with high uncertainty in this work were the solvent and resin consumption for adsorption (**Paper III**), consumption of reagents for cationization (**Paper III**), loss of solvent in residual bark (**Paper IV**) and need

5 Conclusion and outlook

The present work assessed the process design of extraction, pretreatment, and enzymatic hydrolysis of wood and bark from Norway spruce and Scots pine. In addition, the environmental performance of different process choices for extraction and further processing of the extract were evaluated. The work is part of the needed efficiency improvements, utilization of side streams, recycling within biorefineries, and understanding of sustainable process development, essential for the success of the emerging biobased economy. After each chapter, the most important outcomes have been given on a detailed level. To conclude this thesis, some general observations related to the research questions in the first chapter, together with suggestions of progression of this work, are given here.

The susceptibility to pretreatment and enzymatic hydrolysis depends on not only the tree species but also on the tissue type. By adapting the severity of the pretreatment, comparable overall glucose yields can be achieved for sapwood, heartwood, and knots of Scots pine, using today's basic process design. This implies that pretreating different wood tissue types separately can increase the overall yield. However, the enzymatic hydrolysis of Scots pine bark is severely impaired compared with wood, and requires a markedly different process design to succeed. Removing water-soluble extractives from Scots pine and Norway spruce bark prior to acid-catalyzed steam explosion improves the overall glucose yield after subsequent enzymatic hydrolysis. It could be of value to conduct further studies to find the reasons behind the impaired hydrolysis of bark. As described in this work, there are several possible extraction technologies, which may result in a pretreated material with better enzymatic digestibility. In addition, further developed tailored enzyme cocktails may improve the enzymatic hydrolysis of bark. The biorefinery research community also needs to agree on analytical procedures adapted for bark to understand favorable process design for bark better, as today's generally accepted and used methods were developed for materials that are chemically significantly different from bark.

By adapting the severity of the pretreatment, comparable overall glucose yields can be achieved for sapwood, heartwood, and knots of Scots pine, using today's basic process design. This implies that pretreating different wood tissue types separately can increase

the overall yield. However, the enzymatic hydrolysis of Scots pine bark is severely impaired compared with wood, and requires a markedly different process design to succeed. Removing water-soluble extractives from Scots pine and Norway spruce bark prior to acid-catalyzed steam explosion improves the overall glucose yield after subsequent enzymatic hydrolysis. It could be of value to conduct further studies to find the reasons behind the impaired hydrolysis of bark. As described in this work, there are several possible extraction technologies, which may result in a pretreated material with better enzymatic digestibility. In addition, further developed tailored enzyme cocktails may improve the enzymatic hydrolysis of bark.

In the environmental assessments of the extraction and further processing of phenolic compounds from Norway spruce bark, the production of process chemicals used was identified as a significant hotspot in most of the studied impact categories. Simple, water-based systems, therefore, have substantial advantages, even though yields of the desired extract can be lower. If solvents other than water are used, efficient recovery is essential. It is in such cases favorable with solvents that are easy to separate and recover, for instance, supercritical carbon dioxide. Use of chemical reagents always results in a net consumption, since they are incorporated in the product. Therefore, the production routes of these chemicals and corresponding environmental effects are of great importance. Increased access to sustainably produced reagents with high environmental performance on the market will thus be crucial for reaching a sustainable biobased economy.

All studied extraction technologies have a relatively high process heat demand, and after the need for chemicals, the heat production has the most considerable impact on the environmental performance of the extraction systems. The choice of heat sources and utilization of heat integration considerably affect the assessment. This underlines the need to environmentally assess scaled-up processes in an industrially relevant context not to get a skewed picture.

The outcome of an environmental assessment is dependent on the quality and amount of information about the studied emerging production system. With limited information about the product's exact final application and use, it is motivated to use the mass of produced product as a functional unit in initial LCAs of emerging technologies, as in this thesis. The boundaries of technical systems are then limited to cradle-to-gate and not cradle-to-grave. However, since the phenolic extract is produced from a biological and chemically complex matrix, the product may get different properties when produced using different extraction designs. Choosing a functional unit, including the intended use of the product, will account for these properties in the environmental assessment and should be the aim of future LCAs.

More experimentally validated information of the up-scaled extraction of Norway spruce bark would, of course, decrease the uncertainty of calculated impacts for the studied extraction technologies. However, some valuable data could have been recovered during the already reported lab-scale experiments. In the literature, many researchers tend to only report detailed information about the product, such as the extract, and not information on side streams or feedstocks that would help to verify the mass balances needed to evaluate the system. Most importantly, more information about the residual bark as well as indications of the amount of solvent that ends up with the bark residue would have been valuable for the LCAs presented in this thesis. In addition, it would have been favorable if more information and precise data of the feedstock, in this case the softwood bark, such as storage conditions, moisture content, and compositional analysis were provided since there can be considerable variations within woody feedstocks.

Designing efficient and sustainable softwood biorefineries is a complex task but crucial for leaving the fossil era behind. Hopefully, this thesis adds one more piece to the puzzle.

6 References

- Åkerholm, M., Salmén, L., 2001. Interactions between wood polymers studied by dynamic FT-IR spectroscopy. *Polymer* 42, 963–969. [https://doi.org/10.1016/S0032-3861\(00\)00434-1](https://doi.org/10.1016/S0032-3861(00)00434-1)
- Allen, F., Andreotti, R., Eveleigh, D.E., Nystrom, J., 2009. Mary Elizabeth Hickox Mandels, 90, bioenergy leader. *Biotechnology for biofuels* 2, 22. <https://doi.org/10.1186/1754-6834-2-22>
- Almeida, J.R.M., Runquist, D., Sánchez Nogué, V., Lidén, G., Gorwa-Grauslund, M.F., 2011. Stress-related challenges in pentose fermentation to ethanol by the yeast *Saccharomyces cerevisiae*. *Biotechnology Journal* 6, 286–299. <https://doi.org/10.1002/biot.201000301>
- Ameer, K., Shahbaz, H.M., Kwon, J.H., 2017. Green Extraction Methods for Polyphenols from Plant Matrices and Their Byproducts: A Review. *Comprehensive Reviews in Food Science and Food Safety* 16, 295–315. <https://doi.org/10.1111/1541-4337.12253>
- Andrić, P., Meyer, A.S., Jensen, P.A., Dam-Johansen, K., 2010. Reactor design for minimizing product inhibition during enzymatic lignocellulose hydrolysis: I. Significance and mechanism of cellobiose and glucose inhibition on cellulolytic enzymes. *Biotechnology Advances* 28, 308–324. <https://doi.org/10.1016/j.biotechadv.2010.01.003>
- Aro, T., Fatehi, P., 2017. Production and Application of Lignosulfonates and Sulfonated Lignin. *ChemSusChem* 10, 1861–1877. <https://doi.org/10.1002/cssc.201700082>
- Arvidsson, R., Tillman, A., Sandén, B.A., Janssen, M., Nordelöf, A., Kushnir, D., Molander, S., 2018. Environmental Assessment of Emerging Technologies: Recommendations for Prospective LCA. *Journal of Industrial Ecology* 22, 1286–1294. <https://doi.org/10.1111/jiec.12690>
- Attard, T.M., Clark, J.H., Mcelroy, C.R., 2020. ScienceDirect Recent developments in key biorefinery areas. *Current Opinion in Green and Sustainable Chemistry* 21, 64–74. <https://doi.org/10.1016/j.cogsc.2019.12.002>
- Bajpai, P., 2017. Single-Cell Protein from Lignocellulosic Wastes. https://doi.org/10.1007/978-981-10-5873-8_7

- Bello, A., Virtanen, V., Salminen, J.P., Leiviskä, T., 2020. Aminomethylation of spruce tannins and their application as coagulants for water clarification. *Separation and Purification Technology* 242, 116765. <https://doi.org/10.1016/j.seppur.2020.116765>
- Beltrán-Heredia, J., Sánchez-Martín, J., Jiménez-Giles, M., 2011. Tannin-based coagulants in the depuration of textile wastewater effluents: Elimination of anthraquinonic dyes. *Water, Air, and Soil Pollution* 222, 53–64. <https://doi.org/10.1007/s11270-011-0803-z>
- Bergerson, J.A., Brandt, A., Cresko, J., Carbajales-Dale, M., MacLean, H.L., Matthews, H.S., McCoy, S., McManus, M., Miller, S.A., Morrow, W.R., Posen, I.D., Seager, T., Skone, T., Sleep, S., 2020. Life cycle assessment of emerging technologies: Evaluation techniques at different stages of market and technical maturity. *Journal of Industrial Ecology* 24, 11–25. <https://doi.org/10.1111/jiec.12954>
- Berlin, A., Maximenko, V., Gilkes, N., Saddler, J., 2007. Optimization of enzyme complexes for lignocellulose hydrolysis. *Biotechnology and Bioengineering* 97, 287–296. <https://doi.org/10.1002/bit.21238>
- Bermek, H., Eriksson, K., 2009. Lignin, Lignocellulose, Ligninase. *Encyclopedia of Microbiology* 373–384.
- Biometria, 2021. Skogsindustrins virkesförbrukning 2016–2020. Biometria, Uppsala.
- Boerjan, W., Ralph, J., Baucher, M., 2003. Lignin Biosynthesis. *Annual Review of Plant Biology* 54, 519–546. <https://doi.org/10.1146/annurev.arplant.54.031902.134938>
- Bondesson, P.-M., Galbe, M., Zacchi, G., 2014. Comparison of energy potentials from combined ethanol and methane production using steam-pretreated corn stover impregnated with acetic acid. *Biomass and Bioenergy* 67, 413–424. <https://doi.org/10.1016/j.biombioe.2014.05.025>
- Bos, H.L., Broeze, J., 2020. Circular bio-based production systems in the context of current biomass and fossil demand. *Biofuels, Bioproducts and Biorefining* 14, 187–197. <https://doi.org/10.1002/bbb.2080>
- Brander, M., Tipper, R., Hutchison, C., Davis, G., 2008. *Consequential and Attributional Approaches to LCA*. Ecometrica Press.
- Browning, B.L., 1967. *Methods of wood chemistry*. vol I. John Wiley & Sons, New York.
- Bugg, T.D.H., Ahmad, M., Hardiman, E.M., Rahmanpour, R., 2011. Pathways for degradation of lignin in bacteria and fungi. *Natural Product Reports* 28, 1883–1896. <https://doi.org/10.1039/c1np00042j>
- 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>
- Chum, H.L., Johnson, D.K., Black, S.K., Overend, R.P., 1990. Pretreatment-Catalyst Effects and the Combined Severity Parameter. *Applied Biochemistry and Biotechnology* 24–25, 1–14.

- Cooper, D.R., Gutowski, T.G., 2020. Prospective Environmental Analyses of Emerging Technology: A Critique, a Proposed Methodology, and a Case Study on Incremental Sheet Forming. *Journal of Industrial Ecology* 24, 38–51. <https://doi.org/10.1111/jiec.12748>
- Couturier, M., Navarro, D., Chevret, D., Henrissat, B., Piumi, F., Ruiz-Dueñas, F.J., Martinez, A.T., Grigoriev, I. V., Riley, R., Lipzen, A., Berrin, J.G., Master, E.R., Rosso, M.N., 2015. Enhanced degradation of softwood versus hardwood by the white-rot fungus *Pycnoporus coccineus*. *Biotechnology for Biofuels* 8, 1–16. <https://doi.org/10.1186/s13068-015-0407-8>
- Cunha, V.M.B., Silva, M.P. da, Costa, W.A. da, Oliveira, M.S. de, Bezerra, F.W.F., Melo, A.C. de, Pinto, R.H.H., Machado, N.T., Araujo, M.E., Junior, R.N. de C., 2018. Carbon Dioxide Use in High-Pressure Extraction Processes, in: *Carbon Dioxide Chemistry, Capture and Oil Recovery*. InTech. <https://doi.org/10.5772/intechopen.71151>
- Da Porto, C., Decorti, D., Natolino, A., 2014. Water and ethanol as co-solvent in supercritical fluid extraction of proanthocyanidins from grape marc: A comparison and a proposal. *Journal of Supercritical Fluids* 87, 1–8. <https://doi.org/10.1016/j.supflu.2013.12.019>
- 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, 1–28. <https://doi.org/10.1186/s13068-020-01697-w>
- De Carvalho, K.C.C., Montoro, S.R., Cioffi, M.O.H., Voorwald, H.J.C., 2016. Polyhydroxyalkanoates and Their Nanobiocomposites With Cellulose Nanocrystals, Design and Applications of Nanostructured Polymer Blends and Nanocomposite Systems. Elsevier Inc. <https://doi.org/10.1016/B978-0-323-39408-6.00012-1>
- Dekker, E., Zijp, M.C., van de Kamp, M.E., Temme, E.H.M., van Zelm, R., 2020. A taste of the new ReCiPe for life cycle assessment: consequences of the updated impact assessment method on food product LCAs. *International Journal of Life Cycle Assessment* 25, 2315–2324. <https://doi.org/10.1007/s11367-019-01653-3>
- Deshpande, R., Giummarella, N., Henriksson, G., Germgård, U., Sundvall, L., Grundberg, H., Lawoko, M., 2018. The reactivity of lignin carbohydrate complex (LCC) during manufacture of dissolving sulfite pulp from softwood. *Industrial Crops and Products* 115, 315–322. <https://doi.org/10.1016/j.indcrop.2018.02.038>
- Ecoinvent, 2019a. Ecoinvent 3.6 APOS heat and power co-generation, wood chips, 6667 kW, state-of-the-art 2014, SE.
- Ecoinvent, 2019b. Ecoinvent 3.6 APOS electricity, high voltage, production mix, NORDEL.
- Ecoinvent, n.d. ecoinvent history.pdf [WWW Document]. URL <https://www.ecoinvent.org/about/history/history.html> (accessed 9.17.21).

- Eijsink, V.G.H., Petrovic, D., Forsberg, Z., Mekasha, S., Røhr, Å.K., Várnai, A., Bissaro, B., Vaaje-Kolstad, G., 2019. On the functional characterization of lytic polysaccharide monoxygenases (LPMOs). *Biotechnology for Biofuels* 12, 1–16. <https://doi.org/10.1186/s13068-019-1392-0>
- Ek, M., Gellerstedt, G., Henriksson, G., 2009. *Pulp and Paper Chemistry and Technology Volume 1*, Biotechnology.
- Endler, A., Persson, S., 2011. Cellulose synthases and synthesis in arabidopsis. *Molecular Plant* 4, 199–211. <https://doi.org/10.1093/mp/ssq079>
- Eriksson, D., Arshadi, M., Kataria, R., Bergsten, U., 2018. Lipophilic extractives in different tree fractions and forestry assortments of *Pinus sylvestris* due for thinning or final cutting. *Scandinavian Journal of Forest Research* 33, 594–602. <https://doi.org/10.1080/02827581.2018.1434235>
- Eriksson, T., Börjesson, J., Tjerneld, F., 2002. Mechanism of surfactant effect in enzymatic hydrolysis of lignocellulose. *Enzyme and Microbial Technology* 31, 353–364. [https://doi.org/10.1016/S0141-0229\(02\)00134-5](https://doi.org/10.1016/S0141-0229(02)00134-5)
- Fernandes, A.N., Thomas, L.H., Altaner, C.M., Callow, P., Forsyth, V.T., Apperley, D.C., Kennedy, C.J., Jarvis, M.C., 2011. Nanostructure of cellulose microfibrils in spruce wood. *Proceedings of the National Academy of Sciences of the United States of America* 108. <https://doi.org/10.1073/pnas.1108942108>
- Franke, R., Schreiber, L., 2007. Suberin - a biopolyester forming apoplastic plant interfaces. *Current Opinion in Plant Biology* 10, 252–259. <https://doi.org/10.1016/j.pbi.2007.04.004>
- Frankó, B., Galbe, M., Wallberg, O., 2015. Influence of bark on fuel ethanol production from steam-pretreated spruce. *Biotechnology for Biofuels* 8, 1–11. <https://doi.org/10.1186/s13068-015-0199-x>
- Galbe, M., Wallberg, O., 2019. *Biotechnology for Biofuels Pretreatment for biorefineries : a review of common methods for efficient utilisation of lignocellulosic materials*. *Biotechnology for Biofuels* 1–26. <https://doi.org/10.1186/s13068-019-1634-1>
- Gaurav, G., Bihari Singh, A., Mistry, S., Gupta, S., Dangayach, G.S., Meena, M.L., 2021. Recent progress of scientific research on life cycle assessment. *Materials Today: Proceedings*. <https://doi.org/10.1016/j.matpr.2021.06.208>
- Gilbert, H.J., Stålbrand, H., Brumer, H., 2008. How the walls come crumbling down: recent structural biochemistry of plant polysaccharide degradation. *Current Opinion in Plant Biology* 11, 338–348. <https://doi.org/10.1016/j.pbi.2008.03.004>
- Gjerdrum, P., 2003. Heartwood in relation to age and growth rate in *Pinus sylvestris* L. in Scandinavia. *Forestry* 76, 413–424. <https://doi.org/10.1093/forestry/76.4.413>
- Glessinger, E., 1952. *Den kommande träåldern* (Original title: The coming age of wood). AB Gustaf Lindströms Boktryckeri, Stockholm.
- Gode, J., Martinsson, F., Hagberg, L., Palm, D., 2011. *Miljöfaktaboken 2011 Estimated emission factors for fuels, electricity, heat and transport in Sweden*. Stockholm.

- Goedkoop, M., Heijungs, R., Huijbregts, M., Schryver, A. De, Struijs, J., Zelm, R. van, 2013. ReCiPe 2008: A life cycle impact assessment method which comprises harmonised category indicators at the midpoint and the endpoint level.
- Goedkoop, M., Heijungs, R., Huijbregts, M., Schryver, A. De, Struijs, J., Zelm, R. Van, 2009. ReCiPe 2008. Potentials 1–44.
- Gourlay, K., Hu, J., Arantes, V., Andberg, M., Saloheimo, M., Penttilä, M., Saddler, J., 2013. Swollenin aids in the amorphogenesis step during the enzymatic hydrolysis of pretreated biomass. *Bioresource Technology* 142, 498–503.
<https://doi.org/10.1016/j.biortech.2013.05.053>
- Hagberg, L., Särholm, E., Gode, J., 2009. LCA calculations on Swedish wood pellet production chains - according to the Renewable Energy Directive [WWW Document]. URL
<https://www.ivl.se/download/18.343dc99d14e8bb0f58b7597/1445517411880/B1873.pdf> (accessed 12.2.19).
- Halmemies, E.S., Brännström, H.E., Nurmi, J., Läspä, O., Al, R., 2021. Effect of Seasonal Storage on Single-Stem Bark Extractives of Norway Spruce (*Picea abies*).
- Hames, B., Ruiz, R., Scarlata, C., Sluiter, A., Sluiter, J., Templeton, D., 2008. Preparation of Samples for Compositional Analysis NREL/TP-510-42620. National Renewable Energy Laboratory.
- Hänninen, T., Kontturi, E., Vuorinen, T., 2011. Distribution of lignin and its coniferyl alcohol and coniferyl aldehyde groups in *Picea abies* and *Pinus sylvestris* as observed by Raman imaging. *Phytochemistry* 72, 1889–1895.
<https://doi.org/10.1016/j.phytochem.2011.05.005>
- Hauschild, M., Rosenbaum, R.K., Olsen, S.I., Al, E., 2017. Life Cycle Assessment - Theory and Practice. <https://doi.org/10.1007/978-3-319-56475-3>
- Herlet, J., Kornberger, P., Roessler, B., Glanz, J., Schwarz, W.H., Liebl, W., Zverlov, V. V., 2017. A new method to evaluate temperature vs. pH activity profiles for biotechnological relevant enzymes. *Biotechnology for Biofuels* 10, 1–12.
<https://doi.org/10.1186/s13068-017-0923-9>
- Horn, S.J., Vaaje-Kolstad, G., Westereng, B., Eijsink, V.G., 2012. Novel enzymes for the degradation of cellulose. *Biotechnology for biofuels* 5, 45.
<https://doi.org/10.1186/1754-6834-5-45>
- Huijbregts, M., Steinmann, Z.J.N., Elshout, P.M.F.M., Stam, G., Verones, F., Vieira, M.D.M., Zijp, M., van Zelm, R., 2016. ReCiPe 2016. National Institute for Public Health and the Environment. <https://doi.org/10.1007/s11367-016-1246-y>
- Hunt, Robert G., Franklin, W.E., Hunt, R. G., 1996. LCA — How it came about. *The International Journal of Life Cycle Assessment* 1, 4–7.
<https://doi.org/10.1007/BF02978624>
- ISO 14040, 2006. Environmental management - Life cycle assessment - Principles and framework. European Committee for Standardization. Geneva.

- ISO 14044, 2006. Environmental management – Life cycle assessment – Requirements and guidelines – Amendment 1. Geneva.
- Iwata, M., Gutiérrez, A., Marques, G., Sabat, G., Kersten, P.J., Cullen, D., Bhatnagar, J.M., Yadav, J., Lipzen, A., Yoshinaga, Y., Sharma, A., Adam, C., Daum, C., Ng, V., Grigoriev, I. V., Hori, C., 2021. Omics analyses and biochemical study of *Phlebiopsis gigantea* elucidate its degradation strategy of wood extractives. *Scientific Reports* 11, 1–14. <https://doi.org/10.1038/s41598-021-91756-5>
- 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, 726–750. <https://doi.org/10.1016/j.biotechadv.2017.07.007>
- Jeffries, T.W., Jin, Y.S., 2004. Metabolic engineering for improved fermentation of pentoses by yeasts. *Applied Microbiology and Biotechnology* 63, 495–509. <https://doi.org/10.1007/s00253-003-1450-0>
- Jensen, W., Fremer, K.E., Sierilä, P., Wartiovaara, V., 1975. The chemistry of bark, in: Browning, B.L. (Ed.), *The Chemistry of Wood*. Robert E. Krieger, New York, pp. 587–666.
- Jovanovic, A., Petrovic, P., Đordjevic, V., Zdunic, G., Savikin, K., Bugarski, B., 2017. Polyphenols extraction from plant sources. *Lekovite sirovine* 37, 45–49. <https://doi.org/10.5937/leksi1737045j>
- Kačiková, D., Kačík, F., Čabalová, I., Ďurkovič, J., 2013. Effects of thermal treatment on chemical, mechanical and colour traits in Norway spruce wood. *Bioresource Technology* 144, 669–674. <https://doi.org/10.1016/j.biortech.2013.06.110>
- Kadić, A., Lidén, G., 2017. Does sugar inhibition explain mixing effects in enzymatic hydrolysis of lignocellulose? *Journal of Chemical Technology and Biotechnology* 92, 868–873. <https://doi.org/10.1002/jctb.5071>
- Kamm, B., Kamm, M., 2004. Principles of biorefineries. *Applied Microbiology and Biotechnology* 64, 137–145. <https://doi.org/10.1007/s00253-003-1537-7>
- Katakojwala, R., Mohan, S.V., 2021. A critical view on the environmental sustainability of biorefinery systems. *Current Opinion in Green and Sustainable Chemistry* 27, 100392. <https://doi.org/10.1016/j.cogsc.2020.100392>
- Kebbi-Benkeder, Z., Colin, F., Dumarçay, S., Gérardin, P., 2015. Quantification and characterization of knotwood extractives of 12 European softwood and hardwood species. *Annals of Forest Science* 72, 277–284. <https://doi.org/10.1007/s13595-014-0428-7>
- 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>

- Kirker, G.T., Blodgett, A.B., Arango, R.A., Lebow, P.K., Clausen, C.A., 2013. The role of extractives in naturally durable wood species. *International Biodeterioration and Biodegradation* 82, 53–58. <https://doi.org/10.1016/j.ibiod.2013.03.007>
- Klöpffer, W., Grahl, B., 2014. *Life Cycle Assessment (LCA) A Guide to Best Practice*. Wiley-VCH, Weinheim.
- Kristensen, J.B., Felby, C., Jørgensen, H., 2009. Yield-determining factors in high-solids enzymatic hydrolysis of lignocellulose. *Biotechnology for biofuels* 2, 11. <https://doi.org/10.1186/1754-6834-2-11>
- Krogell, J., Holmbom, B., Pranovich, A., Hemming, J., Willför, S., 2012. Extraction and chemical characterization of Norway spruce inner and outer bark. *Nordic Pulp and Paper Research Journal* 27, 6–17. <https://doi.org/10.3183/npprj-2012-27-01-p006-017>
- Kumar, R., Wyman, C.E., 2009. Effect of additives on the digestibility of corn stover solids following pretreatment by leading technologies. *Biotechnology and bioengineering* 102, 1544–57. <https://doi.org/10.1002/bit.22203>
- Laks, P.E., 1991. Chemistry of bark, in: Hon, D.N.-S., Shiraishi, N. (Eds.), *Wood and Cellulosic Chemistry*. Marcel deckker, New York, pp. 257–330.
- Larsen, J., Øtergaard Petersen, M., Thirup, L., Li, H.W., Iversen, F.K., 2008. The IBUS process - Lignocellulosic bioethanol close to a commercial reality. *Chemical Engineering and Technology* 31, 765–772. <https://doi.org/10.1002/ceat.200800048>
- Ljunggren Söderman, M., Kushnir, D., Sandén, B.A., 2014. Will metal scarcity limit the use of electric vehicles?, in: Sandén, B.A. (Ed.), *Systems Perspectives on Electromobility*. Chalmers University of Technology, Gothenburg, Sweden, pp. 76–89.
- Majewicz, T.G., Podlas, T.J., 2000. Cellulose Ethers, in: *Kirk-Othmer Encyclopedia of Chemical Technology*. John Wiley & Sons, Inc., Hoboken, NJ, USA. <https://doi.org/10.1002/0471238961.0305121213011005.a01>
- Martinez, D., Berka, R.M., Henrissat, B., Saloheimo, M., Arvas, M., Baker, S.E., Chapman, J., Chertkov, O., Coutinho, P.M., Cullen, D., Danchin, E.G.J., Grigoriev, I. V., Harris, P., Jackson, M., Kubicek, C.P., Han, C.S., Ho, I., Larrondo, L.F., De Leon, A.L., Magnuson, J.K., Merino, S., Misra, M., Nelson, B., Putnam, N., Robbertse, B., Salamov, A.A., Schmoll, M., Terry, A., Thayer, N., Westerholm-Parvinen, A., Schoch, C.L., Yao, J., Barbote, R., Nelson, M.A., Detter, C., Bruce, D., Kuske, C.R., Xie, G., Richardson, P., Rokhsar, D.S., Lucas, S.M., Rubin, E.M., Dunn-Coleman, N., Ward, M., Brettin, T.S., 2008. Genome sequencing and analysis of the biomass-degrading fungus *Trichoderma reesei* (syn. *Hypocrea jecorina*). *Nature Biotechnology* 26, 553–560. <https://doi.org/10.1038/nbt1403>
- Meng, X., Ragauskas, A.J., 2014. Recent advances in understanding the role of cellulose accessibility in enzymatic hydrolysis of lignocellulosic substrates. *Current Opinion in Biotechnology* 27, 150–158. <https://doi.org/10.1016/j.copbio.2014.01.014>
- Merino, S.T., Cherry, J., 2007. Progress and Challenges in Enzyme Development for Biomass Utilization, in: Olsson, L. (Ed.), *Biofuels*. <https://doi.org/10.1016/j.jbiotec.2007.09.006>

- 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, 395–400. <https://doi.org/10.1016/j.indcrop.2011.10.035>
- Modenbach, A.A., Nokes, S.E., 2013. Enzymatic hydrolysis of biomass at high-solids loadings - A review. *Biomass and Bioenergy* 56, 526–544. <https://doi.org/10.1016/j.biombioe.2013.05.031>
- Monavari, S., Galbe, M., Zacchi, G., 2009. Impact of impregnation time and chip size on sugar yield in pretreatment of softwood for ethanol production. *Bioresource technology* 100, 6312–6316. <https://doi.org/10.1016/j.biortech.2009.06.097>
- Moni, S.M., Mahmud, R., High, K., Carbajales-Dale, M., 2020. Life cycle assessment of emerging technologies: A review. *Journal of Industrial Ecology* 24, 52–63. <https://doi.org/10.1111/jiec.12965>
- Nijland, J.G., Driessen, A.J.M., 2020. Engineering of Pentose Transport in *Saccharomyces cerevisiae* for Biotechnological Applications. *Frontiers in Bioengineering and Biotechnology* 7, 1–13. <https://doi.org/10.3389/fbioe.2019.00464>
- Normark, M., Winstrand, S., Lestander, T. a, Jönsson, L.J., 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>
- Olsen, J.P., Alasepp, K., Kari, J., Cruys-Bagger, N., Borch, K., Westh, P., 2016. Mechanism of product inhibition for cellobiohydrolase Cel7A during hydrolysis of insoluble cellulose. *Biotechnology and Bioengineering* 113, 1178–1186. <https://doi.org/10.1002/bit.25900>
- Østby, H., Hansen, L.D., Horn, S.J., Eijsink, V.G.H., Várnai, A., 2020. Enzymatic processing of lignocellulosic biomass: principles, recent advances and perspectives, *Journal of Industrial Microbiology and Biotechnology*. Springer International Publishing. <https://doi.org/10.1007/s10295-020-02301-8>
- Pallardy, S., 2007a. The Woody Plant Body, in: *Physiology of Woody Plants*. Academic Press, pp. 9–38.
- Pallardy, S., 2007b. Carbohydrates, in: *Physiology of Woody Plants*. pp. 199–215.
- Palmqvist, E., Hahn-Hägerdal, B., 2000. Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. *Bioresource Technology* 74, 25–33. [https://doi.org/10.1016/S0960-8524\(99\)00161-3](https://doi.org/10.1016/S0960-8524(99)00161-3)
- Pedersen, M., Meyer, A.S., 2010. Lignocellulose pretreatment severity - relating pH to biomatrix opening. *New biotechnology*. <https://doi.org/10.1016/j.nbt.2010.05.003>
- Piccolo, C., Wiman, M., Bezzo, F., Lidén, G., 2010. Enzyme adsorption on SO₂ catalyzed steam-pretreated wheat and spruce material. *Enzyme and Microbial Technology* 46, 159–169. <https://doi.org/10.1016/j.enzmictec.2009.11.007>
- Picea, W., Storage, I., Jurczyk, T., 2021. Chemical and Morphological Composition of Norway Spruce.

- Pielhop, T., Amgarten, J., Von Rohr, P.R., Studer, M.H., 2016. Steam explosion pretreatment of softwood: The effect of the explosive decompression on enzymatic digestibility. *Biotechnology for Biofuels* 9, 1–13. <https://doi.org/10.1186/s13068-016-0567-1>
- Pizzi, A., 2008. Tannins: Major Sources, Properties and Applications, in: Belgacem, M., Gandini, A. (Eds.), *Monomers, Polymers and Composites from Renewable Resources*. Elsevier, Oxford, pp. 179–199. <https://doi.org/10.1016/B978-0-08-045316-3.00008-9>
- Popp, J., Lakner, Z., Harangi-Rákos, M., Fári, M., 2014. The effect of bioenergy expansion: Food, energy, and environment. *Renewable and Sustainable Energy Reviews* 32, 559–578. <https://doi.org/10.1016/j.rser.2014.01.056>
- Raitanen, J.E., Järvenpää, E., Korpinen, R., Mäkinen, S., Hellström, J., Kilpeläinen, P., Liimatainen, J., Ora, A., Tupasela, T., Jyske, T., 2020. Tannins of conifer bark as Nordic piquancy—sustainable preservative and aroma? *Molecules* 25, 1–19. <https://doi.org/10.3390/molecules25030567>
- Ralph, J., Lapierre, C., Boerjan, W., 2019. Lignin structure and its engineering. *Current Opinion in Biotechnology* 56, 240–249. <https://doi.org/10.1016/j.copbio.2019.02.019>
- Rocha-Martín, J., Martínez-Bernal, C., Pérez-Cobas, Y., Reyes-Sosa, F.M., García, B.D., 2017. Additives enhancing enzymatic hydrolysis of lignocellulosic biomass. *Bioresource Technology* 244, 48–56. <https://doi.org/10.1016/j.biortech.2017.06.132>
- Rodrigues, S., Fernandes, F.A.N., 2017. Extraction Processes Assisted by Ultrasound, in: Bermudez-Aguirre, D. (Ed.), *Ultrasound: Advances for Food Processing and Preservation*. Elsevier, pp. 351–368. <https://doi.org/10.1016/B978-0-12-804581-7.00014-2>
- Rødsrud, G., Lersch, M., Sjöde, A., 2012. History and future of world's most advanced biorefinery in operation. *Biomass and Bioenergy* 46, 46–59. <https://doi.org/10.1016/j.biombioe.2012.03.028>
- Roffael, E., 2016. Significance of wood extractives for wood bonding. *Applied Microbiology and Biotechnology* 100, 1589–1596. <https://doi.org/10.1007/s00253-015-7207-8>
- Rowell, R.M., Pettersen, R., Han, J.S., Rowell, J.S., Tshabalala, M.A., 2005. Cell wall chemistry, in: Rowell, R.M. (Ed.), *Handbook of Wood Chemistry and Wood Composites*. CRC Press, Boca Raton, pp. 35–74.
- Ruiz, H.A., Galbe, M., Garrote, G., Ramirez-Gutierrez, D.M., Ximenes, E., Sun, S.-N., Lachos-Perez, D., Rodríguez-Jasso, R.M., Sun, R.-C., Yang, B., Ladisch, M.R., 2021. Severity factor kinetic model as a strategic parameter of hydrothermal processing (steam explosion and liquid hot water) for biomass fractionation under biorefinery concept. *Bioresource Technology* 342, 125961. <https://doi.org/10.1016/j.biortech.2021.125961>
- Rusanen, A., Lappalainen, K., Kärkkäinen, J., Tuuttila, T., Mikola, M., Lassi, U., 2019. Selective hemicellulose hydrolysis of Scots pine sawdust. *Biomass Conversion and Biorefinery* 9, 283–291. <https://doi.org/10.1007/s13399-018-0357-z>

- Sala, S., Amadei, A.M., Beylot, A., Ardente, F., 2021. The evolution of life cycle assessment in European policies over three decades. *International Journal of Life Cycle Assessment*. <https://doi.org/10.1007/s11367-021-01893-2>
- Sassner, P., Galbe, M., Zacchi, G., 2008. Techno-economic evaluation of bioethanol production from three different lignocellulosic materials. *Biomass and Bioenergy* 32, 422–430. <https://doi.org/10.1016/j.biombioe.2007.10.014>
- Schmidt, N.D., Peschon, J.J., Segel, I.H., 1983. Kinetics of enzymes subject to very strong product inhibition: Analysis using simplified integrated rate equations and average velocities. *Journal of Theoretical Biology* 100, 597–611. [https://doi.org/10.1016/0022-5193\(83\)90325-9](https://doi.org/10.1016/0022-5193(83)90325-9)
- Seo, M.W., Lee, S.H., Nam, H., Lee, D., Tokmurzin, D., Wang, S., Park, Y.K., 2022. Recent advances of thermochemical conversion processes for biorefinery. *Bioresource Technology* 343, 126109. <https://doi.org/10.1016/j.biortech.2021.126109>
- Shinde, S.D., Meng, X., Kumar, R., Ragauskas, A.J., 2018. Recent advances in understanding the pseudo-lignin formation in a lignocellulosic biorefinery. *Green Chemistry* 20, 2192–2205. <https://doi.org/10.1039/c8gc00353j>
- Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., 2008a. Determination of Ash in Biomass NREL/TP-510-42622. National Renewable Energy Laboratory.
- Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2012. Determination of Structural Carbohydrates and Lignin in Biomass NREL/TP-510-42618. National Renewable Energy Laboratory.
- Sluiter, A., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., 2008b. Determination of Extractives in Biomass. Technical Report NREL/TP-510-42619. National Renewable Energy Laboratory.
- Sluiter, J., Sluiter, A., 2011a. Summative Mass Closure Laboratory Analytical Procedure (LAP) Review and Integration NREL/TP-510-48087. National Renewable Energy Laboratory.
- Sluiter, J., Sluiter, A., 2011b. Summative Mass Closure Laboratory Analytical Procedure (LAP) Review and Integration: Pretreated Slurries NREL/TP-510-48825. National Renewable Energy Laboratory.
- Söderström, J., Pilcher, L., Galbe, M., Zacchi, G., 2003. Two-step steam pretreatment of softwood by dilute H₂SO₄ impregnation for ethanol production. *Biomass and Bioenergy* 24, 475–486. [https://doi.org/10.1016/S0961-9534\(02\)00148-4](https://doi.org/10.1016/S0961-9534(02)00148-4)
- Sonnemann, G., Gemechu, E.D., Sala, S., Schau, E.M., Allacker, K., Pant, R., Adibi, N., Valdivia, S., 2018. Life Cycle Thinking and the Use of LCA in Policies Around the World, in: *Life Cycle Assessment*. Springer International Publishing, Cham, pp. 429–463. https://doi.org/10.1007/978-3-319-56475-3_18

- Sturm, A., Tang, G.-Q., 1999. The sucrose-cleaving enzymes of plants are crucial for development, growth and carbon partitioning. *Trends in Plant Science* 4, 401–407. [https://doi.org/10.1016/S1360-1385\(99\)01470-3](https://doi.org/10.1016/S1360-1385(99)01470-3)
- Sun, F.F., Hong, J., Hu, J., Saddler, J.N., Fang, X., Zhang, Z., Shen, S., 2015. Accessory enzymes influence cellulase hydrolysis of the model substrate and the realistic lignocellulosic biomass. *Enzyme and Microbial Technology* 79–80, 42–48. <https://doi.org/10.1016/j.enzmictec.2015.06.020>
- Sun, Z., Fridrich, B., De Santi, A., Elangovan, S., Barta, K., 2018. Bright Side of Lignin Depolymerization: Toward New Platform Chemicals. *Chemical Reviews* 118, 614–678. <https://doi.org/10.1021/acs.chemrev.7b00588>
- Swedish National Forest Inventory, 2021. Forest statistics 2021 Official Statistics of Sweden. Swedish University of Agricultural Sciences, Umeå.
- Tanase, C., Cosarcă, S., Muntean, D.L., 2019. A critical review of phenolic compounds extracted from the bark of woody vascular plants and their potential biological activity. *Molecules* 24. <https://doi.org/10.3390/molecules24061182>
- Teter, S.A., 2012. Development of a Commercial Ready Enzyme Application System for Ethanol.
- Therasme, O., Volk, T.A., Cabrera, A.M., Eisenbies, M.H., Amidon, T.E., 2018. Hot water extraction improves the characteristics of willow and sugar maple biomass with different amount of bark. *Frontiers in Energy Research* 6, 1–13. <https://doi.org/10.3389/fenrg.2018.00093>
- Thonemann, N., Schulte, A., Maga, D., 2020. How to conduct prospective life cycle assessment for emerging technologies? A systematic review and methodological guidance. *Sustainability (Switzerland)* 12, 1–23. <https://doi.org/10.3390/su12031192>
- Tischner, U., Masselter, S., Hirschl, B., 2000. How to do EcoDesign? : a guide for environmentally and economically sound design. Verlag form, Frankfurt am Main.
- Tiwari, B.K., 2015. Ultrasound: A clean, green extraction technology. *TrAC - Trends in Analytical Chemistry* 71, 100–109. <https://doi.org/10.1016/j.trac.2015.04.013>
- Turner, P., Mamo, G., Karlsson, E.N., 2007. Potential and utilization of thermophiles and thermostable enzymes in biorefining. *Microbial Cell Factories* 6, 1–23. <https://doi.org/10.1186/1475-2859-6-9>
- 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, 2203–2209. <https://doi.org/10.1016/j.biortech.2009.11.052>
- Van Den Brink, J., De Vries, R.P., 2011. Fungal enzyme sets for plant polysaccharide degradation. *Applied Microbiology and Biotechnology* 91, 1477–1492. <https://doi.org/10.1007/s00253-011-3473-2>
- Varila, T., 2020. From Norway Spruce Bark to Carbon Foams : Characterization , and Applications 15, 3651–3666.

- Várnai, A., Huikko, L., Pere, J., Siika-aho, M., Viikari, L., 2011. Synergistic action of xylanase and mannanase improves the total hydrolysis of softwood. *Bioresource technology* 102, 9096–104. <https://doi.org/10.1016/j.biortech.2011.06.059>
- Venkatesan, T., Choi, Y.W., Kim, Y.K., 2019. Impact of Different Extraction Solvents on Phenolic Content and Antioxidant Potential of *Pinus densiflora* Bark Extract. *BioMed Research International* 2019. <https://doi.org/10.1155/2019/3520675>
- Wang, Z., Jönsson, L.J., 2018. Comparison of catalytically non-productive adsorption of fungal proteins to lignins and pseudo-lignin using isobaric mass tagging. *Bioresource Technology* 268, 393–401. <https://doi.org/10.1016/j.biortech.2018.07.149>
- Weiss, N.D., Felby, C., Thygesen, L.G., 2019. Enzymatic hydrolysis is limited by biomass-water interactions at high-solids: Improved performance through substrate modifications 09 Engineering 0904 Chemical Engineering 09 Engineering 0915 Interdisciplinary Engineering. *Biotechnology for Biofuels* 12, 1–13. <https://doi.org/10.1186/s13068-018-1339-x>
- Wernet, G., Bauer, C., Steubing, B., Reinhard, J., Moreno-Ruiz, E., Weidema, B., 2016. The ecoinvent database version 3 (part I): overview and methodology. *International Journal of Life Cycle Assessment* 21, 1218–1230. <https://doi.org/10.1007/s11367-016-1087-8>
- Wiedenhoeft, A.C., Miller, R.B., 2005. Structure and function of wood, in: Rowell, R.M. (Ed.), *Wood Chemistry and Wood Composites*. CRC Press, pp. 9–33. <https://doi.org/10.5694/j.1326-5377.1996.tb122017.x>
- Willför, S.M., Sundberg, A.C., Rehn, P.W., Saranpää, P.T., Holmbom, B.R., 2005. Distribution of lignans in knots and adjacent stemwood of *Picea abies*. *Holz als Roh- und Werkstoff* 63, 353–357. <https://doi.org/10.1007/s00107-005-0024-5>
- Wiman, M., Dienes, D., Hansen, M. a T., van der Meulen, T., Zacchi, G., Lidén, G., 2012. Cellulose accessibility determines the rate of enzymatic hydrolysis of steam-pretreated spruce. *Bioresource Technology* 126, 208–15. <https://doi.org/10.1016/j.biortech.2012.08.082>
- Xing, W., Yin, M., Lv, Q., Hu, Y., Liu, C., Zhang, J., 2014. Oxygen Solubility, Diffusion Coefficient, and Solution Viscosity, Rotating Electrode Methods and Oxygen Reduction Electrocatalysts. Elsevier B.V. <https://doi.org/10.1016/B978-0-444-63278-4.00001-X>
- Yue, F., Lu, F., Ralph, S., Ralph, J., 2016. Identification of 4-O-5-Units in Softwood Lignins via Definitive Lignin Models and NMR. *Biomacromolecules* 17, 1909–1920. <https://doi.org/10.1021/acs.biomac.6b00256>

Process development and environmental assessment within softwood based biorefineries

We live in a society dependent on fossil resources for energy, fuels, and chemicals. However, there are many reasons, such as limited reserves, self-sufficiency, and not least climate change, calling for a transition from fossil to renewable sources. Biorefineries, converting biomass instead of fossil resources to energy and chemicals, are the key to a less fossil-dependent society. However, although biomass is renewable, the production is inherently restricted. Therefore, to transfer towards a biobased economy, efficiency improvements, utilization of side streams, and recycling within biorefineries are needed. To avoid problem shifting, where the intended solution to one problem leads to new and often ignored problems, an essential tool to guide this evolution is life cycle assessment (LCA).