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Franko, Balazs

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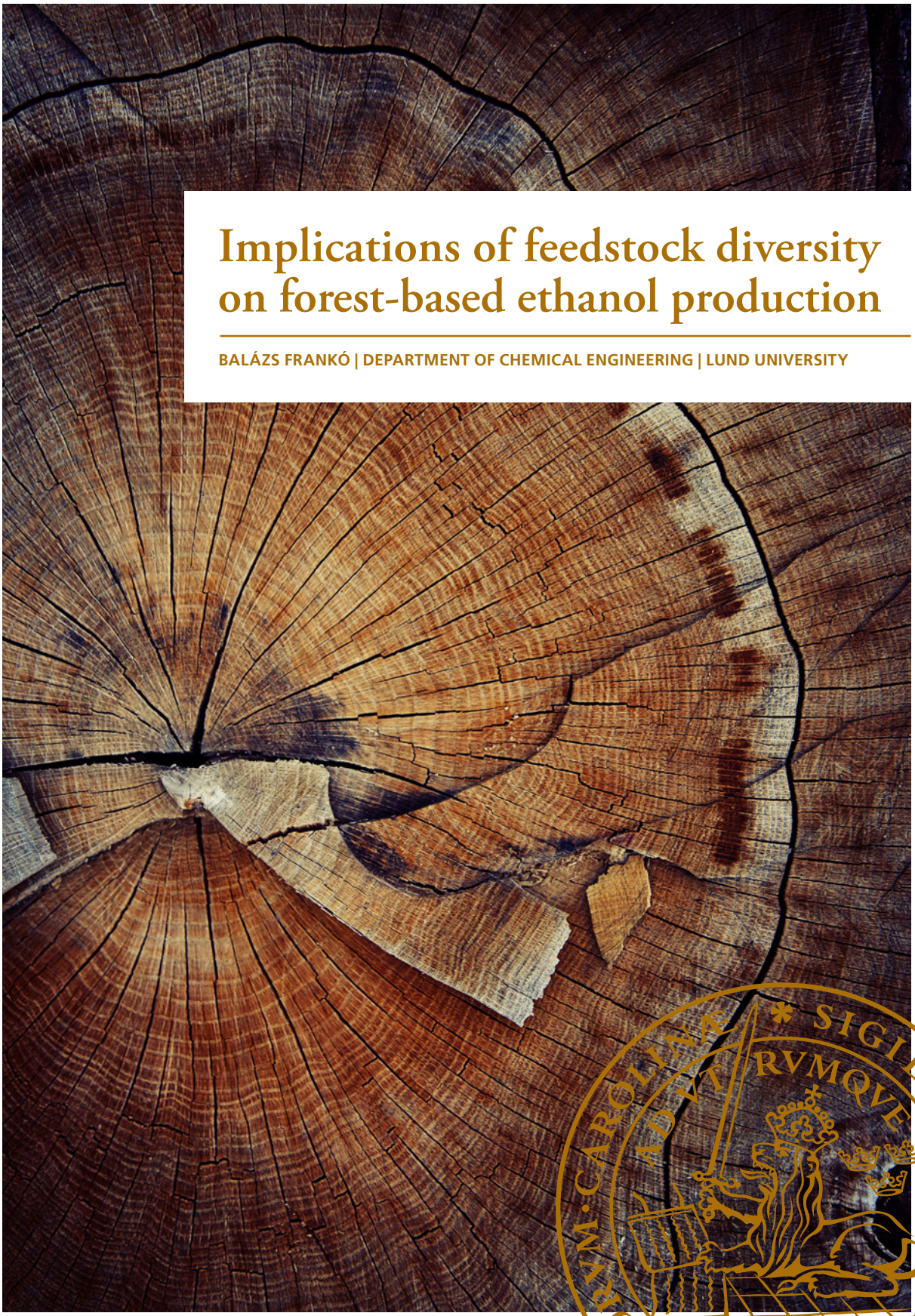

Implications of feedstock diversity on forest-based ethanol production

BALÁZS FRANKÓ | DEPARTMENT OF CHEMICAL ENGINEERING | LUND UNIVERSITY

Implications of feedstock diversity on forest-based ethanol production

Balázs Frankó

LUND
UNIVERSITY

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Faculty opponent
Tom Granström, PhD
St1 Renewable Energy Oy, Helsinki, Finland

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Abstract

The utilization of lignocellulosic biomass to produce biofuels, such as bioethanol, has the potential to provide a sustainable alternative to fossil fuels, and thus mitigate greenhouse gas emissions from the transportation sector. Forest biomass is expected to be a significant source of such biomass, as it can serve as an abundant and sustainable feedstock for bioethanol production. It is unlikely that white wood chips will be used as a sole commercial feedstock for the production of bioethanol, due to increasing feedstock competition and requirements to meet large scale. The high demand for biomass means that other forestry assortments, not traditionally utilized by the forest industry, such as harvesting residues, will have to be exploited. However, the presence of bark in these forest residues is expected to pose a challenge in the traditional wood-to-ethanol process and adversely affect the conversion efficiency.

Ethanol production from softwoods was investigated with the main objective of assessing the potential of expanding the feedstock base of an ethanol plant to include not only white wood, but also other forestry residues from a process perspective. Bark was found to be significantly more difficult to hydrolyze to monomeric sugars than white wood. This could mainly be attributed to the condensation reactions of bark extractives during acid-catalyzed steam pretreatment, which rendered the otherwise water-soluble extractives insoluble, and altered the structure of the solid fraction, resulting in impaired enzymatic hydrolysis. Techno-economic evaluations showed decreasing profitability of ethanol production with increasing bark content in the feedstock. Thus, the utilization of bark-containing forestry residues will not lead to significant cost reductions compared to higher-value pulpwood at current market prices, unless the conversion of cellulose and hemicellulose to monomeric sugars is improved.

Another alternative to increase the future biomass supply for large-scale bioethanol production is the use of fast-growing trees such as willow and poplar. Although the production of ethanol from these hardwood species is well documented, the inclusion of biomass from fast-growing tree species in a softwood feedstock base for bioethanol production has not previously been investigated. The structural differences between hardwood and softwood could be expected to reduce the pretreatment efficacy when treating a mixture of the two. However, it was found that the use of a mixture of poplar and spruce would presumably be constrained more by the performance of the fermenting microorganism, than the efficacy of steam pretreatment, and that the ethanol production process could be sufficiently robust to allow small amounts of hardwood in a softwood-to-ethanol process.

Key words

Ethanol, lignocellulose, softwood, spruce, bark, forest residues, steam pretreatment, enzymatic hydrolysis

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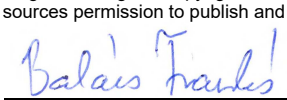
Signature  Date 2018-04-23

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Balázs Frankó

LUND
UNIVERSITY

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Faculty of Engineering | Department of Chemical Engineering
Lund University, Sweden

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"Good judgement comes from experience.
Experience comes from bad judgement."

Mulla Nasrudin

Abstract

The utilization of lignocellulosic biomass to produce biofuels, such as bioethanol, has the potential to provide a sustainable alternative to fossil fuels, and thus mitigate greenhouse gas emissions from the transportation sector. Forest biomass is expected to be a significant source of such biomass, as it can serve as an abundant and sustainable feedstock for bioethanol production. It is unlikely that white wood chips will be used as a sole commercial feedstock for the production of bioethanol, due to increasing feedstock competition and requirements to meet large scale. The high demand for biomass means that other forestry assortments, not traditionally utilized by the forest industry, such as harvesting residues, will have to be exploited. However, the presence of bark in these forest residues is expected to pose a challenge in the traditional wood-to-ethanol process and adversely affect the conversion efficiency.

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Popular Scientific Summary

Carbon dioxide and other greenhouse gases released into the atmosphere from a range of human activities are causing warming of the global climate. In order to limit the increase in temperature to well below 2°C above pre-industrial levels, a goal pledged in the Paris Agreement by nearly 200 countries, the emission of these gases worldwide need to approach zero in the long term. Today, the largest contribution to climate change, in Sweden and around the world, is from the burning of fossil fuels such as oil, coal and natural gas to provide us and our industries with heat and electricity, and to run our vehicles.

Ethanol, a plant-derived renewable fuel, has been identified as an alternative to fossil fuels, with the aim of decreasing the carbon emissions associated with the transport sector. Ethanol, or ethyl alcohol, has the same chemical formula regardless of whether it is in alcoholic beverages or in fuel. It is a colorless, volatile and flammable liquid that can be produced from biomass (and is thus often called bioethanol), and can replace gasoline in our cars. Ethanol has become a price-competitive fuel due to rising global oil prices, however, it is currently mainly produced from edible feedstocks, such as corn, wheat, sugarcane and sugar beet. Research suggests that a greater reduction in greenhouse gas emissions could be achieved by utilizing the residual biomass from industrial, agricultural and forestry activities. While well-established technology can be used to produce ethanol from grains and other sugar-containing crops, the technology required for the production of ethanol from these so-called lignocellulosic biomass feedstocks is still being developed.

Sweden is a country dominated by forests, and sustainable forestry is vitally important for its national economy. With its access to raw materials, the forest industry is well-positioned to diversify its products through wood-to-ethanol production. This would contribute significantly to reaching the goal of zero net emissions of greenhouse gases, which Sweden has pledged to achieve by 2045 at the latest. Increased environmental concerns and technological advances in the production of ethanol from wood biomass make forest-based ethanol an increasingly attractive option, but large-scale implementation requires the efficient utilization of low-cost residues from forest or silvicultural harvesting (e.g., thinnings, branches, low-value decayed trees).

The aim of the work presented in thesis was to assess the feasibility of utilizing various forest-based feedstocks potentially available as raw materials for future ethanol production, and its implications on the wood-to-ethanol conversion process. Different types of forest biomass have different properties (e.g., energy content, moisture content, particle size), and different degrees of heterogeneity, which can affect the conversion process. Moreover, the presence of bark in these feedstocks can also place extra demands on the process and influence conversion efficiencies.

Acid-catalyzed steam pretreatment, one of the pretreatment strategies commonly used for processing wood biomass, was not found to be effective for the pretreatment of bark, and techno-economic evaluations showed decreasing profitability with increasing bark content in the raw material. It was shown that several key aspects of the process need to be further developed and optimized before forest harvest residues can be used to produce ethanol. For instance, fine-tuning of the pretreatment process and the pretreatment conditions based on the feedstock composition is needed to ensure maximum sugar recovery from bark-containing forest residues. This would provide significant cost improvements, and facilitate the implementation of large-scale ethanol production from wood.

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Anya és Apa, köszönöm, hogy mindig és mindenben számíthatok Rátok!

List of publications

This thesis is based on the following papers, which will be referred to in the text by their roman numerals:

I. **Frankó, B.**, Galbe, M., and Wallberg, O.
Influence of bark on fuel ethanol production from steam-pretreated spruce. *Biotechnology for Biofuels*, 8:15, 2015

II. **Frankó, B.**, Galbe, M., and Wallberg, O.
Ethanol production from bark-containing forestry residues: A comparative techno-economic analysis. *Applied Energy*, 184, pp. 727-736, 2016

III. **Frankó, B.**, Carlqvist, K., Galbe, M., Liden, G., and Wallberg, O.
Removal of water-soluble extractives improves the enzymatic digestibility of steam-pretreated softwood barks. *BioEnergy Research*, 184:2, pp. 599–615, 2018

IV. **Frankó, B.**, Jovanovic, H., Galbe, M., and Wallberg, O.
Co-pretreatment of spruce and poplar wood chips for ethanol production. (Manuscript)

My contributions to the publications

Paper I I participated in the conception and design of the study. I planned and performed the experimental work, evaluated the results, and wrote the paper together with the co-authors.

Paper II I designed and performed the study, evaluated the results and wrote the paper with input from the co-authors.

Paper III I participated in the conception and design of the study. I planned and performed all the experimental work, evaluated the results, and wrote the paper together with the co-authors.

Paper IV I designed the study with input from the co-authors. I performed the experimental work together with Hanna Jovanovic. I evaluated the results and wrote the paper together with the co-authors.

Other related publications

I have also contributed to the papers below. However, these publications are not included in this thesis:

I. Erdei, B., **Frankó, B.**, Galbe, M., and Zacchi, G.
Separate hydrolysis and co-fermentation for improved xylose utilization in integrated ethanol production from wheat meal and wheat straw. *Biotechnology for Biofuels*, 5:12, 2012

II. Erdei, B., **Frankó, B.**, Galbe, M., and Wallberg, O.
Glucose and xylose co-fermentation of pretreated wheat straw using mutants of *S. cerevisiae* TMB3400. *Journal of Biotechnology,* 164, pp. 50-58, 2013

Abbreviations

AHP	Alkaline hydrogen peroxide
DP	Degree of polymerization
CHP	Combined heat and power
FPU	Filter paper unit
GHG	Greenhouse gas
HWE	Hot-water extracted
LPMO	Lytic polysaccharide monooxygenases
NPV	Net present value
NREL	National Renewable Energy Laboratory
SHF	Separate hydrolysis and fermentation
SSF	Simultaneous saccharification and fermentation
wt%	Weight percent
WIS	Water-insoluble solids

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1. Introduction

1.1. Background

The Paris Agreement on climate change came into force in 2016, following its historic adoption at the 21st Conference of the Parties to the United Nations Framework Convention on Climate Change [1]. The implementation of the agreement to reduce anthropogenic emissions of carbon dioxide (CO_2) and other greenhouse gases (GHGs) is essential if we are to address climate change and its impacts. This requires a transformative change in the energy sector, which accounts for more than two-thirds of global GHG emissions, in an era when the worldwide energy production has risen continuously from 255 exajoules (EJ) in 1973 to 571 EJ in 2015, largely dominated by fossil fuels (81%) [2]. Although fossil fuels, in particular natural gas and oil, will continue to dominate the energy supply for several decades to come, a major shift towards renewable energy technologies is currently underway [3]. Biomass, hydropower, wind, solar, and geothermal are all major renewable energy sources, currently constituting 14% of the global energy mix, and their share is projected to double by 2040 [3]. Although the power sector is currently leading the change towards renewable electricity generation [3], greater efforts are required to accelerate the implementation of renewables in other fields of energy use, such as transportation, heating and cooling in buildings and industry [4]. The transportation sector, for example, accounts for 30% of the global final energy consumption, and is responsible for the highest CO_2 emissions of all end-use sectors; still, it has the lowest share of renewable energy [5].

Renewable energy can be used in the transportation sector indirectly, through the electrification of transportation, or directly, by replacing gasoline and diesel in internal combustion engines with biofuels. Biofuels is the term used to describe liquid and gaseous fuels derived from renewable biomass resources. Liquid biofuels account for the greatest share of renewable energy in the transportation sector, mostly due to blending mandates, which define the proportion of biofuel that must be used in transportation fuel, often combined with other measures such as tax incentives. Currently, 64 countries have established or planned biofuel mandates and targets [6]. As a result, the consumption of biofuels reached 1.6 million barrels of oil equivalent per day in 2015 [7], while the global stock of electric vehicles passed the 2 million mark

in 2016 [8]. However, it is clear that increasing the share of renewables in the transportation sector requires a considerable intensification of efforts, given that biofuels and electricity today represent only about 3% and 0.1% of the transport sector's total final energy consumption, respectively [5].

1.2. Biofuels

Among renewable energy options, biomass has the potential to provide energy-dense liquid transportation fuels and serve as an alternative to the petroleum-based fuels used in existing infrastructure. Moreover, utilizing local biomass resources for biofuel production can also provide additional benefits, such as increased energy security, by reducing dependency on oil imports, and a contribution to economic development in rural areas, by creating new sources of income in the agricultural and forestry sectors [9].

Biomass is currently converted to liquid biofuels by the fermentation of carbohydrates to ethanol, or by the extraction and refining of plant oils. First-, second- and third-generation biofuels can be classified as conventional or advanced biofuels based on the feedstock used, the technology maturity or GHG emissions balance. First-generation biofuels, such as sugar- or starch-based ethanol and oil-crop based diesel, are being produced on commercial scale with well-established conventional biofuel conversion technologies. Advanced biofuel conversion technologies, on the other hand, are still in different stages of commercialization for the production of second- and third-generation biofuels from lignocellulosic feedstock and algae, respectively; the aim being to circumvent, or at least alleviate, the shortcomings associated with the utilization of food crops for the production of first-generation biofuels [10].

The production of lignocellulosic, sometimes called cellulosic, biofuels has the potential to be superior to that of conventional biofuels and gasoline in terms of energy balance, GHG emissions, land-use requirements and other environmental factors, such as water quality and consumption, air pollution, soil quality and erosion, as well as biodiversity [11, 12]. Whether these environmental benefits are realized will, however, depend on which, where, and how, lignocellulosic biofuels are produced [12-14]. Thus, transparent and stringent sustainability criteria must be widely implemented, as already in the European Union and the United States, covering economic, environmental and social perspectives, in order to assure the overall sustainability of biofuels, and to avoid undesirable externalities of increased biofuel production [15].

Despite the fact that the utilization of lignocellulosic feedstocks have the potential to increase the production of sustainable biofuels, the commercialization of lignocellulosic

biofuel production has been slower than anticipated [7]. An increase in the production and use of advanced biofuels still requires effective and balanced policies that create a stable, long-term investment environment, and promote the commercialization of technologies, efficiency improvements, and further cost reduction throughout the production chain of different biofuels [9].

1.3. Challenges of commercialization

To be economically viable in the long term, advanced biofuels must move toward cost parity with petroleum-based fuels. Despite recent advances in the economic and technical feasibility of conversion technologies, the cost competitiveness of biomass-derived fuels is still recognized as a major impediment to full commercial implementation [16-18]. Although alternative lignocellulosic biofuel products such as hydrocarbons, N-butanol and isobutanol, are being considered, ethanol is one of the most established lignocellulosic biofuels, and commercial-scale production has recently started at a number of 'pioneer' plants (e.g., POET-DSM, DuPont, GranBio, Raizen). Ethanol is an internationally traded commodity with tight margins, which has to compete with gasoline, and the cost-effective large-scale production of lignocellulosic ethanol at the expected production capacity continues to be a challenge [19].

Challenges remain that have to be overcome collectively to achieve the lowest cost combination of feedstock, logistics, and conversion technology [20, 21]. Technical improvements are needed in the production of bioethanol to reduce both the capital cost and production cost of converting lignocellulosic biomass [17, 19]. Simplifying operations, eliminating process steps, speeding up reaction rates or co-locating a new plant with existing industrial facilities, such as conventional bioethanol plants and power plants, could reduce the investment cost [19, 22, 23]. At the same time, production costs could be decreased by, e.g., increasing yields, reducing the use of chemicals and nutrients, improving the energy efficiency, and through the development of enzymes with much higher specific activity [17, 19, 24].

Lignocellulosic biomass is available in a variety of forms with varying levels of quality, supply risk, and harvesting cost. Although current commercial-scale pioneer ethanol plants almost exclusively use agricultural residues (corn stover, wheat straw, sugarcane bagasse), additional facilities could utilize a wide range of feedstocks, from agricultural and forest residues to dedicated energy crops (e.g., switchgrass and miscanthus), short-rotation tree species (e.g., poplar, eucalyptus) and municipal solid waste. Nevertheless, large amounts of biomass will be required for production on an industrial scale, implying a local supply with a wide radius, or imports, which could lead to an increase in feedstock costs, as well as increased competition with other industries [7]. Additional obstacles associated with feedstock production and logistics are the annual

variability and seasonality of biomass, as well as its scattered geographical distribution, making harvesting, preprocessing, transport and storage complex and expensive [25]. Given the low bulk density of lignocellulosic biomass and the significant logistical challenges, the transportation cost of lignocellulosic biomass represents a diseconomy of scale, which is in contrast to the economy of scale associated with advanced conversion technologies [26].

The choice of feedstock is a key factor among the production variables that affect the commercial viability of lignocellulosic biofuel production [27, 28]. The feedstock supply influences profitability in various ways: i) its availability impacts the scale of production that would be necessary to realize economy of scale [26], ii) its procurement cost represents a significant fraction of the total production cost [28], and iii) its quality attributes affect the overall yield [29, 30]. Flexibility of feedstock utilization in the conversion process would be highly beneficial as this would enable the use of a broader range of biomass resources, potentially leading to lower costs (through the use of residues) and reduced price volatility (due to region- and species-specific yield-reducing impacts, such as extreme weather, and pest infestations) [9], as well as minimizing seasonality constraints and storage requirements [26]. However, feedstock quality attributes (compositional, physical and structural) affect its conversion, which could in turn limit feedstock flexibility in the process.

1.4. Scope and outline

Forest biomass is one of the renewable resources that could contribute considerably to the projected total renewable potential. The work described in this thesis is focused on ethanol production from softwoods with the main objective of assessing the feasibility of expanding the 'clean' white wood feedstock base from a process perspective, and assessing the associated challenges and possibilities. Diversification of the feedstock can be achieved by utilizing additional forestry biomass in existing forests (e.g., residues), or by including new plantations of fast-growing trees in the feedstock base.

This thesis is organized in five chapters. Chapter 1 provides a background in the societal context of biofuel production and discusses the status of lignocellulosic ethanol commercialization. In Chapter 2, the recalcitrant nature of lignocellulosic biomass is discussed by briefly describing the chemical and physical features of the plant cell wall. Chapter 3 outlines the biochemical conversion of lignocellulosic biomass into ethanol. The key results reported in Papers I-IV are compiled in Chapter 4 with the aim of setting up a broader perspective of the findings. The utilization of various forest-based assortments potentially available as raw materials for future ethanol production, and their implications on the conversion process, are discussed in greater depth. Concluding remarks and suggestions for future work are presented in Chapter 5.

2. Lignocellulosic biomass

It is important to understand the underlying chemical composition and structure of plant cell walls in order to identify the challenges and potential opportunities associated with the utilization of lignocellulosic biomass. In contrast to sugar- and starch-based crops, the sugars in lignocellulosic biomass are trapped inside a complex, heterogeneous matrix. While starch grains serve as energy storage for plants, providing easily accessible sugars, the lignocellulosic matrix of plants forms the rigid structure that helps the plant withstand the effects of weather and attack by microorganisms and insects. It is thus necessary to break down this matrix and overcome the recalcitrance of the plant cell wall in order to produce bioethanol from lignocellulosic feedstocks.

2.1. Chemical structure

Lignocellulosic material consists mainly of three different types of polymers, namely cellulose, hemicellulose and lignin. Cellulose, which accounts for 30-50% of lignocellulosic biomass on a dry weight basis, is generally the most abundant polymer in plants, followed by hemicellulose (15-35%) and lignin (10-30%). Lignocellulose also contains low amounts of pectin, proteins, inorganic compounds, and non-structural components often referred to as extractives. The chemical composition of lignocellulosic biomass differs not only between different species, but also varies with age, stage of growth, and other conditions within a single plant [31]. Typical compositions of various kinds of lignocellulosic biomass are given in Table 1.

Table 1. Typical composition of various lignocellulosic materials (% dry basis)

	Glucan	Xylan	Galactan	Arabinan	Mannan	Lignin	Ref.
Softwoods							
Spruce	46.5	8.3	1.7	1.2	13.5	27.9	[32]
Pine	43.6	6.6	2.2	1.6	10.8	26.8	[33]
Hardwoods							
Poplar	43.8	14.9	1.0	0.6	3.9	29.1	[34]
Willow	43.0	14.9	2.0	1.2	3.2	26.6	[35]
Agricultural crops							
Wheat straw	38.8	22.2	2.7	4.7	1.7	18.5	[36]
Corn stover	36.8	22.2	2.9	5.5	-	23.1	[37]
Bagasse	40.2	22.5	1.4	2.0	0.5	25.2	[38]
Energy crops							
Miscanthus	41.0	20.0	0.6	1.7	0.1	23.2	[39]
Switchgrass	36.6	21.1	1.0	2.8	0.8	18.3	[40]

Cellulose is a linear homopolymer of D-glucose units linked by β-1,4 glycosidic bonds, where the smallest repetitive unit is cellobiose, a disaccharide consisting of two glucose units (Figure 1). Although glucose is a highly water-soluble molecule, its solubility decreases dramatically with an increase in the degree of polymerization (DP), making cellulose extremely insoluble in water under normal conditions. The DP of cellulose chains varies depending on the source, typically from ~1 000 in agricultural residues to ~5 000 in woods [41].

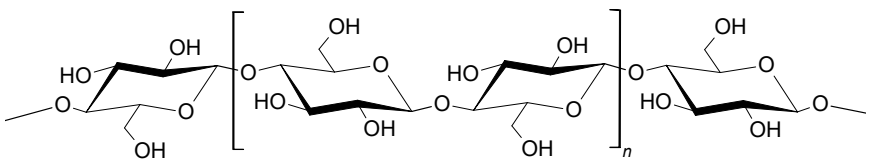

Figure 1. The structure of cellulose. The smallest repetitive unit is cellobiose, a disaccharide consisting of two glucose units linked by β-1,4 glycosidic bond.

Hemicellulose refers to the diverse group of short-chain branched polymers of sugars with a number of substituents. These complex, diverse polysaccharides are made up of hexoses (mainly D-glucose, D-galactose and D-mannose), pentoses (D-xylose and L-arabinose), and sugar acids (D-glucuronic, D-galacturonic and 4-O-methyl-D-glucuronic acids), and their DP is lower than that of cellulose (typically 50-300) [42]. The composition of hemicelluloses differs in the type of glycosidic linkages, side-chain composition, and DP, depending on the plant species and cell tissues [43, 44]. Hemicelluloses are usually classified by the predominant sugars in the β-1,4 linked polysaccharide backbone, e.g., xylans and mannans [45]. The major hemicellulose component in hardwood species is O-acetyl-4-O-methylglucuronoxylan, also called glucuronoxylan, whereas L-arabino-D-xylan is the main component in agricultural

plants such as grasses and straw. In contrast, O-acetyl-galactoglucomannans are the most common components in softwood species, which means that, unlike hardwoods and agricultural feedstocks, softwoods are primarily composed of hexose sugars, which can be readily fermented to ethanol by ordinary baker's yeast.

Lignin is an aromatic heteropolymer consisting of phenylpropane units connected by both ether and carbon-carbon linkages [43]. The three basic monomeric units (monolignols), differing in their degree of methoxylation, are *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol, which produce *p*-hydroxyphenyl, guaiacyl, and syringyl phenylpropanoid units, respectively, when incorporated into the lignin polymer (Figure 2) [46]. The composition of lignin varies between species, cell and tissue type [47]. For instance, softwood lignins are mostly composed of guaiacyl units, hardwood lignins are predominantly guaiacyl and syringyl units with trace amounts of *p*-hydroxyphenyl units, whereas agricultural plants contain significant amounts of all three units at different ratios [48]. Softwoods are generally considered to be the most recalcitrant lignocellulosic feedstock as a result of the higher amount of lignin and greater degree of cross-linking between lignin units as well as to hemicellulose, compared to hardwoods and agricultural residues [46].

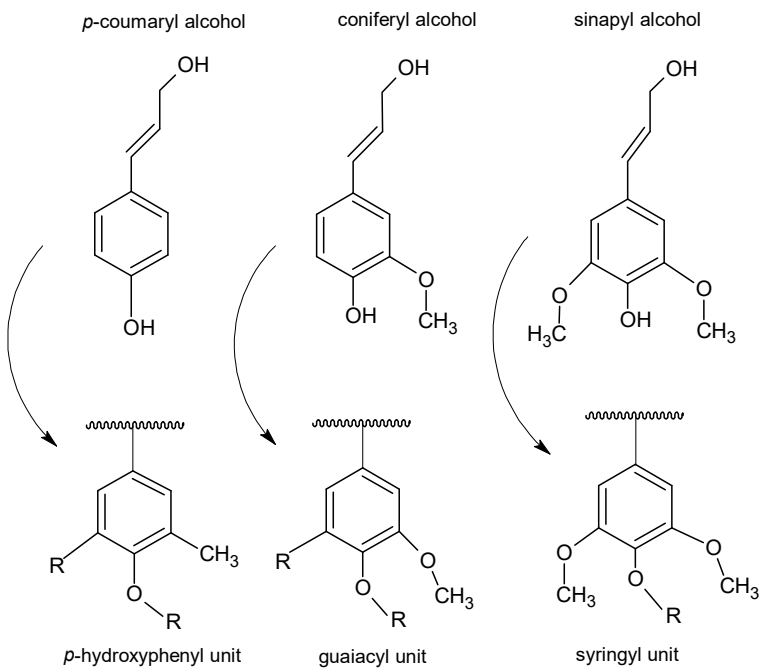

Figure 2. The three lignin units resulting from their respective monolignols.

Lignocellulose also contains other components such as pectin, proteins, ash and extractives. Pectins are the third main structural polysaccharide group of plant cell walls, consisting of homo-galacturonic acid backbones with neutral sugar side-chains consisting of L-rhamnose, L-arabinose, D-galactose and D-xylose [49]. Woody species generally have a much lower ash content than agricultural species, which leads to wood being the preferred feedstock for biomass conversion processes that are particularly sensitive to ash, such as thermochemical conversion [50]. Extractives comprise a large variety of non-structural compounds soluble in neutral organic solvents or water. These extracellular and low-molecular weight compounds consist of both lipophilic (e.g., resin acids, fats and waxes) and hydrophilic (e.g., phenolic compounds, stilbenes) types [51]. The extractives content of wood is usually less than 10%, but can vary from trace amounts up to 40% of the dry weight in the bark fraction [51].

2.2. Morphology

The lignocellulosic matrix is arranged in progressively more complex structures with increasing scale, from bundling of individual cellulose chains, to the macroscopic structure of plants (Figure 3).

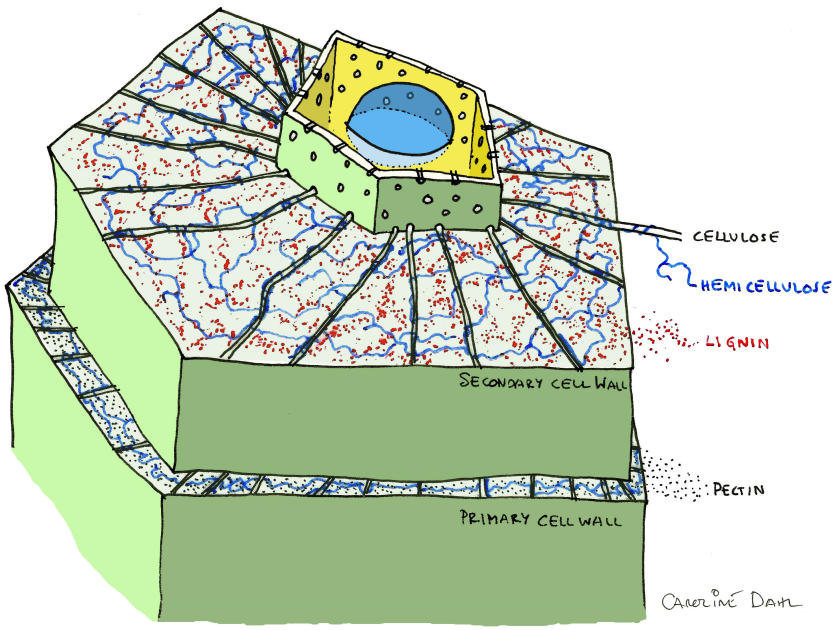

Figure 3. Schematic illustration of the cell wall structure. By Caroline Dahl, from Wikimedia Commons [52].

Cellulose is mainly present in a crystalline structure that is interspersed by some disorganized paracrystalline or amorphous regions. Crystalline cellulose exists in the form of insoluble microfibrils, which are composed of approximately 24 hydrogen-bonded parallel cellulose chains (determined diameters of wood microfibrils could correspond to about 12 and 32 chains) [53]. The inter- and intra-molecular hydrogen bonds between the glucose molecules keep the chains straight and stacked in a sheet-like structure held together by van der Waals forces [50]. Generally, cellulose fibrils are coated with hemicellulose, which functions as a cross-linking agent, binding microfibrils, lignin, cell-wall proteins, pectins, and non-structural polysaccharides through a variety of covalent and non-covalent interactions, to form the rigid cell wall structure [50].

The plant cell wall typically has a multilayered structure composed of three types of layers, namely the middle lamella, the primary wall, and the secondary wall, the last being further divided into three sublayers (S1, outer; S2, middle; and S3, inner). These layers differ from one another in their chemical composition, as well as in their structure [51]. For example, in wood fibers the fractions of cellulose and hemicellulose increase from the middle lamella to the secondary wall (S2 and S3 have the highest cellulose concentration), whereas lignin dominates in the middle lamella, and its fraction decreases with increasing distance from the middle lamella [42].

3. Lignocellulose-based ethanol production

The conversion of lignocellulosic biomass into biofuels, such as ethanol, can be achieved through biochemical and thermochemical (e.g., gasification or pyrolysis) processing routes, or hybrid approaches comprising sequential steps [54, 55]. As there is clearly no universal solution to the challenges associated with the recalcitrant nature of lignocellulosic biomass in producing biofuels, a number of conversion technologies have been explored in many configurations, each approach having its particular advantages and disadvantages.

Biochemical conversion routes rely on biocatalysts, such as enzymes and microbial cells, to convert lignocellulosic biomass into a mixed sugar stream, which can then be fermented to produce ethanol. The conversion process from biomass to ethanol generally consists of four major operations: pretreatment, enzymatic hydrolysis, fermentation, and product recovery. A typical production process is illustrated in Figure 4.

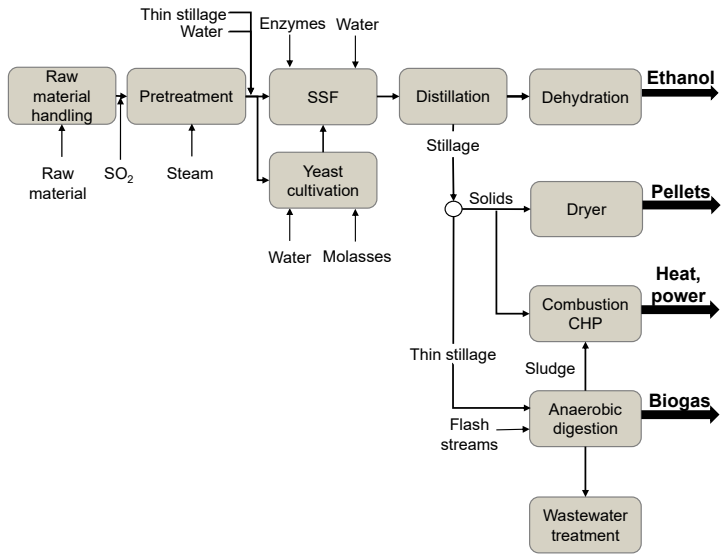

Figure 4. Simplified process overview for ethanol production utilizing lignocellulosic biomass as raw material. Adapted from Paper II. CHP: combined heat and power.

Lignocellulosic biomass is delivered to the ethanol plant, where size reduction is performed prior to pretreatment. For instance, steam pretreatment can be used to break down the lignocellulosic matrix of the biomass and to solubilize much of the hemicellulose to provide monomeric sugars in order to facilitate the subsequent enzymatic hydrolysis. The pretreated slurry can either be enzymatically hydrolyzed separately from the fermentation step, known as separate hydrolysis and fermentation (SHF), or in the presence of the fermentative microorganism, known as simultaneous saccharification and fermentation (SSF). The product is recovered from the fermentation broth using distillation and molecular sieve adsorption to obtain pure ethanol. The stillage from distillation is dewatered to recover the insoluble lignin-rich residue, which can then be burned on-site to produce steam, heat and electricity, or converted to various co-products. The remaining liquor is partly recycled to dilute the pretreated slurry prior to SSF, while the rest is anaerobically digested to produce biogas.

This chapter briefly describes the process steps and depicts the highly intertwined nature of the biomass-to-ethanol conversion process.

3.1. Pretreatment

Pretreatment is widely recognized as a necessary first step in the bioethanol process in order to break down the recalcitrant lignocellulosic matrix of native biomass and facilitate the release of fermentable sugars in the subsequent enzymatic hydrolysis. However, the choice of pretreatment technique is far from trivial. Not only is pretreatment considered to be the most expensive process step, it also affects the performance and cost of essentially all other operations in the conversion scheme, from the choice of feedstock and size reduction requirements, through enzymatic hydrolysis and fermentation, to product recovery and co-product utilization [56]. Thus, the following key factors should be considered to ensure economically viable pretreatment [57]:

- a high yield of the readily digestible cellulosic fraction to enhance the rate and extent of the subsequent enzymatic hydrolysis,
- high overall sugar recovery,
- minimal formation of inhibitors,
- efficient fractionation and recovery of the various biomass components,
- low capital cost (e.g., a reactor with a minimal volume, made of moderately priced construction materials),

- low operating costs (e.g., elimination of the need for extensive size reduction of the raw material, minimized heat and power requirements, minimal need for, and inexpensive, chemicals), and
- minimal contribution to other downstream costs (e.g., low dilution of the pretreated material, and the need for subsequent neutralization conditioning should be minimal and inexpensive).

Although a number of different pretreatments, involving biological, physical and chemical processes, or a combination of these, have been proposed and studied for a wide range of lignocellulosic feedstocks [58, 59], currently only a few achieve the high yields of sugars at a sufficiently low cost to be considered attractive on commercial scale. Steam pretreatment appears to be the technology of choice for a number of pioneer advanced bioethanol facilities utilizing agricultural feedstocks [60], whereas only a few methods, including acid-catalyzed steam pretreatment, alkaline or sulfite pulping and organosolv pretreatment, have seen shown to be suitable for softwoods [61, 62].

3.1.1. Steam pretreatment

Steam pretreatment results in high yields on a wide range of substrates at sufficiently low cost to be economically feasible. Steam pretreatment (also known as steam explosion) refers to the technique in which lignocellulosic biomass is rapidly heated by high-pressure steam, with or without the addition of an acid catalyst, and held under pressure for a certain period of time (from a few seconds to minutes) before the sudden release of pressure [63]. This provides a cellulose-rich water-insoluble fraction amenable to enzymatic hydrolysis, as well as high recovery of hemicellulose, which is essential for the efficient utilization of all the sugars present in the raw material. In contrast, many of the pretreatments related to pulping, which effectively provide a readily digestible cellulosic fraction, often result in the dissolution of hemicelluloses in the lignin-rich liquid fraction, which makes recovery difficult. Steam pretreatment also has the advantage of producing high-consistency slurries, as direct steam injection is possible on a dry biomass feedstock. In addition, feedstocks with a wide range of particle sizes and moisture contents can be treated [64], and the chemical catalyst loading is generally low (less than 5% of the biomass dry weight) [65].

The aim of steam pretreatment is to facilitate enzymatic conversion of the cellulose, and to recover as much hemicellulose in the monomeric form, with the lowest concentration of inhibitors, as possible. This is one of the key challenges, as higher severity pretreatment is required to produce a readily hydrolysable cellulose fraction at low enzyme loadings, but this also leads to considerable degradation of hemicellulosic sugars. The addition of an acid catalyst, such as SO_2 or H_2SO_4, has been shown to be beneficial in promoting hemicellulose and cellulose hydrolysis, with limited

carbohydrate degradation [66, 67]. Using gaseous SO_2 ensures uniform penetration of wood chips [68], and the unabsorbed catalyst can be easily recycled prior to steam pretreatment [69]. Moreover, this process does not cause the same degree of corrosion as pretreatment with H_2SO_4 [68]. The main drawback of SO_2 is its high toxicity, which may pose safety and health risks [70].

Mechanism of steam pretreatment

Steam pretreatment enhances the susceptibility of lignocellulosic materials to enzymatic hydrolysis by changing their structural and compositional organization. From a compositional point of view, the lignin and cellulose are retained in the solid fraction, while the hemicellulose is solubilized. The physical breakdown of the lignocellulosic structure is caused by the adiabatic expansion of absorbed water and hydrolysis reactions involving the cell wall components [71]. Steam pretreatment opens up the cell wall structure and makes a greater surface area accessible to enzymes by: i) reducing the fiber size (fragmentation), ii) removing hemicellulose, and iii) redistributing the lignin [72]. Although steam pretreatment does not remove lignin, its physical reorganization also influence the amenability of the steam-pretreated material to enzymatic hydrolysis, as well as the suitability of the remaining lignin for co-product applications [72].

Lignin appears to cycle between the solid and liquid phase during steam pretreatment through a complex mechanism that may involve phase transition, depolymerization/repolymerization reactions and/or solubilization, which results in both morphological and chemical changes [73]. Lignin was inferred to coalesce on cell walls and migrate into the bulk liquid phase above the lignin glass transition temperature, which results in the formation of droplets on the cell wall surface upon cooling [74]. In addition to these morphological changes, lignin also undergoes chemical reactions during steam pretreatment (Figure 5).

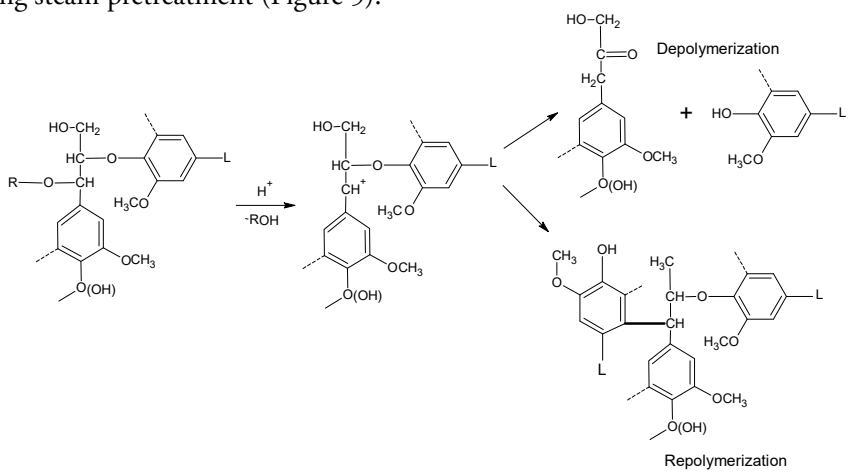

Figure 5. Reaction scheme denoting the competition between depolymerization of a β-O-4 structure and repolymerization with a lignin structure containing a reactive aromatic carbon. Adapted from [75].

Under acidic conditions, carbonium ion intermediates, with a high affinity for nucleophiles within the lignin structure, are formed from benzyl alcohol structures. In β-O-4-linked structures (the most abundant linkage connecting phenylpropane units), the carbonium ion may react further by the cleavage of the ether bond and lignin fragmentation (depolymerization), or by the formation of stable C-C linkages with any adjacent aromatic ring with an electron-rich carbon (polymerization) [76]. The molecular weight distribution of lignin would be expected to decrease sharply if the significant cleavage of β-O-4 linkages were the only mechanism. As this is not the case under typical steam pretreatment conditions, depolymerization is obviously accompanied by comprehensive repolymerization, resulting in an increase in molecular size, and a more condensed polymer structure [75].

Inhibitors

The existence of side reactions resulting in lignocellulose-derived by-products, many of which are inhibitory in the following biochemical processes, is inevitable during pretreatment (Figure 6).

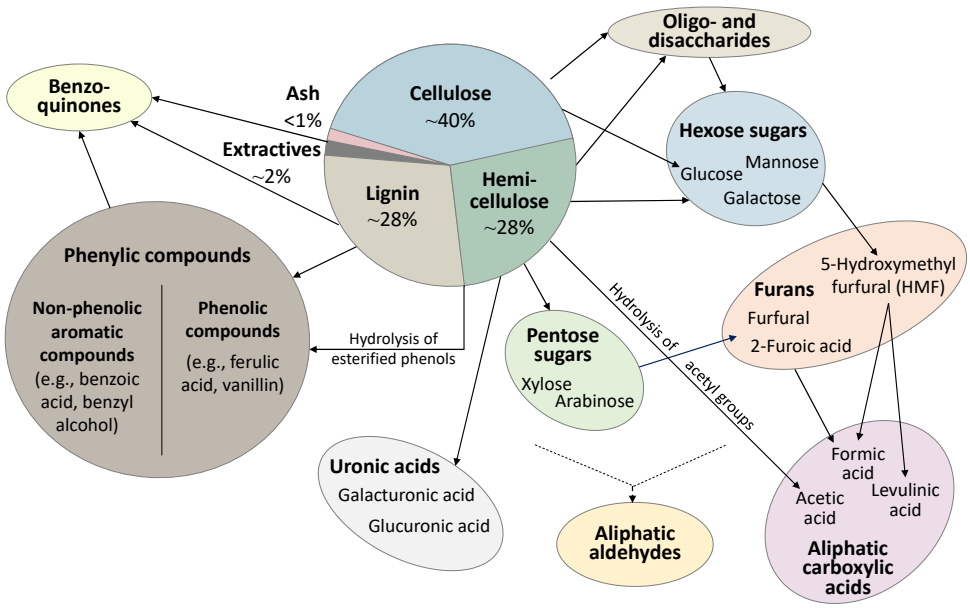

Figure 6. Degradation products from lignocellulose as a result of pretreatment under acidic conditions. Adapted from [77]. Numbers indicate fractions of constituents of wood of *Norway spruce*. Arrows indicate tentative formation pathways.

The amount and variety of the degradation products formed are directly related to the type of biomass, the pretreatment method, and the pretreatment conditions [78]. For instance, the main degradation products of acid-catalyzed steam pretreatment are usually divided into 3 categories based on their origin: furan derivatives, weak acids, and phenolic compounds. Increasing the severity of pretreatment promotes the

degradation of solubilized (mainly hemicellulosic) sugars to furan derivatives (e.g., furfural and 5-hydroxymethyl furfural), or further to secondary degradation products (e.g., levulinic acid and formic acid). Acetic acid and uronic acids are not sugar degradation products, but are released as the result of hemicellulose hydrolysis. A large number of phenolic compounds are also liberated by the partial breakdown of lignin. Some of the most common phenols formed during acid pretreatment of wood are 4-hydroxybenzoic acid, 4-hydroxybenzaldehyde, vanillin, coniferyl aldehyde, syringaldehyde, syringic acid, and Hibbert ketones [79-82]. As some of the extractives are phenolic compounds, some of the phenols in lignocellulosic hydrolysates are likely to originate from extractives [77]. Apart from phenolic compounds, various non-phenolic aromatic compounds (e.g., benzoic acid, benzyl alcohol) are also found in lignocellulosic hydrolysates [78, 82]. The formation of benzoquinones (e.g., hydroquinone and catechol) from phenolic compounds is likely to occur during pretreatment [83]. Although the formation of degradation products can be minimized through optimization of the pretreatment, their inhibitory effects on enzymes and fermenting microorganisms become more apparent at high solids loading and/or as they accumulate due to the recirculation of process water. Although the mechanisms are different and depend on the chemical structure of the inhibitors, the degradation products affect the overall cell physiology of the fermenting microorganism, often resulting in decreased cell viability, ethanol yields, and productivity [84].

Apart from the obvious inhibition problems in the subsequent bioconversion, pretreatment has far-reaching impacts on all the major operations in the process. For example, the solids concentration during pretreatment determines the potential concentration of the released sugars and thus, the final ethanol concentration, which in turn affects the required size of the fermentation vessel and the cost of energy for product recovery. Furthermore, the distribution of sugars between the monomeric and oligomeric forms in the liquid phase can affect the fermenting organism as well as the enzymes, while pretreatment also determines how much of the lignin, and other fractions of biomass, can be recovered, and their suitability for further co-product utilization. Hence, the whole process must be considered when the performance of the pretreatment is evaluated.

3.2. Enzymatic hydrolysis

Efficient enzymatic hydrolysis of the pretreated slurry requires several different enzymes acting (synergistically) to break down the diverse chemical structure of lignocellulosic biomass. The most commonly used commercial enzyme cocktails are produced by the fungus *Trichoderma reesei* (the asexual form of the fungus *Hypocrea jecorina*), genetically engineered for enhanced enzyme production. Enzymatic hydrolysis is usually

performed at a pH of 4.5-5.0 and temperatures in the range 40 to 50°C. The optimal enzyme cocktail (i.e., the required enzymes at the lowest possible concentrations in optimal proportions) must be specifically tailored for a given feedstock, pretreated with a given method.

Cellulases comprise endoglucanases, cellobiohydrolases, and β-glucosidases, which synergistically depolymerize cellulose by hydrolyzing the glycosidic bonds in different regions of the cellulose [85]. While endoglucanases randomly attack the bulk cellulose creating free chain ends, cellobiohydrolases cleave cellobiose from the end of cellulose chains in a processive manner. The soluble cellodextrins and cellobiose released are then further hydrolyzed to glucose by the β-glucosidases. Significant benefits can also be obtained by including enzymes such as hemicellulases and pectinases, which hydrolyze the non-cellulosic polysaccharides [86-88]. Hemicellulases represent a very large number of different enzyme activities that can be divided into depolymerizing enzymes (e.g., endo-xylanases, β-xylosidase, endo-mannanase and β-mannosidase), which cleave the hemicellulose backbone, and debranching enzymes (e.g., α-l-arabinofuranosidases, α-glucuronidase, esterases), which remove substituents connected to the main chains. Hydrolytic enzymes, such as cellulases and hemicellulases, are also supplemented with lytic polysaccharide monooxygenases (LPMOs) to further enhance the synergistic degradation of lignocellulosic biomass components [89]. LPMOs are metalloenzymes that cleave cellulose using a mechanism involving molecular oxygen and an electron donor, which leads to oxidation of one of the carbons in the glycosidic bonds, i.e., oxidation of C1 or C4 [90]. Apart from hydrolytic enzymes and LPMOs, a third class of non-hydrolytic proteins has been implicated in biomass depolymerization [91]. These 'disruptive proteins' or 'amorphogenesis-inducing' proteins appear to be capable of loosening or disrupting cellulosic fibrils without releasing soluble sugars, thereby increasing the accessibility of the cellulose to the enzymes [92, 93].

The factors influencing the rate and extent of enzymatic hydrolysis can be divided into substrate characteristics (e.g., composition, particle size, DP, crystallinity, accessible surface area), enzyme features (e.g., synergism, adsorption, inhibition), and physical factors (e.g., pH and temperature), although many of these factors are inter-related [94]. While it is clear that the sugar yield is ultimately determined by cellulose accessibility and enzyme inhibition, the complexity of biomass and the multiplicity of enzymes make it difficult to differentiate the relative importance of these influencing factors and to fully understand the enzyme-substrate interactions [95].

As was discussed above, the physical, chemical, and morphological characteristics of the pretreated material vary considerably, depending on the nature of the lignocellulosic feedstock, as well as the method of pretreatment and the conditions used. For instance, steam pretreatment removes hemicellulose and redistributes lignin, which generally makes the cellulose more accessible to the enzymes; however, softwoods are more recal-citrant than other types of biomass. The higher lignin content and higher proportion

of guaiacyl lignin subunits, which are thought to be less easily extracted and more easily condensed due to their greater potential for cross linking [96], make softwood biomass inherently more resistant to lignin redistribution during steam pretreatment. The recalcitrance of softwood biomass is thus attributed to both limited cellulose accessibility and non-productive enzyme binding by lignin during enzymatic hydrolysis, resulting in reduced enzymatic digestibility [97, 98]. However, when the accessibility of cellulose in steam-pretreated softwood is increased by post-treatment (e.g., sulfonation), unproductive enzyme binding plays a less prominent role in decreasing the efficiency of enzymatic hydrolysis. This is true even at relatively high lignin concentrations, suggesting that accessibility is the major determinant of hydrolysability [98].

3.3. Fermentation

The solubilization of the cellulose and hemicellulose fractions by pretreatment and enzymatic hydrolysis results in a mixture of hexose (i.e., glucose, galactose and mannose) and pentose (i.e., xylose and arabinose) sugars, together with a wide range of compounds possibly having inhibitory effects on the microorganism used for fermentation. This places extra demands on the fermenting microorganisms, compared to first-generation bioethanol production, to achieve high ethanol yield, productivity and titer, which are necessary to minimize the impact of the fermentation step on capital and operating costs.

Strains of *Saccharomyces cerevisiae* are the most widely used industrially to produce ethanol from hexoses, providing high yields and productivities, in addition to high tolerance to ethanol, low pH and high osmotic pressure [99]. Large-scale ethanol production with *S. cerevisiae* is normally carried out at a pH around 5 and at 30°C under anaerobic conditions (1 mole of glucose is converted into 2 moles of ethanol, which also results in the net production of 2 moles of CO_2 and ATP). However, *S. cerevisiae* is not able to utilize pentose sugars for ethanol production, which is necessary for lignocellulosic feedstocks such as agricultural residues and hardwoods containing large amounts of xylan. Due to its robust industrial background, *S. cerevisiae* was an obvious target for tailoring by metabolic engineering and classical procedures such as random mutagenesis. The introduction of the xylose-fermenting pathway into *S. cerevisiae* has been approached by heterologous expression of either genes encoding xylose isomerase from bacteria, or genes encoding xylose reductase and xylitol dehydrogenase from fungi [84]. Regardless of the inserted xylose pathway, almost all reported industrial strains have overexpressed genes from the pentose phosphate

pathway [100]. In addition to genetic modification, the modified *S. cerevisiae* strains can be further developed (e.g., increased inhibitor tolerance) using evolutionary engineering strategies [101], or adapted to a given lignocellulosic hydrolysate (by on-site cultivation on the liquid hydrolysate) [102], to improve performance in a highly inhibitory environment.

Historically, two main process configurations have been used for enzymatic hydrolysis and fermentation: SHF and SSF. In an SHF configuration, both the enzymatic hydrolysis and the fermentation can be carried out under optimal conditions, which is considered the main advantage, due to the considerably different temperature optima. On the other hand, the end-product inhibition of enzymes and higher investment cost due to the need for two separate vessels are generally considered the main drawbacks of this configuration. SSF integrates the enzymatic hydrolysis and fermentation in one vessel, which means compromising the operating conditions, but eliminates end-product inhibition by the continuous fermentation of glucose to ethanol, which in turn also lowers the risk of contamination. However, many of the advantages of SHF and SSF can be combined by integrating a pre-hydrolysis step into the SSF process. In this hybrid process configuration the enzymes are added to the reactor some time before the fermenting organism is added (i.e., a pre-hydrolysis step). The pre-hydrolysis step is performed at the optimum temperature for the enzymes to increase the hydrolysis rate, before lowering the temperature to accommodate the fermenting microorganism. Although many, more fine-tuned, approaches have been investigated for bioconversion, it is clear that the properties of the pretreated material, the enzyme mixture and the fermenting organism are all important when selecting the most favorable fermentation strategy and appropriate conditions to achieve optimal performance.

3.4. Product and co-product recovery

Downstream processing must be considered an integral part of the whole process, as a variety of energy products (e.g., electricity, solid fuels, biogas and district heating) can be produced from lignocellulosic biomass in combination with bioethanol, which can reduce the minimum ethanol selling price.

At the end of the bioconversion process, the fermentation broth contains mainly ethanol and residual solids, but also a large number of residual low-molecular-weight organic substances, the enzymes, and the fermenting microorganism. In order to avoid ethanol losses, the whole broth is distilled using one or more stripper columns. The ethanol stream can be further concentrated to near azeotropic concentrations by a

rectifier, and then subsequently dehydrated using water adsorption in zeolites. Although these separation steps are more mature and, in most cases, have been proven on large scale, they are in general very energy demanding [103]. The energy demand increases significantly at low ethanol concentrations, even when multiple-column distillation is performed. A high final ethanol concentration after fermentation, at least above 4-6 wt%, is thus required [104].

The separation of the solid fraction from the stillage is usually performed by filtration (e.g., using a filter press) after the stripper. Due to the high energy value of lignin, the thermal conversion of this solid residue can provide the energy required by the entire process. The excess solid residue can either be dried, pelletized and then sold (as a solid fuel), or used to produce surplus electricity that can be sold. The liquid part of the stillage stream can be subjected to anaerobic digestion to produce biogas as a promising alternative to energy-intensive evaporation.

3.5. Process simulation

Due to the complex interdependence of the aforementioned process steps, process simulation is an invaluable tool to evaluate the economic impacts of changes in process design on the overall conversion process; enabling production cost comparisons and providing guidance in subsequent research. Aspen Plus® is a commercial process simulator in which flowsheets can be implemented and processes simulated based on experimental data. This involves rigorous material and energy balance calculations based on tabulated thermodynamic and physical property data specifically developed for lignocellulosic biomass [105]. This can be coupled with economic evaluations to study the economic feasibility of a technical solution using measures such as the net present value (NPV) and the minimum ethanol selling price. However, the results obtained from such techno-economic evaluations should not be regarded as absolute values, but can be used to compare different process scenarios from technical and economic standpoints.

4. Forest-based ethanol production

In this chapter, the key results reported in Papers I-IV are summarized and discussed in a broader perspective. These studies have focused on the utilization of softwoods as forest-based raw materials for ethanol production with the aim of assessing the robustness of the acid-catalyzed steam pretreatment and the bioconversion process to a more diversified feedstock base. In the first part, the effects of bark, which is expected to make up a considerable fraction of forest harvest residues, on the typical softwood-to-ethanol conversion process are discussed, whereas the second part briefly touches upon the inclusion of short-rotation tree species to the use of long-rotation softwoods for ethanol production, through a preliminary study on the steam pretreatment of a mixture of poplar and spruce. Supplementary data that were not included in the papers are also presented.

4.1. Biomass supply from long-rotation forestry

Forests and forestry can play an important role in the transition from a fossil-fuel-based economy to a clean and sustainable bioeconomy, by providing biofuels, biochemicals and bioenergy. Although it is difficult to forecast the extent of the contribution that lignocellulosic biomass could make on a global scale, as it depends on a large number of factors [106], forest biomass is expected to constitute a significant fraction [107]. However, in most cases it is not the global, but the regional or local, feedstock supply that is critical to secure an investment. In the Northern Hemisphere, softwood forests represent one of the largest sources of lignocellulosic biomass; thus, there is considerable interest in the utilization of softwoods for the large-scale production of advanced biofuels in Scandinavia and Canada, for example.

Sweden, where the modeled bioethanol plant described in Paper II was hypothetically located, is home to a large forest industry and extensive forest resources. Swedish forests are found in the boreal and temperate zones, and are managed primarily by a clearcut system with long rotation periods (60-100 years). The total standing volume in productive forest areas is dominated by two softwood species, namely Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*), accounting together for 80% [108]. Two-thirds of the country's land area (28 million hectares) is covered by forests, of

which some 23 million hectares is considered productive [108]. However, efficient and sustainable production of wood for any end use must go hand in hand with the preservation of valuable ecological and socio-cultural values.

Historically, forest resources were heavily exploited during the 18th and 19th centuries, which led to Sweden's first Forestry Act, passed in 1903. The trend of depletion was reversed by requiring land owners to replant after harvesting, and the total standing volume has increased significantly over time [109]. The current forest policy, adopted in 1993, and reinforced through a parliamentary decision in 2008, also integrates ecological and social considerations into modern forestry practices to ensure sustainable forest management. As over-exploitation of forest resources, with annual harvest levels above annual growth, is a non-sustainable short-term solution, attention must be turned to harvesting more of the forest biomass that is available, but not utilized by the traditional forest industry [109]. Potential sources of raw material for biofuel production include by-products of the wood-processing industry (e.g., sawdust and shavings, bark) and forest biomass that has traditionally been left in the forest at stem-wood harvest (e.g., logging residues, stumps and early thinnings). However, it should be borne in mind that the availability and cost of these fractions may be significantly influenced by competing users (e.g., pulp mills, pellet mills). Industrial utilization of these forest residues must be undertaken with as little impact on biodiversity, soil, water and the long-term yield of forest land, as possible. This can be achieved through the choice of suitable logging sites, careful planning, adapted methods, conservation and appropriate compensation measures [110].

Forest biomass can be classified according to its typical end-use and qualities [111]. Tree harvesting in Sweden, as in many other countries, involves felling trees and delimbing stems. Traditionally, the lower part of the stem, with the larger diameter, is sent to a sawmill as saw logs, while the upper, thinner part, with a lower value, is used as pulpwood in pulp and paper mills. Forest harvest residues can be further classified into tree tops and branches, stumps, early thinnings (i.e., small diameter trees) and low-quality non-merchantable logs (not suitable for either lumber or pulp production). These forestry assortments are left in the forest or adjacent to roads in large piles for around a year before being chipped and transported, if intended for bioenergy purposes. Wood processing industries also produce by-products, such as sawdust and shavings, hog fuel and bark.

Different types of forest biomass have different quality attributes (e.g., energy content, moisture content, particle size), which affect their procurement and preprocessing costs, as well as their transportation. The heterogeneity of forest residues and the presence of bark can also place extra demands on the softwood-to-ethanol conversion process, and influence conversion efficiencies. This has mostly been overlooked in previous research on the production of bioethanol from white wood chips. As it is unlikely that only white wood chips will be used for large-scale bioethanol production, one of the goals

of the work presented in this thesis was to assess the suitability of bark-containing forest residues for ethanol production by examining the effects of bark on a conversion process previously optimized for white wood chips only.

4.1.1. Bark as a raw material

Despite being abundant and supposedly inexpensive, the utilization of forest residues for ethanol production presents challenges due to the higher degree of complexity and heterogeneity of these bark-containing feedstocks compared with white wood only. Bark is the outermost layer covering tree stems and branches, amounting to 10-15% of the total weight of the tree [51]. The chemical composition and structure of bark differ significantly from those of wood. Bark is a highly complex, heterogeneous material composed of several kinds of cell. Bark can be roughly divided into living inner bark and dead outer bark [51]. The chemical compositions of spruce wood chips, and spruce and pine barks used as raw material in Papers I, III and IV were determined according to the analytical procedures developed by the National Renewable Energy Laboratory (NREL). Typical values of the composition of lignocellulosic biomass are given in Table 2.

Table 2. Composition of the spruce wood chips and spruce and pine barks presented in Papers I and III (% dry basis)

	Glucan	Xylan	Galactan	Arabinan	Mannan	Lignin	Extractives
Norway spruce							
Wood chips	42.4±1.2	5.6±0.0	1.3±0.2	0.7±0.2	9.9±0.6	33.8±0.1	3.3±0.2
Bark	23.1±0.4	3.6±0.1	0.8±0.1	4.3±1.2	3.4±0.8	33.9±0.1	28.2±0.3
Scots pine							
Bark	20.0±0.1	4.6±0.0	3.0±0.0	4.1±0.0	3.2±0.0	40.9±0.3	19.4±0.2

Although many of the constituents of wood can be found in bark, it has lower cellulose and hemicellulose contents, and typically contains higher amounts of ash, non-cellulosic sugars, and extractives. The extractives are one of the most disparate compositional characteristics of bark. The extractives content can vary considerably, even within the same species, depending on felling season, storage conditions [112], and extraction method [113], but extractives generally account for 20-40 wt% of dry bark. Extractives from Scots pine and Norway spruce barks have recently been characterized by many researchers [112, 114-118]. These can essentially be divided into soluble lipophilic compounds, such as resin acids, and hydrophilic compounds, such as phenolic compounds and stilbenes. It should be noted that although the valorization of extractives for value-added co-products could improve the overall process economics, the scope of this work was limited to the production of ethanol via the sugar platform. The robustness of acid-catalyzed steam pretreatment and bioconversion was assessed to investigate the impact of the presence of bark in the feedstock (Paper I), and the

suitability of utilizing bark-containing forest residues for ethanol production was subsequently assessed by techno-economic analysis (Paper II).

4.1.2. The effects of bark on the overall sugar recovery

Although steam pretreatment has the advantages of requiring limited capital, energy, and chemical input, while being effective for a wide range of biomass feedstocks, softwoods have proved to be one of the most challenging lignocellulosic feedstocks [70]. It has been shown that more severe pretreatment conditions [58], relatively high enzyme dosage [119], and/or a delignification step [120] are needed to overcome the recalcitrance of softwoods and provide a reasonable yield of monomeric sugars. Among lignocellulosic feedstocks, softwood biomass has a higher lignin content as well as a higher proportion of guaiacyl lignin subunits, which are thought to be less easily extracted and more easily condensed due to their greater potential for cross linking [96]. This was exacerbated in the study presented in Paper I, where bark and wood fractions were mixed together prior to pretreatment. A lower proportion of sugars was dissolved in the monomeric form in the liquid fraction after steam pretreatment when bark was included (Figure 7), and lower yields were observed in the enzymatic hydrolysis step with increasing bark content in the wood and bark mixtures (Figure 8).

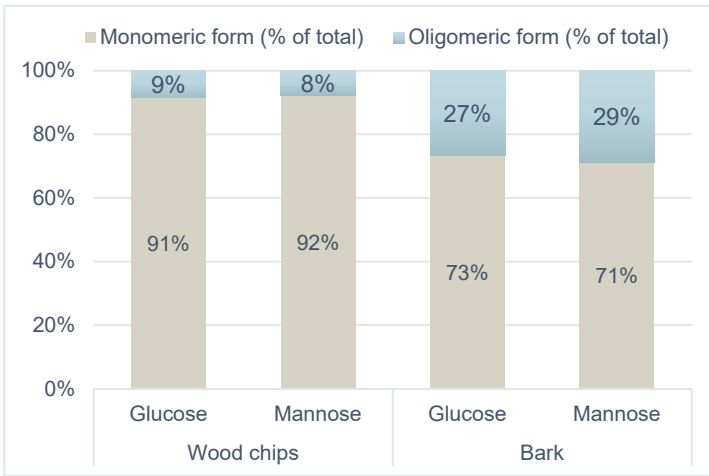

Figure 7. Percentage of glucose and mannose recovered in monomeric and oligomeric form in the liquid fraction of steam-pretreated spruce wood chips and bark. Acid-catalyzed steam pretreatment of spruce wood chips and bark was performed at 210°C, for 5 min, with 2.5% SO_2.

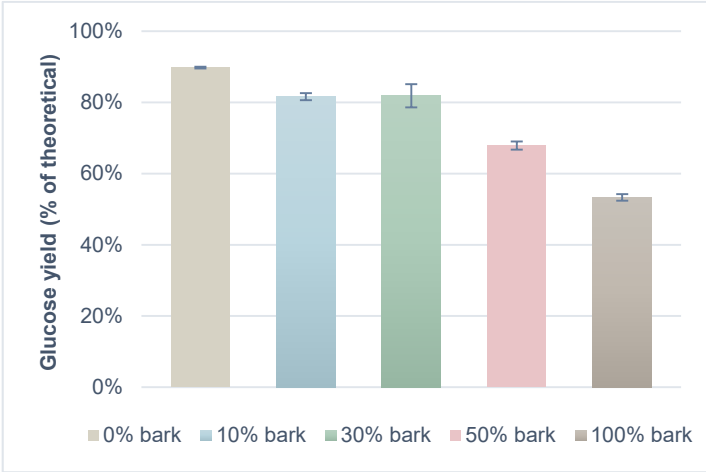

Figure 8. Glucose yield from enzymatic hydrolysis of steam-pretreated mixtures of spruce wood and bark. Enzymatic hydrolysis was performed at 10% WIS loading, 45°C, pH 5 for 96 h using 20 FPU/g WIS Cellic CTec3 enzyme cocktail. Glucose yield is expressed as percentage of the theoretical based on all available glucose in the pretreated materials. Acid-catalyzed steam pretreatment was performed at 210°C, for 5 min, with 2.5% SO_2. FPU: filter paper unit; WIS: water-insoluble solids.

Bark was found to be significantly more difficult to hydrolyze to monomeric sugars under the pretreatment condition previously shown to be effective for spruce wood chips (210°C, 5 min, 2.5% SO_2,) [121]. Kemppainen found that more severe pretreatment was detrimental for spruce bark, as the use of acid catalyst (i.e., H_2SO_4) or higher temperature decreased the rate of enzymatic hydrolysis [122]. However, the results of additional steam pretreatment trials on spruce bark in the present work showed the opposite. Increasing the severity of the steam pretreatment resulted in improved enzymatic hydrolysability of bark (Figure 9).

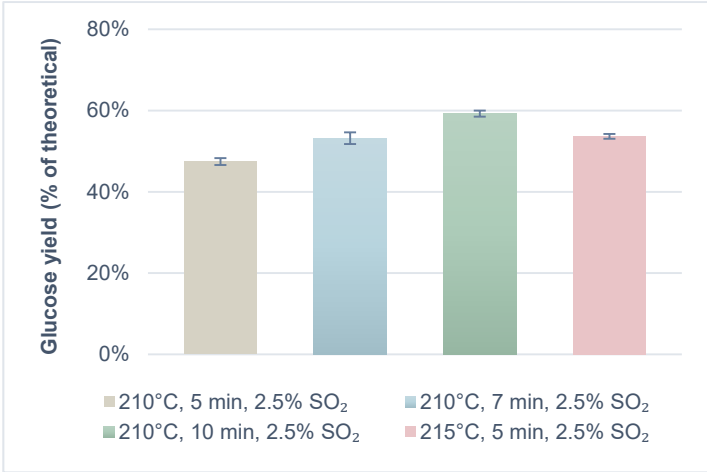

Figure 9. Glucose yield from enzymatic hydrolysis of steam-pretreated spruce bark. Enzymatic hydrolysis was performed at 5% WIS loading, 45°C, pH 5 in 0.05 M acetate buffer solution for 96 h using 20 FPU/g WIS Cellic CTec3 enzyme cocktail. Glucose yield is expressed as percentage of the theoretical based on all available glucose in the pretreated materials.

This could be attributed to the higher extent of hemicellulose removal and the consequent increase in cellulose accessibility. However, the glucose yields obtained were still considerably lower for bark than for white wood. Soluble compounds generated during pretreatment are known to impair the hydrolytic performance of the enzymes. Inhibitory effects of monomeric and oligomeric sugar components [123, 124], and non-sugar components [98, 125-127] such as degradation products of sugars, lignin, and extractives, have been reported previously. Phenolic compounds, either in monomeric or oligomeric form, derived from bark can have an inhibitory effect on the enzymes [128], which makes the enzymatic hydrolysis of bark more challenging than that of spruce wood chips. However, decreasing enzymatic digestibility with increasing proportions of bark in SO_2-catalyzed steam-pretreated spruce bark and wood mixtures was observed not only on whole slurry, but also on washed fibers (Figure 10).

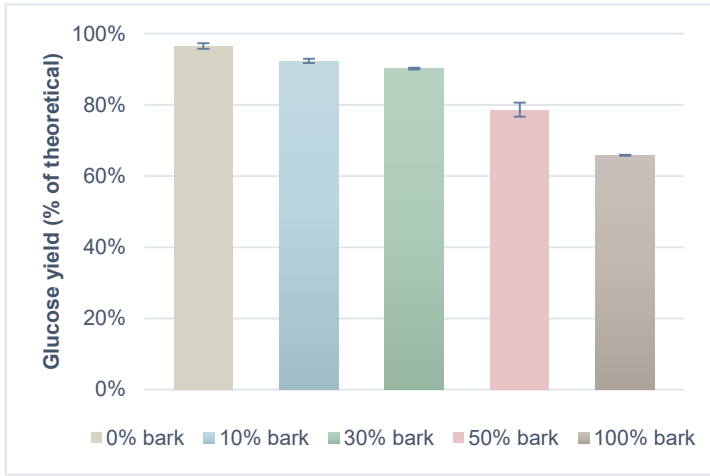

Figure 10. Glucose yield from enzymatic hydrolysis of washed fibers obtained from steam-pretreated mixtures of spruce wood and bark. Enzymatic hydrolysis was performed at 5% WIS loading, 45°C, pH 5 in 0.05 M acetate buffer solution for 96 h using 20 FPU/g WIS Cellic CTec3 enzyme cocktail. Glucose yield is expressed as percentage of the theoretical based on all available glucose in the washed pretreated fibers. Acid-catalyzed steam pretreatment was performed at 210°C, for 5 min, with 2.5% SO_2.

This suggests that the soluble inhibitory compounds liberated during steam pretreatment of bark are not the sole reason for the impaired enzymatic digestibility of bark compared to the wood fraction. The residual extractives together with the lignin play a critical role in limiting cellulose hydrolysis.

Post-treatment, such as alkaline extraction and alkaline hydrogen peroxide (AHP) treatment, were investigated after steam pretreatment of bark with the aim of (partially) removing lignin to enhance cellulose accessibility (Figure 11). Alkali treatment has been shown to be effective on hardwoods, but not on softwoods, supposedly due to the more even redistribution of guaiacyl lignin in softwoods, which restricts access to cellulose microfibrils [129]. In contrast, AHP treatment has been shown to be one of the most effective post-treatment methods for fast and complete hydrolysis of steam-pretreated softwoods [130].

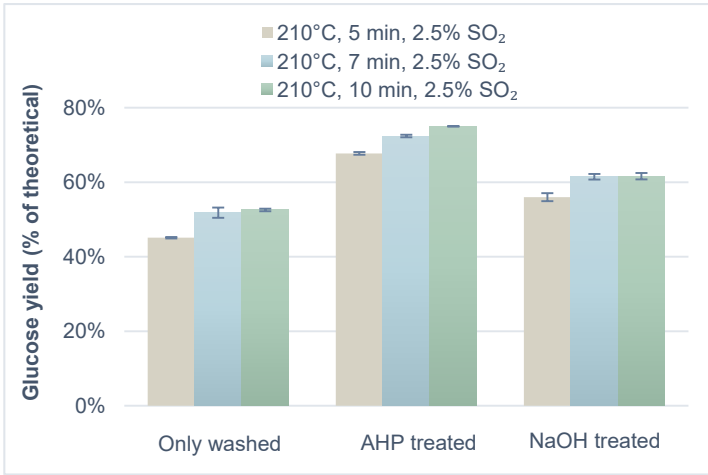

Figure 11. Glucose yield from enzymatic hydrolysis of steam-pretreated spruce bark after post-treatments. Enzymatic hydrolysis was performed at 5% WIS loading, 45°C, pH 5 in 0.05 M acetate buffer for 96 h using 20 FPU/g WIS Cellic CTec3 enzyme cocktail. Glucose yield is expressed as percentage of the theoretical based on all available glucose in the washed post- and/or pretreated materials. AHP post-treatment was performed with 1% H_2O_2, at a solid:liquid ratio of 1:50, 80°C pH 11.5 for 45 min on washed steam-pretreated spruce bark. NaOH treatment was performed with 4% NaOH solution, at a solid:liquid ratio of 1:20, 121°C for 30 min on washed steam-pretreated spruce bark. The post-treated materials were thoroughly washed prior to enzymatic hydrolysis.

The extent of delignification was found to be higher using AHP treatment than NaOH treatment of steam-pretreated bark, in accordance with previous findings [130], which resulted in a greater improvement in the efficiency of enzymatic hydrolysis following AHP treatment (Figure 11). However, the residual lignin present in the treated barks was still quite high, despite post-treatments. The difficulty in removing lignin could have resulted from condensation reactions, which probably occurred between the lignin and the extractives, and between different lignin moieties during the steam pretreatment of bark, as AHP and NaOH post-treatment only decreased the lignin content of the steam-pretreated barks, from about 60% to 36% and 50%, respectively.

Condensation reactions of bark extractives during acid-catalyzed steam pretreatment have been suggested to render the otherwise water-soluble extractives insoluble, and alter the structure of the solid fraction, which in turn can impair enzymatic hydrolysis [122]. Although the removal of extractives has previously been performed to valorize the extracted compounds [112, 131], the effect of hot-water extraction on acid-catalyzed steam pretreatment has not been examined. A simple hot-water extraction step was found to remove more than half of the water-soluble extractives from spruce bark (57%) and pine bark (51%) (Paper III). The compositional analysis of the steam-pretreated non-extracted/extracted softwood barks revealed that the acid-insoluble lignin content of the pretreated materials decreased as more water-soluble phenolic compounds were removed from the barks by hot-water extraction

prior to steam pretreatment. This supports the hypothesis that water-soluble bark phenolics are rendered insoluble by acid-catalyzed treatment, and are subsequently analyzed as insoluble lignin residue [113, 122, 132]. The partial removal of water-soluble extractives by hot-water extraction before the steam-pretreatment step improved the enzymatic digestibility of barks, and the positive effect was significantly greater when steam pretreatment was performed with an acid catalyst (Figure 12).

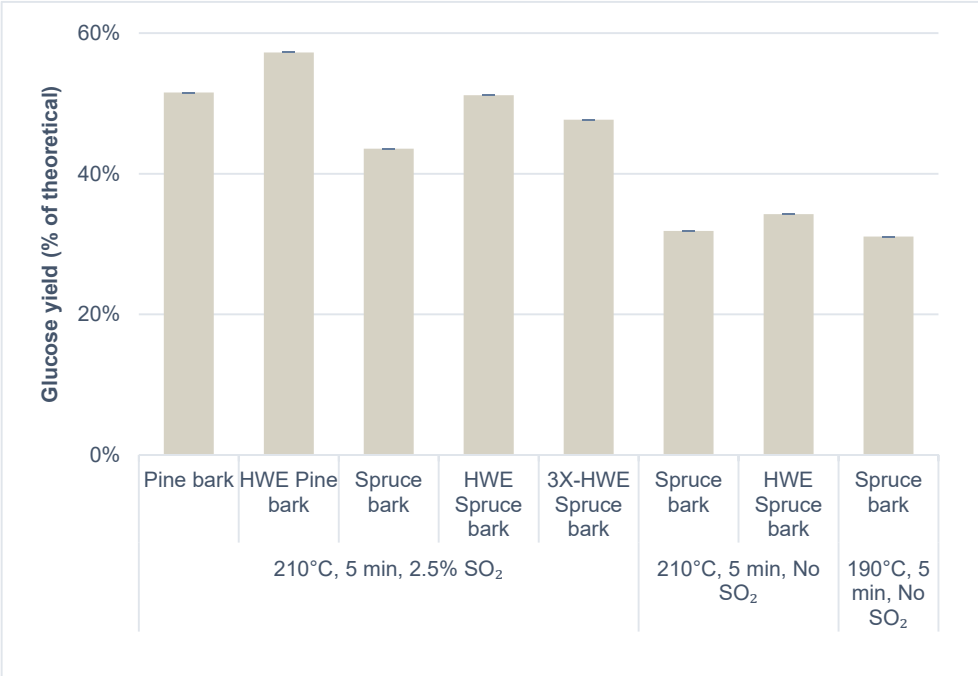

Figure 12. Glucose yield after enzymatic hydrolysis of steam-pretreated spruce and pine barks. Enzymatic hydrolysis of barks, non-extracted or hot water-extracted (HWE), steam-pretreated under various conditions, was performed at 10% WIS loading, 45°C, pH 5 for 96 h using Cellic CTec3 enzyme cocktail at a dose of 5 wt%, based on WIS, corresponding to approximately 9 FPU/g WIS. Glucose yield is expressed as percentage of the theoretical based on all available glucose in the pretreated materials. Hot-water extraction was performed with water at 80°C for 2 h at a stirring rate of 200 rpm. 3X-HWE spruce bark was obtained by repeating the hot water extraction step 3 times.

These results further confirm the hypothesis that the water-soluble extractives fraction contributes to detrimental changes during steam pretreatment of bark that impair the subsequent enzymatic hydrolysis, especially when steam pretreatment is carried out in the presence of an acid catalyst. They also show that the removal of water-soluble extractives improves the digestibility of bark. However, this effect was found to be more pronounced for spruce bark than for pine bark, as evidenced by the 30% and 11% improvements in glucose yield, respectively (Figure 12). Moreover, more thorough hot-water extraction (i.e., hot water extraction repeated three times, '3X-HWE'), resulting in the removal of an additional 15% of water-soluble extractives before the

acid-catalyzed steam pretreatment, did not result in any further improvement in enzymatic digestibility (Figure 12).

In addition to the type of biomass, the physical characteristics of the feedstock, such as the particle size and moisture content, can also influence the optimal severity of steam pretreatment, and thus the overall sugar recovery. For instance, smaller particle size facilitates mass transfer, and thus slightly increases the observed severity of pretreatment, whereas larger particle size could lead to uneven cooking [133]. Moisture content influences the ability of heat and chemicals to penetrate the raw material, and seems to have a non-linear effect on the required severity of steam pretreatment: a minimum critical moisture is essential for effective steam pretreatment, while excessive moisture reduces the rate of heating and increases the energy requirement [64]. For the assessment of the efficacy of steam pretreatment on different feedstocks, as described in Papers I, III and IV, the particle size and moisture content of all biomass substrates were adjusted prior to steam pretreatment, to allow reliable comparisons to be made between the samples.

4.1.3. The effects of bark on fermentation

Fermentation of the hydrolysates (i.e., the liquid fraction of the pretreated material) and the hydrolysates after enzymatic hydrolysis showed that the inclusion of bark did not impair fermentability, as ethanol yields were comparable or higher with increasing bark content in the pretreated mixtures (Paper I). The concentration of inhibitory degradation products formed during steam pretreatment was lower with increasing bark content in the mixtures, due to the lower initial carbohydrate content of bark. Either the higher extractives content was not inhibitory to the yeast, or the condensation or polymerization of water-soluble extractives during pretreatment eliminated their inhibitory effect. The latter has been reported in previous research where hydrolysates of bark-containing softwood feedstock prepared at higher pretreatment severity showed comparable or better fermentability than the bark-free feedstock, whereas the hydrolysate obtained from low-severity steam pretreatment was found to be inhibitory to the yeast [134]. The inhibitory effect was attributed to the water-soluble extractives recovered in the liquid fraction, and could be alleviated by additional acid hydrolysis of the hydrolysate obtained from low-severity pretreatment. One possible explanation of this is that the bark-containing hydrolysates may contain some compounds that are inhibitory to *S. cerevisiae*, which are transformed under more severe steam pretreatment, thus becoming less inhibitory during fermentation [134].

4.1.4. Techno-economic implications

Although the availability and price of different kinds of forest biomass can vary significantly depending on the demand from competing sectors, biomass transportation systems, biomass supply sources, accessibility, and the scale of production [135], the cost of feedstock is the single greatest expense in the biomass-to-ethanol conversion process [136, 137]. Therefore, it is necessary to maximize the yield of sugar and/or ethanol from biomass to ensure the efficient utilization of the feedstock, while high final ethanol titers reduce the energy required for distillation. There could be substantial differences in the theoretical ethanol yield (defined as the amount of ethanol that can be produced per dry metric ton of raw material) between different forestry assortments due to the lower content of carbohydrates in bark compared to white wood. Moreover, problems associated with enzymatic hydrolysis of bark, due to its complex structure, may further deteriorate the conversion of bark-containing feedstocks to ethanol, possibly increasing the cost of production. A techno-economic analysis was performed, assuming the same plant design and operating conditions in all cases, based on the results presented in Paper I, to assess the effects of including bark on the whole conversion process, and the effects on the cost of ethanol production using different forestry assortments with different bark contents (Paper II).

Ethanol production from different forestry assortments containing different amounts of bark was assumed to take place in an energy-driven biorefinery producing solid pellets, biogas and electricity, besides ethanol as the main product. It was found that the profitability, evaluated in terms of the NPV assuming an 11% discount rate and an investment life of 20 years, decreased with increasing bark content of the feedstock (Figure 13).

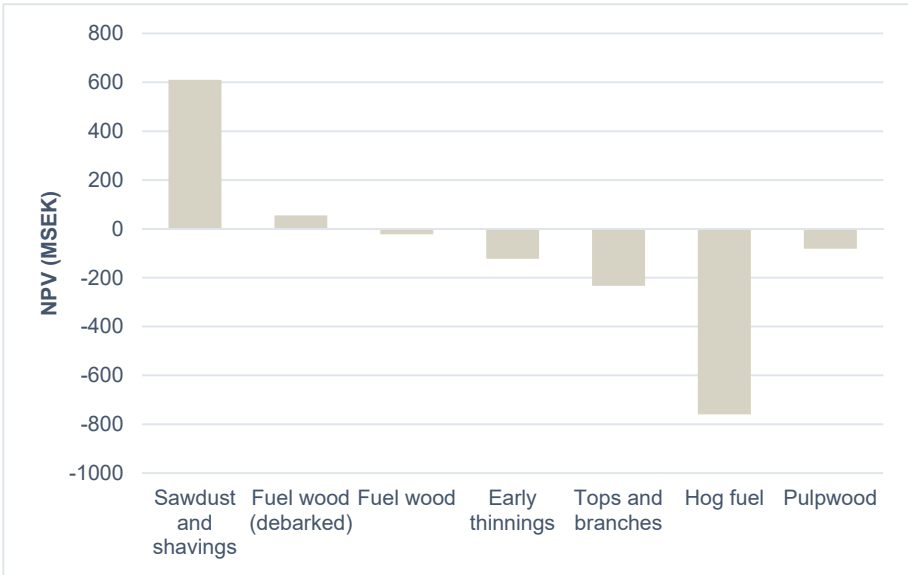

Figure 13. Net present value (NPV), calculated assuming a discount rate of 11% for 20 years, in scenarios utilizing different forestry assortments with different bark contents for ethanol production.

Under the basic assumptions applied in the study presented in Paper II, only the utilization of sawdust and shavings gave a significant positive NPV, as this was the cheapest white wood feedstock among those investigated, and exhibited the highest ethanol potential and the highest overall ethanol yield. Furthermore, the sensitivity analyses indicated the following: i) that white-wood sawdust and shavings was superior as a feedstock for ethanol production over a wide range of raw material prices; ii) there is a need to improve the enzymatic hydrolysability of bark-containing forest harvest residues, such as early thinnings, tops and branches, to achieve significant cost improvement compared with the utilization of pulpwood, and iii) the production of ethanol from hog fuel (i.e., mostly bark) is not economically feasible using the process investigated here.

Positive NPVs were obtained when assuming the same sugar yield after the enzymatic hydrolysis of bark-containing forest harvest residues as for white wood, implying potential feasibility. The decreasing ethanol potential with increasing bark content of the feedstock would not undermine the economic production of ethanol for bark contents up to 30%. As the future biomass potential lies mainly in the increased removal of forest harvest residues in Sweden [138], it is, first and foremost, necessary to tailor the enzyme cocktail and the pretreatment conditions to achieve higher conversions of cellulose and hemicellulose to monomeric sugars in enzymatic hydrolysis. For instance, considerable improvements have been achieved in the hydrolysis of spruce bark when pectinase enzymes were used as a supplement to cellulolytic enzymes, due to the presence of pectin in bark [122]. On the other hand,

the 20-year NPV was still found to be negative for hog fuel, even when assuming the same sugar yield as for white wood. This leads to the conclusion that extractives and/or lignin components must be utilized in higher-value co-products to improve the overall process economics. Therefore, tailoring the pretreatment process and its conditions to ensure the recovery of all biomass components in a reactive form at high yields should be an important objective of further research.

4.2. Biomass supply from short-rotation forestry

Fast-growing tree plantations have also been identified as possible sources of biomass for large-scale biofuel and bioenergy production in Sweden [138, 139]. Willow (*Salix* species) and poplar (*Populus* species) are the most commonly considered candidates for short-rotation forestry. Plantations of both *Populus* (including both poplar and hybrid aspen) and *Salix* species have been established during recent decades, grown predominantly on arable land in small plantations, where research has been conducted to assess both their productivity and their environmental effects [140]. In general, these hardwoods are characterized by fast growth, high survival rates, and high production potential [141].

Expanding the feedstock base of an ethanol plant could result in cost reduction by maximizing the economy of scale through increased feedstock volume, as well as hedging the sensitivity to the volatility of feedstock costs [142]. Although a production process able to utilize a wide range of feedstocks could considerably facilitate the large-scale production of ethanol, the conversion of mixed biomass feedstocks to fermentable sugars and ethanol without compromising the efficiency of the process is extremely challenging. The different attributes of the different feedstocks place distinct challenges on each conversion step for efficient raw material utilization. For instance, most feedstocks have different established optimal pretreatment conditions, efficient enzymatic hydrolysis could require different accessory enzymes depending on the feedstock used, while the different pattern of sugars released and inhibitory compounds formed during pretreatment could influence the performance of the fermenting microorganism. This indicates that the best way to utilize different types of feedstocks is to process them in successive campaigns. Research has traditionally focused on the pretreatment of single feedstocks, and there are few studies on the pretreatment of mixtures of lignocellulosic feedstocks [143].

Steam pretreatment of mixed substrates has previously been performed on mixed hardwoods [144, 145], mixed softwoods [121] and on mixtures of hybrid poplar and wheat straw [146]. The separate steam pretreatment of spruce and poplar is well documented, but steam pretreatment of a mixture of these species has not previously been investigated. The optimal steam pretreatment conditions, for the highest glucose

and xylose recovery, for poplar are reported to be 195-200°C, for 5-15 min, with 2.5-3% SO_2 [147, 148], which are close to the optimal conditions reported for steam pretreatment of spruce [121]. Under the pretreatment conditions investigated in the final study (Paper IV), overall sugar recoveries after steam pretreatment, defined as the amount of sugar in the pretreated material divided by the amount of sugar in the raw material, for the 50:50 wt% mixture of spruce and poplar could be predicted to within 2% by linear interpolation of the results obtained for the pure species. The recovery of monomeric hexose sugars after steam pretreatment and enzymatic hydrolysis for the 50:50 blend could be predicted to within 4% by linear interpolation of the pure species results (Table 3). This suggests that there are no synergistic or antagonistic interactions during steam pretreatment and enzymatic hydrolysis when mixing spruce and poplar, and that linear interpolation gives accurate results for the total sugar recovery.

Table 3. Predicted and experimental combined hexose yield for mixed feedstocks after steam pretreatment and enzymatic hydrolysis. Enzymatic hydrolysis of steam-pretreated materials was performed at 10% WIS loading, 45°C, pH 5 for 96 h using Cellic CTec3 enzyme cocktail at a dose of 5 wt% based on WIS.

Pretreatment conditions	Combined hexose yield (g monomeric hexose sugars/100 g dry raw material)				
	Spruce	Poplar	50:50 Blend		
			Predicted	Measured	Difference
210°C, 5 min, 2.5% SO_2	37.13	35.40	36.26	36.77	1.4%
205°C, 5 min, 2.5% SO_2	38.11	36.12	37.12	38.59	3.8%
200°C, 5 min, 2.5% SO_2	38.85	39.80	39.32	38.99	-0.8%

As expected, spruce proved to be more recalcitrant than poplar, as lower sugar recoveries were achieved after enzymatic hydrolysis of spruce than poplar steam pretreated at the same conditions (Figure 14), despite the fact that the amount of monomeric hexose sugars after steam pretreatment and enzymatic hydrolysis per 100 g dry raw material was found to be similar for all pretreated materials (Figure 15).

Figure 14. Combined hexose recovery after steam pretreatment and enzymatic hydrolysis of spruce, poplar and a 50:50 blend. Enzymatic hydrolysis of the steam-pretreated feedstocks was performed at 10% WIS loading, 45°C, pH 5 for 96 h using Cellic CTec3 enzyme cocktail at a dose of 5 wt% based on WIS. Combined hexose recovery is expressed as percentage of all available hexoses in the original raw materials.

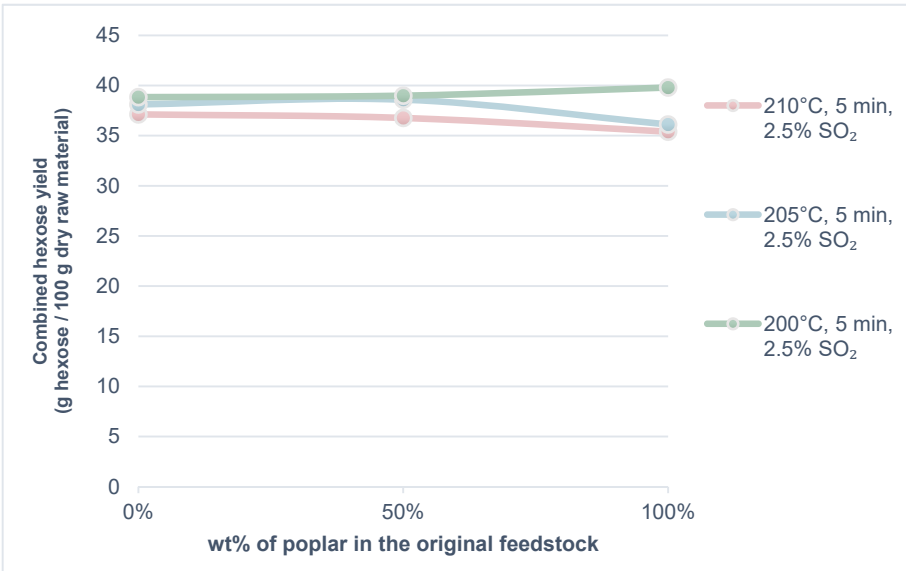

Figure 15. Combined hexose yield after steam pretreatment and enzymatic hydrolysis of spruce, poplar and a 50:50 blend. Combined hexose yield is expressed as g hexose recovered in monomeric form after steam pretreatment and enzymatic hydrolysis/100 g dry raw material. Enzymatic hydrolysis of the steam-pretreated feedstocks was performed at 10% WIS loading, 45°C, pH 5 for 96 h using Cellic CTec3 enzyme cocktail at a dose of 5 wt% based on WIS.

Yet, the ethanol production rate and ethanol yield are not only dependent on the sugar yield, but also on the fermentability of the hydrolysate. The fermentability of the hydrolysates could be expected to deteriorate with increasing poplar inclusion, due to the high amount of acetic acid liberated together with other inhibitors during steam pretreatment of poplar (Table 4).

Table 4. Concentration of inhibitory compounds measured in the hydrolysates of steam-pretreated spruce, poplar and a 50:50 blend

	WIS content (wt%)	Formic acid (g L^{-1})	Acetic acid (g L^{-1})	Levulinic acid (g L^{-1})	HMF[1] (g L^{-1})	Furfural (g L^{-1})
210°C, 5 min, 2.5% SO_2						
Spruce	12.7	2.4	6.2	1.9	3.4	2.0
50:50 blend	13.8	2.1	11.4	1.0	2.3	2.8
Poplar	13.7	1.9	16.9	0.6	1.6	3.6
205°C, 5 min, 2.5% SO_2						
Spruce	13.2	2.6	6.2.	1.8	3.0	2.2
50:50 blend	12.8	2.2	11.7	1.3	2.4	3.5
Poplar	12.3	1.8	17.5	0.8	1.6	4.4
200°C, 5 min, 2.5% SO_2						
Spruce	15.2	2.0	6.3	0.9	2.2	1.5
50:50 blend	14.5	1.7	11.4	0.4	1.4	2.0
Poplar	14.5	1.6	16.1	0.4	0.9	2.8

[1] HMF = 5-hydroxymethyl furfural

5-Hydroxymethyl furfural, formed by the degradation of hexoses, was found at higher concentrations in the hydrolysate of spruce, whereas the concentration of furfural, formed by the degradation of pentoses, was higher in the poplar hydrolysate. This can be exemplified by the fermentation test performed on the hydrolysates obtained after pretreatment at 205°C, for 5 min, with 2.5% SO_2 (i.e., the pretreatment condition that resulted in the pretreated materials with the highest enzymatic hydrolysability). The highest ethanol yield was achieved with the spruce hydrolysate, and the lowest with the poplar hydrolysate, indicating a lower fermentability with increasing poplar content (Figure 16).

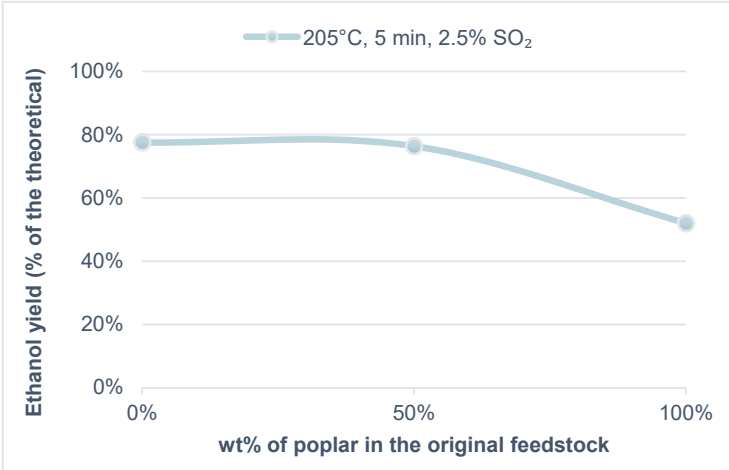

Figure 16. Ethanol yield from hydrolysate fermentation. Fermentation was performed with Ethanol Red®, an industrial hexose-fermenting *S. cerevisiae* strain, at 30°C, pH 5 for 96 h, at a yeast load of 5 g L^{-1} in shake flasks with a working volume of 50 mL, containing 0.5 g L^{-1} $(NH_4)_2HPO_4$ and 1 g L^{-1} yeast extract. Ethanol yield is expressed as percentage of the theoretical based on all available hexose sugars (i.e., glucose, galactose and mannose) in the hydrolysates obtained from steam-pretreated spruce, poplar and a 50:50 blend.

The inhibitory effect has been found to be even more pronounced on genetically engineered pentose-fermenting yeasts [149], which should be used to also ferment the significant fraction of xylose released from poplar. The addition of low amounts of poplar to spruce might even be a beneficial strategy to lower the concentration of inhibitors (e.g., acetic acid), thereby reducing the need for an expensive chemical detoxification process. These results suggest that the concurrent utilization of poplar and spruce would be constrained more by the performance of the yeast, than the efficacy of steam pretreatment, and the production process could prove to be sufficiently robust to allow the addition of low amounts of hardwood (10-20% on dry basis) in a softwood-to-ethanol process.

5. Concluding remarks

Large-scale production of second-generation bioethanol has recently started, and an intensive learning period is expected in the near future. However, the success of future bioethanol plants lies in being able to produce ethanol efficiently from a wide range of feedstocks. In the case of forest biomass, this requires the utilization of more heterogeneous and complex forest harvest residues such as early thinnings, tops and branches, for ethanol production, besides the well-defined white wood chips.

The studies presented in this thesis have focused on the utilization of softwoods as raw materials, with special attention devoted to the pretreatment step of the ethanol production process. In the first part, the effects of bark, which is expected to make up a considerable fraction of forest harvest residues, on the typical softwood-to-ethanol conversion process were investigated. The second part briefly touched upon the inclusion of short-rotation hardwood species to the utilization of established long-rotation softwoods for ethanol production, through a preliminary study on the steam pretreatment of a mixture of poplar and spruce.

Acid-catalyzed steam pretreatment, one of the typical pretreatments used for processing lignocellulosic biomass such as softwood chips, was found not to be effective for the pretreatment of bark, which contains high amounts of extractives. Some of the water-soluble extractives of bark precipitated during acid-catalyzed steam pretreatment, which contributed significantly to the impaired enzymatic hydrolysability of steam-pretreated bark. The removal of water-soluble extractives by simple hot-water extraction prior to steam pretreatment was, therefore, found to be beneficial.

Techno-economic evaluation showed decreasing profitability with increasing bark content of the raw material, due to the lower amount of carbohydrates available in bark and its poorer enzymatic digestibility. Improved conversion of cellulose and hemicellulose to monomeric sugars would thus be necessary if bark-containing forest harvest residues are to be used for ethanol production.

No synergistic or antagonistic interactions were observed in the steam pretreatment of a mixture of spruce and poplar, as linear interpolation gave accurate results (to within 4%) based on the results of the pure species, for overall sugar recovery after steam pretreatment and/or enzymatic hydrolysis of the 50:50 blend. However, the high amount of acetic acid liberated during steam pretreatment of poplar had a detrimental

effect on the fermentability of the hydrolysate, which in turn limits the amount of hardwood that a softwood-to-ethanol process could endure.

The work presented in this thesis was concerned with ethanol production via the sugar platform as part of an energy-driven biorefinery concept. In this context, it is apparent that several key aspects of the process need to be further developed and optimized before forest harvest residues can be used in this bioconversion process. For instance, fine-tuning of the pretreatment process and the pretreatment conditions to suit the feedstock composition is needed to ensure maximum sugar recovery. Although the production of co-products, apart from bioethanol, could change the optimal configuration of the process, the effective fractionation of lignocellulosic biomass into its main constituents (cellulose, hemicellulose, lignin, and extractives) is essential. The valorization of each biomass component to provide higher-value products could improve the overall process economics, which in turn could promote the utilization of forest biomass in a biorefinery process for the production of biofuels and biochemicals.

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Paper I

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Influence of bark on fuel ethanol production from steam-pretreated spruce

Balázs Frankó*, Mats Galbe and Ola Wallberg

Abstract

Background: Bark and bark-containing forest residues have the potential for utilization as raw material for lignocellulosic ethanol production due to their abundance and low cost. However, the different physical properties and chemical composition of bark compared to the conventionally used wood chips may influence the spruce-to-ethanol bioconversion process. This study assesses the impact of bark on the overall bioconversion in two process configurations, separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF), utilizing steam-pretreated spruce bark and wood mixtures.

Results: Mixtures of different proportions of spruce bark and wood chips were subjected to SO_2-catalyzed steam pretreatment at 210°C for five minutes, which has been shown to be effective for the pretreatment of spruce wood chips. The final ethanol concentration was the highest without bark and decreased significantly with increasing proportions of bark in both process configurations. However, this decrease cannot be attributed solely to the lower availability of the carbohydrates in mixtures containing bark, as the ethanol yield also decreased, from 85 to 59% in SSF and from 84 to 51% in SHF, as the mass fraction of bark was increased from 0 to 100%.

Conclusions: The results show that it was significantly more difficult to hydrolyse spruce bark to monomeric sugars than wood chips. Bark had an adverse effect on the whole bioconversion process due to its lower enzymatic hydrolyzability. On the other hand, bark inclusion had no detrimental effect on the fermentability of steam-pretreated spruce wood and bark mixtures. It was also observed that lower amounts of inhibitory degradation products were formed during the steam pretreatment of spruce bark than during the steam pretreatment of wood chips.

Keywords: Ethanol, Softwood, Spruce, Bark, Steam pretreatment, SSF, SHF

Background

The driving force behind the exploitation of renewable energy sources is the necessity to shift from a fossil-fuel dependent economy to one based on renewable resources. Biomass can be used to efficiently produce renewable liquid or gaseous fuels, providing alternatives to fossil fuels [1]. Ethanol, for instance, is already being produced from sugar and starch crops, and is used worldwide, as a consequence of policies promoting ethanol production [2-5]. However, the controversy of ethanol production from sugar and starch crops (first-generation ethanol) has led to the development of technologies employing lignocellulosic biomass as raw material [6-8].

The utilization of lignocellulosic biomass to produce ethanol provides an alternative to sugar and starch crops. However, the additional cost of the lignocellulosic ethanol production process, resulting from the necessity of pretreatment and enzymes for lignocellulosic biomass refining, has led to limited profitability in comparison with sugar- and starch-based ethanol production [9]. Thus, there is a need to further decrease the production cost of lignocellulosic ethanol in order for it to become competitive with the first-generation ethanol [10].

One possible means of cost reduction is to utilize abundant low-cost lignocellulosic raw materials such as bark [11]. Bark and bark-containing forest residues could serve as a potential feedstock for ethanol production, although bark is considered to be an inferior raw material to higher-value wood chips due to its composition. Compared with spruce wood chips, spruce bark has a lower content of carbohydrates, and contains significantly more extractives and ash [12]. The lower content of carbohydrates results in decreased sugar concentration after hydrolysis, and thus

* Correspondence: Balazs.Franko@chemeng.lth.se
Department of Chemical Engineering, Lund University, PO Box 124, Getingevägen 60, Lund SE-221 00, Sweden

a lower ethanol concentration after fermentation. The pre-hydrolyzates obtained from pretreated bark can contain elevated amounts of water-soluble extractives and polyphenols, which may have inhibitory effects on fermenting microorganisms and cellulolytic enzymes [13-15]. Therefore, the combined utilization of bark and wood chips for ethanol production might pose an even greater challenge than the use of softwood chips, which is already demanding. In this case, not only the inherent recalcitrance of the material must be overcome, but also the problems resulting from the significant difference in the physical properties and chemical composition of bark and wood chips. However, if these limitations could be overcome, then abundantly available, low-value forestry residues could be exploited, and existing spruce-to-ethanol production processes could be simplified by not having to debark the material. Ultimately, it is likely that forestry residues available for bioethanol production will contain varying amounts of bark, and it is therefore important to investigate the effects of bark on production processes previously optimized for wood chips only.

Previous studies have mainly focused on the effects of bark on fermentability. Boussaid *et al.* found that including bark led to decreased fermentability of pre-hydrolyzates prepared under low-severity pretreatment conditions, while pre-hydrolyzates prepared under higher severity conditions could be fermented comparably well to ethanol when 9% bark was included [13]. Similar results were obtained by Robinson *et al.*, who found that up to 30% bark, on a dry basis, had negligible effects on the fermentability of pre-hydrolyzates obtained from SO_2-catalyzed steam-exploded Douglas fir whitewood [16]. Although the enzymatic hydrolyzability of bark has been investigated previously [17], there are few reports on the influence of bark on enzymatic hydrolysis and the overall ethanol yield of the ethanol bioconversion process when performed at higher water-insoluble solids (WIS) content [18]. Enzymatic hydrolysis and fermentation must be performed at a higher WIS loading in order to increase the ethanol concentration after fermentation, which is essential to reduce the cost of distillation and thus the marginal production cost production [19].

The aim of the present study was to assess the feasibility of utilizing bark together with spruce wood chips for the fermentative conversion of biomass to ethanol at 10% WIS content, using a commercial enzyme cocktail to hydrolyse the steam-pretreated material, and an industrial strain of *Saccharomyces cerevisiae* as the fermenting microorganism. Spruce bark mixed with wood chips at different ratios ranging from 0 to 100% were subjected to SO_2-catalyzed steam pretreatment at 210°C for five minutes, which has previously been shown to be effective for spruce wood chips [20]. The effects of bark inclusion on the spruce-to-ethanol bioconversion process were investigated by performing separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) of the steam-pretreated wood and bark mixtures.

Results and discussion

Steam pretreatment of wood and bark mixtures

The composition of the spruce wood chips and bark is given in Table 1. The content of carbohydrates, with the exception of arabinan, was lower in the bark than in the wood chips. The amount of hexose sugars available in the bark feedstock was only about half of that in the wood feedstock. Even though only neutral carbohydrates were analyzed, spruce bark also contains a significant amount of other polysaccharides, such as pectin [21,22]. The bark contained significantly more extractives and ash than were found in the wood chips. The content of acid-soluble lignin may be slightly overestimated for both feedstocks due to possible interference from other non-lignin components [23].

The compositions of the water-insoluble solid fractions of the steam-pretreated materials were determined, and the results are presented in Table 2. As a result of the lower glucan content of the bark feedstock, the glucan content of the steam-pretreated mixtures decreased with increasing proportions of bark (Table 2). Detectable amounts of sugars originating from the hemicellulose were observed in the solid fraction after pretreatment of 100% bark. Steam pretreatment dissolved most of the hemicelluloses in all other steam-pretreated materials. This could be due to the higher recalcitrance of bark or the possible neutralization of the SO_2 added to the raw material by the higher ash and extractives content of the bark feedstock. This indicates that bark requires more SO_2 or higher severity steam pretreatment conditions to dissolve hemicellulose to the same extent as in wood chips. Interestingly, the acid-insoluble lignin content of

Table 1 Composition of the spruce wood and bark feedstocks as a percentage of dry matter (% of DM)

Feedstock	Carbohydrates						Lignin		Extractives[c]	Ash
	Glucan	Xylan	Galactan	Arabinan	Mannan	Sum of neutral carbohydrates	ASL[a]	AIL[b]		
Wood	42.4	5.6	1.3	0.7	9.9	59.9	7.6	26.2	3.3	0.2
Bark	23.1	3.6	0.8	4.3	3.4	35.2	13.3	20.5	28.2	2.2

[a]Acid-soluble lignin.
[b]Acid-insoluble lignin.
[c]Water and ethanol extractives.

Table 2 Composition of the water-insoluble fraction of steam-pretreated wood and bark mixtures as a percentage of dry matter (% of DM)

Bark content (% of DM)	Carbohydrates						Lignin		Ash
	Glucan	Xylan	Galactan	Arabinan	Mannan	Sum of neutral carbohydrates	ASL[a]	AIL[b]	
0	54.5	n.d.	n.d.	n.d.	0.7	55.2	3.1	42.9	0.3
10	51.8	n.d.	n.d.	n.d.	0.5	52.3	2.9	43.1	0.5
30	48.4	n.d.	n.d.	n.d.	0.5	48.9	2.7	46.3	0.8
50	44.5	1.4	n.d.	n.d.	0.4	46.3	2.8	48.5	1.2
100	36.7	2.1	0.3	0.2	0.8	40.1	3.6	48.8	2.5

[a]Acid-soluble lignin.
[b]Acid-insoluble lignin.
n.d. Not detected.

the water-insoluble fractions increased as the bark content increased in the feedstock (Table 2), although the acid-insoluble lignin content was found to be higher in the wood chips than in the bark (Table 1). This has been found in previous studies reporting that bark contains water-soluble phenolic compounds that can condense with lignin during acid-catalyzed steam pretreatment and appear as acid-insoluble lignin in the subsequent compositional analysis [18,24]. This phenomenon could play a significant part in the structural changes of the bark during the acid-catalyzed steam pretreatment carried out at the optimal condition for the wood chips.

The composition of liquid fractions obtained from the steam-pretreated materials is presented in Table 3. As a consequence of the lower carbohydrate content of bark, the concentration of total sugars (expressed in monomeric form) in the liquid fraction decreased with increasing proportions of bark, with the exception of arabinose due to the higher arabinan content of bark. As it can be seen in Figure 1, the addition of bark seemed to have a negligible effect on the overall recovery of glucose (94 to 96% for all steam-pretreated materials) and mannose (80 to 82% for all steam-pretreated materials) in the steam pretreatment step, although a lower proportion of sugars was dissolved in monomeric form in the liquid fraction as bark was added. This confirms the hypothesis that the pretreatment conditions previously found to be optimal for spruce wood chips may be too mild to overcome the recalcitrance of bark.

The amount of degradation products generated during steam pretreatment is a function of the severity of the pretreatment and the concentration of carbohydrates present in the feedstock. Therefore, the most likely explanations of the decreasing concentrations (Table 3), and also the amount of all measured inhibitors expressed as grams of inhibitors formed per 100 g dry raw material (Figure 2) with increasing bark inclusion, are the lower carbohydrate content and the higher recalcitrance of the bark feedstock.

Fermentability of pre-hydrolyzates

In order to evaluate the effect of the inhibitory compounds present in the liquid fractions obtained from the steam-pretreated materials, the pre-hydrolyzates were subjected to a fermentation test. As shown in Figure 3, all the pre-hydrolyzates showed similar high degrees of fermentability to ethanol, and the ethanol yields were in the same range as in the control solution, which contained only pure monomeric glucose and mannose.

Although bark contained more extractives than wood chips (Table 1), these had no detrimental effect on the fermentability of the pre-hydrolyzates. Boussaid *et al.* reported decreased fermentability of pre-hydrolyzates following low-severity steam pretreatment of bark-containing softwood, and attributed it to extractives, which were recovered in the water-soluble fraction [13]. However, additional acid hydrolysis of the pre-hydrolyzates increased the ethanol yield significantly, and they believed this to be due to the condensation and polymerization of water-soluble phenolic compounds. Moreover, they also showed that pre-hydrolyzates obtained from steam pretreatment at a

Table 3 Composition of the liquid fraction of the steam-pretreated wood and bark mixtures

Bark content (% of dry matter)	Total sugars (expressed as monomeric sugar) (g/L)					Inhibitors (g/L)				
	Glucose	Xylose	Galactose	Arabinose	Mannose	HMF[a]	Furfural	Formic acid	Acetic acid	Levulinic acid
0	26.5	9.7	5.1	2.7	21.7	2.0	1.3	1.5	1.9	1.0
10	21.8	9.2	4.0	3.3	20.7	1.8	1.1	1.3	1.7	0.7
30	20.1	9.2	4.2	4.8	17.8	1.4	0.8	0.7	1.4	0.4
50	18.3	8.0	4.1	5.9	13.7	1.0	0.7	0.4	1.2	0.3
100	18.2	6.0	4.3	10.0	5.6	0.4	0.4	0.4	0.6	0.2

[a]5-Hydroxymethylfurfural.

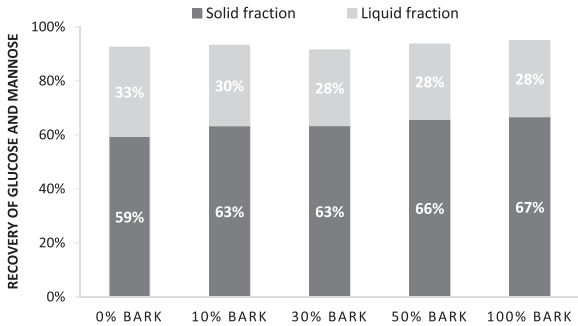

Figure 1 Recovery of glucose and mannose after steam pretreatment of wood and bark mixtures. Recovery expressed as percentage of the theoretical based on the glucan and mannan content of the raw materials.

severity factor above three (as defined by Overend *et al.* [25]) fermented well to ethanol despite the inclusion of 9% bark. This indicates that the relatively high severity of the steam pretreatment applied in our study also made the condensation and polymerization of water-soluble phenolic compounds possible, hence possibly eliminating their inhibitory effect. These results support previous findings that lower amounts of inhibitory compounds are generally formed during steam pretreatment of softwood bark than in the case of wood chips, and as a consequence, prehydrolyzates of steam-pretreated spruce bark can be fermented comparably well into ethanol [12,16,18].

Separate hydrolysis and fermentation of wood and bark mixtures

Previous studies have mostly been devoted to the investigation of the fermentability of steam-pretreated softwood bark [12,13,16,17], while little has been reported on the effect of including bark on the enzymatic hydrolyzability of steam-pretreated softwoods. Furthermore, most previous studies have been performed at lower WIS contents [17,18]. The implementation of enzymatic hydrolysis and fermentation, either separately or simultaneously, at a higher WIS content is driven by the possible energy savings in the distillation step [26]. In order to investigate the effects of bark on enzymatic hydrolyzability and fermentability of the hydrolyzed pretreated materials separately, SHF experiments were performed at 10% WIS content. The major advantage of SHF is that hydrolysis and fermentation can be carried out under their optimal conditions. However, SHF in general requires longer overall process time in comparison with SSF [27], and the end-product inhibition of enzymes by glucose and cellobiose results in a reduced rate of saccharification [28].

Figure 4 shows the concentration profiles for glucose during the enzymatic hydrolysis of steam-pretreated wood and bark mixtures and final glucose yields. The highest final glucose concentration (80.6 g/L) was achieved with

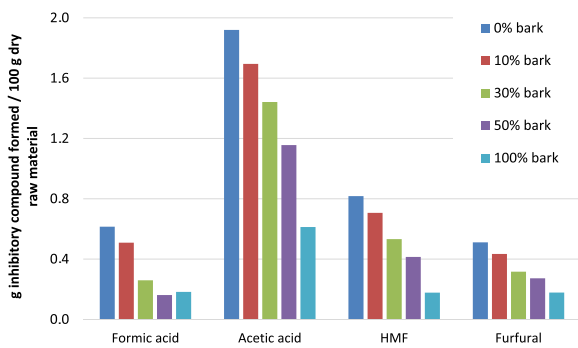

Figure 2 The amounts of inhibitory compounds formed during steam pretreatment of wood and bark mixtures. HMF, 5-hydroxymethylfurfural.

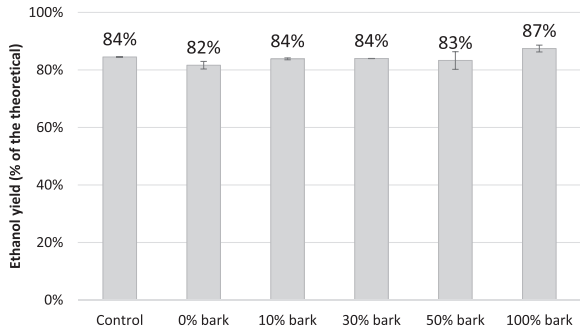

Figure 3 Ethanol yields of pre-hydrolyzate fermentation. Ethanol yields expressed as percentage of the theoretical based on glucose and mannose. Fermentation was performed with *Saccharomyces cerevisiae* at 30°C, pH 5.5 for 24 hours.

no bark addition, and decreased to 34 g/L with 100% bark. Furthermore, the highest glucose yield (90% based on all available glucose) and the highest rate of hydrolysis were obtained when no bark was added. The significant extractives content of bark can be a possible explanation for the lower glucose yields with bark addition. Phenolic compounds, either in monomeric or oligomeric form, deriving from bark can have inhibitory effect on the enzymes [14,15,29], which makes the enzymatic hydrolysis of bark more challenging than in the case of spruce wood chips. As can be seen in Figure 4, the glucose yield decreased significantly with increasing proportions of bark, and reached 53% at 100% bark. However, the same trend of decreasing hydrolyzability was also observed with increasing proportions of bark at 5% WIS loading, both on whole slurry and on washed fibre (data not shown). This suggests that the underlying reason for the lower enzymatic hydrolyzability is not the inhibitory effect of phenolic compounds in the liquid phase, but rather the higher recalcitrance or the structural changes caused by the relocation of bark extractives during acid-catalyzed steam pretreatment.

Previous studies have reported higher sugar yields from bark-containing softwood or bark as the only raw material. However, these enzymatic hydrolysis experiments were performed at a lower WIS content. For instance, Kemppainen *et al.* reported between 70 and 80% yields, depending on the steam pretreatment conditions, after 48 hours of enzymatic hydrolysis of spruce bark at 1% dry matter content [18]. A glucose yield of 79.6% was obtained by Robinson *et al.* in the enzymatic hydrolysis of softwood containing bark pretreated with steam and an additional alkaline peroxide treatment [17]. Another possible way can be to use additional accessory enzymes in order to increase the yield of the enzymatic hydrolysis of bark. For instance, 24% improvement was observed in hydrolysis of spruce bark

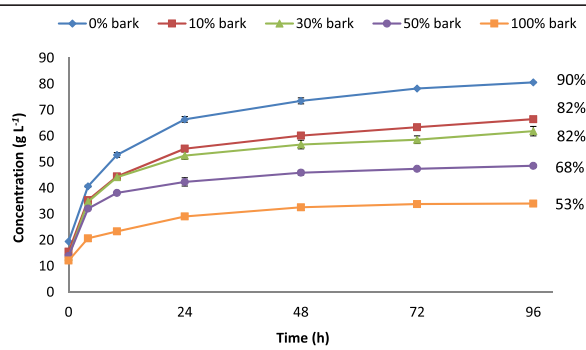

Figure 4 Concentration profiles for glucose during enzymatic hydrolysis and the final glucose yields. Enzymatic hydrolysis of steam-pretreated wood and bark mixtures was performed at 10% WIS loading, 45°C, pH 5 for 96 hours using 20 FPU/g WIS Cellic CTec3. Final glucose yields expressed as percentage of the theoretical based on all available glucose. FPU, filter paper unit; WIS, water-insoluble solids.

after 48 hours when pectinase enzymes were used as a supplementation to the cellulolytic enzymes [18]. Due to the presence of pectin in bark, the pectinase activity of the applied enzyme cocktail may also significantly affect the hydrolysis yields.

After the removal of the solid fraction of enzymatically hydrolyzed mixtures by filtration, the liquid fractions were subjected to fermentation. Figure 5 shows the concentration profiles for total hexose sugars and the ethanol produced during fermentation, together with the final ethanol yields (percentage of the theoretical maximum stoichiometric yield based on all available hexose sugars). The ethanol concentrations reached their maximum values after 24 hours in all cases. However, the final ethanol concentrations were considerably lower as the amount of bark was increased, due to the lower concentration of hexose sugars available for ethanol production in the enzymatically hydrolyzed bark-containing mixtures. As can be seen in Figure 5, almost all hexose sugars were consumed by the yeast and fermented into ethanol. The ethanol yields were largely unaffected by the addition of bark, and were in the same range; above 90%.

Table 4A shows the final concentrations of the substrates and products, together with the ethanol yield and the initial volumetric ethanol productivity in the SHF experiments. The volumetric ethanol productivity during the first two hours increased with bark inclusion, from 1.7 g/L·h to 3.2 g/L·h as the bark content was increased from 0 to 100%. A possible explanation for the higher initial volumetric ethanol productivity could be the lower concentration of 5-hydroxymethylfurfural (HMF) and furfural in the mixtures with higher bark content (Table 3). HMF and furfural are known to be inhibitory to yeast [30], and the fermentability of pre-hydrolyzates is significantly decreased with increasing concentrations of HMF and furfural. Taherzadeh *et al.* found that the rate of fermentation decreased considerably when the combined amount of HMF and furfural exceeded approximately 2 g/L [12]. In the present study, the HMF and furfural concentrations were highest when no bark was added to the spruce chips (1.6 g/L and 1.0 g/L, respectively), which were completely detoxified by the yeast in the first four hours of fermentation. As a consequence of the inhibitory effect of these degradation products, lower initial volumetric ethanol productivity was observed with the pretreated mixtures containing wood chips, than that for 100% bark, which contained the lowest concentrations of HMF and furfural.

The results of the SHF experiments showed that bark had no detrimental effects on the fermentability, which is in agreement with the results of the pre-hydrolyzates fermentation. However, the addition of bark has an adverse effect on enzymatic hydrolyzability, which limits the amount of ethanol that can be produced by the yeast in the bioconversion process.

Simultaneous saccharification and fermentation of wood and bark mixtures

In the SSF process configuration, enzymatic hydrolysis and fermentation are performed simultaneously in the same vessel, and the end-product inhibition during hydrolysis is minimized by the continuous conversion of glucose to ethanol by the fermenting microorganism [31]. However, enzymatic hydrolysis and fermentation are performed under sub-optimal conditions. In the present study, SSF was performed at 10% WIS content to assess the effect of including bark on both fermentability and enzymatic hydrolyzability. Figure 6 shows the concentration profiles for total hexose sugars and the

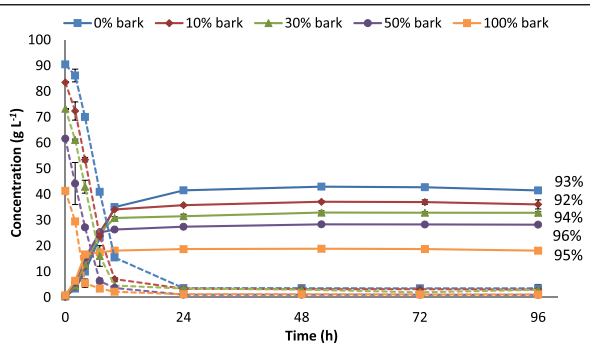

Figure 5 Concentration profiles for total hexose sugars (dashed lines) and ethanol (solid lines) during fermentation and the final ethanol yields. Fermentation of the supernatants obtained from the enzymatic hydrolysis of steam-pretreated wood and bark mixtures was performed at 10% WIS loading, 30°C, pH 5 for 96 hours using Ethanol Red yeast (5 g/L). Final ethanol yields expressed as percentage of the theoretical based on all available hexose sugars.

Table 4 Substrate, product and by-product concentrations and yields obtained from SHF (A) and SSF (B) experiments

A)						
	Bark content (% of DM)	0	10	30	50	100
Enzymatic hydrolysis step in SHF	Glucose concentration (g/L)	80.6 ± 0.1	66.5 ± 0.7	61.8 ± 1.8	48.5 ± 0.5	34.0 ± 0.5
	Glucose yield (% of the theoretical)	89.8 ± 0.3	81.6 ± 1.0	81.9 ± 3.3	67.9 ± 1.1	53.3 ± 0.9
Fermentation step in SHF	Total hexose sugar concentration (g/L)	3.5 ± 0	3.1 ± 0	2.9 ± 2.1	1.0 ± 0.7	1.1 ± 0
	Glycerol concentration (g/L)	5.2 ± 0	4.5 ± 0.1	4.2 ± 0.1	3.6 ± 0	3.0 ± 0.1
	Volumetric ethanol productivity[a] (g/L·h)	1.7 ± 0	2.0 ± 0	2.3 ± 1.2	2.8 ± 0.1	3.2 ± 0
	Ethanol concentration (g/L)	41.5 ± 0.8	36.1 ± 1.8	32.8 ± 0.4	28.2 ± 0.1	18.1 ± 0
	Ethanol yield (% of the theoretical)	93.1 ± 0.1	92.0 ± 0	93.8 ± 0.8	96.2 ± 0.9	95.0 ± 0.2
SHF	Overall ethanol yield (% of the theoretical)	83.6	75.1	76.8	65.3	50.7
B)						
	Bark content (% of DM)	0	10	30	50	100
SSF	Total hexose sugar concentration (g/L)	4.2 ± 0.3	1.9 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	3.3 ± 0.1
	Glycerol concentration (g/L)	4.8 ± 0.1	4.2 ± 0.2	3.8 ± 0.1	3.4 ± 0	3.6 ± 1.4
	Volumetric ethanol productivity[a] (g/L·h)	1.7 ± 0.1	1.8 ± 0.3	2.5 ± 0	2.9 ± 0.1	4.0 ± 0.1
	Ethanol concentration (g/L)	45.8 ± 0.8	39.3 ± 0.9	34.5 ± 0.4	29.4 ± 0.1	20.9 ± 0
	Overall ethanol yield (% of the theoretical)	85.4 ± 1.9	81.1 ± 2.3	77.5 ± 1.3	70.5 ± 0.4	59.3 ± 0.1

[a]Calculated for the first two hours.
SSF and SHF experiments of steam-pretreated wood and bark mixtures were performed at 10% WIS loading, pH 5 using Cellic CTec3 enzyme cocktail (20 FPU/g WIS) and an industrial *S. cerevisiae* strain, Ethanol Red (5 g/L). DM, dry matter; SHF, separate hydrolysis and fermentation; SSF, simultaneous saccharification and fermentation; WIS, water-insoluble solids.

ethanol produced during SSF, together with the ethanol yields obtained (percentage of the theoretical based on all available hexose sugars).

As can be seen in Figure 6, the highest final ethanol concentration (46 g/L) was obtained when no bark was added, and it decreased significantly with increasing additions of bark. However, this decrease cannot be attributed solely to the lower amount of carbohydrates available in the bark-containing mixtures, as the ethanol yield also decreased, from 85 to 59%, as the proportion of bark was increased from 0 to 100%.

As can be seen in Table 4B, the volumetric ethanol productivity during the first two hours of SSF increased from 1.7 to 4.0 g/L·h as the amount of bark was increased from 0 to 100%. Furthermore, accumulation of the hexose sugars was also observed during the same time period in all SSF experiments, with the exception of 100% bark, where no accumulation of hexose sugars

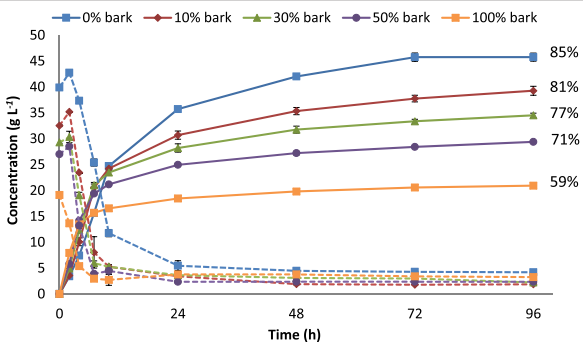

Figure 6 Concentration profiles for total hexose sugars (dashed lines) and ethanol (solid lines) during SSF and the final ethanol yields. SSF of the steam-pretreated wood and bark mixtures was performed at 10% WIS loading, 35°C, pH 5 for 96 hours using 5 g/L Ethanol Red yeast and 20 FPU/g WIS Cellic CTec3. Final ethanol yields expressed as percentage of the theoretical based on all available hexose sugars. FPU, filter paper unit; SSF, simultaneous saccharification and fermentation; WIS, water-insoluble solids.

occurred during SSF (Figure 6). Similarly to the SHF experiments, the lower concentrations of inhibitory compounds with increasing amounts of bark contributed to higher initial volumetric ethanol productivities than without bark. This indicates that the fermentability was not affected negatively by the inclusion of bark. This is also confirmed by that the addition of bark had no noticeable negative effects on the sugar utilization of the yeast in the SSF experiments (Figure 6). All the available glucose and mannose were consumed by the yeast, and only galactose was detected at low concentrations after 96 hours. Glycerol was the main by-product produced by the yeast in all cases (Table 4), and there was no significant difference in the yield of glycerol based on all available hexose sugars (approximately 4% in all SSF experiments).

The ethanol yield obtained in the SSF experiments with no bark was in the same range as reported for spruce chips in previous studies [32,33], while Kemppainen *et al.* reported an ethanol yield of 66.4% of the theoretical in SSF of sequentially hot-water extracted and steam-pretreated spruce bark [18]. This higher yield might be explained by the removal of the extractives from the bark prior steam pretreatment. Moreover, the six-hour prehydrolysis applied before SSF, and the possible structural differences between industrial bark and the freshly processed bark used in the present study, should not be neglected.

Comparing the two process configurations, it is apparent that SSF was superior to SHF in all cases, since SSF resulted in higher overall yields regardless of the bark content (Table 4). It is also evident that the decreased enzymatic hydrolyzability of bark is a decisive factor behind the declining ethanol yields, with increasing amounts of bark in both process configurations. Bark was found to be significantly more difficult to hydrolyse to monomeric sugars than wood chips. Lower amounts of monomeric sugars were recovered in the liquid fraction after steam pretreatment, and lower yields were observed in the enzymatic hydrolysis step with increasing bark content. Although, it appears that bark might require more severe steam pretreatment to overcome its inherent recalcitrance, the possible unfavorable structural changes in the steam-pretreated bark could also hamper the enzymatic hydrolysis. The relocation of extractives and bark lignin during the acid-catalyzed steam pretreatment might reduce the accessibility for the enzymes to cellulose, which can result in lower enzymatic hydrolyzability. Delignification methods might also be an alternative to achieve higher yields in enzymatic hydrolysis, and thus provide more sugars for ethanol fermentation; however, chemical delignification operations are expensive and would constitute an additional burden on the already sensitive economics of second-generation ethanol production [34]. Thus, further research is needed to improve the enzymatic hydrolyzability of bark in order to achieve higher yields at lower enzyme dosages.

Conclusions

The effect of including bark in the spruce-to-ethanol production process has been assessed. The results showed that adding bark had no detrimental effects on the fermentability of steam-pretreated spruce bark and wood mixtures, and it was observed that lower amounts of degradation products were formed during the steam pretreatment of spruce bark than spruce wood chips. However, the addition of bark had an adverse effect on the whole bioconversion process due to the low hydrolyzability of bark. This was reflected by the decreasing overall ethanol yield with increasing proportions of bark in both process configurations. SSF proved to be more efficient than SHF for all wood and bark mixtures, since this process configuration resulted in higher overall yields, regardless of bark content.

Materials and methods

Materials

Fresh spruce, *Picea abies*, was debarked and kindly provided by a local sawmill (ATA Timber Widtskövle AB, Everöd, Sweden), together with the bark fraction. The bark and the bark-free chipped wood were further chipped using a knife mill (Retsch GmbH, Haan, Germany) and sieved in order to obtain the fraction with a size range between 2 and 10 mm. The spruce had a dry matter content of 40%, while the bark had a somewhat lower dry matter content of 35%. The raw materials were stored in plastic bags at 4°C until used.

The enzyme preparation used was Cellic CTec3, kindly provided by Novozymes A/S (Bagsværd, Denmark). The yeast used in both the SSF and SHF experiments was Ethanol Red, kindly provided by Leaf Technologies (Marcq-en-Baroeul Cedex, France). The yeast used to determine the fermentability of the pre-hydrolyzates was prepared on an agar plate from ordinary baker's yeast, *Saccharomyces cerevisiae*, produced by Jästbolaget (Rotebo, Sweden). Vitahop, kindly provided by BetaTec (Schwabach, Germany), was used in the SSF and SHF experiments to avoid bacterial contamination. All chemicals used were of reagent grade quality.

Feedstock preparation and steam pretreatment

The bark and wood fractions were mixed to obtain batches containing 0, 10, 30, 50 and 100% bark on a dry weight basis. Each batch had a total dry weight of 700 g. The mixtures were impregnated with gaseous SO_2 (2.5% w/w, based on the water content of the mixtures) in tightly sealed plastic bags for 20 minutes at room temperature, and then subjected to steam pretreatment.

Steam pretreatment was performed at 210°C for five minutes in a 10 L reactor (Process- & Industriteknik AB, Kristianstad, Sweden), as described previously by Palmqvist *et al.* [35]. The pretreated materials were stored at 4°C before subsequent analysis and experiments.

Fermentation of pre-hydrolyzates

Yeast that was used to evaluate the fermentability of pre-hydrolyzates was aerobically cultivated. The inoculum culture was prepared by adding yeast cells, previously grown on a YPG agar plate (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose and 15 g/L agar) for three days at 30°C, to two 250 mL Erlenmeyer flasks, together with 70 mL of an aqueous solution containing 23.8 g/L glucose, 10.8 g/L $(NH_4)_2SO_4$, 5.0 g/L KH_2PO_4 and 1.1 g/L $MgSO_4{\cdot}7\ H_2O$. The solution also contained 14.4 mL/L trace element solution and 1.4 mL/L vitamin solution, prepared according to Taherzadeh *et al.* [36]. The pH was adjusted to pH 5 with 2.5 M NaOH solution. The Erlenmeyer flasks were sealed with cotton plugs and incubated at 30°C on a rotary shaker (Adolf Kühner AG, Basel, Switzerland) for 20 hours. Aerobic batch propagation was performed in a 2 L LABFORS fermentor (Infors AG, Bottmingen, Switzerland) at 30°C for 24 hours, with a working volume of 1 L. Propagation was initiated by the addition of 140 mL inoculum cultures to an autoclaved medium containing 20 g/L glucose, 22.5 g/L $(NH_4)_2SO_4$, 10.5 g/L KH_2PO_4 and 2.2 g/L $MgSO_4{\cdot}7\ H_2O$, 60.0 g/L trace element solution and 6.0 g/L vitamin solution. The aeration rate was 1 L/min, corresponding to 1 vvm (gas volume flow per unit working volume per minute). The stirrer speed was 700 rpm and the pH was maintained at pH 5 with 2.5 M NaOH solution. The dissolved oxygen concentration was monitored continuously with an O_2-sensor (Mettler-Toledo GmbH, Urdorf, Switzerland). When all the sugars had been consumed as indicated by the O_2-sensor, cultivation was stopped and the cells were harvested by centrifugation in 700 mL bottles at 3,600 × g for 10 minutes. The supernatant was discarded and the dry matter content of the harvested cells was determined. The time between cell harvesting and the initialization of the fermentation tests was less than two hours.

Fermentation tests were carried out on the pre-hydrolyzates to assess their fermentability and the extent of inhibition by the compounds formed during steam pretreatment. Pre-hydrolyzates were obtained from the steam-pretreated materials by vacuum filtration using grade five filter paper (Munktell Filter AB, Falun, Sweden). The pre-hydrolyzates were then diluted with deionized water to obtain an equivalent solids concentration (the concentration of inhibitors corresponding to an SSF with a certain WIS load) corresponding to a WIS load of 10% mass fraction. The initial concentrations of fermentable sugars were adjusted to 30 g/L glucose and 20 g/L mannose in order to obtain comparable fermentation results. A reference solution was prepared with the same sugar concentrations to serve as a control. Fermentation was performed anaerobically on a rotary shaker in shake flasks with a working volume of 100 mL, containing 0.5 g/L $(NH_4)_2HPO_4$, 0.025 g/L $MgSO_4{\cdot}7\ H_2O$ and 0.2 mL/L Vitahop. Fermentation tests were conducted at 30°C and pH 5.5 for 24 hours with a yeast concentration of 5 g/L. The fermentation experiments were performed in duplicate.

Separate hydrolysis and fermentation

Enzymatic hydrolysis of the whole pretreated slurry was performed in 2 L LABFORS bioreactors with a working weight of 1.2 kg. A WIS load of 10% mass fraction and Cellic CTec3 enzyme cocktail at a load of 20 FPU/g WIS based on the final weight, were applied. The hydrolysis experiments were performed at 45°C, with a stirring rate of 400 rpm, at pH 5 maintained with 2.5 M NaOH solution. After 96 hours of enzymatic hydrolysis the supernatants were separated by vacuum filtration using grade five filter paper (Munktell Filter AB). The supernatants obtained from the duplicates were mixed and stored at −20°C prior to fermentation.

Fermentation of the supernatant was performed in 2 L LABFORS bioreactors with a working weight of 0.55 kg. Ethanol Red yeast was added at a dry weight concentration of 5 g/L based on the final weight. The supernatant was supplemented with $(NH_4)_2HPO_4$ solution at a concentration of 0.5 g/L and 0.125 mL/L Vitahop. Fermentation was carried out at 30°C, with a stirring rate of 250 rpm for 96 hours, at pH 5 maintained with 2.5 M NaOH solution. All experiments were performed in duplicate.

Simultaneous saccharification and fermentation

The SSF experiments using the whole pretreated slurry were performed in sterilized 2 L LABFORS bioreactors with a working weight of 1 kg. A WIS load of 10% mass fraction, the Cellic CTec3 enzyme cocktail at a load of 20 FPU/g WIS and Ethanol Red yeast at a dry weight concentration of 5 g/L based on the final amount, were applied. The experiments were carried out at 35°C, with a stirring rate of 400 rpm for 96 hours, at pH 5 maintained with 2.5 M NaOH solution. The SSF media were supplemented with $(NH_4)_2HPO_4$ solution at a concentration of 0.5 g/L and 0.125 mL/L Vitahop. All experiments were performed in duplicate.

Analysis

The total solids content of biomass materials and total dissolved solids content of liquid samples were determined according to the National Renewable Energy Laboratory (NREL) standardized laboratory analytical procedure [37].

The structural carbohydrates, lignin, extractives and ash content of the solid fractions and the composition of the liquid fractions were determined according to NREL standardized laboratory analytical procedures [38-41].

All samples obtained from experiments or compositional analysis were centrifuged in 2 mL Eppendorf tubes at 16,000 × g for 10 minutes. The supernatant was filtered using 0.2 μm syringe filters (GVS Filter Technology Inc., Indiana, United States), and filtered samples were stored at −20°C prior to high-performance liquid chromatography (HPLC) analysis. Sugars, ethanol, organic acids and other by-products were analyzed using a Shimadzu LC-20 AD HPLC system equipped with a Shimadzu RID 10A refractive index detector (Shimadzu Corporation, Kyoto, Japan). Monomeric sugars were quantified with isocratic ion-exchange chromatography using an Aminex HPX-87P column with a De-Ashing Bio-Rad micro-guard column at 85°C (both from Bio-Rad Laboratories, Hercules, California, United States) using reagent grade water as the mobile phase at a flow rate of 0.5 mL/min. Ethanol, organic acids and other by-products were determined using an Aminex HPX-87H chromatography column with a Cation-H Bio-Rad micro-guard column at 50°C (Bio-Rad Laboratories, Hercules, California, United States), with a mobile phase of 5 mM sulfuric acid at a flow rate of 0.5 mL/min.

Yield calculations

The glucose yield in the enzymatic hydrolysis experiments was calculated on the basis of total available glucose in the liquid and the solid fraction of the steam-pretreated materials. The theoretical amount of glucose released during enzymatic hydrolysis is 1.11 times the amount of glucan in the solid fraction of the steam-pretreated materials (due to the addition of water in hydrolysis). The ethanol yield is expressed as a percentage of the theoretical stoichiometric ethanol yield (0.51 g/g), based on total available hexose sugars, namely glucose, mannose and galactose, in the solid and/or the liquid fraction of the steam-pretreated materials.

Abbreviations

AIL: Acid-insoluble lignin; ASL: Acid-soluble lignin; DM: Dry matter; FPU: Filter paper unit; HMF: Hydroxymethylfurfural; HPLC: High-performance liquid chromatography; NREL: National Renewable Energy Laboratory; SHF: Separate hydrolysis and fermentation; SSF: Simultaneous saccharification and fermentation; WIS: Water-insoluble solids; YPG: Yeast peptone glucose.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

BF, MG and OW designed and coordinated the study. BF carried out the experiments, analyzed the results and prepared the manuscript. MG and OW reviewed the manuscript. All authors read and approved the final manuscript.

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Paper II

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Bioethanol production from forestry residues: A comparative techno-economic analysis

Balázs Frankó, Mats Galbe, Ola Wallberg *

Department of Chemical Engineering, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden

HIGHLIGHTS

- A proposed cellulosic ethanol biorefinery in Sweden was simulated with Aspen Plus.
- Forestry residues with different bark contents were evaluated as raw materials.
- The bark content negatively influenced the minimum ethanol selling price.
- Sensitivity analyses were performed to assess the influence of raw material cost.

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ABSTRACT

A techno-economic analysis was conducted to assess the feasibility of using forestry residues with different bark contents for bioethanol production. A proposed cellulosic ethanol biorefinery in Sweden was simulated with Aspen Plus. The plant was assumed to convert different forestry assortments (sawdust and shavings, fuel logs, early thinnings, tops and branches, hog fuel and pulpwood) to ethanol, pellets, biogas and electricity. The intention was not to obtain absolute ethanol production costs for future facilities, but to assess and compare the future potential of utilizing different forestry residues for bioethanol production. The same plant design and operating conditions were assumed in all cases, and the effect of including bark on the whole conversion process, especially how it influenced the ethanol production cost, was studied. While the energy efficiency (not including district heating) obtained for the whole process was between 67 and 69% regardless of the raw material used, the ethanol production cost differed considerably; the minimum ethanol selling price ranging from 0.77 to 1.52 USD/L. Under the basic assumptions, all the forestry residues apart from sawdust and shavings exhibited a negative net present value at current market prices. The profitability decreased with increasing bark content of the raw material. Sensitivity analyses showed that, at current market prices, the utilization of bark-containing forestry residues will not provide significant cost improvement compared with pulpwood unless the conversion of cellulose and hemicellulose to monomeric sugars is improved.

1. Introduction

Biomass energy, or bioenergy, is considered to be an important source of renewable energy in mitigating greenhouse gas emissions and replacing fossil fuels [1]. The use of biomass residues, such as forestry residues, is strongly advocated under European Union (EU) legislation in order to help achieve the climate and energy targets of the EU for 2020 and beyond [2,3]. Forestry residues represent a potentially large source of lignocellulosic biomass, which can be used to produce bioenergy in the form of electricity, heat and liquid transportation fuels [4,5]. For instance, bioenergy from forest and agricultural residues accounts for most of the renewable fuel in Sweden, where the bioenergy use in 2013 was around 129 TWh, corresponding to 22–23% of the total national energy consumption [6]. Furthermore, the Swedish Forest Agency estimates that the recovery of forest harvest residues can be further increased without negatively affecting the environment [7]. Consequently, considering that softwoods are one of the major

Abbreviations: AD, anaerobic digestion; CHP, combined heat and power; COD, chemical oxygen demand; DM, dry matter; FPU, filter paper unit; HHV, higher heating value; LHV, lower heating value; MESP, minimum ethanol selling price; NPV, net present value; NREL, National Renewable Energy Laboratory; SSF, simultaneous saccharification and fermentation; WIS, water-insoluble solids.

* Corresponding author.
E-mail addresses: Balazs.Franko@chemeng.lth.se (B. Frankó), Mats.Galbe@chemeng.lth.se (M. Galbe), Ola.Wallberg@chemeng.lth.se (O. Wallberg).

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lignocellulosic feedstocks in the uppermost northern hemisphere, forest harvest residues constitute an abundant, sustainable supply of biomass for bioenergy production in geographical areas such as Scandinavia and the Pacific Northwest [8].

While forest bioenergy is already a feasible choice for large-scale heat and power production [9], the utilization of forestry residues for the production of liquid biofuels, such as ethanol, is hindered by economic and technical challenges [10–12]. Softwoods are generally considered to be the most recalcitrant lignocellulosic feedstock for biochemical conversion to ethanol, primarily due to their structure and high lignin content [13]. As a result, particular attention must be paid to the process steps associated with the breakdown of the biomass by pretreatment and enzymatic hydrolysis. It has been shown that more severe pretreatment conditions [14], relatively high enzyme dosage [15] and/or a delignification step [16] are needed to overcome the inherent recalcitrance of softwoods and provide a reasonable yield of monomeric sugars for the subsequent fermentation step. Furthermore, the potentially broad heterogeneity of the incoming biomass and the presence of bark make the utilization of forest harvest residues for ethanol bioconversion even more challenging.

Forestry residues include the by-products of pulp- and sawmills (sawdust and shavings; bark) and forest harvest residues from logging operations (tops and branches; nonmerchantable fuel logs), which can contain significant amounts of bark. The chemical composition and structure of bark differ significantly from those of wood [17]. Bark contains considerably less carbohydrates, but more extractives and ash [18]. These physical and chemical properties can influence the ethanol production process and its feasibility in various ways. For instance, the high content of inorganics in bark may partially neutralize the acid used for impregnation prior to pretreatment [19]. The condensation reaction of extractives during pretreatment can lead to structural changes that impair the enzymatic hydrolysis by possibly reducing the accessibility of cellulose [20], while phenolic compounds and other extractives liberated may inhibit the enzymes [21] and the fermenting microorganism [22]. In addition, the amount of ethanol that can be produced per dry metric ton of bark is lower than for wood due to the lower content of carbohydrates in bark. As a consequence, bark is generally not considered a favorable source of fermentable sugars. Although the aforementioned factors might not be as pronounced for forest harvest residues as for bark only, the theoretical ethanol potential and the overall ethanol yield are strongly influenced by the bark content of forest residues [23]. Since debarking of logging residues may be technically difficult or uneconomic, the influence of including bark must be investigated more thoroughly.

As was shown by Stephen et al. [24], the economic viability of bioenergy options, including bioethanol production, is very sensitive to changes in the type of feedstock, as the feedstock accounts for a significant part of the production cost [25,26]. Besides that the bark content of forestry residues significantly influences the softwood-to-ethanol bioconversion process, the market price of various forestry assortments also varies considerably based on their typical end use. For instance, hog fuel from debarking operations, composed mostly of bark, might be competitive with debarked whole roundwood due to its lower price [27]. In previous techno-economic evaluations of bioethanol production from softwood, processes consisting of SO_2-catalyzed steam pretreatment followed by enzymatic hydrolysis and fermentation, performed either simultaneously or separately, have been studied from several aspects [28–31]. However, the effect of including bark in the feedstock on the ethanol production cost to our knowledge has not been investigated.

This study was therefore carried out to evaluate the feasibility of utilizing forestry residues with different bark contents for bioethanol production and focused on determining the effect of bark content on the production process and the ethanol production costs. The intention was not to calculate absolute ethanol production costs for future facilities, but to assess and compare the future potential of utilizing different forestry residues for ethanol production in terms of economic performance within the context of the wood-to-ethanol bioconversion process. Overall, the attained results will help understand how the bark content of the raw materials influences the economic viability of bioethanol production from different forestry assortments.

2. Methods

The feasibility of utilizing forestry residues with different bark contents for bioethanol production was assessed by comparing the cost of production through a techno-economic analysis based on process simulation and economic evaluation of ethanol production from 6 different forestry assortments. Flowsheets were implemented and simulated in the commercial software Aspen Plus version 8.2 (Aspen Technology Inc., Massachusetts, USA) to perform rigorous thermodynamic calculations for mass and energy balances. The capital and operation costs were estimated using Aspen Process Economic Analyzer and vendors' quotations. These data were then imported and used in an Excel spreadsheet to calculate the overall investment cost and ethanol production cost, expressed as the minimum ethanol selling price (MESP), for each forestry residue.

2.1. Process simulations

The model used in this study is an updated and modified version of the model developed and previously described by Sassner et al. [31], Wingren et al. [28] and Joelsson et al. [32]. NRTL-HOC was selected in Aspen Plus as the standard method for all simulations. It was complemented with the STEAMNBS model that was used in the steam cycle in the heat and power production stage. The physical properties of the lignocellulosic biomass components, such as cellulose and lignin, and other complex components, such as yeast and enzymes, were taken from the National Renewable Energy Laboratory (NREL) database for biofuel components [33].

The energy recovery, based on the lower heating values (LHVs) calculated in Aspen Plus, was defined as the energy output in the products (ethanol, pellets, biogas, electricity and carbon dioxide) divided by the energy input, comprising the raw material, molasses and enzymes.

2.2. Conceptual design

An overview of the assumed design of the ethanol production process, which was the same in all cases regardless of the forestry residue utilized, is shown in Fig. 1, while more detailed flow diagrams of the main parts of the process have also been added to the Supplementary materials. As each process step has been described in detail previously [28,31,32,34], only a brief summary will be provided here, focusing mainly on the slight modifications made.

The proposed bioethanol plant was assumed to be located in Sweden, with the capacity to process 200,000 dry metric ton of raw material annually, being operated for 8000 h per year. It was assumed that the forestry residues are transported to the plant by truck and stored in a stack before being fed to the pretreatment area. The biomass was first impregnated with sulfur dioxide (0.015 kg SO_2/kg dry material) and then preheated to 95 °C by direct injection of low-pressure secondary steam prior to steam pretreatment. Steam pretreatment was modeled as a continuous

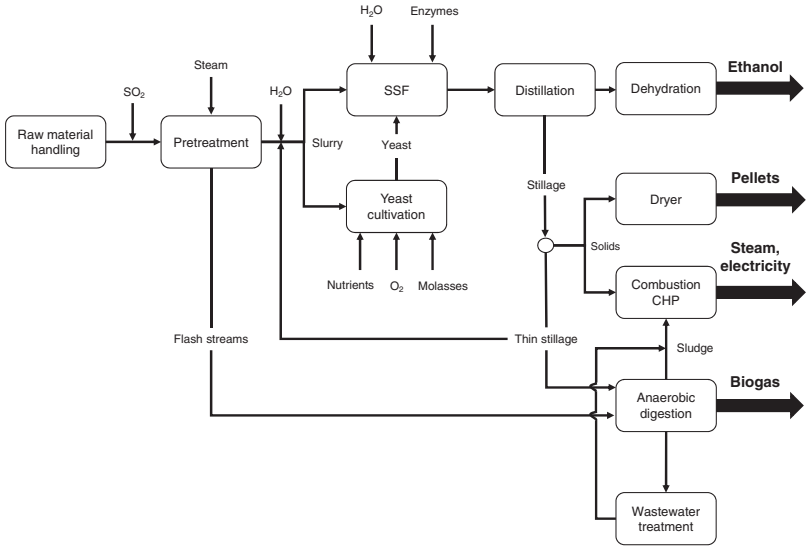

Fig. 1. Simplified process overview for the proposed ethanol plant utilizing different assortments of forestry residues as raw material.

reactor heated to 205 °C by injecting saturated steam at 20 bar. Heat losses were assumed to be 10% of the adiabatic heat demand. The pretreated material was flashed in two pressure reduction steps (at 4 and 1 bar), and the flash vapors obtained were condensed to heat other streams in the facility. Fresh water was added to the pretreated slurry to adjust its water-insoluble solids (WIS) content to 18 wt% before a liquid stream for yeast cultivation was separated in a filter press. Yeast, *Saccharomyces cerevisiae*, was produced in a yeast cultivation fermentor, while a commercial cellulolytic enzyme cocktail was purchased. The yeast was cultivated on hydrolysate supplemented with molasses, and the pH adjusted with NH_3. The amount of molasses added differed depending on the raw material, as the amount of sugar released during pretreatment varied. Biomass conversion of 0.4 g biomass/g fermentable sugars was assumed in the yeast cultivation step.

Prior to simultaneous saccharification and fermentation (SSF), fresh water and part of the thin stillage were added to the pretreated slurry to further adjust its WIS content to 12 wt%. The recirculation of the thin stillage was set to reduce the amount of fresh water required in the SSF step by a maximum of 50% [35]. SSF was assumed to be carried out at a WIS load of 12% by mass at 35 °C for 96 h, 3 g dry yeast/L, and an enzyme dosage of 15 FPU (filter paper units)/g WIS. Conversion factors for both steam pretreatment and SSF were based on results obtained from experimental work described previously by Frankó et al. [23].

The product was recovered using distillation and molecular sieve adsorption to obtain pure (99.8 wt%) ethanol from the fermentation broth leaving the SSF fermentor. The distillation unit used to concentrate ethanol to 92.5 wt% consisted of two parallel stripper columns (25 trays each; with a Murphree efficiency of 50%) and a rectifier (35 trays; with a Murphree efficiency of 70%), which were heat integrated by operating them at different pressures. Top stage pressures were 3, 1.25 and 0.3 bar, respectively. Ethanol recovery was designed to be 99.5% in each column.

The stillage of the two stripper columns was separated in a filter press (where the retention of solid particles was assumed to be 95%), resulting in a solid fraction with a dry matter (DM) content of around 50 wt% and a thin stillage with a DM content of 56 wt%. Part of the thin stillage was recycled to dilute the pretreated slurry prior to SSF, and the rest was led to the wastewater treatment unit. The solid fraction was divided into two parts: the amount of solids required to meet the steam demand of the process was burnt in a steam boiler to generate fresh steam, while the excess solid residue was dried to produce pellets that can be sold as a solid fuel co-product. The drying step was modeled as a steam dryer working at 4 bar, with superheated steam as the drying medium. The secondary steam generated was used in other parts of the process.

The thin stillage together with the rectifier stillage and the condensed steam from the dryer and the pretreatment step were treated by anaerobic digestion (AD) in order to produce biogas. An average value of biogas production was calculated based on an assumed reduction in chemical oxygen demand (COD) of the substrate. Based on personal communication (Åsa Davidsson, personal communication, 2015) the assumed degradation factors during AD were set to: (i) 90% for easily digested compounds, such as monomeric sugars and organic acids; (ii) 50% for compounds that were assumed to require additional hydrolysis, such as polysaccharides and degradation products from steam pretreatment; and (iii) 0% for other materials that were considered inert or difficult to degrade, such as lignin and extractives. Methane and sludge productions were set to 0.25 kg methane/kg COD removed and 0.03 kg DM/kg chemical oxygen demand fed, respectively. Sludge produced during AD was pressed to 30% DM, and then sent for incineration.

A combined heat and power (CHP) plant operating on process streams from the ethanol production process was assumed to be co-located with the ethanol facility. Steam and electricity are

generated by combusting part of the solid fraction separated from the stillage and the sludge obtained from AD in a boiler. The superheated steam generated (90 bar, 470 °C) was allowed to expand to 4 bar through a high-pressure turbine system. However, part of the steam was withdrawn at 20 bar for pretreatment and drying. District heating was not included in the cases, but the excess electricity produced was assumed to be sold to the grid. The isentropic and mechanical efficiencies of the turbines were set to 90% and 97%, respectively.

2.3. Feedstocks

The following forestry residues were included in the study: sawdust and shavings, fuel logs (non-merchantable wood, i.e., decayed or damaged logs), logging residues (tops and branches, early thinnings) and hog fuel (residues from debarking) as well as pulpwood as a reference case. The compositions of the forestry assortments, given in Table 1, were obtained by the linear combination of the composition of the bark and spruce samples previously determined by Frankó et al. [23]. Proportional normalization of the feedstock composition was performed to satisfy mass balance constraints within the process model.

The overall lower heating values (LHV) of the raw materials with different bark content, obtained from the weighted sum of each compound's LHV, were in accordance with literature values for logging residues, bark and sawdust [36]. The DM contents of the raw materials were assumed to be 45% in all cases.

The cost of the different forestry residues delivered to the plant was assumed to be between 20.66 and 31.29 USD/MWh, based on statistics provided by the Swedish Forest Agency [37] and the Forestry Research Institute of Sweden [38]. An additional 1.48 USD/MWh (~7.38 USD/dry metric ton) was included in the raw material cost of fuel logs and pulpwood to account for debarking [39].

2.4. Economic calculations

The economic evaluation consisted of four main parts: capital cost estimation, operating cost estimation, revenue summary, and calculations of the net present value (NPV) and MESP. The same methodology as described by Sassner et al. [31], Wingren et al. [28] and Joelsson et al. [32] was used.

Fixed capital investment costs were estimated either with Aspen Process Economic Analyzer, based on the results (mass and energy balances) obtained from Aspen Plus simulations, or from vendors' quotations updated according to the price index for 2012. The costs for the following equipment were estimated based on vendors' quotations; boiler, dryer and pelletizing equipment, molecular sieves, filter presses, anaerobic digestion and pretreatment units. The variable operating costs, for example, for chemicals, enzymes, utilities, etc., together with the assumed revenues from ethanol and co-products are summarized in Table 2. The costs of chemicals have been slightly altered from the references or the Indicative Chemical Prices to reflect that biorefineries are large consumer of chemicals.

The fixed operational cost included insurance, maintenance, working capital and labor. The plant was assumed to be operated by a total of 28 employees. 88,556 USD per person per year, including social security contributions, was assumed as a Swedish average wage for workers. The cost of working capital was determined according to recommendations in the literature [44], and was assumed to be equivalent to an interest rate of 11%. Ethanol, biogas, electricity and heat were produced in all cases, but heat was not considered to be a source of income. The prices of all raw materials and products were assumed to be subject to the same rate of inflation. All prices were adjusted to inflation and currency exchange rates to 2012 US dollars (USD).

Economic analysis was performed for an "n^{th} plant" (mature technology) to obtain an NPV that provides a measure of the investment value. The MESP was also calculated in each case by assigning a value of zero to the NPV. The NPV of each case was calculated using the expression:

$$NPV = -I_0 + \sum_{n=1}^{n} \frac{CF_n}{(1+r_d)^n},$$

where I_0 is the capital cost, CF is the cash flow, r_d is the discount rate of 11% and n is the lifetime in years, which was set to 20. Cash flow was calculated by deducting the sum of fixed and variable operational costs from the sum of revenues from ethanol and other co-products. The MESP can thus be defined as the price required (at the factory level, without sales taxes) for a zero NPV for each raw material when the cash flows are discounted at 11%, considering 20 years of investment period.

Table 1
Properties, composition and cost of the different forestry residues utilized as raw material.

Case Raw material	A Sawdust and shavings	B Fuel logs (debarked)	C Fuel logs	D Early thinnings	E Tops and branches	F Hog fuel	G Pulpwood (debarked)
DM content (wt% wet basis)	45	45	45	45	45	45	45
Bark content (wt% of DM)	0	10 (0[a])	10	20	30	80	10 (0[a])
Composition (wt% of DM)							
Glucan	42.8	41.4	41.4	40.0	38.5	31.4	41.4
Xylan	5.6	5.4	5.4	5.2	5.1	4.2	5.4
Galactan	1.5	1.4	1.4	1.4	1.4	1.2	1.4
Arabinan	0.9	1.2	1.2	1.5	1.8	3.3	1.2
Mannan	10.1	9.4	9.4	8.7	8.0	4.5	9.4
Lignin	34.3	34.2	34.2	34.2	34.1	33.9	34.2
Extractives	3.3	5.3	5.3	7.2	9.2	18.9	5.3
Ash	0.3	0.6	0.6	0.8	1.1	2.3	0.6
Acetate	1.2	1.1	1.1	1.0	0.8	0.3	1.1
Ethanol potential (L/kg DM)[b]	0.35	0.31	0.34	0.33	0.31	0.24	0.31
Ethanol yield (% of theoretical)[d]	85	85	81	79	77	64	85
Cost of raw material (USD/MWh)	22.88	28.78[c]	28.78	27.30	25.83	20.66	31.29[c]
Lower heating value (MJ/kg DM)	18.38	18.38	18.41	18.43	18.45	18.58	18.38

[a] Assumed to contain 10% bark; debarked at the ethanol plant and bark used for pellet production.
[b] Based on C6 sugar content of the raw materials subjected to ethanol production.
[c] Additional cost of debarking assumed to be 1.48 USD/MWh.
[d] Theoretical ethanol yield in the SSF step based on the C6 sugar content of the steam-pretreated materials.

Table 2
Input (chemical and utility costs per unit) and output (revenues per unit) values used in economic calculations.

	Value	Unit
Input		
Chemicals		
SO_2 [40]	0.22	USD/kg
NH_3 (25 wt%)	0.30	USD/kg
$(NH_4)_2HPO_4$	0.22	USD/kg
$MgSO_4$ [40]	0.65	USD/kg
Antifoam [41]	2.95	USD/kg
CO_2 [42]	0.004	USD/kg
Molasses	0.15	USD/kg
Enzymes	2.07	USD/FPU $\times 10^6$
Utilities		
Cooling water [43]	0.02	USD/m^3
Process water [43]	0.21	USD/m^3
Output		
Ethanol	0.96	USD/L
Pellets	29.52	USD/MWh
Biogas	51.66[a]	USD/MWh
Electricity	88.56[b]	USD/MWh

[a] The average price of unrefined biogas, i.e., not upgraded and pressurized for use as vehicle gas.
[b] The total price of electricity, including both the selling price (59.04 USD/MWh) and the income from green electricity certificate (29.52 USD/MWh).

3. Results and discussion

In this study, we have assessed the feasibility of utilizing forestry residues with different bark contents for ethanol production, by comparing the cost of production through techno-economic analysis. The investigated assortments can be classified according to their typical end uses and qualities as follows: by-products from pulp- and sawmills, such as sawdust and shavings (Case A) and hog fuel (Case F), as well as forest harvest residues in the form of whole non-merchantable fuel logs, either debarked (Case B) or not (Case C), chipped early thinnings (Case D) and tree tops and branches (Case E). Pulpwood (Case G) was also considered as a possible raw material for ethanol production as a reference case. The DM and bark content of these different forestry residues were pegged at a specific value, which may appear unrealistic in the operation of a large-scale ethanol production plant due to the inherent variations in the properties of incoming biomass. However, this simplification was necessary in order to assess the influence of bark content, as the moisture content of the feedstock (as well as the chip size) can also influence the enzymatic hydrolysability of the raw material [45]. Assuming the same plant design and operating conditions in all cases allowed us to study the effect of bark content on the whole conversion process, and thereby the variation in ethanol production cost utilizing forestry residues containing different amounts of bark.

3.1. Mass and energy flows

Data for some of the key streams involved in the ethanol production process are listed in Table 3. For the proposed process with an intake of 200,000 metric ton of dry raw material annually, sawdust and shavings (Case A) resulted in the highest ethanol production of 5.8 metric ton/h, which corresponds to 58,500 m^3/year. Ethanol production decreased significantly as the bark content of the raw material increased, and only 2.5 metric ton/h ethanol was produced from hog fuel, which corresponds to 24,900 m^3/year. However, the severe conditions required in the acid-catalyzed steam pretreatment for efficient release of sugars might be detrimental to the hog fuel. Results published by Kemppainen et al. [20] indicate that acid-catalyzed steam pretreatment of spruce bark reduces the hydrolysability of the biomass due to unfavorable condensation of phenolic compounds. Therefore, it is important to consider that employing different pretreatment conditions or forms of pretreatment for bark might result in better yields, and thus higher ethanol production. The ethanol concentration in the SSF broth varied in the different cases from 2.1 to 5.3 wt%, which corresponds to 20.9 to 52.5 g/L. Concurrent with the decrease in ethanol production, the production of co-products, such as pellets and excess electricity, increased with increasing bark content of the raw material (Table 3). The electricity produced in the process (based on the steam needed) was always sufficient to cover the need and provided a surplus. Although the production of excess heat for district heating can be a viable option in Sweden [32], it was not included as a source of income in this study.

Despite the same raw material input, there were slight differences in the input of other materials depending on the forestry assortment used (Table 3). For instance, as the liquid phase of the steam-pretreated materials contained less sugars with increasing bark content of the raw material, the need for the addition of molasses increased to cultivate the same amount of yeast for the fermentation step. The enzyme requirement was the same in all cases, except Cases B and G, where only the debarked wood fraction was used for ethanol production resulting in a lower enzyme consumption in relation to the raw material input (the fuel logs and pulpwood were assumed to be debarked at the ethanol plant prior to steam pretreatment and the bark used for pellet production directly). From a production process point of view, there was no difference between debarked fuel logs (Case B) and debarked pulpwood (Case G), and these two cases thus had the same input and output values.

3.2. Comparison of energy efficiencies and total heat and power demands

The overall energy efficiency for the whole process was between 67 and 69% in all cases (Fig. 2). In contrast, the energy input recovered in the form of ethanol was only 13–31%, which emphasizes the importance of co-products in an energy-efficient process. Although the overall energy recovery of the process was essentially unaffected by the kind of forestry residue used, the ratio of ethanol to pellets produced changed considerably as the bark content of the raw material increased, as can also be seen in Table 3. The decrease in ethanol yield was compensated for by an increase in the amount of pellets produced. Thus, the energy output in the form of ethanol decreased as the bark content of the raw material increased, and more energy was recovered as pellets. This shift was also reflected in the increasing total energy demand with respect to the produced ethanol (Table 3). Energy demands previously reported were in the range of 1019 MJ/L ethanol [28,32,46], which is comparable with the results obtained from raw materials with lower bark content.

3.3. Economics

Although the overall energy efficiency of the process varied only slightly with the different raw materials, the same energy efficiency did not translate into the same economic performance. The ethanol production cost, expressed as MESP in Fig. 3, differed considerably with the kind of forestry residue, from 0.77 USD/L ethanol to 1.52 USD/L ethanol. These values are in agreement with those found in previous studies on softwood, where MESP values ranged from 0.68 to 0.89 USD/L ethanol for different scales and configurations [32], and Stephen et al. [27] estimated the ethanol production cost for a 50 ML/y softwood facility to be 0.98 USD/L ethanol (converted and recalculated

Table 3
The main mass flows in the production of ethanol utilizing various forestry residues.

Case Raw material	A Sawdust and shavings	B Fuel logs (debarked)	C Fuel logs	D Early thinnings	E Tops and branches	F Hog fuel	G Pulpwood (debarked)
Input							
Raw material (metric ton/h)	25	25	25	25	25	25	25
Molasses (metric ton/h)	0.46	0.51	0.74	0.82	0.90	1.30	0.51
Enzymes (metric ton/h)	1.2	1.0	1.2	1.2	1.2	1.2	1.0
Products							
Ethanol (metric ton/h)	5.8	5.2	5.1	4.7	4.3	2.5	5.2
Methane (metric ton/h)	0.81	0.73	0.79	0.77	0.75	0.67	0.73
Pellets (metric ton/h)	6.6	7.9	7.6	8.2	8.9	12.0	7.9
Electricity produced (MW)	5.1	4.8	5.3	5.4	5.4	5.6	4.8
Electricity for sale (MW)	1.5	1.4	1.7	1.8	1.8	2.0	1.4
Carbon dioxide (metric ton/h)	6.1	5.5	5.5	5.1	4.7	2.9	5.5
Energy demand[a] (MJ/L ethanol)	13.5	14.4	15.7	17.3	19.0	34.6	14.4

[a] The energy demand was defined as the produced ethanol divided by the energy input (heat and electricity used in the process).

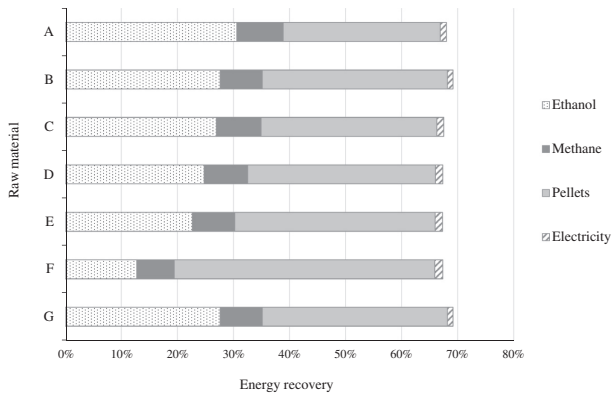

Fig. 2. Energy recovery of the production process. The energy recovery, based on the lower heating value (LHV), calculated in Aspen Plus, was defined as the energy output in the products divided by the energy input of the raw material, molasses and enzymes. The raw materials are: A: Sawdust and shavings; B: Debarked fuel logs; C: Fuel logs; D: Early thinnings; E: Tops and branches; F: Hog fuel; G: Debarked pulpwood.

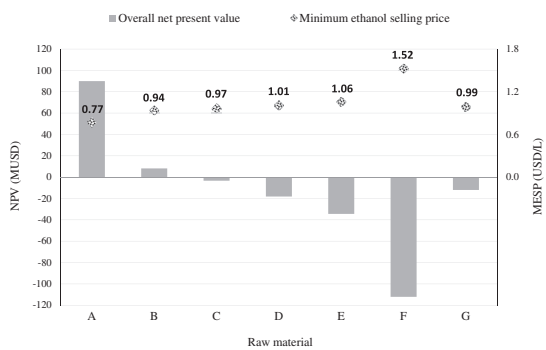

Fig. 3. Overall NPV and MESP for each raw material. The NPV was calculated at 11% discount rate for an investment period of 20 years. The raw materials are: A: Sawdust and shavings; B: Debarked fuel logs; C: Fuel logs; D: Early thinnings; E: Tops and branches; F: Hog fuel; G: Debarked pulpwood.

according to the consumer price index of the same year) at an 8% discount rate and an investment lifetime of 20 years.

The MESP was lowest for Case A (0.77 USD/L ethanol) as sawdust and shavings was the cheapest white wood feedstock (no bark) among the assortments investigated, exhibiting the highest ethanol potential and highest overall ethanol yield. Pulpwood, included as a reference, had a MESP of 0.99 USD/L ethanol. Hog fuel proved to be the least suitable feedstock for ethanol production in the process considered here, and its MESP, of 1.52 USD/L ethanol, was significantly higher than those of the other forestry residues investigated. Utilizing other forestry residues resulted in a MESP in the same range as that of pulpwood, and an increase in MESP with increasing bark content was observed at the current market prices. This means that lower feedstock costs would not offset the yield loss due to the impaired enzymatic hydrolysability with increasing bark content.

Comparison of the MESPs between fuel logs, debarked or not (Cases B and C), pinpoints the detrimental effect of bark on the ethanol production process (Fig. 3). Although the theoretical amount of ethanol produced per unit dry raw material (ethanol potential) is higher when the whole fuel logs together with the bark are included in the ethanol production process (Table 1), a lower MESP was obtained when only the debarked wood fraction was used for ethanol production, due to differences in the enzymatic hydrolysability of wood and bark (lower ethanol yield). A MESP of 0.94 USD/L ethanol was obtained for debarked fuel logs (Case B), while including the bark in the process (Case C) resulted in an increase in the MESP to 0.97 USD/L ethanol. This shows that the ethanol potential alone should not be used as the basis for assessing which feedstock is most suitable, and that the cost for debarking, which was assumed to be 1.48 USD/MWh (~7.38 USD/dry metric ton), was justified.

Stephen et al. [24] previously estimated a MESP of 0.86 USD/L ethanol and a significant 20 year net income loss when utilizing whole logs (roundwood) for ethanol production as a base case at a plant of the same scale (200,000 dry metric ton per year, assuming an 8% discount rate and a 20-year amortization period). Switching to any forestry residues resulted in improved economics compared to high-value roundwood. They also found that sawdust and shavings gave the greatest improvement as the cost of this feedstock had the lowest contribution to the ethanol production cost among the raw materials investigated, as was found in the present study. Forest harvest residues, pulp chips and hog fuel had the same, somewhat lower, positive impact on the ethanol production cost, as they assumed the same yield for these feedstocks. However, taking the effect of bark content on the conversion process into account enables a more detailed and accurate comparison between forestry residues with different bark contents. Thus, three conclusions can be drawn from the results in Fig. 3. Firstly, sawdust and shaving residues from sawmills are the most favorable forestry assortment for ethanol production at current market prices. Secondly, the utilization of bark-containing forestry residues will not lead to any significant cost improvement compared with pulpwood unless yield improvements are possible in the future. Lastly, it is not economically feasible to produce ethanol from hog fuel or bark only in the investigated process configuration. Although changing the pretreatment conditions or employing different kinds of pretreatment might increase the sugar conversion for bark, the use of bark extractives to produce high-value co-products would significantly decrease the production cost.

The profitability of the different cases was also evaluated in terms of the NPV. The results are presented in Fig. 3. A negative NPV was obtained for all cases except Case A (sawdust and shavings) and B (debarked fuel logs) at current market prices. As expected, the NPV correlates well with the MESP, and varied between 90.08 MUSD for sawdust and shavings and −112.1 MUSD for hog fuel. This clearly indicates that lignocellulosic ethanol from softwoods will have a difficult time competing with conventional fossil fuels or first-generation bioethanol at current market prices, regardless of the forestry assortment, as has been pointed out previously by Stephen et al. [27].

The cash flow NPV for each raw material was further broken down into operational costs and revenues, and are shown together with the total capital costs in Fig. 4 (a more detailed breakdown of costs and revenues is also available in the Supplementary material). The estimated total capital costs and the operational costs were in the same range, as the same plant design and operating conditions were assumed in all cases, and the main differences are found in the annual revenues from the product and co-products, and the cost of the raw material. Although the co-product revenues are important for the overall economics, it can be clearly seen from Fig. 4 that ethanol is the main product in terms of revenues; therefore, the overall ethanol yield has a high impact on the process economics.

3.4. Sensitivity analysis of raw material cost and enzymatic hydrolysability of forestry residues

The cost of forestry residues can vary considerably depending on the location, season, and the highly complex supply chain [47,48], as well as the changing demand from other industries competing for the same feedstock (i.e., heat and power or pellet production) [49,50]. Although the assessment of the effect of geographical location is outside the scope of this study, investigating the effect of bark content at different raw material costs could provide a broader picture of the feasibility of ethanol production from forestry residues. Similarly, assuming the same process ethanol yield for bark-containing forestry residues as for white wood, although this seems unrealistic at no additional cost, indicates the future potential of each raw material for the production of ethanol at certain market conditions. Sensitivity analyses were performed to evaluate the influence of the raw material cost alone (Fig. 5), and together with the effect of an improved process ethanol yield (Fig. 6), on the ethanol production cost for each raw material.

The results shown in Fig. 5A indicate the superiority of white wood sawdust and shavings as a feedstock for ethanol production (Case A) over a wide range of raw material prices. It is also clear that the production of ethanol from hog fuel is not feasible using the process configuration investigated here. Furthermore, as long as the gap between the cost of forest harvest residues and pulpwood is modest, the gain in utilizing lower-cost residues will become less pronounced (Cases B, C, and D) or will vanish completely (Case E), if the total sugar yield of bark-containing forest harvest residues is not improved. MESP changed linearly with the raw material cost for all cases (Fig. 5B), however the effect was more prominent with higher bark content. The choice of feedstock may be a single forestry assortment, or a combination of several, depending on availability and the current market prices, but using the process configuration investigated here, the bark content is a factor that should be considered in future investigations.

As the impaired conversion of cellulose and hemicellulose to monomeric sugars was the main reason for the higher MESP of bark-containing forestry residues, improvements in the pretreatment and enzymatic hydrolysis steps to achieve the same yields as for white wood feedstocks will be necessary to exploit the lower raw material costs. Fig. 6 shows the results of the sensitivity analysis of raw material costs for each raw material assuming the same enzymatic hydrolysis yield as in Case A. Comparison of the MESPs for Cases B and C (Fig. 6A) shows that producing ethanol from fuel logs without debarking will be more profitable if the enzymatic

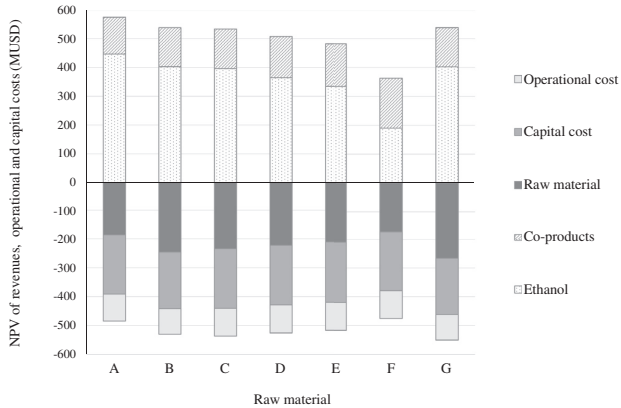

Fig. 4. NPV of revenues, operational and capital costs. The NPV of different revenues, operational and capital costs was calculated at a discount rate of 11% for an investment period of 20 years. The raw materials are: A: Sawdust and shavings; B: Debarked fuel logs; C: Fuel logs; D: Early thinnings; E: Tops and branches; F: Hog fuel; G: Debarked pulpwood.

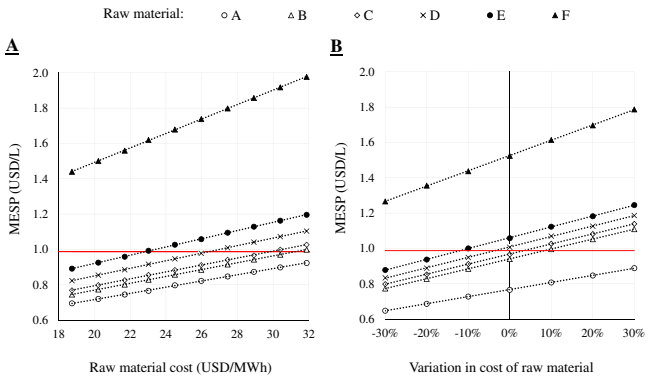

Fig. 5. Effect of variations in the cost of raw material (A: in absolute terms; B: in relative terms) on the MESP. The red line represents the MESP of pulpwood at the current market conditions (0.99 USD/L ethanol) as a reference. The raw materials are: A: Sawdust and shavings; B: Debarked fuel logs; C: Fuel logs; D: Early thinnings; E: Tops and branches; F: Hog fuel. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

hydrolysis yield can be maintained at that for white wood at no further cost. One possible way of improving the enzymatic hydrolysability may be the use of a feedstock-tailored enzyme cocktail, as both the structure and chemical composition of bark and wood differ. Similarly, the pretreatment method or conditions could have also been further tailored for bark to achieve higher conversion of cellulose and hemicellulose to monomeric sugars in enzymatic hydrolysis. By achieving the same process ethanol yield, all forestry residues would give lower MESPs at current market prices than pulpwood (Fig. 6B). Cases C, D and E resulted in the same MESP, and all exhibited positive NPVs (data not shown). The decreasing ethanol potential with increasing bark content of the feedstock did not undermine the economic production of ethanol with a bark content up to 30%, assuming the same enzymatic hydrolysis yield as for white wood. However, at higher bark content (Case F) the lower available sugar content of the raw material resulted in a higher MESP, even at the same sugar yield, and the 20 year NPV was still negative. It is also important to bear in mind that the same yield translates into different yield-improvement requirements for each forestry residue; being highest for hog fuel (40% improvement). This suggests that the production of ethanol from hog fuel will not be feasible in the future unless other high-value by-products can be extracted from the bark during the process.

4. Conclusions

The feasibility of ethanol production from different forestry residues containing different amounts of bark has been studied. While the raw materials differ in their theoretical ethanol potential and also in their overall ethanol yield depending on the bark content of the feedstock, their prices also vary considerably based on

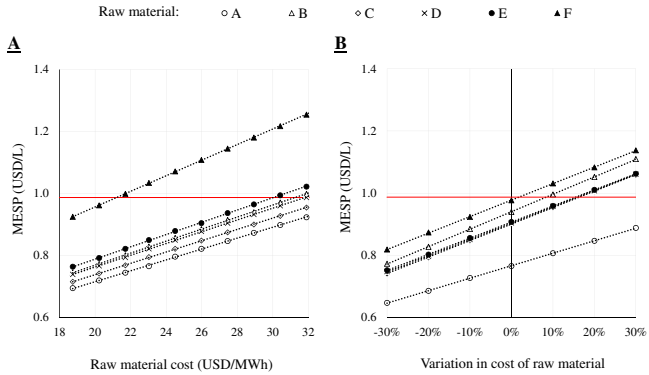

Fig. 6. Effect of variations in raw material costs (A: in absolute terms; B: in relative terms) on the MESP at the same enzymatic hydrolysis yield. The conversion factor for enzymatic hydrolysis during SSF was set to reach the ethanol yield of Case A (85% of the theoretical based on C6 sugars) for all cases. The red line represents the MESP of pulpwood at the current market conditions (0.99 USD/L ethanol) as a reference. The raw materials are: A: Sawdust and shavings; B: Debarked fuel logs; C: Fuel logs; D: Early thinnings; E: Tops and branches; F: Hog fuel. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

their typical end use and the current demand. Under the basic assumptions employed here, only sawdust and shavings, the cheapest white wood feedstock with the highest ethanol potential and overall ethanol yield, exhibited a considerably positive NPV at current market prices. The bark content of the feedstock was shown to affect the MESP, and the lower cost of bark-containing forestry residues could not offset the yield losses due to impaired enzymatic hydrolysis. However, if the conversion of cellulose and hemicellulose to monomeric sugars could be increased to the same level as for white wood feedstock, the NPV would be positive, and not even the decreasing ethanol potential with increasing bark content of the feedstock could undermine the economic production of ethanol from raw materials containing up to 30% bark. However, ethanol production from hog fuel, containing 80% bark, was found not to be feasible, as the NPV was still negative at the same sugar yield as for white wood.

Authors' contributions

BF, MG and OW designed and coordinated the study. BF carried out the simulations, analyzed the results and prepared the manuscript. MG and OW reviewed the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apenergy.2016.11.011.

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Paper III

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Removal of Water-Soluble Extractives Improves the Enzymatic Digestibility of Steam-Pretreated Softwood Barks

Balázs Frankó[1] · **Karin Carlqvist**[1] · **Mats Galbe**[1] · **Gunnar Lidén**[1] · **Ola Wallberg**[1]

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Abstract Softwood bark contains a large amounts of extractives—i.e., soluble lipophilic (such as resin acids) and hydrophilic components (phenolic compounds, stilbenes). The effects of the partial removal of water-soluble extractives before acid-catalyzed steam pretreatment on enzymatic digestibility were assessed for two softwood barks—Norway spruce and Scots pine. A simple hot water extraction step removed more than half of the water-soluble extractives from the barks, which improved the enzymatic digestibility of both steam-pretreated materials. This effect was more pronounced for the spruce than the pine bark, as evidenced by the 30 and 11% glucose yield improvement, respectively, in the enzymatic digestibility. Furthermore, analysis of the chemical composition showed that the acid-insoluble lignin content of the pretreated materials decreased when water-soluble extractives were removed prior to steam pretreatment. This can be explained by a decreased formation of water-insoluble "pseudo-lignin" from water-soluble bark phenolics during the acid-catalyzed pretreatment, which otherwise results in distorted lignin analysis and may also contribute to the impaired enzymatic

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✉ Balázs Frankó
Balazs.Franko@chemeng.lth.se

Karin Carlqvist
Karin.Carlqvist@chemeng.lth.se

Mats Galbe
Mats.Galbe@chemeng.lth.se

Gunnar Lidén
Gunnar.Liden@chemeng.lth.se

Ola Wallberg
Ola.Wallberg@chemeng.lth.se

[1] Department of Chemical Engineering, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden

digestibility of the barks. Thus, this study advocates the removal of extractives as the first step in the processing of bark or bark-rich materials in a sugar platform biorefinery.

Keywords Softwood · Bark · Extractives · Steam pretreatment · Enzymatic saccharification

Abbreviations

AIL	acid-insoluble lignin
ASL	acid-soluble lignin
DM	dry matter
EH	enzymatic hydrolysis
FPU	filter paper unit
HPLC	high-performance liquid chromatography
HWE	hot water extracted
NREL	National Renewable Energy Laboratory
STEX	steam explosion
WIS	water-insoluble solids

Introduction

Large amounts of bark are produced and are readily available worldwide at sawmills and pulp mills, as bark is removed from the logs during the manufacturing process. In Sweden, 80% of the total standing volume in productive forest lands comprises Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*) species, and an estimated 7.7 million m^3 of bark is produced annually, based on industrial wood consumption [42]. Today, most bark is combusted at mill sites or district heating plants to produce heat and electricity, although upgrading bark constituents to value-added fuels and chemicals could be beneficial economically and environmentally [30].

Although many of the constituents in wood also occur in bark, the chemical composition and structure of bark differ significantly from those of wood [35]. For example, bark has a lower cellulose and hemicellulose content but typically contains higher amounts of ash, non-cellulosic sugars, and extractives. One of the most disparate compositional characteristic of bark is its large amounts of extractives—i.e., soluble lipophilic (such as resin acids) and hydrophilic components (phenolic compounds, stilbenes) [35]. Extractives from Scots pine and Norway spruce barks have recently been characterized by Bianchi et al. [4, 5], Co et al. [8], Kemppainen et al. [15], Krogell et al. [17], Normand et al. [26], and Vernarecová et al. [48]. Extractives have both traditional (e.g., tannins in the leather industry) as well as a range of new uses—for instance, to produce adhesives, resins, and foams [9, 21]. Certain extractives also have pharmaceutical applications [22, 29].

The emergence of second-generation biofuels has increased the interest in assessing the suitability of softwood barks as a feedstock for renewable fuel production [6, 31, 32, 43]. Unfortunately, the structural complexity and heterogeneity of bark render it more difficult to utilize than wood fractions. Softwoods are generally considered the most recalcitrant type of lignocellulosic feedstock for the production of monomeric sugars by pretreatment and enzymatic hydrolysis [11, 19], but the breakdown of softwood barks to generate monomeric sugars from the carbohydrate part has proved to be even more challenging [10, 52].

The lower holocellulose content of bark inevitably lowers theoretical sugar/ethanol yields; furthermore, extractives can potentially have adverse effects on the biochemical conversion of

pretreated material. It has previously been shown that elevated amounts of soluble extractives can impair the hydrolytic performance of the enzymes [16, 50], whereas Kemppainen et al. [14] hypothesized that the condensation reactions of bark extractives during acid-catalyzed steam pretreatment, rendering the otherwise water-soluble extractives insoluble and altering the structure of the solid fraction, results in impaired enzymatic hydrolysis. Thus, more severe pretreatment of spruce bark—through the use of an acid catalyst or higher temperature—resulted in a material that elicited a lower hydrolysis rate and sugar yield when subjected to enzymatic hydrolysis. This finding has negative implications in cases where debarking proves to be technically difficult or uneconomic, but severe pretreatment would also be required to provide reasonable sugar yields (e.g., forest harvest residues). The removal of extractives has mainly been investigated with the idea to valorize the extracted compounds [15, 21], but it also generates a holocellulose-enriched residual and might also improve the enzymatic digestibility [14]. However, the effect of hot water extraction followed by acid-catalyzed steam pretreatment was not examined.

In this study, the effects of hot water extraction of softwood barks on subsequent acid-catalyzed steam pretreatment and enzymatic hydrolysis were assessed. The composition of the non-extracted and the hot water-extracted barks of Norway spruce and Scots pine, as well as the steam-pretreated materials, was analyzed. The enzymatic digestibilities of the barks were determined after steam pretreatment and acid-catalyzed steam pretreatment, with or without prior hot water extraction, to examine the possibility of utilizing water extraction to enhance sugar recovery. The results have implications for bark biorefineries and the pretreatment of softwood forest harvest residues—an abundant raw material that is expected to contain bark.

Methods

Raw Materials

The bark of Scots pine, *P. sylvestris*, was obtained from a tree that was sampled from long-term field trials in the Svartbergets experimental forests, Unit of Field-Based Research, Swedish University of Agricultural Sciences (SLU). The bark fraction was separated, chipped to approximately 100 × 10 mm, and stored in plastic bags at −20 °C. The bark of Norway spruce, *P. abies*, was kindly provided by a local sawmill (ATA Timber Widtskövle AB, Everöd, Sweden). The pine and spruce barks were chipped further using a knife mill (Retsch GmbH, Haan, Germany) and sieved to obtain a 2- to 10-mm fraction. Pine bark had a dry matter content of 44 wt%, whereas that of spruce bark was 33 wt%. The raw materials were stored in plastic buckets at 4 °C until use.

Hot Water Extraction

Water extraction of the raw materials was performed in a 60-L stirred tank in 2 consecutive steps: a 2-h cold water extraction at 6% consistency, followed by a 3-h hot water extraction after the primary extracts were drained and replaced with hot tap water (decreasing the consistency to 5.1%). The conditions of hot water extraction were chosen to facilitate effective removal of water-soluble extractives [15] but to avoid intense hemicellulose removal [17], as well as to provide comparability with previous results on hot water-extracted spruce bark [14, 15, 21]. The temperature was maintained at 25 °C during the cold water extraction, whereas

after being heated for 1 h, it was kept at 80 °C for 2 h in the hot water extraction step. The stirring rate (200 rpm) was the same in both steps. More thorough water extraction was performed by repeating the hot water extraction step three times. After extraction, the extracts were drained, and the extracted barks were collected. The extracted materials were filter-pressed at a maximum pressure of 5 bar using a hydraulic press (HP5M, Fischer Maschinenfabrik, Neuss, Germany) to adjust the DM content to 30–35 wt% prior to steam pretreatment.

Steam Pretreatment

Prior to steam pretreatment, each batch, with a total dry weight of 600 g, was impregnated with gaseous SO_2 (2.5 wt%, based on the moisture content of raw material) in tightly sealed plastic bags for 20 min at room temperature. Excess SO_2 was vented before the steam pretreatment by leaving the plastic bags open for 30 min. Steam pretreatment was performed in batches at 210 °C for 5 min in a 10-L reactor, per Palmqvist et al. [28]. Steam pretreatments were also conducted without SO_2 impregnation at 190 or 210 °C for 5 min. The pretreated slurries were stored at 4 °C prior to subsequent analysis and experiments.

Enzymatic Hydrolysis

Enzymatic hydrolysis of the pretreated slurry was performed in 2-L Labfors bioreactors (Infors AG, Bottmingen, Switzerland) with a working weight of 1 kg. A water-insoluble solids (WIS) load of 10% mass fraction and Cellic CTec3 enzyme cocktail, kindly provided by Novozymes A/S (Bagsvaerd, Denmark), at a load of 5% mass fraction of WIS, were applied, corresponding approximately to 9 FPU/g WIS. The hydrolysis experiments proceeded for 96 h at 45 °C, with a stirring rate of 400 rpm, at pH 5, maintained with 2.5 M NaOH solution. Samples from the hydrolysis liquid were separated in a centrifuge (Galaxy 16 DH, VWR International, Radnor, PA, USA), Germany) in 2-mL Eppendorf tubes at 16,000x*g* for 8 min. The supernatant was passed through 0.2-μm filters (GVS Filter Technology, Morecambe, UK) and stored at −20 °C. The enzymatic hydrolysis experiments were performed in duplicate.

Analyses

The total solids content of biomass materials and the total dissolved solids content of liquid samples were determined per the National Renewable Energy Laboratory (NREL) [36]. The WIS content of pretreated slurries was measured using the no-wash method of Weiss et al. [46]. The extractives, structural carbohydrates, lignin, and ash contents of the solid fractions and the composition of the liquid fractions were determined per NREL methods [37–40].

Sugars, organic acids, and other degradation products were quantified by high-performance liquid chromatography (HPLC) on a Shimadzu LC 20AD HPLC system that was equipped with a Shimadzu RID 10A refractive index detector (Shimadzu Corporation, Kyoto, Japan). Samples for sugar analysis were pH-adjusted to 5, if necessary, with $CaCO_3$ and centrifuged in 2-mL Eppendorf tubes (16,000×*g* for 5 min). All samples were passed through 0.2-μm filters (GVS Filter Technology) and stored at −20 °C until analysis. Sugars were analyzed on a Bio-Rad Aminex HPX-87P column with a De-Ashing Bio-Rad micro-guard column (Bio-Rad Laboratories, Hercules, CA, USA) at 85 °C using degassed deionized water as the eluent at a flow rate of 0.5 ml/min. Organic acids and other degradation products were analyzed on a Bio-

Rad Aminex HPX-87H chromatography column with a Cation-H Bio-Rad micro-guard column at 50 °C, with a mobile phase of 5 mM sulfuric acid at a flow rate of 0.5 mL/min.

Yield Calculation

The glucose yield in the enzymatic hydrolysis experiments was calculated, based on the total available glucose in the liquid and the solid fraction of the steam-pretreated materials per the following equation. The nomenclature for the equations is presented in Table 1.

$$Yield_{glucose} = \frac{c_g \times \frac{m}{\rho_L} \times (1-\mathrm{WIS})}{m \times \mathrm{WIS}_0 \times \varphi_{glucan} \times 1.11 + c_{total} \times \frac{m_{hydrolysate}}{\rho_{hydrolysate}}}$$

$$= \frac{monomeric\ glucose\ after\ 96\ \mathrm{h}\ enzymatic\ hydrolysis\ (g)}{glucose\ in\ the\ solid\ phase + glucose\ (monomeric + oligmeric)\ in\ the\ liquid\ phase\ of\ the\ pretreated\ material\ (g)}$$

The degree of enzymatic hydrolysis was calculated as:

$$Degree\ of\ \mathrm{EH} = \frac{c_g \times \frac{m}{\rho_L} \times (1-\mathrm{WIS}) - c_{g_0} \times \frac{m_{hydrolysate}}{\rho_{hydrolysate}} \times (1-\mathrm{WIS}_0)}{m \times \mathrm{WIS}_0 \times \varphi_{glucan} \times 1.11 + (c_{total} - c_{monomeric}) \times \frac{m_{hydrolysate}}{\rho_{hydrolysate}}}$$

$$= \frac{monomeric\ glucose\ released\ during\ enzymatic\ hydrolysis\ (g)}{glucose\ in\ the\ solid\ phase + oligomeric\ glucose\ in\ the\ liquid\ phase\ of\ the\ pretreated\ material\ (g)}$$

Results and Discussion

The digestibility of pretreated softwood barks has been reported to be rather low [10, 51]. One factor that has been suggested to contribute to this is the condensation of water-soluble phenolic compounds during acid-catalyzed steam pretreatment. These compounds remain in the fiber fraction—they are in fact analyzed as acid-insoluble lignin—and can reduce the accessibility to cellulose during enzymatic hydrolysis [14]. As a result, less severe pretreatment

Table 1 Nomenclature for parameters in the equations

c_g	Glucose concentration (g/L)
c_{g_0}	Initial glucose concentration (g/L)
WIS	Mass fraction of water-insoluble solids (%)
WIS_0	Initial mass fraction of water-insoluble solids (%)
m	Working weight of the reactor (g)
$m_{\mathrm{hydrolysate}}$	Weight of the liquid fraction of the pretreated material added (g)
ρ_L	Liquid density (g/L)
$\rho_{\mathrm{hydrolysate}}$	Liquid density of the liquid fraction of the pretreated material (g/L)
c_{total}	Total glucose (both monomeric and oligomeric forms) concentration in liquid fraction of the pretreated material (g/L)
$c_{\mathrm{monomeric}}$	Monomeric glucose concentration in liquid fraction of the pretreated material (g/L)
$\varphi_{\mathrm{glucan}}$	Mass fraction of glucan in the water-insoluble solids of the pretreated material (%)
1.11	Molecular ratio of glucose to glucan (180/162 = 1.11)

(i.e., without acid catalyst) was found to be beneficial for spruce bark. Alternatively, water-soluble extractives can be removed before pretreatment in order to avoid detrimental condensation reactions and enable pretreatment conditions that are sufficiently severe to break down softwood bark. In this study, this step was performed by hot water extraction of softwood barks, after which the extracted materials were subjected to acid-catalyzed steam pretreatment and enzymatic hydrolysis.

Removal of Water-Soluble Extractives

Hot water extraction was used to remove extractives of spruce and pine barks, and raw material analyses were performed before and after the hot water extraction to determine the total amount removed (Table 2). Spruce bark had a higher total extractives content (24.0%) than pine bark (19.4%), the primary difference between which was the content of water-soluble extractives—the ethanol-soluble extractives content of spruce bark was slightly higher than that of pine bark.

Extractives contents between studies should be compared with caution, even for the same species, because they also depend on age, felling season, storage conditions [4, 15], and extraction method [7]. A wide range of extractives content has consequently been reported for spruce and pine barks, ranging from 4.5 to 28.2% for spruce bark [10, 14, 24] versus 3.5 to 19.3% for pine bark [24, 27, 45]. The results of this study are consistent with the extractives content for spruce and pine barks using similar extraction schemes. For spruce bark, Frankó et al. [10] reported 28.2% of total (water- and ethanol-soluble) extractives, whereas Valentín et al. [45] obtained a 13.7% water-soluble extractives content for pine bark.

A major compositional difference between spruce and pine barks that this study noted, apart from the extractives content, was the considerably higher lignin content of pine bark. The total lignin content was 40.9% for pine bark, in contrast to 29.9% for spruce bark. These results are comparable with the reported values for pine (33.7 and 44.9%) and spruce barks (27.9%; 32.8 and 33.8%) [10, 14, 24, 45]. Spruce bark had higher glucan content than pine bark, whereas the contents of the other main carbohydrates were similar between spruce and pine barks. Accordingly, the total content of carbohydrates was higher in spruce versus pine bark. The proportion of C6 carbohydrates to total carbohydrates was nearly the same in both softwood barks (80 and 75%). Similar carbohydrate contents were also reported for spruce and pine barks by Miranda et al. [24]. The ash content was also comparable with the range in the literature [10, 14, 24, 33, 47].

The water extraction scheme removed more than half of the water-soluble extractives from spruce (57%) and pine bark (51%) (Table 2). Consequently, the levels of other bark constituents, such as carbohydrates, lignin, and ash, increased in hot water-extracted barks compared with the non-extracted raw materials. A variety of research approaches and analytical methods have been used to characterize hydrophilic extractives of softwood barks [4, 5, 15, 17, 21]. The extraction yields vary with different factors (e.g., extraction temperature, time, solid loading, particle size, etc.) but water extracts of softwood barks are mainly composed of condensed tannins, stilbene glucosides, and mono- and polysaccharides (e.g., pectic polysaccharides). The chemical composition of water extracts from spruce and pine barks, among other European softwood species, has been analyzed by Bianchi et al. [5]. Although the ratio of condensed tannins relative to total phenolic compounds was high in the water extracts for spruce and pine barks, Bianchi et al. [5] found that the proportion of total phenolic compounds was significantly lower in water extracts from pine bark versus spruce bark (13.0 and 34.1%, respectively).

Table 2 Composition of raw and the hot water-extracted (HWE) spruce (*Picea abies*) and pine (*Pinus sylvestris*) barks as a percentage of dry matter (% of DM)

Material	Carbohydrates						Lignin		Extractives		Ash
	Glucan	Xylan	Galactan	Arabinan	Mannan	Total	ASL[a]	AIL[b]	Water	Ethanol	
Spruce bark	25.2 ± 0.1	3.8 ± 0.0	2.4 ± 0.0	4.0 ± 0.1	2.3 ± 0.0	37.7	5.5 ± 0.2	24.4 ± 0.2	17.1 ± 0.1	6.9 ± 0.2	2.3 ± 0.1
HWE Spruce bark	27.6 ± 0.6	4.2 ± 0.1	2.6 ± 0.1	4.7 ± 0.2	2.5 ± 0.0	41.6	6.1 ± 0.1	28.1 ± 0.6	7.3 ± 0.5	6.5 ± 0.4	2.7 ± 0.1
3X-HWE Spruce bark[c]	27.2 ± 0.0	5.0 ± 0.0	3.3 ± 0.1	5.1 ± 0.1	2.5 ± 0.0	43.1	6.5 ± 0.1	30.7 ± 0.3	4.7 ± 0.2	6.3 ± 0.1	3.1 ± 0.0
Pine bark	20.0 ± 0.1	4.6 ± 0.0	3.0 ± 0.0	4.1 ± 0.0	3.2 ± 0.1	34.9	4.0 ± 0.1	36.9 ± 0.2	13.2 ± 0.1	6.2 ± 0.1	0.9 ± 0.0
HWE Pine bark	22.0 ± 0.2	5.1 ± 0.0	3.5 ± 0.0	4.9 ± 0.1	3.4 ± 0.1	38.9	4.5 ± 0.1	39.4 ± 0.7	6.4 ± 0.1	6.4 ± 0.2	1.1 ± 0.1

[a] Acid-soluble lignin

[b] Acid-insoluble lignin

[c] Hot water extraction performed three times

More thorough water extraction, performed by repeating the hot water extraction 3 times (3X-HWE), removed an additional 15% of the water-soluble extractives from spruce bark, but complete removal of extractives was not achieved. Even though hot water extraction can efficiently remove tannins from bark, condensed tannins cannot be completely extracted due to covalent bonds between the condensed tannins and the cellulose matrix [9, 13].

Steam Pretreatment

Steam pretreatment with SO_2 as the acid catalyst is considered a suitable pretreatment method for recalcitrant lignocellulosic feedstocks, such as softwood [12], and was chosen in the current study for the barks. The composition of the water-insoluble solids fractions of the steam-pretreated materials were determined (Table 3). As a result of its lower initial glucan content, the glucan content of steam-pretreated pine barks—non-extracted and hot water-extracted—was considerably lower than in spruce barks. Steam pretreatment removed most of the hemicelluloses in all steam-pretreated materials, but sugars that originated from the hemicellulose, primarily xylose and mannose, were still detected in the solid fraction of the pretreated slurries. No significant difference in holocellulose content was observed between non-extracted and hot water-extracted barks pretreated under the same conditions.

In contrast, the acid-insoluble lignin (AIL) content of the water-insoluble fractions was higher in steam-pretreated barks that were not hot water-extracted, regardless of species (Table 3), although the AIL content was originally lower in the non-extracted raw materials than in hot water-extracted barks (Table 2). The total lignin recovery over steam pretreatment was 116 and 112% for non-extracted spruce and pine barks, respectively, compared with 101 and 107% for the hot water-extracted spruce and pine barks. This difference was most likely due to larger formation of "pseudo-lignin" in the steam pretreatment of non-extracted barks. The lowest total lignin recovery over steam pretreatment (94%) was obtained with 3X-HWE spruce bark. The apparent AIL content of the pretreated materials decreased as more water-soluble phenolic compounds were removed from the barks by hot water extraction prior to steam pretreatment, supporting the hypothesis that water-soluble bark phenolics are rendered insoluble in acid-catalyzed treatments and are subsequently analyzed as insoluble lignin residue [7, 10, 14, 44]. Further, the AIL content was considerably lower for the barks—both non-extracted and hot water-extracted—that were steam-pretreated without the addition of an acid catalyst (i.e., under milder conditions). In the absence of an acid catalyst, the extent of degradation of hemicellulosic sugars during steam pretreatment is lower, which also results in a lower formation of lignin-like compounds ("pseudo-lignin") [34].

The composition of liquid fractions that were obtained from the steam-pretreated materials (Table 4) did not differ significantly between the non-extracted and hot water-extracted barks, regardless of species. The concentrations of total sugars (expressed in monomeric form) were slightly lower in the liquid fraction of pretreated pine barks than in the corresponding spruce barks; however, the ratios of monomeric and oligomeric sugars were the same for all steam-pretreated materials that were subjected to the same pretreatment conditions—5 to 10% of all dissolved sugars were in oligomeric form after steam pretreatment at 210 °C for 5 min with 2.5% SO_2. Omitting the acid catalyst in the pretreatment step significantly increased oligomeric sugar levels (55 to 60% of all dissolved sugars). Decreasing the severity of the pretreatment by performing the steam pretreatment at 190 °C shifted the ratio further, with nearly 70% of all dissolved sugars in oligomeric form. Moreover, as a consequence of milder

Table 3 Composition of water-insoluble fractions of steam-pretreated spruce and pine barks as a percentage of dry matter (% of DM)

Conditions of steam pretreatment	Material	Carbohydrates						Lignin		Ash
		Glucan	Xylan	Galactan	Arabinan	Mannan	Total	ASL[a]	AIL[b]	
210 °C; 5 min; 2.5% SO_2	Spruce bark	40.4 ± 1.8	1.5 ± 0.2	n.d.	n.d.	0.3 ± 0.0	42.2	2.8 ± 0.2	54.8 ± 0.1	2.4 ± 0.1
	HWE Spruce bark	40.1 ± 0.1	1.3 ± 0.1	n.d.	n.d.	0.2 ± 0.0	41.6	2.5 ± 0.0	53.8 ± 0.2	2.3 ± 0.0
	3X-HWE Spruce bark[c]	41.7 ± 0.0	1.1 ± 0.0	n.d.	0.2 ± 0.0	0.1 ± 0.0	43.1	2.6 ± 0.1	50.9 ± 0.7	2.1 ± 0.0
	Pine bark	26.2 ± 0.0	0.3 ± 0.1	n.d.	0.1 ± 0.1	0.1 ± 0.0	26.7	2.2 ± 0.1	69.3 ± 0.1	0.6 ± 0.0
	HWE Pine bark	27.1 ± 0.2	0.3 ± 0.1	n.d.	0.1 ± 0.1	0.1 ± 0.0	27.6	2.1 ± 0.0	68.3 ± 0.1	0.7 ± 0.1
210 °C; 5 min; No SO_2	Spruce bark	40.5 ± 0.2	3.4 ± 0.0	0.7 ± 0.0	0.2 ± 0.0	0.5 ± 0.1	45.3	3.4 ± 0.3	48.0 ± 0.6	1.8 ± 0.1
	HWE Spruce bark	42.5 ± 0.5	3.5 ± 0.0	0.5 ± 0.0	0.1 ± 0.0	0.7 ± 0.0	47.3	3.4 ± 0.1	47.8 ± 0.2	1.9 ± 0.0
190 °C; 5 min; No SO_2	Spruce bark	44.0 ± 0.5	4.2 ± 0.0	0.9 ± 0.0	0.3 ± 0.0	1.4 ± 0.0	50.8	3.4 ± 0.1	47.8 ± 0.2	2.3 ± 0.1

n.d. not detected

[a] Acid-soluble lignin

[b] Acid-insoluble lignin

[c] Hot water extraction performed three times

Table 4 Composition of liquid fractions of steam-pretreated spruce and pine barks

Conditions of steam pretreatment	Material	Total sugars (expressed as monomeric sugar) (g L^{-1})					Inhibitors (g L^{-1})		
		Glucose	Xylose	Galactose	Arabinose	Mannose	HMF[a]	Furfural	Acetic acid
210 °C; 5 min; 2.5% SO_2	Spruce bark	13.3	4.3	4.7	7.1	5.1	0.6	0.7	1.7
	HWE Spruce bark	13.1	5.4	5.3	8.5	5.3	0.6	0.9	2.0
	3X-HWE Spruce bark[b]	10.2	5.0	5.3	7.8	4.3	0.3	0.6	1.4
	Pine bark	7.3	2.9	5.2	4.7	4.2	1.1	1.1	1.6
	HWE Pine bark	8.1	3.8	6.2	6.4	4.6	1.1	1.0	1.8
210 °C; 5 min; No SO_2	Spruce bark	6.9	2.2	3.4	4.4	4.9	0.1	0.3	2.2
	HWE Spruce bark	4.8	2.3	2.9	4.1	4.2	0.1	0.1	2.0
190 °C; 5 min; No SO_2	Spruce bark	6.6	2.4	4.4	8.4	5.4	0.2	0.0	0.7

[a] Hydroxymethylfurfural

[b] Hot water extraction step performed three times

pretreatment conditions, the concentrations of dissolved sugars were slightly lower in the liquid fractions of materials pretreated without the addition of acid catalyst.

The levels of degradation products (1.4–2.0 g/L acetic acid and 0.3–1.1 g/L HMF and furfural) were similar for hot water-extracted and non-extracted barks pretreated under the same conditions, consistent with earlier studies that found that softwood barks generate less inhibitors during acid-catalyzed steam pretreatment than bark-free softwoods (2–3 g/L acetic acid, 2–6 g/L HMF, and ~ 1.5 g/L furfural) [10, 43]. Steam pretreatment without the addition of an acid catalyst (lower severity) resulted in even lower concentrations of inhibitory compounds (less than 0.3 g/L HMF or furfural) in the liquid fraction of steam-pretreated spruce barks, because the amount of degradation products that are generated during steam pretreatment is a function of the severity of the pretreatment.

Effects on Enzymatic Digestibility

The glucan content of spruce barks was, as discussed, higher than that of pine barks (Table 3), as was the glucose concentration after enzymatic hydrolysis of spruce barks (Fig. 1). However, the final glucose yields were higher for pine barks than the corresponding spruce barks. The proportion of glucose that was released during steam pretreatment was similar between softwood barks (14.4 and 16.6% for spruce and pine barks, respectively); thus, pine bark showed better digestibility based on the difference in the degree of hydrolysis (32.8 and 43.4% for spruce and pine barks, respectively) (Fig. 2).

In general, softwoods are recalcitrant to biochemical conversion and require high-severity pretreatment conditions [12], high enzyme doses [2], and possibly an additional delignification step [19] to provide a reasonable yield of monomeric sugars. Overcoming the inherent

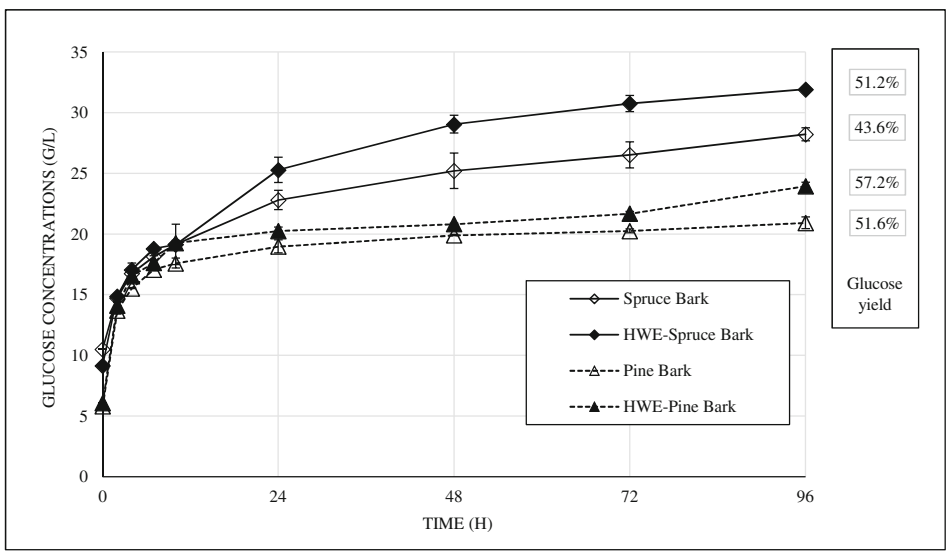

Fig. 1 Concentration profiles of glucose during enzymatic hydrolysis and final glucose yields. Enzymatic hydrolysis of spruce (*diamonds*) and pine barks (*triangles*), non-extracted (*open symbols*), and hot water-extracted (HWE) (*filled symbols*), steam-pretreated under the same conditions (210 °C, 5 min, 2.5% SO_2) at 10% WIS loading, 45 °C, pH 5 for 96 h using Cellic CTec3 enzyme cocktail at a dose of 5 wt% based on WIS. The *error bars* show the lowest and highest concentrations. Total glucose yields expressed as percent of the theoretical value, based on all available glucose in the pretreated materials

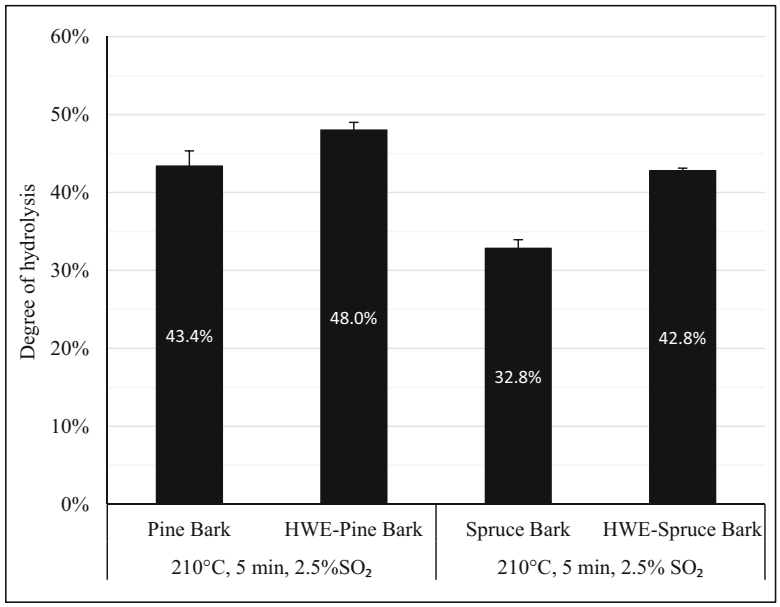

Fig. 2 Degree of hydrolysis after 96 h of enzymatic hydrolysis of steam-pretreated pine and spruce barks. Enzymatic hydrolysis of steam-pretreated (210 °C, 5 min, 2.5% SO_2) pine and spruce barks, non-extracted or hot water-extracted (HWE), at 10 wt% WIS loading, 45 °C, pH 5 for 96 h using Cellic CTec3 enzyme cocktail at a dose of 5 wt% based on WIS. The degree of hydrolysis was calculated based on the sum of oligomeric glucose in the liquid fraction and glucose available in the solid fraction of the steam-pretreated materials. The *error bars* show the lowest and highest values

recalcitrance of the bark fractions of spruce and pine has been more challenging for these types of wood fractions [10, 27]. These results are consistent with the glucose yields that were obtained in this study (Fig. 1). For instance, using twice the amount of the same enzyme cocktail, but at the same solids loading as in the current study, the glucose yield was 53% for spruce bark that was pretreated under the same conditions [10]. Higher glucose yields—up to 80%—were reported by Kemppainen et al. [14] for spruce bark but at a significantly lower solids loading (1% dry matter) and an enzyme loading of 25 FPU/g solid Celluclast 1.5 L.

Soluble compounds generated during the pretreatment of softwoods are known to impair microbial fermentation [1] and also the hydrolytic performance of the enzymes. The inhibitory effects of monomeric [49] and oligomeric [20] sugar components and non-sugar components, such as degradation products of sugars, lignin, and extractives [3, 16, 18, 50], have been previously examined. However, decreasing enzymatic digestibility has previously been observed both on whole slurry and on washed fibers with increasing proportions of bark in SO_2-catalyzed steam-pretreated spruce bark and wood mixtures [10], suggesting that the soluble inhibitory compounds that are liberated during steam pretreatment of bark are not the main cause of the significantly lower enzymatic digestibility of bark versus the wood fraction.

One of the goals of this work was to determine whether enzymatic digestibility can be improved by removing extractives prior to acid-catalyzed pretreatment. Regardless of the species, hot water extraction positively affected the digestibility of the pretreated materials (Fig. 2). However, this favorable effect was more pronounced for spruce bark versus pine bark. The degree of hydrolysis rose from 32.8 to 42.8% and from 43.4 to 48.0% for spruce and pine barks, respectively, from the hot water extraction prior to steam pretreatment. Although barks still remain challenging substrates for enzymatic hydrolysis, this increase in enzymatic digestibility of steam-pretreated spruce and

pine barks corresponds to 30 and 11% glucose yield improvement, respectively. The hot water extraction step was more efficient for spruce bark—i.e., a slightly higher proportion of water-soluble extractives was removed. However, because spruce bark originally contained more water-soluble extractives than pine bark, the hot water-extracted barks harbored approximately the same fraction of water-soluble extractives prior to steam pretreatment. This result suggests that there are differences in the chemical structure of the water-soluble extractives fraction of the barks of these softwood species, contributing to the disparate enzymatic digestibilities. Thus, the total amount of remaining water-soluble extractives is not the sole determinant.

Because the effect of hot water extraction on enzymatic digestibility was more prominent with spruce bark and also because its holocellulose content makes it more relevant as a sugar platform than pine bark, additional experiments were performed with spruce bark, including a more extensive hot water extraction (i.e., repeated three times) and steam pretreatments without the addition of SO_2 (Fig. 3). The steam pretreatment of non-extracted spruce bark at 210 °C for 5 min with 2.5% SO_2 catalyst, which has been shown to be effective for the pretreatment of spruce wood chips [41], resulted in the lowest yield of glucose that was released during enzymatic hydrolysis. Steam pretreatment without the acid catalyst and a decrease in temperature (lowering the severity of the steam pretreatment) did not significantly improve this yield. These results somewhat contradict a previous study, in which more severe steam pretreatment decreased the rate and yield of hydrolysis [14]. This trend, however, was

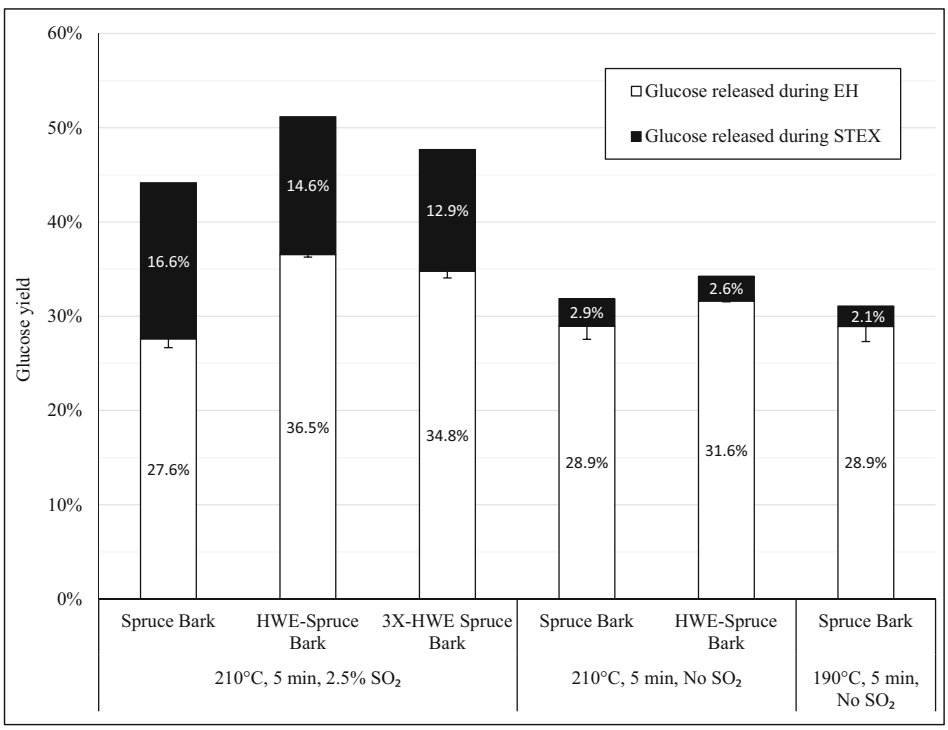

Fig. 3 Glucose yield after enzymatic hydrolysis of steam-pretreated spruce barks. Enzymatic hydrolysis of spruce barks, non-extracted or hot water-extracted (HWE), steam-pretreated under various conditions at 10% WIS loading, 45 °C, pH 5 for 96 h using Cellic CTec3 enzyme cocktail at a dose of 5 wt% based on WIS. The *filled bars* show the glucose released during steam pretreatment (STEX) step, while the *unfilled bars* represent the glucose released during the enzymatic hydrolysis (EH) step. The *error bars* show the lowest and highest values

not seen at higher enzyme doses in that study, and there was no significant difference in the final glucose yields of enzymatic hydrolysis observed after 48 h, regardless of the use of acid catalyst in the pretreatment step. Although the enzyme dose in our experiments was comparable with the low dose in the aforementioned study, the newer, more effective commercial enzyme cocktail that was used in our study might explain the improved, similar enzymatic digestibility, regardless of the addition of acid catalyst or the decrease in temperature in the steam pretreatment.

However, with regard to total glucose yields (Fig. 3), it is apparent that the use of an acid catalyst during the steam pretreatment was highly beneficial when the monomeric glucose that was released during the steam pretreatment was included. Total glucose yield of 31.9% was obtained after enzymatic hydrolysis of non-extracted spruce bark that was steam-pretreated for 5 min without acid catalyst at 210 °C, whereas addition of the acid catalyst increased the total glucose yield to 43.6%. When comparing hydrolysis data with the results of Kemppainen et al. [14], it should be noted that the acid catalyst and the impregnation method differed in the former study (soaking in 0.5% sulfuric acid solution), which might also have contributed to the difference in total glucose yields. Nevertheless, the total amount of monomeric glucose that was liberated from non-extracted spruce bark by steam pretreatment and enzymatic hydrolysis was considerably higher when acid catalyst was used in the pretreatment step in the present study.

A detailed analysis of interactions between extractives that have been isolated from various wood fractions and cellulose surfaces has previously shown that deposition of the phenolic extractives fraction from pine wood on microcrystalline cellulose negatively affected the glucose release during enzymatic hydrolysis [23]. The partial removal of water-soluble extractives by hot water extraction before the steam pretreatment step improved the enzymatic digestibility of spruce bark. The degree of enzymatic hydrolysis and total glucose yields were greater with hot water-extracted spruce bark in all cases, but the positive effect was significantly better when the steam pretreatment was performed with an acid catalyst (32 and 9% improvement in the degree of hydrolysis with and without an acid catalyst in the pretreatment step, respectively). This result is consistent with the explanation that water-soluble extractives undergo detrimental changes during steam pretreatment that impair the subsequent enzymatic hydrolysis, especially when steam pretreatment is performed in the presence of acid catalyst. Despite the improvements in the enzymatic digestibility of both barks by hot water extraction prior to pretreatment, the total glucose yields remained lower than previous results on the stem wood fraction of spruce [10, 25]. Additionally, a more thorough hot water extraction step, resulting in the removal of an additional 15% of water-soluble extractives before the acid-catalyzed steam pretreatment, did not result in further improvements in the degree of hydrolysis or total glucose yield (Fig. 3). Clearly, bark remains a challenging substrate for enzymatic hydrolysis.

Conclusions

The use of acid catalyst during steam pretreatment was found to be beneficial in reducing the recalcitrance of softwood barks from spruce and pine. However, the formation of water-insoluble “pseudo-lignin” from water-soluble bark extractives during acid-catalyzed steam pretreatment resulted in distorted lignin analysis of the pretreated materials and potentially contributed to an impaired enzymatic digestibility. The acid-insoluble lignin content of the pretreated materials decreased as more water-soluble phenolic compounds were removed from the barks by hot water extraction prior to steam pretreatment, whereas no significant difference

in holocellulose content was observed between non-extracted and hot water-extracted barks pretreated under the same conditions. Partial removal of water-soluble extractives by hot water extraction improved the enzymatic digestibility of steam-pretreated softwood barks. The obtained increase in enzymatic digestibility of steam-pretreated spruce and pine barks after extraction corresponded to 30 and 11% glucose yield improvement, respectively.

Compliance with Ethical Standards

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Conflict of Interests The authors declare that they have no conflict of interest.

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Paper IV

Co-pretreatment of spruce and poplar wood chips for ethanol production

Balázs Frankó*, Hanna Jovanovic, Mats Galbe, and Ola Wallberg
Department of Chemical Engineering, Lund University
P.O. Box 124, SE-221 00 Lund, Sweden

Email addresses:
BF: Balazs.Franko@chemeng.lth.se
HJ: Hanna.Jovanovic@chemeng.lth.se
MG: Mats.Galbe@chemeng.lth.se
OW: Ola.Wallberg@chemeng.lth.se

* Corresponding author

Abbreviations

AIL: acid-insoluble lignin; ASL: acid-soluble lignin; DM: dry matter; HMF: 5-hydroxymethyl furfural; NREL: National Renewable Energy Laboratory; WIS: water-insoluble solids

ABSTRACT

The biochemical production of ethanol from various softwood and hardwood species is well documented, but little has been reported on the impact of mixing these woody feedstocks on the conversion process. In this study, 3 steam pretreatment conditions were applied to characterize the interactions between spruce (softwood) and poplar (hardwood) that were concurrently processed by SO_2-catalyzed steam pretreatment and enzymatic hydrolysis. No synergistic or antagonistic interactions were observed in the concurrent use of spruce and poplar—our linear interpolation model accurately predicted the overall sugar recovery after steam pretreatment and enzymatic hydrolysis for a 50:50 blend to within 3%, based on the results of the individual feedstocks. The combined sugar yields after steam pretreatment and enzymatic hydrolysis ranged from 58% to 71%, wherein poplar generated higher yields than spruce. Conversely, the significant amount of acetic acid liberated during the steam pretreatment of poplar had a detrimental effect on the ethanol productivity and yield in the fermentation of the poplar hydrolysate. These results suggest that the concurrent use of poplar and spruce for ethanol production would be constrained to a greater extent by the performance of the yeast than the efficacy of the conversion of cellulose to glucose by steam pretreatment and enzymatic hydrolysis.

Keywords:
mixed feedstocks, steam pretreatment, ethanol production, softwood, hardwood, enzymatic hydrolysis

1. Introduction

The use of lignocellulosic feedstocks has the potential to provide a sustainable means of increasing the share of renewables in the transportation sector. Lignocellulosic biofuels, such as ethanol, can be produced from a wide range of biomass—from agricultural and forest residues to dedicated energy crops and short-rotation tree species (e.g., poplar, eucalyptus)—but the selection of feedstock is a key factor that influences their commercial viability (Chovau et al., 2013; Hess et al., 2007).

The availability of feedstock impacts the scale of production that is needed to realize economies of scale (Richard, 2010). Further, its cost of procurement is a significant fraction of the total production cost (Chovau et al., 2013), whereas its quality attributes (e.g., composition, particle size) affect the overall yield (Li et al., 2016; Williams et al., 2016). Although current commercial-scale pioneer ethanol plants almost exclusively use single-biomass feedstocks (Brethauer & Studer, 2015), the ability to process diverse feedstocks efficiently to produce biofuels would be beneficial toward realizing full commercial deployment (Shi et al., 2013). The conversion of mixed biomass feedstocks to fermentable sugars and ethanol without any compromise in the efficiency of the conversion process could lower the production cost by maximizing the scale with increased feedstock volume and hedging the sensitivity to price volatility, thus minimizing the cost of the feedstock supply (Nielsen, 2016).

The conversion of mixed biomass feedstocks to ethanol, however, is challenging. Biomass-to-ethanol conversion is a highly intertwined, multistep process that comprises a pretreatment step to disrupt the compact structure of plant cells, enzymatic depolymerization of polysaccharides to monomeric sugars, and the subsequent fermentation of sugars to ethanol. Consequently, the chemical and structural diversity of lignocellulosic biomass and hence the various processing optima might compromise overall performance and limit the possibility to blend feedstocks.

Steam pretreatment, a leading pretreatment technology with regard to technical and economic considerations, is effective in processing various types of lignocellulosic biomass (Yang & Wyman, 2008), but there are limited data on pretreating heterogeneous mixtures of feedstocks. Concurrent steam pretreatment of diverse feedstocks has been performed for biomass combinations that have been obtained by mixing: i) several parts of the same plant [e.g., sugarcane bagasse and sugarcane straw (Pereira et al., 2015) or bark and wood (Frankó et al., 2015; Kim et al., 2005)], ii) various plants among agricultural (e.g., wheat straw and corn stover) or forestry biomass [e.g., mixed hardwoods (Lim & Lee, 2013; Schultz et al., 1983) and mixed

softwoods (Stenberg et al., 1998)], and iii) combinations between these categories [e.g., poplar and wheat straw (Vera et al., 2015)].

Although the production of ethanol from many wood species is well documented, the inclusion of a fast-growing tree species in the use of softwood feedstock base has not been examined. Thus, the aim of this study was to assess the robustness of steam pretreatment and explore the possibility of processing spruce and poplar concurrently for ethanol production. To evaluate the efficacy of steam pretreatment and the effects of mixed biomass feedstocks, the mixtures were compared with the individual feedstocks in terms of composition, enzymatic digestibility, and fermentability.

2. Materials and Methods

2.1 Raw materials

Debarked Norway spruce, *Picea abies*, was kindly provided by a Swedish pulpmill (Södra Cell Mörrum, Mörrum, Sweden). Poplar, *Populus trichocarpa*, was harvested 4 years after being planted in a field trial by the Swedish University of Agricultural Sciences (SLU) in Alnarp, Sweden. Branches with leaves were removed, and the stems were debarked manually before the material was cut into smaller pieces using a turbine cut system (Bosch AXT 25 TC). The spruce and poplar white-wood pieces were chipped further using a knife mill (Retsch GmbH, Haan, Germany) and sieved to obtain a 2–10-mm fraction. The dry matter (DM) content was determined to be 72 wt-% for spruce and 40 wt-% for poplar. To adjust the DM content of the raw materials to 50 wt-%, the poplar chips were air-dried, whereas the spruce chips were soaked in water at room temperature for 2 hours, filter-pressed at a maximum pressure of 20 bar using a hydraulic press (HP5M, Fischer Maschinenfabrik, Neuss, Germany) to remove the excess water, and then air-dried until the desired DM content was reached. The raw materials were stored in plastic buckets at 4°C until use.

2.2 Feedstock preparation and steam pretreatment

SO_2-catalyzed steam pretreatment was applied in batches of individual feedstocks and their 50:50 blends (1:1 ratio of spruce and poplar, based on dry weight) in a 10-L reactor, per Palmqvist et al. (1996). Prior to the steam pretreatment, each batch, with a total dry weight of 600 g, was impregnated with 2.5 wt-% gaseous SO_2, based on the moisture content of the raw material, in tightly sealed plastic bags for 20 minutes at room temperature. Excess SO_2 was vented before the steam pretreatment by leaving the plastic bags open for 30 min. The pretreatment conditions were based on the optimal settings for the individual feedstocks (i.e., 200°C, 5 min, 2.5% SO_2 and 210°C, 5 min, 2.5% SO_2 and for poplar and spruce, respectively) (Kumar et al., 2009; Stenberg et al., 1998) and 1 additional condition between these levels (i.e., 205°C, 5 min, 2.5% SO_2). The pretreated slurries were stored at 4°C prior to subsequent analysis and experiments.

2.3 Enzymatic hydrolysis

Enzymatic hydrolysis of the pretreated slurries was performed in 2-L Labfors bioreactors (Infors AG, Bottmingen, Switzerland) with a working weight of 1 kg. A water-insoluble solids (WIS) load of 10% mass fraction and Cellic CTec3 enzyme

preparation, kindly provided by Novozymes A/S (Bagsværd, Denmark), at a load of 5% mass fraction of WIS, were applied, corresponding to approximately 9 filter paper units/g WIS. The hydrolysis experiments proceeded for 96 h at 45°C, a stirring rate of 400 rpm, and pH 5, maintained with 2.5 M NaOH solution. Samples from the hydrolysis liquid were separated by a centrifuge (Galaxy 16 DH, VWR International, Radnor, PA, USA), in 2-mL Eppendorf tubes at 16,000 x *g* for 8 minutes. The supernatant was passed through 0.2-µm filters (GVS Filter Technology, Morecambe, United Kingdom) and stored at -20°C. The enzymatic hydrolysis experiments were performed in duplicate.

2.4 Fermentation

Fermentation test was performed on the liquid fraction of the materials steam-pretreated at 205°C for 5 min with 2.5% SO_2 to evaluate the extent of inhibition by the compounds that were formed during the steam pretreatment. Hydrolysates were obtained from the steam-pretreated materials by vacuum filtration using No. 5 filter paper (Munktell Filter AB, Falun, Sweden). The hydrolysates were then diluted with deionized water to obtain an equivalent solids concentration (i.e., the concentration of inhibitors in a simultaneous saccharification and fermentation at a certain WIS load) that corresponded to a WIS load of 10% mass fraction. Fermentation was performed on an orbital shaker (Lab-Therm, Adolf Kühner AG, Basel, Switzerland) anaerobically at 30°C, 180 rpm, and pH 5 for 72 h with Ethanol Red, an industrial hexose-fermenting yeast (kindly provided by Lesaffre Advanced Fermentations, Marcq-en-Baroeul Cedex, France), at a concentration of 5 g L^{-1} in shake flasks with a working volume of 50 mL, containing 0.5 g L^{-1} $(NH_4)_2HPO_4$ and 1 g L^{-1} yeast extract. The fermentation experiments were performed in duplicate.

2.5 Analyses

The total solids content of the biomass materials and the total dissolved solids content of the liquid samples were determined per the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008a). The WIS content of the pretreated slurries was measured using the no-wash method of Weiss et al. (2010). The extractive, structural carbohydrate, lignin, and ash contents of the solid fractions and the composition of the liquid fractions were determined per NREL protocols (Sluiter et al., 2008b; Sluiter et al., 2008c; Sluiter et al., 2006; Sluiter et al., 2005).

Sugars, organic acids, and other degradation products were quantified by high-performance liquid chromatography on a Shimadzu LC 20AD system that was equipped with a Shimadzu RID 10A refractive index detector (Shimadzu Corporation, Kyoto, Japan). Samples for sugar analysis were pH-adjusted to 5, if

necessary, with $CaCO_3$ and centrifuged in 2-mL Eppendorf tubes (16,000x *g* for 5 min). All samples were passed through 0.2-µm filters (GVS Filter Technology) and stored at -20°C until analysis. Sugars were analyzed on a CarboSep CHO 782 column (Concise Separations, San Jose, CA, United States) with a De-Ashing Bio-Rad microguard column (Bio-Rad Laboratories, Hercules, CA, United States) at 70°C using degassed deionized water as the eluent at a flow rate of 0.6 ml min^{-1}. Ethanol, organic acids, and other degradation products were analyzed on a Bio-Rad Aminex HPX-87H column with a Cation-H Bio-Rad microguard column at 50°C, with a mobile phase of 5 mM sulfuric acid at a flow rate of 0.5 mL min^{-1}.

2.6 Calculations

Pretreatment yield was expressed as the amount of sugars that were recovered in the pretreated materials per 100 g dry raw material. The overall sugary recovery after the pretreatment was expressed as a percentage of the initial carbohydrate content in the raw material. Glucose yield in the enzymatic hydrolysis was calculated, based on the total available glucose in the liquid and the solid fraction of the steam-pretreated materials, per Frankó et al. (2017). Combined sugar yield was expressed as the amount of monomeric sugars that were recovered after the steam pretreatment and enzymatic hydrolysis per 100 g dry raw material. The ethanol yield in the fermentation experiments was expressed as a percentage of the theoretical stoichiometric ethanol yield (0.51 g g^{-1}), based on total available hexose sugars (i.e., glucose, mannose, and galactose) in monomeric form in the hydrolysates.

3. Results

The sugar recovery and enzymatic hydrolyzability of steam-pretreated spruce, poplar, and their 50:50 blend (i.e., spruce and poplar mixed prior to pretreatment at a ratio of 1:1, based on dry weight) were examined to test the hypothesis that overall sugar yields from the mixed feedstock could be predicted by linear interpolation, based on the behavior of the individual species. Further, the fermentability of selected hydrolysates was assessed.

3.1 Raw materials

The chemical composition of the raw materials is presented in Table 1. Although the total carbohydrate content, based on DM, was similar between spruce and poplar (62.8% and 64.4%, respectively), poplar contained considerably less lignin than spruce. The carbohydrate and lignin contents for spruce and poplar were within the ranges of previous studies (Martín-Davison et al., 2015; Monavari et al., 2009; Negro et al., 2003; Wyman et al., 2009).

3.2 Steam pretreatment

The individual feedstocks and their 50:50 blend were steam-pretreated under 3 conditions: at 200°C, 205°C, and 210°C for 5 min with 2.5% SO_2. The chemical compositions of the steam-pretreated materials are summarized in Table 2. Essentially, the solid fraction of the pretreated materials contained only glucan and lignin. All pretreatment conditions resulted in nearly complete dissolution of hemicellulose. In the spruce hydrolysates, glucose and mannose had the highest concentrations, whereas xylose was the most abundant sugar in the poplar hydrolysates under all pretreatment conditions. Steam pretreatment also led to the degradation of solubilized hexoses and pentoses—primarily to 5-hydroxymethyl furfural (HMF) and furfural, respectively, and then to formic acid and levulinic acid.

The concentration of degradation products was lowest in the hydrolysate that was obtained from materials that had been pretreated at 200°C for 5 min with 2.5% SO_2. Additionally, ~2 and ~5 g of acetic acid per 100 g dry raw material was liberated during the steam pretreatment from spruce and poplar, respectively, by the hydrolysis of hemicellulose. Whereas the concentration of acetic acid ranged from 6–7 g L^{-1} in the spruce hydrolysates, poplar hydrolysates contained acetic acid at a concentration of 16–17 g L^{-1}. Similar chemical compositions for the solid and liquid fractions have been reported for spruce and poplar that have been steam-pretreated

under comparable conditions (Hoyer et al., 2013; Negro et al., 2003; Schütt et al., 2013; Tengborg et al., 2001; Vera et al., 2015).

The pretreatment yields and overall sugar recoveries are presented in Figure 1. As expected, pretreatment yields and overall sugar recoveries decreased with greater pretreatment severity, regardless of feedstock. However, the overall sugar recoveries were higher with poplar versus spruce under the same steam pretreatment conditions, irrespective of the severity of the pretreatment. Despite the lower sugar recoveries for spruce, more hexose sugars were recovered from spruce than poplar under the same pretreatment conditions.

3.3 Enzymatic hydrolysis

Whole slurry was subjected to enzymatic hydrolysis to assess the susceptibility of steam-pretreated materials to enzymatic degradation. Higher glucose yields were obtained in the enzymatic hydrolysis of poplar than spruce when steam-pretreated under the same conditions (Figure 2). The highest glucose yield in the enzymatic hydrolysis (73.5%) was achieved with poplar that was pretreated at 200°C for 5 min with 2.5% SO_2, whereas steam pretreatment at 205°C for 5 min with 2.5% SO_2 resulted in the highest glucose yield (67.5%) with spruce. Similar ranges of glucose yields from enzymatic hydrolysis have been reported for steam-pretreated spruce and poplar (Cantarella et al., 2004; Negro et al., 2003; Schütt et al., 2013; Tengborg et al., 2001; Vera et al., 2015), although different solid and enzyme loadings make the comparisons difficult.

3.4 Sugar recovery model for biomass mixtures

Based on the pretreatment yields, overall sugar recoveries, and combined sugar yields for the individual feedstocks, predicted values were calculated for the 50:50 blends by linear interpolation, as shown in Table 3. This model predicted pretreatment yields and overall sugar recoveries after pretreatment to within approximately 2% of the experimental values of the 50:50 blends of spruce and poplar for all pretreatment conditions. The combined sugar recovery after steam pretreatment and enzymatic hydrolysis was predicted to within 3% of actual values by the linear interpolation model. The combined sugar yields from spruce and poplar in this study are similar to previous results for similar pretreatment conditions (Schütt et al., 2013; Stenberg et al., 1998).

3.5 Fermentation

To evaluate the effects of inhibitory compounds in the hydrolysates, fermentation test was performed for the hydrolysates that were obtained from the pretreatment condition of 205°C, 5 min, 2.5% SO_2 (i.e., the condition that resulted in pretreated materials with the highest enzymatic hydrolysability). As shown in Figure 3, the hydrolysates exhibited disparate fermentabilities. Whereas nearly all hexose sugars were consumed after 24 h for the spruce hydrolysate, sugar consumption was slower and incomplete with the hydrolysates of the 50:50 blend and poplar. The ethanol yield was highest with the spruce hydrolysate (78%) and lowest with the poplar hydrolysate (52%). Although the production of volumetric ethanol during the first 4 h was similar for all hydrolysates (0.4—0.65 g L^{-1} h^{-1}), the rate of ethanol production differed considerably after 24 h (0.7, 0.3, and 0.2 g L^{-1} h^{-1} for spruce, the 50:50 blend, and poplar, respectively). The ethanol concentration reached its maximum after 24 h for the spruce hydrolysate (17.5 g L^{-1}), whereas it did not level off even after 72 h for the 50:50 blend and poplar hydrolysate.

4. Discussion

The compositional analysis of spruce and poplar feedstocks before and after steam pretreatment revealed typical characteristic differences between the chemical composition of softwoods and hardwoods. The major hemicellulose component of softwood species is (galacto)glucomannan, whereas glucuronoxylan is the most common hemicellulose constituent in hardwoods. Accordingly, mannan and xylan were the second most abundant carbohydrates after glucan in spruce and poplar, respectively. Consequently, spruce has a higher content of hexose sugars that can be readily fermented to ethanol by wild-type *S. cerevisiae*. Conversely, largely due to the higher lignin content and the greater degree of crosslinking between its lignin units (Boerjan et al., 2003), spruce is more recalcitrant to steam pretreatment and enzymatic hydrolysis, as reflected by the higher-severity conditions that were required for the pretreatment of spruce versus poplar.

The optimal pretreatment conditions for poplar has been reported to be in the range of 195–200°C, 5–15 min, and 2.5% to 3% SO_2 to maximize glucose and xylose recovery after steam pretreatment (Dou et al., 2017; Kumar et al., 2009; Schütt et al., 2013), whereas SO_2-catalyzed steam pretreatment at 210°C for 5 min has previously been shown to be effective for spruce (Stenberg et al., 1998). Thus, 200°C, 205°C, and 210°C for 5 min with 2.5% SO_2 were chosen as the conditions for examining the concurrent use of spruce and poplar.

All pretreatments resulted in the near-complete dissolution of hemicellulose and the enrichment of glucan and lignin in the solid fractions of the pretreated materials. Due to the complete dissolution of hemicellulose, the sugar composition of the hydrolysates reflected the distribution of hemicellulosic carbohydrates in the raw materials. The amount and variety of degradation products that are generated during steam pretreatment are functions of the severity of the pretreatment and the type of biomass (e.g., the concentration of carbohydrates or acetyl groups in the original feedstock). Thus, as expected, the concentration of degradation products was lowest in the hydrolysates that were obtained from materials steam-pretreated at 200°C for 5 min with 2.5% SO_2 (i.e., the lowest-severity pretreatment condition). With increasing pretreatment severity, the formation of degradation products rose and sugar recoveries declined.

For all of the pretreatment conditions, increasing pretreatment severity resulted in greater glucose yields in the enzymatic hydrolysis for spruce, whereas no such improvement was observed for poplar. Moreover, the glucose yields were higher with steam-pretreated poplar versus spruce, regardless of pretreatment condition. These results show that softwood biomass is inherently more resistant to steam pretreatment and enzymatic hydrolysis than hardwood and that a harsher pretreatment is needed to overcome the recalcitrance of spruce.

Based on the experimental overall sugar recovery values for the individual feedstocks, predicted values were also calculated for the steam-pretreated 50:50 blends by linear interpolation. The amount of sugar in the pretreated 50:50 blend of spruce and poplar could be predicted to within 2% of the experimental values by linear interpolation of the results for the pure species under all pretreatment conditions. The linear interpolation model predicted sugar recoveries for the blends accurately, even after enzymatic hydrolysis. The combined sugar recoveries were within approximately 3% of the experimental data, based on the individual feedstocks. These findings suggest that no synergistic or antagonistic interactions occur from blending spruce and poplar during steam pretreatment or enzymatic hydrolysis, as the linear interpolation gave accurate results for the combined sugar yields and recoveries under the pretreatment conditions tested.

Yet, the ethanol production rate and ethanol yield are not only dependent on the sugar yield, but also on the fermentability of the hydrolysate. Although similar ethanol yields were obtained for the hydrolysates from spruce and the 50:50 blend, the fermentability was impaired with the poplar hydrolysate, even with the high yeast pitch (5 g L^{-1}) used in this study. This result could be attributed to the high amount of acetic acid that was liberated during the steam pretreatment of poplar, as all the other inhibitory compounds measured were in the same concentration range for spruce and the blend. Although low acetic acid concentrations have been shown to be beneficial for the anaerobic conversion of glucose to ethanol by *S. cerevisiae*, increasing ethanol yields and lowering by-product yields (Taherzadeh et al., 1997), the undissociated form of acetic acid in this study was close to the concentration (5 g L^{-1}) that has been reported to inhibit growth of *S. cerevisiae* completely (Taherzadeh et al., 1997). Additionally, the inhibitory effects of HMF are believed to be weaker than those of furfural (Sanchez and Bautista, 1988), which explains the lower fermentability of the poplar hydrolysates. Furfural and HMF inhibit CO_2 production by *S. cerevisiae* (Banerjee et al., 1981) and, consequently, ethanol production under fermentative conditions, although the yeast is able to convert furfural and HMF to their corresponding, less inhibitory alcohols (Taherzadeh et al., 1999).

Toxicity problems in ethanol fermentation of steam-pretreated poplar have previously been reported by Cantarella et al. (2004), suggesting that concurrent use of poplar and spruce is constrained to a greater extent by the performance of the yeast than the efficacy of the conversion of cellulose to glucose by steam pretreatment and enzymatic hydrolysis. The inclusion of low amounts of poplar in the use of spruce might even be an effective means of lowering the concentrations of inhibitors (e.g., acetic acid) and thereby reducing the need for costly chemical detoxification.

5. Conclusions

Spruce and poplar can be converted to sugars through steam pretreatment and enzymatic hydrolysis and subsequently to ethanol; however, the impact of mixing these woody feedstocks on these process steps has not been studied extensively. A simple linear interpolation model, based on the experimental values of the individual feedstocks, accurately predicted pretreatment yields, overall sugar recoveries over steam pretreatment, and combined sugar yields after steam pretreatment and enzymatic hydrolysis for the 50:50 blends to within approximately 3% under all pretreatment conditions. Conversely, lower ethanol yield and productivity was observed in the fermentation of the poplar hydrolysate, most likely due to the significant amount of acetic acid liberated during the steam pretreatment.

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Conflict of Interests

The authors declare that they have no conflict of interest.

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Table 1 Chemical composition of the untreated raw materials as a percentage of dry matter (% of DM)

Material	Carbohydrates						Lignin		Extractives		**Total**
	Glucan	Xylan	Galactan	Arabinan	Mannan	Total	ASL[a]	AIL[b]	Water	Ethanol	
Spruce	42.2±0.9	5.9±0.2	1.9±0.0	1.3±0.0	11.5±0.5	62.8	4.0±0.4	25.7±1.3	2.8±0.7	1.4±0.1	**96.7**
Poplar	43.5±0.5	15.0±0.0	1.3±0.1	0.8±0.0	3.5±0.3	64.1	6.1±0.0	15.2±0.3	4.6±0.2	1.7±0.2	**91.7**

[a] Acid-soluble lignin
[b] Acid-insoluble lignin

Table 2. Chemical composition of the steam-pretreated materials

Steam pretreatment	**210°C, 5 minutes, 2.5% SO_2**			**205°C, 5 minutes, 2.5% SO_2**			**200°C, 5 minutes, 2.5% SO_2**		
Material	Spruce	50:50 blend	Poplar	Spruce	50:50 blend	Poplar	Spruce	50:50 blend	Poplar
Solid fraction (% of DM)									
Glucan	52.6±0.8	59.5±0.3	69.6±0.1	54.4±0.0	59.5±0.0	64.1±0.6	54.3±0.8	62.4±0.2	68.1±0.5
Xylan	0.2±0.0	0.6±0.0	1.1±0.0	0.4±0.0	0.4±0.0	0.4±0.0	0.5±0.0	1.0±0.2	1.6±0.1
Galactan	0.3±0.2	-	0.1±0.0	-	0.1±0.0	0.1±0.0	0.6±0.0	0.4±0.0	0.2±0.0
Arabinan	0.1±0.0	-	-	0.1±0.1	0.1±0.0	-	0.1±0.0	0.1±0.0	0.1±0.1
Mannan	0.1±0.0	-	0.2±0.1	0.1±0.1	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.1
Lignin	47.6±1.1	39.8±0.6	29.2±1.2	48.4±0.1	42.1±0.2	36.7±0.2	46.6±1.6	35.9±0.8	29.3±0.4
Liquid fraction (g L^{-1})									
Glucose[a]	37.4	29.1	25.2	37.0	37.8	34.3	29.2	24.4	19.8
Xylose[a]	9.5	10.9	32.2	13.4	23.3	34.5	13.5	26.0	36.3
Galactose[a]	5.2	4.7	1.9	6.8	6.5	5.9	6.4	5.4	4.0
Arabinose[a]	3.7	3.0	2.0	3.8	3.6	3.0	3.9	3.9	2.3
Mannose[a]	27.2	20.4	8.7	24.0	18.0	10.1	27.6	24.1	9.7
Formic acid	2.4	2.1	1.9	2.6	2.2	1.8	2.0	1.7	1.6
Acetic acid	6.2	11.4	16.9	6.2	11.7	17.5	6.3	11.4	16.1
Levulinic acid	1.9	1.0	0.6	1.8	1.3	0.8	0.9	0.4	0.4
HMF	3.4	2.3	1.6	3.0	2.4	1.6	2.2	1.4	0.9
Furfural	2.0	2.8	3.6	2.2	3.5	4.4	1.5	2.0	2.8

[a] Both oligomeric and monomeric included (expressed as monomeric sugar)

Table 3. Predicted and experimental sugar yields and recoveries for the 50:50 blend of spruce and poplar.

	50:50 blend of spruce and poplar								
	210°C, 5 minutes, 2.5% SO_2			205°C, 5 minutes. 2.5% SO_2			200°C, 5 minutes, 2.5% SO_2		
	Predicted	Experimental	Diff.	Predicted	Experimental	Diff.	Predicted	Experimental	Diff.
Pretreatment yield	55.7	55.9	0.4%	58.8	59.9	1.8%	62.9	63.5	0.9%
Overall sugar recovery	77.2%	77.5%	0.4%	81.5%	83.1%	1.9%	87.3%	88.1%	0.9%
Combined sugar yield	43.3	44.0	1.6%	45.5	46.8	2.7%	47.8	47.8	0.0%
Combined hexose yield	36.3	36.8	1.4%	37.1	38.6	3.8%	39.3	39.0	-0.8%

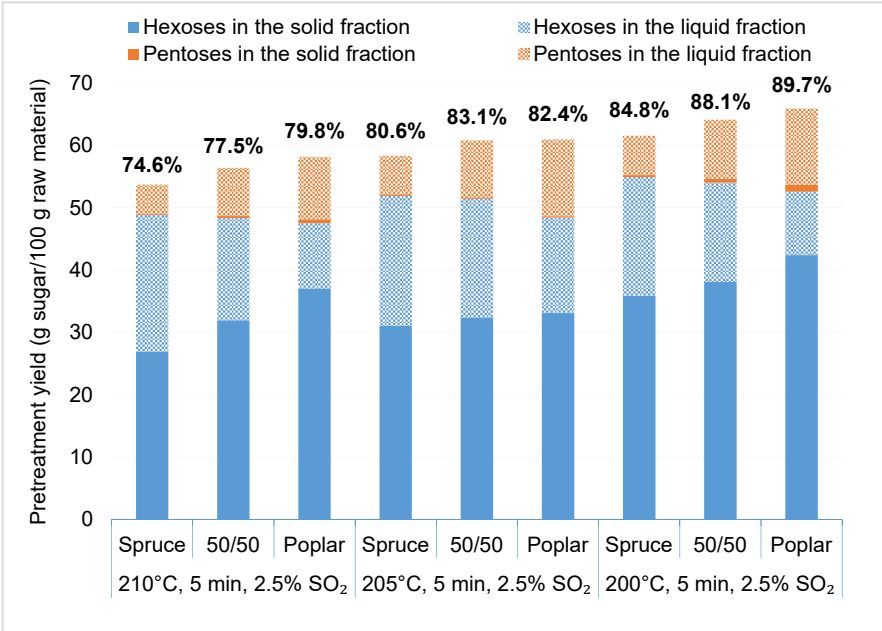

Figure 1. *Pretreatment yield and overall sugar recovery.*
Pretreatment yield is expressed as g hexose and pentose sugars recovered in the steam-pretreated material per 100 g dry raw material. Overall sugary recovery is expressed as the percentage of all available sugars in the raw material.

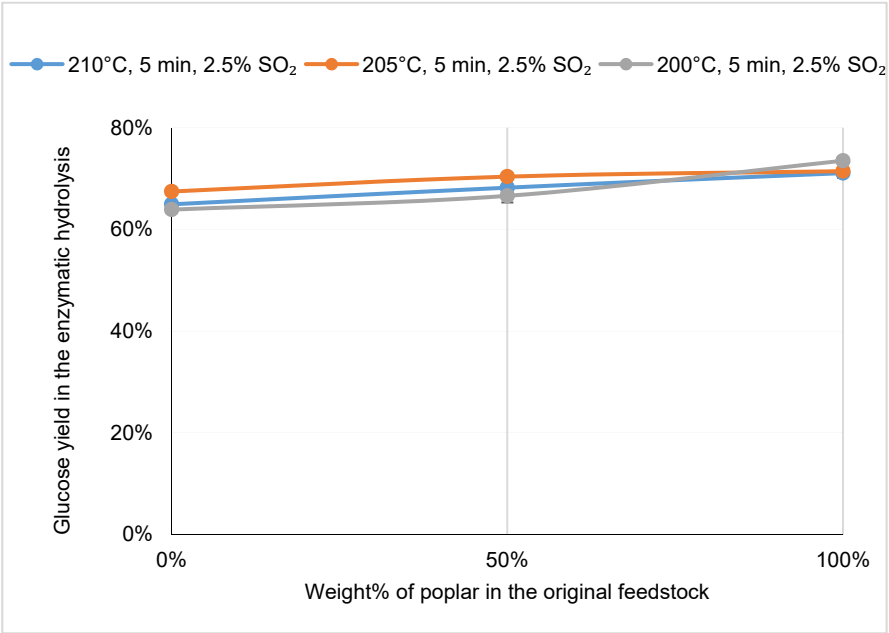

Figure 2. *Glucose yields of steam-pretreated spruce, poplar, and their 50:50 blend in the enzymatic hydrolysis.*
The glucose yield was calculated based on total available glucose in the liquid and solid fractions of the steam-pretreated materials.

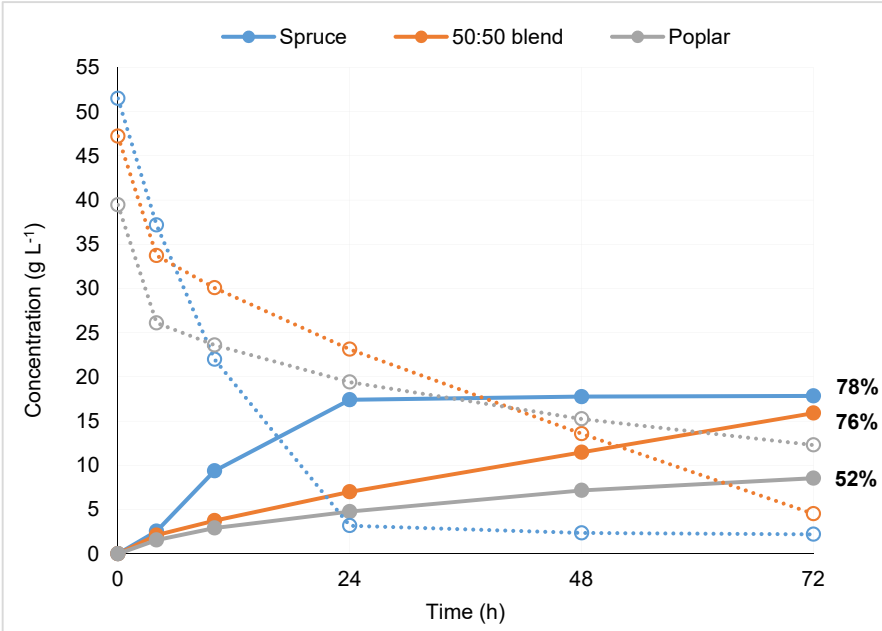

Figure 3. *Concentration profiles for total hexose sugars (dashed lines) and ethanol (solid lines) during fermentation and final ethanol yields.*
Fermentation was performed for the hydrolysates obtained from the materials pretreated at 205°C, for 5 min, with 2.5% SO_2. Final ethanol yields expressed as % of the theoretical maximum stoichiometric yield, based on all available hexose sugars.

The utilization of lignocellulosic biomass to produce biofuels, such as bioethanol, has the potential to provide a sustainable alternative to fossil fuels, and thus mitigate greenhouse gas emissions from the transportation sector. Forest biomass is expected to be a significant source of such biomass, as it can serve as an abundant and sustainable feedstock for bioethanol production.

Sweden is a country dominated by forests, and forestry is vitally important for its national economy. With its access to raw materials, the forest industry is well-positioned to diversify its products through wood to ethanol production. This would contribute significantly to reaching the goal of zero net emissions of greenhouse gases, which Sweden has pledged to achieve by 2045. Increasing environmental concerns, and technological advances in the production of ethanol from wood biomass make forest-based ethanol an attractive option. However, large-scale implementation requires the efficient utilization of low-value residues from forest or silvicultural harvesting (e.g., thinnings, branches, low-value decayed trees).

The aim of the work presented in this thesis was to assess the feasibility of utilizing various forest-based feedstocks potentially available as raw materials for future ethanol production, and its implications on the wood-to-ethanol conversion process. Different types of forest biomass have different properties, and different degrees of heterogeneity, which can affect the conversion process. The robustness of acid-catalyzed steam pretreatment and the bioconversion process was evaluated to investigate the impact of the presence of bark in the feedstock. Further, the inclusion of a fast-growing tree species in the use of established forest feedstock was explored through a study on the steam pretreatment of a mixture of poplar and spruce for bioethanol production.

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Department of Chemical Engineering
Faculty of Engineering, LTH
Lund University