MONASH University

Microwave Assisted Pretreatment of Lignocellulose to Overcome Barriers to the Sugar Platform

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Abstract

The main motivation behind this thesis is the need for sustainable and effective methods for pre-treating recalcitrant biomass for its conversion into a versatile sugar platform. Improved methods for pretreatment could lead to a breakthrough in production of biofuels and chemicals from biomass-derived sugars. This research project addressed this aim by exploring three applications of microwave pretreatment of highly recalcitrant woody biomass.

The purpose of the first investigation was to develop a mild, cost-effective and environmentally benign pretreatment for woody lignocellulose sawdust that maximised sugar yield via enzymatic saccharification. Microwave irradiation (MW) of *Eucalyptus regnans* sawdust in water was investigated and compared directly against conventional liquid hot water (LHW) pretreatment. Following 30 min microwave irradiation at 180°C, the sugar yield was 3.5 times higher using MW than LHW pretreatment under the same conditions. Complete C_5 and C_6 sugar release was achieved after the two-step method of MW pretreatment followed by enzymatic hydrolysis, compared with only 4% without pretreatment and 31% after LHW. Removal of 'lignin droplets' formed on the surface of the pretreated fibers via flowing hot water showed only minor improvement in the yield from enzymatic saccharification. Our results support the hypothesis that lignin prevents access of enzymes rather than inhibits their activity. MW pretreatment accelerated depolymerization of hemicellulose, opening the structure more than LHW pretreatment.

The driver for the subsequent investigation was to improve the efficiency of microwave pretreatment of woody sawdust through the addition of polar solvents which could intentionally be used to manipulate the dipolar polarisation mechanism of microwave heating. Many researchers have reported on ionic liquids for microwave pretreatment of biomass, thus investigating the ionic conduction mechanism, however, limited research has been conducted on polar chemicals to investigate the dipolar mechanism. This part of the project was split into two sections, in the first we report microwave irradiation of *Eucalyptus regnans* (hardwood) and *Pinus radiata* (softwood) sawdust in the presence of different concentrations of isopropyl alcohol, ethylene glycol or formalin solution containing 3.7wt% formaldehyde (FA) examined the effectiveness of pretreatment at 180°C for 10 minutes. Dilute formalin solution released more than 75% of hemicellulose sugars under short microwave treatment for both

wood species. In contrast, just 32 and 55% of the hardwood and softwood hemicellulose was released, respectively, in water only. Furthermore, enzyme hydrolysis of the FA pretreated fibres were unaffected by the solvent pretreatment demonstrating that FA is very effective combination with microwave heating. Lignin from the pretreated fibres was extracted and qualitatively examined using 2D NMR spectroscopy. It was found that FA stabilised the lignin monomers during pretreatment. In the second part, woody biomass was treated with the same solvents under conventional LHW pretreatment under non-stirred conditions. The conventional heating approach confirmed hardwoods as more amenable to pretreatment and FA as the best solvent choice. In addition, high enzyme loading used for enzyme hydrolysis of both water and FA pretreated fibres achieved complete sugar release with the microwave pretreatment, demonstrating no inhibitive effects on the enzymes by the FA.

The final section investigated further aspects of microwave pretreatment of biomass that need to be addressed for future implementation of the technology to reduce energy or capital costs. In particular, the two aspects investigated in this chapter were larger biomass samples (e.g. sizes from sawdust to wood chips) and use of mild acid catalysts. Exceptional sugar yields has been achieved for woody sawdust microwave pretreated in water or FA solutions in the previous chapters. However, milling of biomass to fine particles requires large energy inputs that increase costs but the performance of microwave for larger particles was unknown. Furthermore, this study investigated the use of mild acid catalysts to potentially eliminate the need for high-grade steel for process equipment. Dilute acids with a range of pk_a strengths were added to wood samples of various surface areas, and heated by microwaves. Water alone was not sufficient to release hemicellulose sugars in wood, regardless of the particle size over short heating times. Whereas, when acid catalysts were used there was a significant increase in sugar yield for all samples. Using 50 mM sulphuric acid over short duration microwave exposure (10 min) in the presence of *Eucalyptus nitens* as a benchmark, the experiments indicated that 100 mM formic acid was similarly as effective in wood chip pretreatment. Enzyme hydrolysis of all wood samples revealed that formic acid is on par with sulphuric in terms of total sugar release for the largest chip samples.

This research, which has been guided by sustainable principles, has opened up the potentials of manipulating dipolar solvents for efficient microwave pretreatment of lignocellulose.

General Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Signature:

Print Name: Negin Amini

Date: 28/01/2019

Published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes 1 original papers published in peer reviewed journals and 2 unpublished publications. The core theme of the thesis is development of a sustainable pretreatment method for recalcitrant biomass. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the *Faculty of Engineering, Department of Chemical Engineering* under the supervision of Associate Professor Akshat Tanksale and Associate Professor Victoria Haritos.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of *chapter 3* my contribution to the work involved the following:

Thesis Chapter	Publication Title	Status *(published, in press, accepted or returned for revision)*	Nature and % of student contribution	Co-author name(s) Nature and % of Co-author's contribution*	Co-author(s), Monash student Y/N
3	*Microwave assisted pretreatment of eucalyptus sawdust enhances enzymatic saccharification and maximizes fermentable sugar yield*	Published	*70%. Initiation, experimental work, analysis of data, development and writing up of first draft*	1) *Victoria Haritos. Key ideas, reviewing and editing of paper. 15%* 2) *Akshat Tanksale. Initiation, key ideas, reviewing and editing of paper. 15%*	N

I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student signature: **Date:**   28/01/2019

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor signature: **Date:**   28/01/2019

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List of Publications

Journal Publications

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Conference Proceedings

Amini, N., Haritos, V. and Tanksale A. (2017). Poster Presentation "Microwave Assisted Removal of Xylan and Lignin from Eucalyptus", World Congress of Chemical Engineering 2017, Barcelona, Spain.

Amini, N., Haritos, V. and Tanksale A. (2016). Oral Presentation "Microwave Assisted Removal of Xylan and Lignin from Eucalyptus", Bioenergy Australia 2016, Brisbane, Australia.

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Abbreviations

AC	Autoclave
ATR	Attenuated Total Reflectance
DAD	Diode Array Detector
DLS	Dynamic Light Scattering
DL	Delignification
FA	Formaldehyde
FoA	Formic Acid
FT-IR	Fourier Transform Infrared Spectroscopy
FPU	Filter Paper Units
GC	Gas Chromatography
HPLC	High Pressure Liquid Chromatography
HSQC	Heteronuclear Single Quantum Coherence
ISM	Industrial, Scientific and Medical
LA	Levulinic Acid
LCC	Lignin-Carbohydrate Complex
LHW	Liquid Hot Water
LSC	Ligno-Sulphonate Complex
MW	Microwave
NMR	Nuclear Magnetic Resonance
RID	Refractive Index Detector
SA	Sulphuric Acid
SEM	Scanning Electron Microscope
TOC	Total Organic Carbon
TOF	Turnover Frequency
TON	Turnover Number

Nomenclature

cm	Centimetre
°C	Degrees Celsius
C_p	Heat capacity
ε'	Dielectric constant
ε''	Dielectric loss factor
eV	Energy
GHz	Gigahertz
g	Gram
Hz	Hertz
Kg	Kilogram
λ_o	Wavelength at vacuum conditions
M_w	Molecular weight
m	Meters
mm	Millimeter
mm^2	Surface area
min	Minutes
mg	Milligram
ml	Millilitre
mM	Micromolar
mol%	Molar percentage
η	Efficiency
N	Normality
ρ	Density
p	power
P	Pressure
s	Seconds
σ	Dielectric conductivity
tanδ	Dielectric loss tangent/Dissipation factor

ΔT	Temperature difference
t	Time
T	Temperature
v/v%	Volume by volume percentage
wt%	Weight percentage of solute in solution (by mass)
x	penetration depth

Chapter 1. Introduction

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1.1 Background

Due to depleting fossil reserves, the rising concern for climate change and decreasing levels of easily accessible crude oil, the need for manufacture of renewable fuels and chemicals using green and energy-efficient methods has become an imperative for the planet. Although unconventional oil wells from shale deposits have been developed [1, 2], the drawbacks associated with this source and extraction methods, such as carbon emissions, groundwater contamination and risk of enhanced seismic activity, are not sustainable in the long term [3]. Lignocellulosic biomass is a renewable resource, which can be utilised for future industrial fuels and value-added chemicals production and does not contribute to net carbon emissions.

Various types of lignocellulose can be utilised for this purpose. An extensive amount of research has been reported on the utilisation of food crops and non-woody biomass as they have a lower lignin content which makes them easier to convert, requiring less energy [4]. However, this has sparked a debate by some researchers who argue that food crops should not be used for such purposes. Furthermore, policies worldwide are beginning to shift towards the use of non-food crops for production of chemicals such as ethanol [5]. Wood and wood waste from forestry operations such as branches, mill and pulp operations and thinning's from reducing the risk of fire are an ideal feedstock as they are inexpensive and available abundantly worldwide [6]. However, due to their recalcitrance there has not yet been a breakthrough in establishing an energy efficient process for their conversion into value-added chemicals and fuels.

There are two main treatment methods for the conversion of woody biomass as shown in Figure 1-1: classified as thermochemical and chemical treatments. The well-established thermochemical treatment comprises of combustion, gasification, liquefaction and pyrolysis. The products of these routes range from flue gases, synthesis gas to mixture of oils [7]. However, they require significant energy input as they use high temperatures and pressures. The economic feasibility of gasification processes have been investigated in literature, with the overall efficiency of wood conversion to energy reported to be 36% [8].
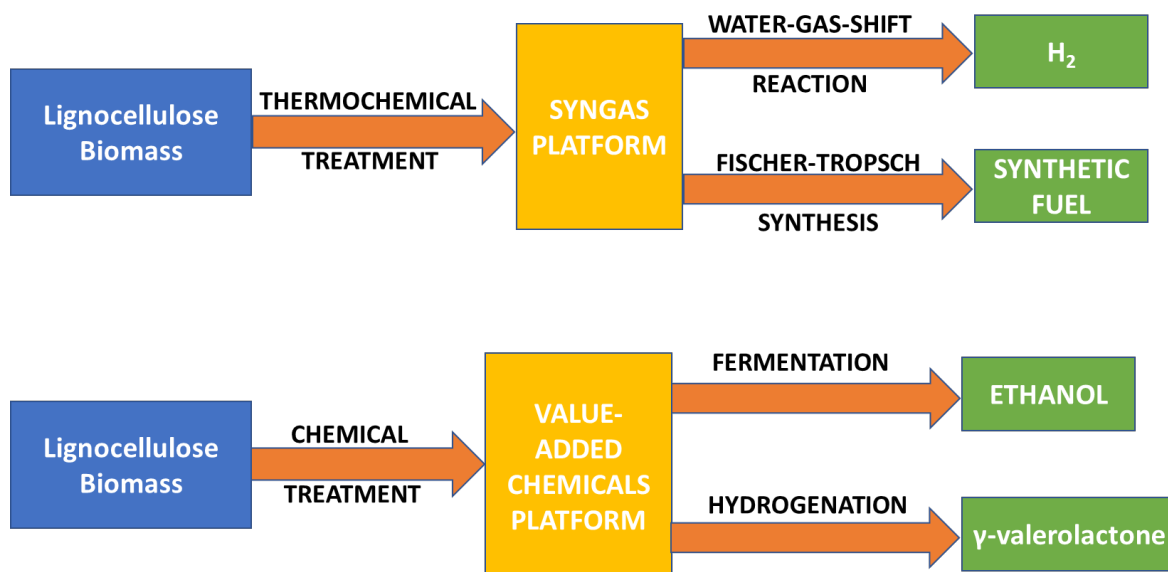
Figure 1-1. Lignocellulose biomass treatment methods

Combustion is the most common process for heat production where biomass is fed to a furnace (450 – 600 °C) subsequent to drying and size reduction [10]. In this process, inorganic elements, as well as CO produced due to incomplete combustion, contribute to pollution and formation of mineral deposits on the process equipment [10]. Gasification of biomass is closely related to combustion but occurs at higher temperatures (750 - 910°C) and in oxygen limited atmosphere [11]. Furthermore, in the steam gasification process energy is introduced into the gaseous mixture by the addition of hydrogen to increase the H/C ratio [12]. Whereas, in the combustion process bonds are broken to release energy and oxidisation of the hydrogen and carbon occur to yield water and carbon dioxide [12]. Pyrolysis (450 – 600 °C) occurs in the absence of oxidizing agents and converts the biomass to liquid hydrocarbons as opposed to gas and char [12]. Hydrothermal liquefaction of biomass involves the heating of wet biomass at milder temperatures (280 – 370 °C) compared to the previously discussed thermochemical approaches mainly due to the presence of water which can act as a catalyst in the process [13]. The products formed here are biocrude (which is bio-oil), char and gas.

Aside from thermochemical approaches, there are chemical treatment technologies which utilise lower temperatures (160 to 240 °C) and may directly transform the wood into chemicals and fuels such as furfural, lactic acid and succinic acid [14]. The most common chemical treatments are acid and alkaline which typically use H_2SO_4 and NaOH [15, 16].

Alternatively, to preserve the wood macromolecules (polysaccharides and/or lignin) for transformation into a wider range of useful chemicals and fuels downstream, chemical pretreatment is required prior to downstream conversion. The preserved macromolecules can then be transformed into any desired chemical through various treatment processes. Pretreatment alters the physical and chemical structure of wood and improves enzymatic hydrolysis rates of cellulose [16]. Thus, the main goal of the pretreatment step is to make the cellulose accessible to hydrolysis for conversion which is illustrated in Figure 1-2.

Current pretreatment methods are categorised into oxidative delignification, physicochemical and organosolv pretreatment; of which hydrogen peroxide delignification, steam explosion and SPORL are the prevailing technologies for each category, respectively. The main disadvantages associated with chemical pretreatment methods are that they consume a large amount of time, cost, and energy [25]. For instance, the pretreatment energy efficiency of spruce using steam explosion and SPORL methods were reported to be 0.26 and 0.35 kg/MJ, respectively [26, 27].

Dilute acid treatment requires between 0.5 to 5% acid loading and temperatures between 160 to 220°C [17]. These mild approaches can be taken, referred to as a chemical pretreatment, and provides a powerful sugar platform intermediate capable of conversion to value-added chemicals. Whereas concentrated acid treatment entails acid loading between 10 to 30% at temperatures below 160°C [18]. Various solid acid catalysts can also be employed for lignocellulose depolymerisation such as Amberlyst, $-SO_3H$ functionalized amorphous carbon or mesoporous silica and H-form zeolites to name a few [19]. The need for high grade steel material resistant to corrosion by acids and pH adjustment of the highly acidic products suited to downstream processing conditions are some of the factors which add to the cost of chemical treatment [17].

Currently, in the lignocellulosic biofuels process, monosaccharide release which incorporates pretreatment and enzyme digestion, can contribute up to 45% of the total costs [20] and this cost is one of the main factors hindering commercial implementation of the biofuels [21]. Development of an energy efficient pretreatment process for woody biomass could catapult renewable fuels and chemicals production worldwide. It is important to note that the pretreatment stage can alter the chemical properties of the biomass in such a way that it can have a negative economic impact later in the lifecycle of the process [22]. Therefore the key

criteria listed below need to be met to ensure optimal results are obtained for lowest cost and energy consumption [23].

- The deconstruction of the lignocellulosic complex

- Reducing the crystallinity of the cellulose structure

- Increasing the porosity of the material

- Preventing the formation of by-products that may hinder the hydrolysis or fermentation

- Minimising the amount of hazardous wastes created
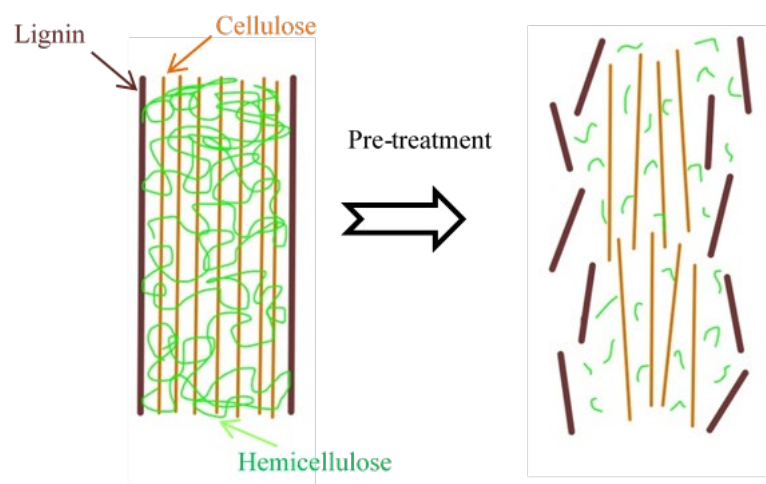
Figure 1-2. Pretreatment process objective

Microwave radiation is an energy efficient alternative heating method, which can potentially be used on a large scale for chemicals and fuels production from lignocellulose biomass. Furthermore, microwave radiation can be employed as both a pretreatment, for lignin removal and disruption of the biomass structure, and treatment, for fast conversion of the macromolecules to value added products, depending on the reagents and conditions used. There have been many reports in the literature for woody biomass pretreatment using microwaves mostly in combination with ionic liquids, of which the results are dramatically enhanced by microwave heating and result in complete dissolution of cellulose or lignin at relatively low temperatures [28]. Despite the numerous reports on the effectiveness of microwave-assisted treatment of woody biomass using ionic liquids, the high purchase cost and problematic regeneration of ionic liquids hinder the upscaling of such pretreatment

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process. There is potential to utilise microwave radiation as an energy efficient pretreatment for woody biomass using non-ionic liquid solvents.

1.2 Research Aims

There has been limited research reported on microwave assisted woody biomass pretreatment using solvents other than ionic liquids. Furthermore, the research to date has used high severity conditions in combination with time and energy consuming processes. This provides an opportunity for an investigation on alternative solvents for microwave pretreatment of woody biomass. The main challenge of this project is the recalcitrance of the wood due to the lignin and the recalcitrant structure of biomass. Therefore, penetrative microwave heating in combination with the right parameters may be the key to efficient biomass pretreatment and then unlocking the scale-up of this process.

The scope of this project is to develop a sustainable and efficient pretreatment process using microwave irradiation for disrupting the structure of recalcitrant woody biomass allowing ready hydrolysis of cellulose to a monosaccharide platform. In addition, to perform a comparative analysis with conventional heating in order to determine the conditions where microwaves are favoured and whether there are any limiting barriers for either heating route. Conditions that may favour microwave-assisted pretreatment over conventional include inclusion of polar solvents into the reaction mixture and larger surface area of wood biomass particles. Furthermore, this project investigated factors affecting the downstream conversion of the pre-treated cellulose fibres into glucose by enzymatic hydrolysis and determined the effectiveness of the different pretreatments. The following specific research aims were developed to be addressed in this thesis:

- To investigate microwave assisted hydrothermal pre-treatment of woody biomass and understand the effect of lignin on downstream enzymatic conversion.
- To investigate readily available solvents with high microwave absorption capacity to exploit the dipolar polarisation mechanism to enhance microwave conversion into heat. To study the effect of these solvents on the chemistry of the pre-treated oligomers and monomers. To investigate the mechanism of depolymerisation of wood under microwave-assisted solvolysis method.

- To investigate alternatives to sulphuric acid catalysed wood chip pretreatment under microwave irradiation. Understand the effects of wood chip particle surface area, catalyst loading and temperature on the extent of pretreatment and downstream depolymerisation.

1.3   Thesis Structure and Chapter outline

This thesis is presented in six chapters, which include three experimental chapters.

Chapter 1: Introduction

This chapter provides important background information and the motivation for this research project. It also addresses the specific research aims, the thesis structure and outline for each chapter.

Chapter 2: Literature Review

This chapter provides the necessary background information such as structure of lignocellulose biomass, the fundamentals and key parameters of microwave radiation as well as limitations of this heating mechanism. A critical review of the relevant literature surrounding conventional and microwave pretreatment methods of woody biomass are presented in this chapter. It was found that prevalent technologies for wood pretreatment, such as steam explosion, have high energy consumption and as such make them a costly choice when implemented on an industrial scale. Microwave irradiation has been proposed to be a more energy efficient alternative pretreatment due to the direct substrate heating, however, a majority of the research investigation has been on its combination with ionic liquids. Thus, the main gap identified for this research project is using microwave pretreatment using water only or readily available solvents and catalysts other than ionic liquids, with the intention to optimise the energy efficiency and sustainability of the overall process.

Chapter 3: Microwave assisted pretreatment of eucalyptus sawdust enhances enzymatic saccharification and maximizes fermentable sugar yield

This chapter provides experimental findings on the microwave (MW) pretreatment of Eucalyptus sawdust in water only and compares it with its competing conventional technology, liquid hot water pretreatment (LHW). Formation of lignin globules were observed on the surface of the pretreated wood fibres; further characterisation confirmed its composition to be a mixture of lignin and carbohydrate complex (LCC). In an attempt to remove the LCC using liquid water at elevated temperature of 120 °C, 120 mg/g of the LCC, which correlates to 60% of the lignin in the raw biomass was successfully removed. Downstream conversion of the 'pretreated only' and 'pretreated and delignified' fibres for both MW and LHW pretreated samples were performed using *Trichoderma reesei*. The major findings demonstrated that MW pretreated fibres provide greater access to enzymes compared with conventionally pretreated samples. In addition, it was proposed that the presence of lignin in the biomass limits downstream conversion via steric hindrance as opposed to enzyme inhibition.

Chapter 4A: Polar solvents facilitate efficient microwave pretreatment of woody biomass

This chapter is presented in two parts. Chapter 4A investigates microwave pretreatment of woody biomass in the presence of polar solvents to intentionally exploit the dipolarisation mechanism and augment the electromagnetic conversion to heat. Hardwood and softwood sawdust were microwave pre-treated in dilute ethylene glycol, isopropanol, formalin and water (as the control solvent). Solutions containing 3.7wt% formaldehyde released double the hemicellulosic sugar yield compared with water only at a short microwave exposure of 10 min. In addition, it was also found that by increasing concentration of formaldehyde, less time was required in the microwave to reach the reaction temperature compared with water only. A solution containing 10wt% formaldehyde took 52% less time to heat to 180°C when compared with water only solution. Further investigation found that the effect of formalin on the pretreated wood macromolecules stabilised the lignin monomers, potentially preventing the condensation of lignin monomers.

Chapter 4B: Comparison against conventional pretreatment

This section compared microwave pretreatment of woody biomass against non-stirred conventional liquid hot water pretreatment. The findings supported the conclusions from Chapter 3 that microwave heating opens up access the wood to improve enzyme yield compared with conventional heating. The findings further supported the enzyme hydrolysis results from Chapter 4A in that, in both cases, formalin pretreated wood had a higher saccharification yield compared with water only suggesting that a combination of microwave and formalin reduces the heating time and further opens up the wood matrix for enzyme digestion, ultimately requiring lower enzyme loading.

Chapter 5: Effect of surface area using various acids in microwave pretreatment of wood chips

Here the project investigated homogenous acid catalysed microwave pretreatment of wood and investigated the effectiveness of different acids and the effect of wood chip surface area under microwave treatment. Sulphuric acid is most commonly used in industry for recalcitrant biomass pretreatment since it provides a significant sugar yield and as such, the lowest concentration reported in literature was set as the benchmark for this study. The effectiveness of sulphuric acid microwave pretreatment was compared with either formic or levulinic acid at concentrations that yielded complete hemicellulose depolymerisation. A range of wood surface areas at constant loading of 4wt% was investigated with microwave heating in the presence of one of the acids or water only. Contrary to effect of acids, water only treatment did not increase the yield of sugar with increasing wood surface area. Formic acid was proposed as a better catalyst for wood pretreatment due to a number of benefits, which could ultimately lead to lower cost when implemented in an industrial scale. The advantages of formic acid compared with the other catalysts include; less acidic hydrolysate pH, no consumption of the acid while still achieving sugar yield similar to sulphuric acid.

Chapter 6: Conclusions and Recommendations

In lignocellulosic biofuels production the total cost associated with sugar release, which includes pretreatment and enzyme digestion, can account up to 45% of the total cost. As such, efficient and low-cost methods are necessary for its commercialisation. The experimental results in this project explore a range of reaction conditions and provide optimised microwave pretreatment methods for woody biomass. In addition, it assists in the understanding of dipolarisation mechanism which on a large scale can be implemented for a faster conversion of electromagnetic waves into heat energy, thus potentially reducing the total pretreatment time and cost. For future studies, it is proposed that a continuous microwave reactor be used to validate the batch scale findings. This will also be of paramount importance for the commercialisation of woody biomass microwave pretreatment.

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Chapter 2.   Literature Review

Abstract

This chapter provides the necessary background information such as structure of lignocellulose biomass, the fundamentals and key parameters of microwave radiation as well as limitations of this heating mechanism. A critical review of the relevant literature surrounding conventional and microwave pretreatment methods of woody biomass are presented in this chapter. It was found that current/common technologies for wood pretreatment, such as steam explosion, have high energy consumption and as such make them a costly choice when implemented on an industrial scale. Microwave irradiation has been proposed to be a more energy efficient alternative pretreatment due to direct substrate heating, however, majority of the research focus to date with microwaves has been in ionic liquid media. Thus, the main gap identified for this research project is the potential of microwave irradiation for woody biomass pretreatment in water only or readily available solvents or in the presence of catalysts with the intention of optimising the energy efficiency of the overall process.

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2.1 Introduction

A breakthrough is required for the pretreatment of recalcitrant woody biomass which can be upscaled for commercial application. Since its introduction by Ooshima et al. in the mid 1980's [1], microwave heating for biomass pretreatment has led to a vast amount of research in this field. The main advantage of microwave heating over conventional heating is the direct substrate-microwave interaction which leads to dissipation of heat as opposed to reliance on convection and conduction [2]. Microwave pretreatment of various types of biomass in ionic liquids have received the most attention due to the exploitation of ionic conduction, which is one of two mechanisms of microwave heating [3, 4]. Verma et al. reported pretreatment of beech wood with ammonium molybdate activated by H_2O_2 assisted with microwave or autoclave heating. Maximum sugar yield of 59.5% was obtained by microwave irradiation at 140°C for 30 min, while heating in an autoclave yielded only 41.8% [5]. Furthermore, complete dissolution and partial delignification of wood has been achieved using ([C$_2$mim]oAc) in the microwave [6]. Despite these impressive results, there are numerous drawbacks associated with ionic liquids which include unknown toxicology, regeneration problems and high operating costs that make it an undesirable method for industrial scale application [7].

The alternate dipolar polarisation mechanism of microwave heating has potential to be explored for woody biomass pretreatment. An in-depth investigation into the utilisation of various polar solvents may lead to a breakthrough in overcoming the recalcitrance of woody biomass in an energy efficient process. To set up the background for this research, the structure of lignocellulose and its evolution during pretreatment was studied. Moreover, the current prevailing conventional technologies for woody biomass pretreatment, the fundamental concepts of microwave heating and the current status of woody biomass microwave pretreatment are discussed. Based on the information in this review the gaps for this research were identified.

2.2 Lignocellulose Biomass

Bioethanol production has reached commercial production from either sugarcane or corn in the USA and Brazil, and potato or wheat in Europe[8]. These fuels are referred to as "first generation" biofuels and have been controversial as they considered to be competing with agricultural land used for the food industry. Thus, worldwide focus is shifting towards the generation of "second generation" biofuels from biomass [9]. There have been optimistic research advances for biomass pretreatment; attracting funding for additional research globally and pilot scale and demonstration scale in USA and Italy [10-13]. By 2025, many countries have set objectives to substitute their current petroleum derived fuels with renewable fuels, Brazil being the leading nation in this change predominantly from non-woody biomass [14].

There are 3271×10^6 m^3 of wood fuel used globally per year, approximately 40% of these can be used as an industrial raw material and the remainder used as waste for energy production [15]. However, efficient pretreatment process for renewable fuels and chemicals from woody biomass have not been developed, or are still too costly, despite the large availability of feedstock.

2.2.1 Structure and composition of wood

Wood is classified into two main types; hardwood and softwood. Common plantation-grown examples of each of these species are *Eucalyptus globulus* and *Pinus radiata*, respectively. The components found in the cell walls of lignocellulose biomass are outlined in Figure 2-1 and their compositional breakdown are shown in Table 2-1. The structure and composition of the cell walls can vary for any given species depending on factors such as age, location, humidity, amount of rainfall and quality of soil [16]. The major components of the cell walls are the macromolecular substances, whereas the minor components are the low molecular weight substances. Each component is discussed in detail in this chapter.

Table 2-1. Eucalyptus and Pinus species average composition [17, 18]

Chemical Composition	Component	Eucalyptus globulus	Pinus radiata
		wt%	wt%
Glucans	Cellulose	44.7	48.1
Xylans		16.0	6.0
Mannans	Hemicellulose	-	11.9
Arabinans		1.09	1.9
Galactans		1.2	3.2
Lignin	Lignin	24.7*	26.2*
Ash	Inorganic matter	0.23	0.3
Extractives	Organic matter	2.96	2.2
Other	Other	9.12	7.5

* Refers to Klason lignin only (acid insoluble)
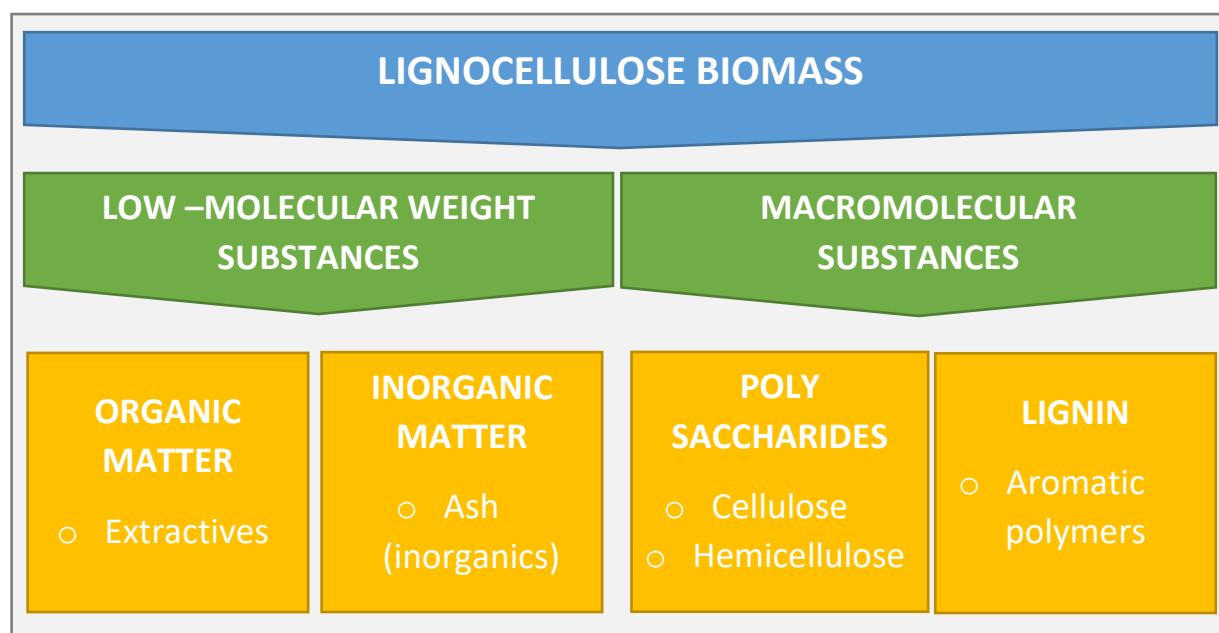
Figure 2-1. Chemical components found in lignocellulose biomass

2.2.1.1 Polysaccharides

Cellulose

Cellulose is comprised of D-glucose monosaccharides chemically bound together by a type of covalent bond referred to as a β-(1,4)-glycosidic bond [19]. An individual glucose molecule has 4 hydroxyl groups which aid its ability to react with other molecules [20]. The hydroxyl groups form intermolecular hydrogen bonds within cellulose and intramolecular hydrogen bonds with the surrounding macromolecules [21]. This contributes to the rigidity and crystalline structure of the cellulose illustrated in Figure 2-2 [20]. In the crystalline region cellulose has a higher density while in the amorphous region it has a lower density with some free hydroxyls [22]. Cellulose strands are bundled together to form microfibrils with an individual microfibril diameter approximately 3 - 5 nm [23]. More than 45% of the wood is comprised of cellulose that is protected by the hemicellulose and lignin. This acts as a shield for the cellulose from microbial and pathogen attack during the lifespan of the cell [24]. In turn, the bonding between the components of wood also make it difficult to access the cellulose easily. Hemicellulose and lignin wrap around the microfibrils and are linked together via various covalent crosslinks [25]. Within the chain, polysaccharide linkages are mainly via glycosidic bonds between the hemiacetal hydroxyl of one monosaccharide and the hydroxyl of another monosaccharide [26]. In the pretreatment stage, it is difficult to disrupt the structure of wood under mild conditions and the best outcome is the disruption of the hemicellulose and/or lignin components, opening up access to cellulose and the sugars within.
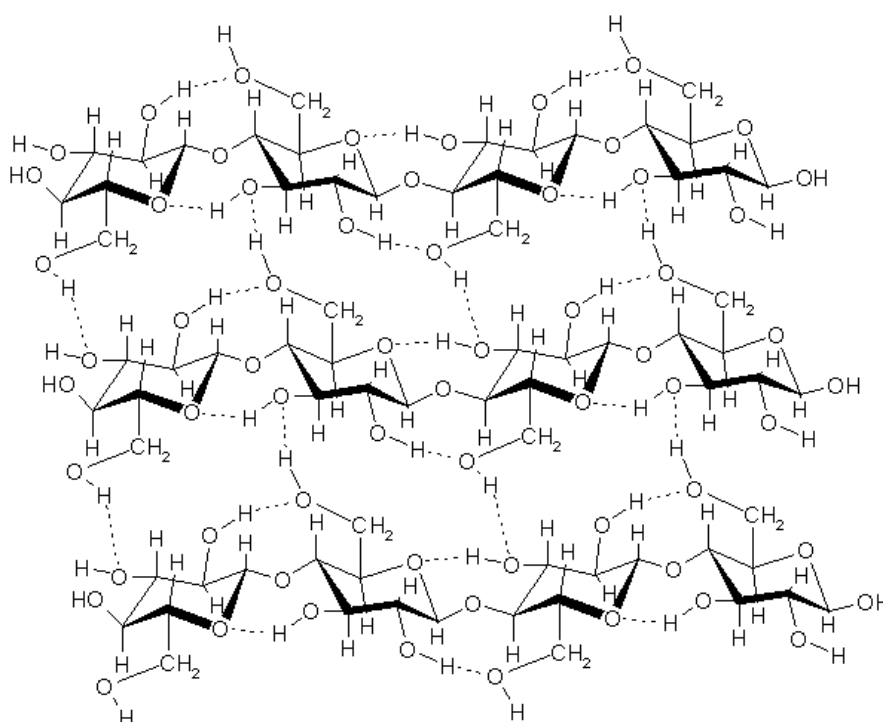
Figure 2-2. **Chemical structures of the cellulose polymer showing the beta-1,4-glycosidic linkages between glucose monosaccharides and hydrogen bonds between cellulose chains (Reprinted from source [27] with permission from Elsevier)**

Hemicellulose

Hemicellulose consists of an heterogeneous polysaccharide made up of both pentoses and hexoses [28]. Furthermore, uronic acids and acetyl groups may also be connected in the hemicellulose polymer. This component of wood can vary between different species. Hemicellulose in softwood has a branched structure with various side chain residues, whereas, for hardwood they comprise of xylan backbone and more uronic acids and acetyl groups [26]. Coupled with the low degree of polymerisation (~200) hemicelluloses are more soluble and readily hydrolysed during pretreatment processes [28, 29]. Figure 2-3 shows the different types of monosaccharides that can make up hemicellulose. The carboxylic acid present in uronic acids of hemicellulose can form direct ester linkages with hydroxyl groups of neighbouring polysaccharides or lignin [26]. Furthermore, there are ester-ether crosslinks between the hydroxycinnamate esters on the hemicellulose polysaccharide and the hydroxyl groups on the lignin [23]. Lastly, there are benzyl ether linkages between the lignin and the hydroxyl groups on the hemicellulose polysaccharides [23]. The cleavage of these bonds

during the pretreatment would be more likely and result in release of hemicellulose monosaccharides.
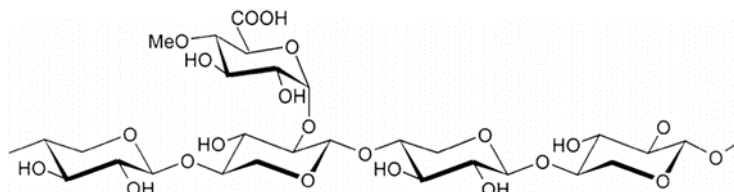
Figure 2-3. **Hemicelullose structure 4-O-methyl-D-glucurono-D-xylan typically found in hardwood (Republished with permission of Anna Ebringerová from [30])**

2.2.1.2 Lignin

There are also significant differences in the lignin structure and composition between hardwoods and softwoods as described in Table 2-2**.** More than two thirds of interconnecting bonds in lignin are through ether bonds with the main linkage of lignin units via β-O-4 aryl ether bonds [31]. Softwoods comprise of up to 50% β-O-4 bonds and hardwoods 62% [31]. This bond can be cleaved by 2 main reaction types: electrophilic and nucleophilic [25]. Table 2-4 illustrates the 3 main phenylpropane units in lignin; Guaiacyl (G), Syringyl (S) and p-hydroxyphenyl (H) with their respective number of methoxy units [31]. The ratio of these units vary between wood species and the stereochemistry of the units is the biggest factor in their reactivity [32]. Hardwoods have a more branched lignin matrix due to the high number of methoxy groups on the benzene ring causing steric hindrance [32]. The phenolic monomers provide the solid structural support of biomass and make it insoluble in most solvents [25]. The different types of ether bonds in lignin consist of phenol-ether, alkyl-ether and diaryl-ether bonds to mention a few as well as internal hydrogen bonding [25, 33]. The complicated structure and various bond types in lignin make it difficult to break apart.

Table 2-2. Percentage abundance of aromatic constituents present in lignin hardwood and softwood [34]

Lignin precursors	% Abundance		No. of methoxy Groups on benzene ring
	Softwood	Hardwood	
G unit (coniferyl alcohol)	<95	25-50	2
S Unit (sinapyl alcohol)	Trace amount	46-75	3
H unit (p-coumaryl alcohol)	<5	0-8	1
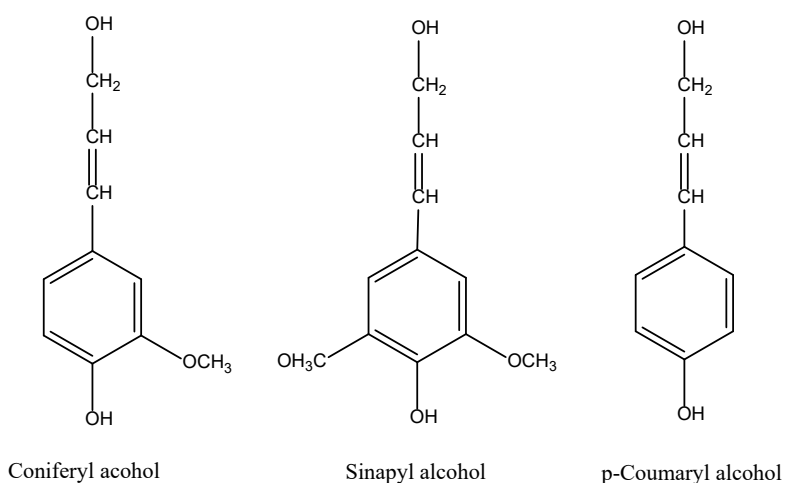
Figure 2-4. **Structure of lignin monomers; Coniferyl alcohol, Sinapyl alcohol and p-Coumaryl alcohol**

2.2.1.3 Inorganic Matter

The inorganic matter of wood accounts for a small percentage of the total wood composition. It is usually referred to as ash. Typically, the amount of ash in woods vary between 0.2 to 0.5% [35]. By incineration of the wood at high temperatures between 600 to 850 °C, the organic materials in the wood would burn, leaving behind the 'ash' [35]. The ash content is mainly elements such as potassium, calcium, magnesium and silicon in some tropical woods [35].

2.2.1.4   Extractives

The extractives content makes up a small percentage of the wood and can encompass a wide variety of components. The main components found in extractives are terpenes, fatty acids, colouring matter and carbohydrates [35]. Various extraction methods are available which are aimed at removing components of the extractives, as some components are water soluble and others insoluble [23].

2.2.2 Mechanical Size Reduction of Wood

Prior to performing pretreatment the wood requires to undergo mechanical size reduction; from the tree form to various smaller sizes. Common size reduction methods which are utilised are; cutting (cm size range), coarse milling (cm to 500 µm size range), fine grinding (<100µm) and ultra-fine grinding (<30µm) [36]. However, there is a trade-off, where the further the size of the wood is reduced, the more energy is required [36]. Therefore, it may be beneficial to utilise a process which requires minimum energy requirement and consequently utilises larger wood chip. There are a number advantages associated with the size reduction of the wood on the pretreatment. One advantage is that the total accessible surface area is increased, which ultimately can improve accessibility and subsequent constituent yields [37]. Another advantage is it can reduce mass and heat transfer limitations and potentially additional energy requirements [38].

2.2.3 Chemical Pretreatment

For an ideal pretreatment, it is necessary to remove the hemicellulose and disrupt the lignin so that there is direct access to the cellulose polymers. The severity of the method conditions (temperature, pH, time) distinguish between whether the process is classified as a pretreatment or treatment. Due to the milder nature of pretreatment, complete solubilisation of the wood does not occur. The pretreatment methods that have been reported for recalcitrant lignocellulose biomass are discussed below in more detail: organosolv, oxidative and physicochemical pretreatment.

2.2.3.1 Organosolv Process

Organosolv process involves fractionation of biomass macromolecules with the aid of an organic solvent as the pretreatment medium. This method operates based on the principle of solubilisation and partitioning of the macromolecules components and disrupts the hydrogen bonding [39]. The main advantage is that relatively pure lignin is obtained as a by-product without the need of a catalyst [40]. In order to keep the method viable, high volatility, full miscibility in water and low boiling point of the solvents make them an ideal choice for regeneration [41]. A summary of the process parameters is highlighted in Table 2-3.

Table 2-3. **Organosolv Process Parameters**

Pretreatment	Process Parameters	Solvent	Catalyst	Ref
Catalysed Organosolv	Temp: 100-180°C	○ Methanol ○ Ethanol ○ Glycerol ○ Ethylene Glycol	○ HCl ○ H_2SO_4 ○ H_3PO_4 ○ Oxalic acid ○ Salicylic acid	[40, 41]
Organosolv	Temp: 185-210°C		-	[39]

Chum et al investigated a range of methanol concentrations (30-70% v/v%) and catalysts (0.01-0.05M H_2SO_4 and 0.05M H_3PO_4) at 165 °C for 2.5 hrs. After enzyme hydrolysis 70-88% of the original C_6 sugars from the Aspen wood chips [42]. In another study, Hallac et al investigated 50-65v/v% ethanol in 1.25-2w/w% H_2SO_4 and oven-dried *Buddleja davidii* between 170-195 °C and 40-60 min. After enzyme hydrolysis of the pretreated wood, 85% of the original glucose from the wood was recovered [43]. Furthermore, Sun et al investigated aqueous glycerol autocatalytic organosolv pretreatment of wheat straw at 220°C for 3 h. After enzyme hydrolysis the pretreated substrate 90% of the cellulose was converted to glucose [44]. The reported literature all provide exceptional C_6 sugar yields, between 70 to 90% of the original sugar available in the wood. However, a common shortfall amongst all of them is the heavy use of solvents, high temperatures and exhaustive exposure time required to achieve this outcome.

2.2.3.2 Oxidative Delignification

Oxidative delignification requires the use of a powerful oxidant, most commonly hydrogen peroxide, to convert the lignin into acids [40]. The concept of this method is that the oxidising agent will attack the aromatic structure in the lignin, which then turns it into acid [45]. Table 2-4 summarizes the pretreatment methods, their process parameters and the C_6 sugar yield obtained after enzyme hydrolysis. Oxidative delignification typically requires a short exposure time and low temperatures, as opposed to other chemical pretreatments. However, the use of oxidising agent carries a number of disadvantages. The major drawbacks of these methods are the high costs associated with the specific reaction vessels required to endure the severe treatment conditions, the purchase of the expensive oxidising agents as well as safety considerations with respect to the upscale of the process [46].

Table 2-4. **Oxidative Delignification Process Parameters**

Pretreatment	Process Parameters	Solvent	C_6 Sugar Yield after Enzyme Hydrolysis	Ref
Hydrogen Peroxide	Loading: 2% H_2O_2 Temp: 30°C Time: 8 hours	H_2O_2	95% glucose from pretreated sugarcane bagasse	[47, 48]
Ozonolysis	Room temperature and pressure	O_3	88.6% and 57% from pretreated wheat and rye straw	[40, 49, 50]
Wet Oxidation	Temp: 17 to 200°C Pressure: 10 - 12 bar Time: 10 - 15 minutes	O_2 or air	85% glucose from pretreated wheat straw	[40, 51]

2.2.3.3   Physicochemical

Physiochemical pretreatment methods utilise high temperature and pressure to alter the physical properties of biomass. These methods are desirable as the chemical structure of the macromolecules are not subjected to high severity through exposure to harsh reagents while achieving high C_6 sugar yields after enzyme hydrolysis. However, the major drawback is the high consumption of energy required to produce the necessary conditions [52]. Table 2-5 describes the process, specific parameters and the outcome from the respective pretreatments.

Steam explosion has been one of the leading prevalent pretreatment technologies for woody biomass. A study by Brownell et al reported the steam explosion of *Populus tremuloides* wood chips and investigated a number of parameters including the effect of sugar yield from the mechanical effect of the explosion. It was reported that the explosion part of the pretreatment has no contribution to the accessibility to the enzymes. This is contrary to some studies that have reported explosion improves the surface area of the biomass, and consequently improving enzyme access to provide higher C_6 sugar yield [53]. The process conditions also lead to degradation of the saccharides, forming compounds such as furfural, which is inhibiting for downstream fermentation [54]. Thus, development of a more advanced pretreatment could be a breakthrough for effective wood depolymerisation.

Carbon dioxide pretreatment operates in a similar manner to steam explosion, the CO_2 causes the hydrolysate to be acidic which contributes to the hemicellulose hydrolysis [54]. Both steam and CO_2 explosion utilise high temperatures and pressures and require mere seconds to complete. Liquid hot water (LHW), utilises liquid water at elevated temperatures to solubilise the hemicellulose. The higher water input in this method leads to lower concentration of degradation products, as opposed to steam explosion [54]. Ammonia fibre explosion (AFEX) requires milder temperatures to operate and 1:1 ratio by weight of ammonia and biomass [54]. Another advantage of this method is that the lignin also gets solubilised due to the pH of the reagent. However, the additional costs as a result of the significant amount of reagent is considered a disadvantage.

Table 2-5. **Physicochemical Process Descriptions**

Pretreatment	Process Description	Outcome of Pretreatment	Ref
Steam Explosion	Biomass subjected to high pressure saturated steam at 160 to 240 °C and 0.7 to 4.8 MPa for some time then pressure is released	Solubilisation of hemicellulose.	[55]
Carbon Dioxide Explosion	Supercritical CO_2 enters into a high-pressure vessel or delivered at a high pressure (7 to 27 MPa) to biomass at temperature of 200°C	Solubilisation of hemicellulose. Disrupts the crystalline structure of cellulose.	[55, 56]
Ammonia Fibre Explosion (AFEX)	Liquid anhydrous ammonia at 60-100°C is subjected to high pressures for variable amounts of time and then pressure is released	Depolymerises hemicellulose and lignin.	[57]
Liquid Hot Water	Pressure applied to keep liquid state of water at raised temperatures 160 – 240 °C	Solubilisation of hemicellulose.	[40]

2.3 Microwave Assisted Pretreatment

An understanding of the fundamentals of microwave heating is required before reviewing the reported pretreatment data for woody biomass. The electromagnetic spectrum illustrates the different types of electromagnetic radiation in increasing wavelength (m) and frequency (Hz), as shown in Figure 2-5. Microwaves are found between radio waves and infrared waves in the electromagnetic spectrum. The most common frequencies utilised for microwave heating are 2.45 GHz (λ = 12.2 cm) for domestic appliances and 915 MHz (λ = 32.8 cm) for industrial application [58]. It has been reported, with much debate, that microwave radiation at such high frequencies, contains thermal and non-thermal effects in an aqueous setting [58].

Wavelength (m)

| 10^{-12} | 10^{-9} | 10^{-6} | 10^{-3} | 1 | 10^{3} |

Frequency (Hz)

| 10^{21} | 10^{18} | 10^{15} | 10^{12} | 10^{9} | 10^{6} |
| Gamma | X-ray | UV | Vis | IR | Micro | Radio |
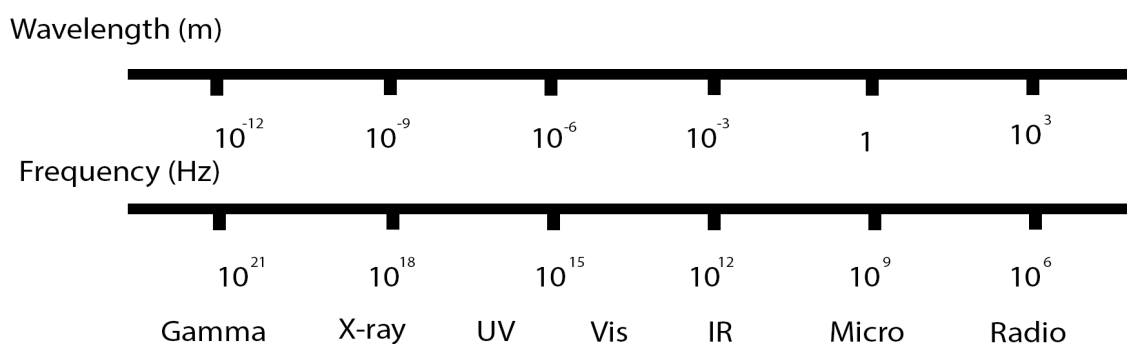
Figure 2-5. **Electromagnetic spectrum outlining the position of microwaves**

These waves are composed of an electric and magnetic field that oscillates. Forces are applied by the electric and magnetic field components of the microwaves; the value has been calculated to be at 2.4 x 10^9 times per second [59]. This rapid movement causes intermolecular interactions producing heat and consequently accelerating the process [59]. The two mechanisms which define the interactions that occur in this process are dipolar polarisation and ionic conduction [60]. Ionic conduction is the stronger mechanism and refers to the increase in interaction between the ions and the microwaves, forming heat through kinetic energy [61]. Ionic conduction mechanism will not be covered in this review. Since the forces of the electric field are so high, the molecules cannot align themselves to the constant changing direction of the field and thus a friction is created that is exhibited as heat, this is known as dipolar polarisation as illustrated in Figure 2-6 [59].

Dielectric properties of solvents are an indication of their dipole strength, which are exhibited through the presence of hydrogen bonds found in hydroxyl groups [62]. The higher the

dielectric constant of a material, the higher the dipole bond/ which in turn makes it an ideal heating accelerant for microwave irradiation. Water has the highest dielectric constant of 80.1 at 20°C as it is fully hydrogen bonded [63]. Polar molecules are better absorber of microwave energy as they can align themselves faster than other molecules, thus making them a favourable solvent choice.
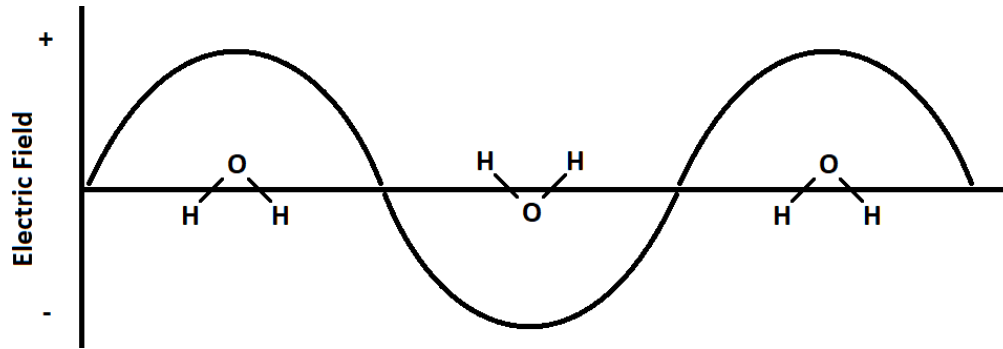
Figure 2-6. **Dipolar polarization mechanism with reference to the electric field**

In comparison to conventional heating, microwave radiation is favoured because of its uniform and selective heating and ability to instantaneously stop and start the heating process [2]. Industrial microwave processing has great potential and been already applied to and become successful in a number of industries e.g. food and rubber [2]. The main advantages of microwave processing are the ability of rapid volumetric heating of polar materials and energy efficient processing [59] and efficiency in time and energy consumption are important goals in designing and upscaling woody biomass pretreatments. In case of microwave-assisted pretreatment of biomass, in-depth understanding and optimisation of the following parameters is vital to minimise energy consumption and duration of pretreatment process of biomass.

2.3.1.1 Dielectric Loss Tangent

The interaction between microwaves, woody biomass and the solvent is governed by the dielectric properties of each which are made up of the dielectric constant and dielectric loss factor [62]. In brief, the dielectric constant is a measure of a material's ability to store electromagnetic energy and the dielectric loss factor is a materials ability to convert that electromagnetic energy into heat [58]. As shown in Equation 1, the dielectric loss tangent (also referred to as the dissipation factor) is a ratio of these two dielectric properties. The variables that determine the reproducibility are: frequency of the electromagnetic wave, temperature and physical state and composition of the material [58]. Table 2-6 lists the dielectric properties of a number of solvents with various microwave absorption capacities. The solvents lacking a difference in polarity have a low microwave absorption capacity and consequently do not heat well. In contrast, solvents with a dipole are absorbers of microwaves and can heat under microwave irradiation. An observation from the data in Table 2-6 shows the solvents with a dipole have dielectric constant greater than 30, these solvents are more in tune with the electromagnetic waves at the frequency of 2.45 GHz, thus exhibit a high loss tangent. Whereas solvents lacking a dipole have lower dielectric constants and subsequently lower loss tangents. It is important to note that there is a lack of dielectric property data at different temperatures and frequencies for many materials.

$$tan\delta = \frac{\varepsilon'}{\varepsilon''} \qquad\qquad (1)$$

Table 2-6. **Polar solvent profiles under microwave radiation (for 2.45 GHz, 20°C) [64-67]**

Solvents	Dielectric Constant (ε)	Loss Tangent (tanδ)	Microwave Absorption Capacity
Ethylene glycol	74.6	1.350	High
Formic Acid	57.9	0.722	High
Methanol	33.1	0.659	High
Water	80.1	0.123	Medium
Acetone	19.1	0.054	Low
Hexane	1.8	0.020	Low

2.3.1.2 Penetration depth

In order to have efficient biomass depolymerisation by the means of microwave heating, it is important to ensure sufficient MW penetration into the material which would lead to volumetric heating of the material, rather than surface heating only. Hence, it is necessary to have an understanding of the penetration depth into a material. The technical definition of penetration depth is the point where only 37% of the initially irradiated microwave power is still present within the material [68] and the value is dependent on heating temperature and frequency. The dissipation factor of microwave irradiation is inversely proportional to the penetration depth, as shown in equation 1 [68]. As an example, water at 25°C and 95°C has a penetration depth of 1.4 and 5.7 cm, respectively. Beyond this temperature heating would be due to convection and conduction [68].

$$tan\delta = \frac{1}{x} \qquad\qquad\qquad\qquad (2)$$

2.3.1.3 Power absorption

The microwave power absorbed per unit volume is represented in equation 2; where E represents the internal electric field magnitude and σ represents the total conductivity which takes into consideration the dielectric properties of the material. Therefore, the dielectric properties of the material have a significant impact on the amount of electromagnetic waves absorbed and consequently on the power input required [59].

$$P = \sigma |E|^2 \qquad\qquad (3)$$

2.3.1.4 Microwave Efficiency

Microwave energy efficiency can be considered the electrical energy converted to microwave energy and microwave energy converted into thermal energy. For the former, it has been reported that efficiency can vary from 0.5 to 0.65 [69]. For the latter, it is dependent on the dielectric properties of the sample being heated. This can be calculated using equation 3 and the properties for both the wood and the reagents. From this equation it is evident that higher efficiency is achieved with larger volumes [69]. Thus, the process becomes inefficient when carried out with smaller volumes and does not represent the efficiency at a larger scale [2].

$$\eta = \frac{m\, C_p \Delta T}{P_{mw} t} \qquad\qquad (4)$$

2.3.2 Review of Microwave Pretreatment for Woody Biomass

Microwave-assisted pretreatment provides a faster route and more efficient heating mechanism, compared with conventional heating methods [61]. Thus, microwave-assisted pretreatment of woody biomass can potentially be an effective pretreatment which utilises a recalcitrant waste in an efficient manner. There are numerous pretreatment methods which can be performed with microwave radiation, a comprehensive review of these can be found in literature [59, 70].

Table 2-7 shows the most relevant literature which use water only for microwave pretreatment of woody biomass. The earliest study of water only microwave pretreatment of wood was by Azuma et al, where various types of hardwood and softwood species were pretreated and their susceptibility to enzyme attack compared. It was reported that the monosaccharide yield was low and mainly xylan oligomers were formed, thus the whole microwave pretreated sample was used for enzyme digestion. The enzyme susceptibility of softwoods was generally lower than that of hardwoods [71]. In another study by Mihiretu et al on microwave hot water pretreatment of aspen sawdust, two thirds of the original xylan were recovered, with majority (90%) of the xylan in oligomer form [72]. Palm and Zacchi also investigated water only pretreatment of barked spruce wood chips to yield xylan oligomers for the production of hydrogels [73]. The reported literature all utilise water at elevated temperatures which allows splitting of the hemiacetal linkage in the wood due to its slightly 'acidic' nature [55, 74]. Furthermore, the pretreatments solubilised the hemicellulose to yield primarily oligosaccharides. These studies lack a direct comparison with the comparative conventional pretreatment to understand if a difference in product yield exists by different heating routes. Moreover, by optimising the microwave pretreatments to yield monomeric saccharides, a sugar platform, the production of endless value added chemicals could be obtained.

Table 2-7. **Hydrothermal microwave pretreatment of wood**

Feedstock	Reaction Conditions	Solvent	Product Yield	Ref
Aspen	• 170 to 200 °C for 8 to 22 min	• Water only	• Maximum xylan yield of 66% and 78% cellulose digestibility	[72]
Eucalypt Slash pine Ezomatsu Hinoki Karamatsu Sugi Todomatsu Loblloly pine Metasequoia Bald cypress Shirakanba	• 170 to 230 °C for 2 to 7 min	• Water only	• Eucalypt and Shirakanba had higher enzyme susceptibility (88-93%) compared to softwoods • Slash pine had highest and Sugi had lowest softwood enzyme susceptibility	[71]
Spruce	• 180 – 230 °C for 2-10 min	• Water only	• 12.5g/100g hemicellulose (70% of the theoretical mannan)	[73]

In line with the predominant microwave heating mechanisms, it would be best to utilise solvents which not only have the ability to absorb the electromagnetic waves efficiently to heat, but can catalyse the biomass depolymerisation as well. Polar solvents and ionic liquids (IL) have the capacity to convert EM waves to heat efficiently; the most relevant research utilising these solvents for microwave pretreatment of woody biomass have been outlined in Table 2-8.

Zhang and Zhao studied microwave conversion of pinewood to furans using the catalysed ionic liquid [bmim]HSO_4. 5-hydroxymethyfurfural and furfural yields of 45-52% and 23-31% respectively were achieved within 3 minutes of microwave exposure [4]. The conditions of this treatment are not designed to yield a sugar platform, however they do exhibit the powerful potential of ILs that convert wood polysaccharides directly to furans [4]. A study by Verma et al investigated microwave pretreatment of beechwood in various ammonium salts activated by hydrogen peroxide. Ammonium molybdate achieved the highest sugar yield, 59.5% at 140 °C [5]. The ammonium ion accelerated the wood depolymerisation and hydrogen peroxide contributed to delignification. However, the extraction of the solvent and catalyst and toxicity towards the downstream conversion of the remaining pulp are shortfalls of this method.

In another study, Sun et al reported complete dissolution of southern yellow pine and red oak in [C2mim]OAc in an oil bath, whereby the process was accelerated with the aid of microwave pulses [6]. Red oak demonstrated a faster dissolution compared with yellow pine. This method did not yield a sugar platform as it requires a catalyst for the cleavage of the covalent bonds between the lignin and the carbohydrates. Furthermore, it did not utilise microwave irradiation as the main source of pretreatment. Xu et al investigated microwave pretreatment of eucalyptus catalysed by [bmim]HSO_4. This pretreatment partially removed the hemicellulose, lignin and the amorphous regions in the cellulose; the pretreatment at 190 °C yielded 49.6 mg/g xylose and enzyme conversion yield of 89.2% glucose [75]. This method did not employ the use of acid catalysts or peroxides; thus, the milder conditions produced a higher oligomer yield. To the best of our knowledge there have been no reports in literature on the microwave pretreatment of woody biomass using polar solvents to specifically manipulate the dipolar polarisation mechanism for a fast, effective and sustainable sugar platform.

Table 2-8. Microwave compatible solvents for microwave pretreatment of woody biomass

Feedstock	Reaction Conditions	Solvent and/or catalyst	Product Yield	Ref
Pinewood	• MW irradiation at 400W for 3 min	• [bmim]HSO_4	• HMF yield 45-52% • Furfural yield 23-31%	[4]
Beechwood	• MW irradiation at 140 °C for 20 min	• 1mM ammonium molybdite • 0.88M hydrogen peroxide	• Hemicellulosic sugar yield 59.5%	[5]
Southern yellow pine Red oak	• Heating in oil bath at 40°C for 1 hour followed by 30-100 MW pulses of 3 s each	• [C2mim]OAc	• Complete dissolution of wood • Partial delignification	[6]
Eucalyptus	• MW irradiation at 190 °C	• [bmim]HSO_4	• Xylose yield 49.6 mg/g • Enzymatic Digestibility (Glucose): 89.2%	[76]

For larger particle size wood chips, severe parameters are necessary for depolymerisation and ultimately hydrolysis of the polysaccharides in the hemicellulose. However, a balance is required to preserve the sugar platform and minimise the formation of degradation products. Thus, the use of catalysts are required for the pretreatment of wood chips to achieve a sugar platform [77]. Microwave heating has not been utilised for the acid catalysed pretreatment of wood chips with water as the co-solvent, there have been reports which utilize a mixture of ethylene glycol or glycerol with various acids for fractionation of the wood macromolecules. Table 2-9 summarises the studies and their sugar yields.

Mitani et al developed a prototype for a continuous flow microwave pretreatment of Japanese cedar sapwood chips in a mixture of ethylene glycol and phosphoric acid for the production of bioethanol. After pretreatment the total sugar yield was reported to be 45.9 wt% [78]. The focus of this study was to reduce the energy consumption compared to conventional heating, which was achieved in part via the enhancement of microwave absorption by the addition of acid catalyst. Optimisation of the final sugar yield was not attempted. In another account, Lui et al investigated microwave pretreatment of Japanese cedar chips in eight acid catalysts. Optimisation of the sugar yield was investigated by varying the pk_a value of the acids and the pretreatment temperature. The highest sugar yield of 53.1% was achieved in aqueous glycerol and 0.1% hydrochloric acid (pk_a -6) at 180 °C for 6 min [79]. A linear correlation was found between the pretreatment efficiency and the pk_a value of the acids, with the exception of phosphoric and malonic acid. The use of glycerol for the organosolv fractionation of the wood chips can lead to additional cost requirement associated with its purchase and removal. Although glycerol has a high tanδ which means it can heat efficiently under microwave irradiation, its presence may not contribute efficiently towards wood depolymerisation due to its stable nature. Thus, investigation of other solvents and catalysts which could eliminate requirement of additional costs while at the same time provide reasonably high sugar yields is needed.

Table 2-9. **Acid catalysed pretreatment of wood chips via microwave heating**

Feedstock	Pretreatment Method	Reaction Conditions	Product Yield	Ref
Japanese cedar	• Continuous flow through MW pretreatment	• Ethylene glycol and H_3PO_4 (95:5)	• 45.9% total sugar yield	[78]
Japanese cedar	• Organosolv MW pre-treatment	• Aqueous glycerol • 0.1% HCl	• 53.1% total sugar yield	[79]

2.4   Research Gaps

There were several gaps surrounding the topic of chemical pretreatment of biomass identified within the literature.

- o The prevailing process for wood pretreatment is steam explosion, which requires significant energy input and produces high concentrations of degradation products. Microwave irradiation provides direct substrate heating mechanism and hence could prove to be an effective alternative to steam explosion. Until now, an in-depth examination of hydrothermal microwave pretreatment of woody biomass and its comparison to conventional liquid hot water has not been reported.

- o Effects of microwave pretreatment on downstream enzymatic hydrolysis process is not well understood. Pretreatment process may produce side products, such as leftover lignin droplets and traces of polar solvents on the pretreated fibres. Inhibition effects of these are not established.

- o To date, many studies have investigated the use of ionic liquids for wood pretreatment, however, exploitation of dipolar polarisation has not been investigated. This is partly due to the lack of dielectric data available for many solvents to assist in the investigation. There are different classes of polar solvents, with various reactivities and different microwave absorption that could be used for microwave pretreatment of woody biomass. A fundamental understanding of polar solvents and their interaction with the wood oligomers, their depolymerisation mechanism and the electromagnetic waves is required.

- o Extensive milling is common to increase the surface area of wood, which increases interaction with the solvent. However, it also increases the overall pretreatment cost. Large particle size, such as wood chips require catalysts (typically, sulphuric acid) to make pretreatment effective. However, alternative bio-based organic catalysts, such as formic and levulinic acids have not been investigated much.

2.5   References

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Chapter 3.   Microwave assisted pretreatment of eucalyptus sawdust enhances enzymatic saccharification and maximizes fermentable sugar yield

Abstract

There is an urgent need of sustainable and efficient methods for the production of biofuels and chemicals from lignocellulosic feedstock. The purpose of this study was to develop a mild, cost-effective and environmentally benign pretreatment for woody lignocellulose to maximize sugar yield via enzymatic saccharification. Microwave irradiation (MW) of *Eucalyptus regnans* sawdust in water was investigated and compared directly against conventional liquid hot water (LHW) pretreatment. Following 30 min microwave irradiation at 180 ₋C, the sugar yield was 3.5 times higher using MW than LHW pretreatment under the same conditions. Complete release of C_5 and C_6 sugars was achieved after the two-step method of MW pretreatment followed by enzymatic hydrolysis, compared with only 4% without pretreatment and 31% after LHW. Removal of 'lignin droplets' formed on the surface of the pre-treated fibers via flowing hot water showed only minor improvement in the yield of enzymatic saccharification. Our results support the hypothesis that lignin prevents access of enzymes rather than inhibits their activity. MW accelerated depolymerization of hemicellulose, opening the structure more than LHW pretreatment.

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3.1 Introduction

The production of fuels and fine chemicals from biomass may help mitigate anthropogenic greenhouse gas emissions and fulfil our demand for energy and materials [1]. Sustainably sourced lignocellulosic biomass, composed of the aromatic polymer lignin, and polymers of C_5 and C_6 sugars, hemicellulose and cellulose, is a widely available and relatively inexpensive feedstock at $50 per ton [2-4]. Thus, the processing of lignocellulosic biomass is of benefit across multiple industries and a green alternative to greenhouse gas emitting sources. Pretreatment is essential to open up the complex lignocellulose structure and allow access to the sugar polymers for cellulolytic enzymes [5]. An impeding factor for lignocellulose pretreatment is its recalcitrance demands a high energy input. One such example is in the lignocellulosic biofuels process, where sugar release, including pretreatment and enzyme digestion, can contribute up to 45% of the total costs [6] and is one of the main factors hindering its full commercialization [7]. Agricultural residues such as corn stover, wheat straw and bagasse have been widely investigated and some are currently used as feedstocks for lignocellulosic ethanol production [8-10]. Forestry species, in particular, eucalypts, are cultivated world-wide but have not been widely considered, due in part to their recalcitrance.

While many pretreatment approaches including hydrothermal/chemical, physical and biological have been studied for biomass, a step change in energy-efficiency and green approach is needed to improve the attractiveness of woody lignocellulose as a feedstock. The more common pretreatment technologies for lignocellulosic biomass include steam explosion (acid catalyzed/uncatalyzed) and liquid hot water (LHW) [11]. While effective, these processes generally need large energy input for heating. Water is an ideal medium for lignocellulose pretreatment due to its low cost, relative simplicity of product and by-product formation and its use avoids the need for corrective action downstream. However, when water alone is used for pretreatment, as in the LHW process, it is not as effective in comparison to acid-catalyzed steam explosion [12]. Variations of the standard LHW pretreatment method have been reported to be more effective at releasing both hemicellulose and cellulose-derived sugars following enzyme treatment of woody feedstocks but these have required two sequential LHW steps [13], increasing the energy requirement.

Microwave irradiation is energy efficient as it directly heats substances via dipole polarization and ionic conduction, as opposed to convection and conduction mechanisms that transfer heat in conventional processes [14]. Industrial microwave processing has great potential and been already applied to and become successful in the food and rubber industries [15]. The main benefits of microwave processing are the rapid and uniform volumetric heating of polar materials and energy efficient processing [15, 16]. In the near future, with the expected growing demand for green energy sources combined with the requirement for more energy efficient processes, alternative treatments such as microwave heating will play a crucial role. To date, most research into microwave treatment of biomass has been undertaken on agricultural by-products and grasses, converting them into polysaccharides and lignin in the presence of acids, alkali, salts, ionic liquids and other high boiling point solvents [17] as substrates for further hydrolysis and conversion into chemicals and fuels [18]. A recent publication by Aguilar-Reynosa et al showed that the microwave pretreatment of corn residues in water resulted in marginal increase in the rate of saccharification and fermentation but achieved similar yield compared with conduction-convection heating at similar energy intensity and enzyme loadings [19]. However, woody biomass is significantly more recalcitrant due to higher lignin content and hence direct comparison of microwave pretreatment of woody biomass in water-only medium against conventional heating is necessary.

Here we report the effectiveness of pretreatment of *Eucalyptus regnans* in water only, comparing microwave irradiation with conventional heating methods under identical reaction conditions. To our knowledge, this is the first detailed study to investigate non-thermal effects of microwave pretreatment of woody biomass in water and demonstrates that microwave heating is significantly more effective than LHW. In microwave pretreatment, hemicellulose was hydrolyzed by up to 90% and lignin droplets were formed on the surface of the fibers. Pretreated biomass was further treated with flowing hot water to remove the lignin droplets, which marginally improved the enzymatic sugar yield. Following lignin droplet removal, which accounted for ~60% of the lignin, complete sugar release was achieved for the microwave pre-treated samples after enzymatic hydrolysis. The same result was achieved for microwave pre-treated samples only (without lignin droplet removal). This was over 3 times higher yield than enzymatic hydrolysis of LHW pretreated samples. These results suggest that microwave

pre-treated biomass samples are sufficiently disrupted to allow enzyme access and efficient sugar release without the need for lignin droplet removal.

3.2 Materials and Methods

3.2.1 Materials

Eucalyptus regnans sawdust (average D_p = 0.5 mm, Pollards Sawdust, Australia) was used for all experiments. The moisture content of the sawdust was determined according to standard method AS/NZS 1800; in brief, 10.0 g of the sawdust was weighed in an analytical balance and placed in an oven at 105°C for 24 h. The sawdust was removed and immediately re-weighed, the mass loss divided by the initial sawdust weight gave a moisture content of 9.5 wt%.

3.2.2 Microwave and liquid hot water pretreatment of biomass

Eucalyptus sawdust (0.4 g) and 10 ml of Milli-Q water were added to a Teflon microwave reactor vessel with 100 ml capacity and heated using a QLab Pro Microwave Digester (Questron Technology Corp, Canada) at 500 W until the desired temperature is reached. Microwave frequency of 2.45GHz is commonly used, as it was in this study, to prevent interference with telecommunication, wireless networks and cellular phone frequencies [20]. The set temperature for the experiments was 180°C and the average time taken to heat the sample to this temperature was 17 min. Once the temperature was reached, the sample was maintained at 180°C for periods between 5 and 60 min. On completion of the microwave irradiation, the vessel was cooled to room temperature, the contents were filtered using Filtech filter paper CAT NO 1830-055 and washed with water to recover two fractions: unreacted solid residue and liquid supernatant. The solid residue was air dried and stored in a sealed tube. Microwave reactions were carried out in duplicate.

For LHW pretreatment, 0.4 g of eucalyptus sawdust and 10 mL of Milli-Q water were introduced into a Swagelok stainless steel single-ended 50 mL sample cylinder equipped with a thermocouple. The cylinder was capped and placed in an oil bath and heated with approximately 90% of the sample cylinder submerged into the oil bath and covered with aluminum foil. The average time taken to reach 180°C in the biomass solution was 15 min. Once the intended temperature was reached, samples were maintained at the set temperature for 15 or 30 min with the temperature being controlled within ±5°C. On

completion of the experiment, the vessel was cooled in a room temperature oil bath, the contents removed and centrifuged to recover the unreacted solid residue and supernatant.

3.2.3 Delignification by removal of lignin droplets on biomass using flowing hot water

The microwave and LHW pre-treated fibers were washed with flowing liquid hot water in a pressurized fixed bed reactor as shown in Figure 3-1. The flowing hot water (1.25 ml/min for 2 h) at 120°C and 2 bar was used to remove the lignin droplets deposited onto the surface of the pretreated samples. The wash-water containing the lignin droplets was collected periodically from the vapor-liquid phase separator and filtered using Whatman™ qualitative filter paper, Grade 1 with 11 μm pore size. The mass fraction of the solid lignin droplets was calculated according to equation 1 to give mg of lignin droplet per gram of dry biomass.

Mass Fraction Removed Lignin $(mg/g) =$

$$\frac{dry\ mass\ lignin\ droplet}{dry\ mass\ of\ original\ sawdust} \quad (1)$$
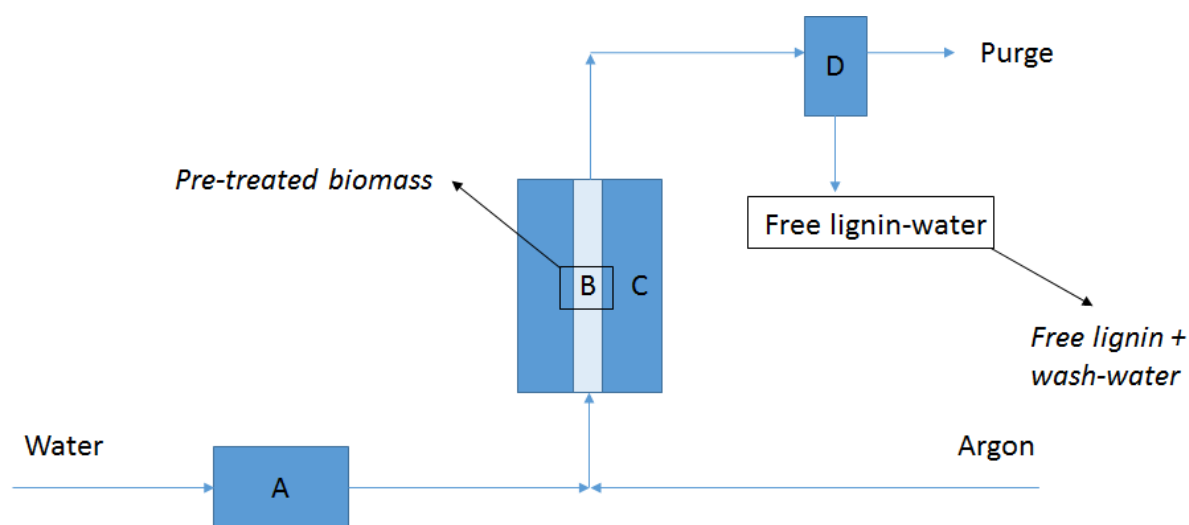
Figure 3-1. Scheme of a pressurized fixed bed reactor used to remove lignin droplets on pretreated biomass (A) HPLC pump, (B) fixed bed reactor, (C) furnace and (D) vapor-liquid phase separator.

3.2.4 Analysis of supernatant and solid residue from delignification of pretreated biomass

The supernatant liquid fraction from the delignification process were centrifuged and analyzed on a TOC-L Shimadzu Total Organic Carbon Analyzer to quantify the carbon compounds present. A 0.2 μm syringe filter was used to remove suspended particles in the solution. Of the 150 ml collected, 5 ml of sample was transferred to a TOC vial and diluted 8-fold prior to analysis.

3.2.5 Hydrolysis of xylo-oligomers to xylose monosaccharides in the pretreatment supernatants

Following the 15 and 30 min microwave and LHW pretreatments, 1 ml of the supernatant was combined with 20 μl of endo-1,4-β-xylanase M6 (rumen microorganism, Megazyme E-XYRU6) and incubated for 20 h at 40°C without shaking. HPLC analysis of the supernatant before and after xylanase digestion was performed to determine the extent of xylo-oligomers in the supernatant and to allow determination of the sugar concentration of those oligomers as monomeric sugars following enzymatic hydrolysis.

3.2.6 Enzyme -catalyzed hydrolysis of cellulose from biomass

Enzymatic hydrolysis of pretreated and raw biomass was performed with cellulase mixtures from *Trichoderma reesei* ATCC 26921 (Sigma-Aldrich, >=700 UI/g) supplemented with cellobiase from *Aspergillus niger* (Sigma-Aldrich, ≥250 UI/g) at two levels of application; high enzyme load of 40 FPU/g (Filter paper units per gram of biomass) which is an overload to reflect industry practice to release the most sugars and low enzyme load of 10 FPU/g which was intentionally done to study the effectiveness of microwave exposure time and the effect of delignification. The biomass samples were prepared by mixing 30 mg of dried, pretreated or raw biomass, 1 ml 50 mM sodium citrate buffer pH 4.7 and the requisite volume of enzyme solution to achieve the required loading, followed by incubation for 48 h at 50°C. Following digestion, supernatant sugar concentrations were determined by HPLC as described below and by the DNS assay [21]. For the DNS assay, glucose standards were prepared by dilution of a stock solution of anhydrous glucose (10 mg/ml) and DNS reagent was added to sample and standard solutions and incubated at 90°C for 10 min. The absorbance of suitably diluted standards and samples was determined at 540 nm using a Shimadzu UV-2450 UV-Vis spectrophotometer. The concentration of reducing sugars in the samples was determined

from the glucose standard curve. All experiments were carried out in duplicate and the results presented as the average value. There was close agreement between the sugar concentrations obtained by HPLC analysis and the DNS assay with ≤6% standard deviation.

3.2.7 HPLC analysis of sugars and chemicals from treated biomass

Samples obtained from microwave and LHW pretreated supernatants, the wash-water from the delignification experiments and the supernatants from enzyme treatment of biomass were analyzed by HPLC using an Agilent Technologies 1220 Infinity instrument equipped with REZEX monosaccharide RHM column using diode array (DAD) and refractive index (RID) detectors. The flow rate for the mobile phase (water) was set at 0.5 ml/min and a sample injection volume of 10 µl. The temperature of the column was set at 65°C and temperature of the RID at 45°C. A standard curve was created for the sugars, furfural and 5-hydroxymethylfurfural each with 4 data points.

3.2.8 Calculation of sugar and chemical yields

The composition of the *Eucalyptus regnans* used in the experiments, as shown in Figure 3-2, was determined according to the NREL method at Queensland University of Technology in Brisbane, Australia. The molar yield of sugars was calculated by dividing the experimental number of moles of sugar by the actual number of moles present in biomass.
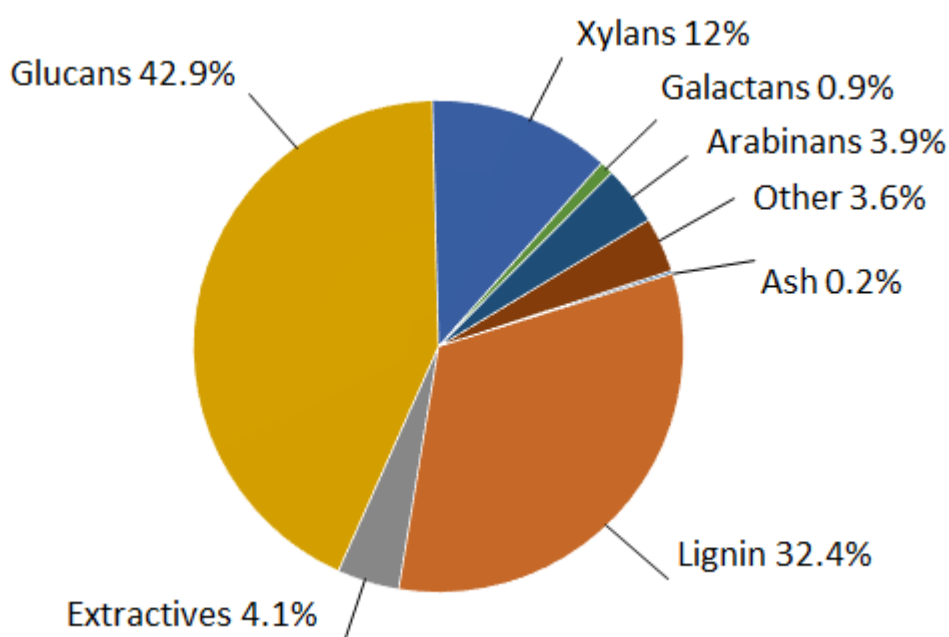
Figure 3-2. **Chemical composition of *Eucalyptus regnans* (Melbourne, Australia)**

The yield of sugars on the mass and mole basis were calculated according to equations 2 and 3, respectively. The percentage molar yield of furfural (FUR) and 5-hydromethylfurfural (5-HMF) are described in equations 4 and 5, respectively. There have been reports of other reaction pathways for the decomposition of glucose, one such pathway is the isomerization of glucose to fructose followed by the decomposition to furfural [22]. Therefore, the molar yield of furfural takes into account both carbon five and six sugars.

$$\textbf{Mass Yield of Sugars (mg/g)} = \frac{\text{mass of sugar released}}{\text{mass of sawdust on dry basis}} \tag{2}$$

$$\textbf{Molar Yield of Sugars (\%)} = \frac{\text{Moles of sugar released}}{\text{initial moles of sugar in sawdust}} \times \textbf{100} \tag{3}$$

$$\textbf{Molar yield (FUR) (\%)} = \frac{\text{moles FUR released}}{\text{initial moles of glucose + xylose}} \times \textbf{100} \tag{4}$$

$$\textbf{Molar yield (5-HMF) (\%)} = \frac{\text{moles 5-HMF released}}{\text{initial moles of glucose}} \times \textbf{100} \tag{5}$$

3.2.9 Biomass particle and lignin droplet size measurement

The average diameters of the untreated and pretreated biomass samples were measured on a Malvern Instruments Master Sizer 3000 using the dry method. In brief, dried pretreated biomass was placed in a compartment and air used to carry the sample through a cell. The diameter (mm) of the detected particles were recorded with respect to the percentage volume distribution and the samples assessed in triplicate experiments.

Dynamic light scattering (DLS) measurements were performed using the Malvern Instruments Nano series Zetasizer to detect the size of the colloidal lignin droplets found in the supernatant after delignification (ESI). The supernatant liquid was ultrasonicated using a 700 W probe for 2 min at 20MHz to break up aggregates and 1 ml of the sample was diluted in 10 ml water and placed in a cuvette for analysis by the % volume method.

3.2.10 Analysis of lignin droplets by FT-IR

An Agilent Technologies Cary 630 FTIR Spectrometer with diamond crystal was used to analyze the solid fraction removed from the delignification process. A small fraction of the

air-dried sample was placed on the crystal and pressed down. The spectra obtained was scanned across 750 to 3800 cm^{-1}.

3.2.11 Microstructure analysis of pretreated biomass

SEM images were taken using a FEI Nova Nano SEM 450 FEG SEM and prepared by placing on a carbon tape attached to stubs and coated with approximately 1 nm of iridium. Samples of raw, microwave, LHW, the respective delignified fibers and lignin droplets were air dried and directly imaged.

3.3 Results and Discussion

3.3.1 Microwave pretreatment fully hydrolyzes hemicellulose

Eucalyptus regnans sawdust samples in aqueous medium were exposed to microwave irradiation or LHW heating at 180°C, the temperature selected was based on the reported thermal softening temperature of hard and softwoods [23]. Microwave treatment selectively hydrolyzed hemicellulose from the biomass without significantly hydrolyzing the cellulose network as the main identified sugar was xylose Figure 3-3A. This result is expected since hemicellulose polymers in hardwood are comprised mainly of xylans that have a backbone of β-1, 4-D-xylopyranose [24, 25]. Hydrolysis of the glycosidic linkages within hemicellulose was likely catalyzed by the hydronium ions generated in water at high temperatures [17, 26]. In addition, the depolymerization of hemicellulose releases acetyl and uronic acids bound to the xylan backbone in eucalyptus, which creates an acidic environment that promotes further hemicellulose hydrolysis and sugar dehydration into furfural[26, 27]. The optimal exposure time was 30 min at which xylose yield peaked at 88.7 mol%; increasing the heating time resulted in dehydration of xylose to furfural with 15 mol% yield at 60 min (Figure 3A). The branching characteristic of hemicellulose polymers coupled with the low degree of polymerization, allows them to be more readily hydrolyzed than cellulose [28].

An earlier study by Mihiretu et al. [29] examined microwave pretreatment of woody biomass in water only and found two thirds of the xylan hydrolyzed in oligomer form from aspen wood heated at 195°C by microwave, whereas in this study more than 90% of the xylan was released, all in monomeric form, at a lower temperature. Similarly, Xu *et. al.* [30] examined the

pretreatment of eucalyptus biomass in water plus acidic ionic liquid [bmim]HSO$_4$ as a catalyst. The maximum xylose yield achieved by Xu *et. al.* was 49.6 mg/g after treatment at 190°C, whereas 107.1 mg/g was achieved in this study at 180°C in water only. In Xu *et al.,* the addition of acidic ionic liquid to the microwave pretreatment led to significant formation of acetic acid, furfural and lactic acid thereby reducing the yield of sugar [30]; these products can negatively affect downstream fermentation. In our case, no solvent removal was required after treatment and production of dehydrated sugar products was minimal up to 30 min microwave exposure time. Compared with microwave pretreatment, LHW pretreatment at identical conditions resulted in lower yields of all the products, indicating lesser extent of deploymerization Figure 3-3B**.**
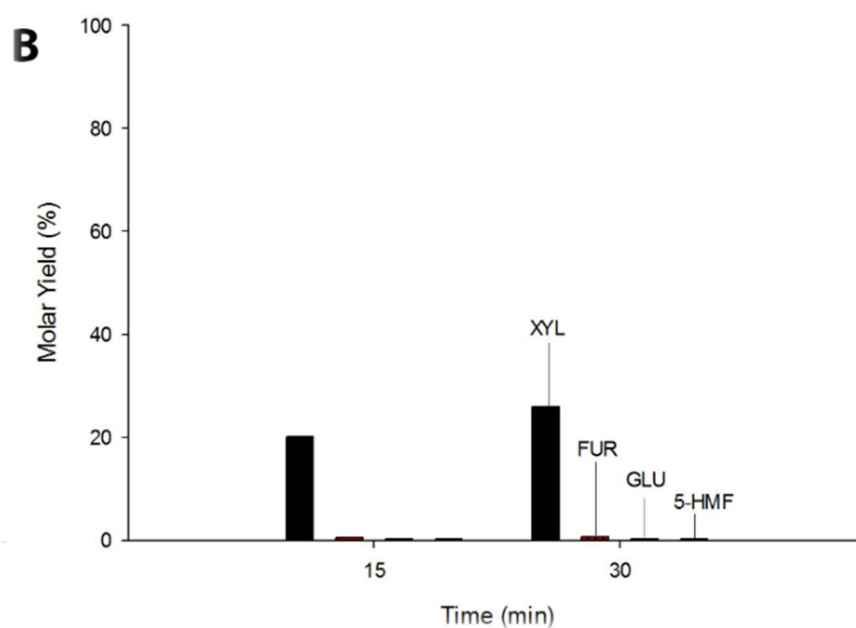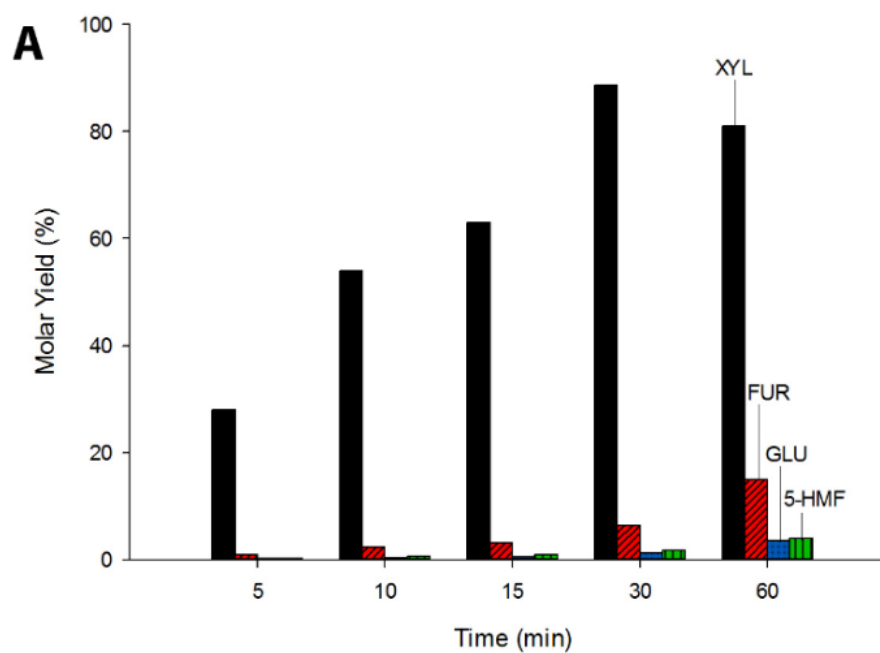
Figure 3-3. Molar yields of products detected in the supernatants of pretreated *Eucalyptus regnans* sawdust in water only at 180°C versus exposure time. (A) Microwave-irradiated and (B) Liquid hot water. Reaction conditions: 4wt% eucalyptus sawdust loading. Xylose (XYL), furfural (FUR), glucose (GLU) and 5-hydroxymethylfurfural (5-HMF)

LHW pretreatment released 3-3.5 times lower xylose, the yield of xylo-oligosaccharides was higher and increased with treatment time in the LHW samples (Table 3-1). Since xylo-oligomers remaining in hemicellulose can interfere with the action of cellulolytic enzymes, it is desirable to remove as much of the hemicellulose fraction as possible prior to enzyme application [31].

Table 3-1. Yields of dehydrated sugar products and monomeric sugar release following *Eucalyptus* biomass pretreatments at 180°C.

Pretreatment Duration (min)	Mass Yield (mg/g$_{db}$)				
	Xylose	Water soluble C$_5$ oligomers[#]	Glucose	Furfural	5-HMF
Microwave					
15	75.9 ± 2.7	27.0 ± 0.1	2.2 ± 0.0	10.9 ± 0.6	3.0 ± 0.5
30	107.1 ± 2.2	22.7 ± 7.5	5.0 ± 0.2	21.8 ± 1.8	6.1 ± 0.9
Liquid hot water					
15	24.2 ± 21.7	34.2 ± 0.9	0.9 ± 0.4	1.7 ± 1.5	0.6 ± 0.4
30	31.4 ± 13.9	67.0 ± 0.0	0.9 ± 0.1	2.5 ± 1.3	1.0 ± 0.4

Product calculation is based on a bone dry biomass basis. The yields of sugars for each treatment includes the products that have undergone dehydration at 180°C. Note: Furfural can derive from both carbon 5 and -6 sugars. # Calculated by difference between the after- and before-xylanase treatment.

Xylan depolymerization into xylose was accelerated in microwave pretreatment, with more than 80% of the xylan hydrolyzed to xylose and the remainder as soluble xylo-oligomers after 30 min pretreatment. By contrast, 31% of xylan was hydrolyzed into xylose after 30 min LHW pretreatment, and ~60% in xylo-oligomer form. Our LHW results were similar to those obtained by Yu *et. al.* on eucalyptus wood chips treated in a batch stirred reactor at 180°C for 20 min which gave a total xylose recovery of 84.4% of which most of the xylose was recovered as oligosaccharides (46.8%) [32]. Unlike conventional heating, microwave irradiation can disrupt the polymeric sugar structure due to both thermal and non-thermal effects [33]. Furthermore, it can accelerate the rate of chemical reactions through ionic conduction and dipolar polarization and reduce overall reaction time [14, 34]. The current work demonstrates that microwave irradiation is a faster and efficient form of pretreatment compared with liquid hot water, accelerating the hydrolysis of xylan and xylo-oligomers into xylose [31, 35].

Raw eucalyptus sawdust used in this study had a particle size distribution of 0.3 – 2.2 mm with a peak size at approximately 1 mm (ESI) and following 30 min microwave pretreatment in water, the residual solids were reanalyzed for particle size (ESI). The results showed a minor reduction in the intensity of the peak, around 0.5 – 0.9 mm, and a new peak at 0.09 – 0.25 mm, which accounted for 1.2% volume distribution of the whole sample. This result clearly demonstrates that the biomass is not changing substantially during microwave treatment and that some smaller particles in the starting material are breaking down during microwave pretreatment.

3.3.2 Pretreated Biomass shows "Lignin Droplet" Deposits

SEM images of the samples before and after pretreatment showed that samples pretreated for 5 min or longer had spherical complexes deposited on the surface of the fibers (Figure 4) and their number and size increased with pretreatment exposure time. Previous studies of pretreated biomass have also described the appearance of these spherical complexes after acid-catalyzed or hydrothermal pretreatment and termed them "lignin droplets" or "pseudo lignin". The mechanism of the formation of lignin droplets observed on corn stover has been hypothesized to be due to the coalescence of lignin during the pretreatment in aqueous conditions at temperatures above the lignin melting temperature [36].The coalescence is thought to happen within the cell wall and migrate to the surface after thermal expansion [36]. Pseudo-lignin, by contrast, are droplets that form on biomass treated under aqueous acid conditions at moderate temperatures [37], and have a similar appearance to lignin droplets. The observation of lignin droplets on woody biomass under microwave treatment in this study (Figure 3-4) is important as their presence has been considered to be inhibitory for the enzymatic hydrolysis of cellulose [38] therefore an effective and efficient method of removal of these droplets may be needed for optimal sugar release from pretreated biomass.
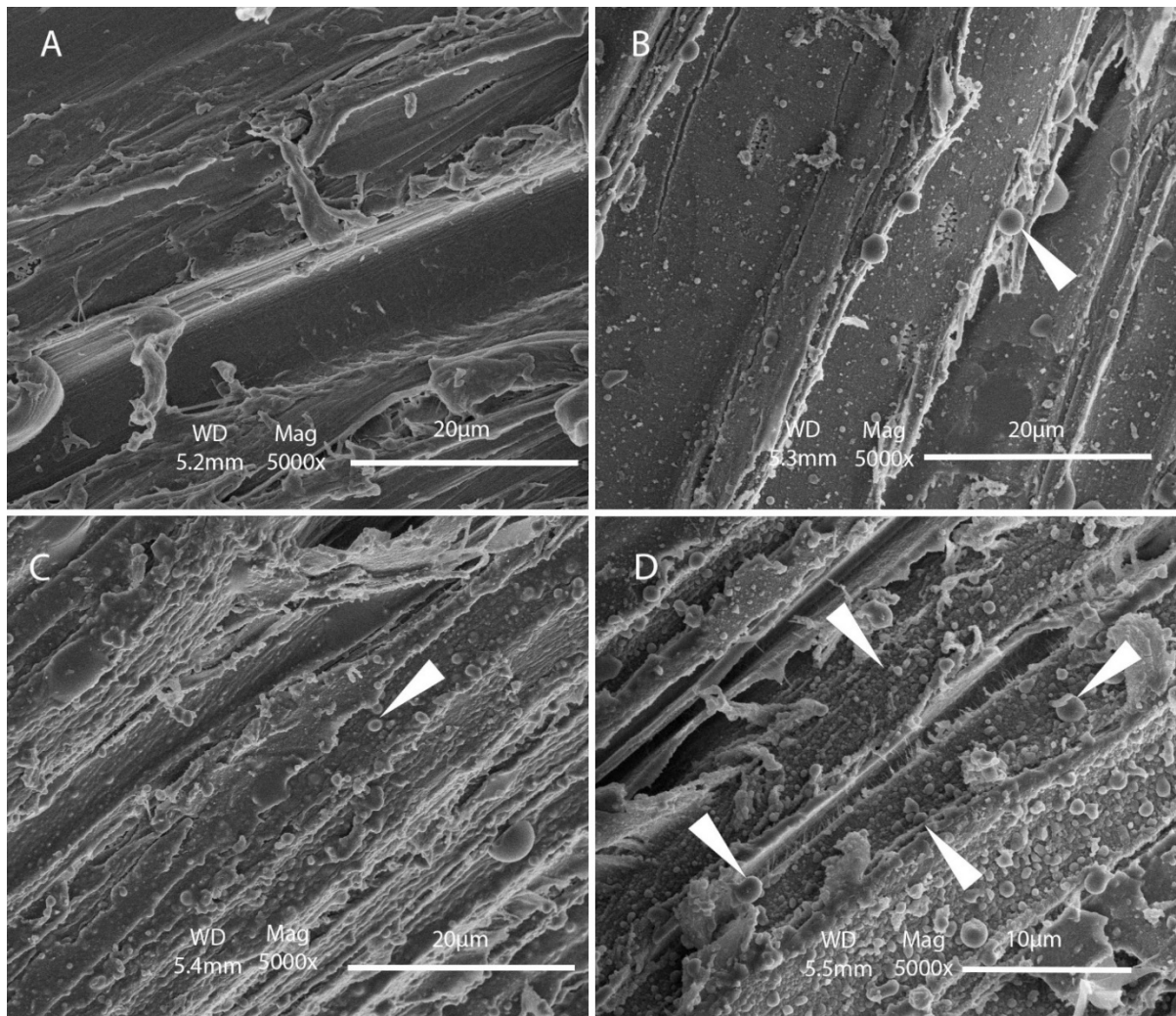
Figure 3-4. SEM micrographs showing the morphological structure of *Eucalyptus regnans* sawdust and the effect of microwave heating in water (A) untreated eucalyptus sawdust, and heated for (B) 15, (C) 30 and (D) 60 min at 180°C.

3.3.3 Flowing Compressed Hot Water Removes Lignin Droplets from Pretreated Biomass

Lignin droplets from microwave and LHW pretreated samples were removed, and the resulting free lignin was characterized. SEM images of the pretreated biomass samples show that microwave and LHW treated eucalyptus wood had similar coverage of lignin droplets on the surface of biomass (Figure 3-5A and Figure 3-5D), respectively). The lignin droplets were successfully removed from the biomass surfaces via flowing compressed hot water (Figure 3-5B and Figure 3-5E) yielding free insoluble lignin (Figure 3-5C and Figure 3-5F) which were collected from wash-water by filtration. Figure 3-5B and Figure 3-5E suggest that the pressurized hot water process was effective for removing the larger lignin droplets, however, some smaller droplets can be observed on the surface. The maximum amount of lignin droplet removed during the delignification step was similar for both microwave and LHW treated samples at approximately 180 mg/g, which corresponds to roughly 60% of lignin in the raw biomass sample.

There has been an ongoing debate about the role of lignin and its effect on enzymatic hydrolysis. Lignin is an intricate network of aromatic polymers connected via carbon to carbon or carbon to oxygen bonds; with aryl ether bonds being their most significant internal linkage [26]. During pretreatment, these linkages are broken and the lignin either breaks down to fragments or forms lignin globules [26]. Previous studies have proposed that there is a correlation between the enzyme loading and presence of lignin as the enzymes are either adsorbed or associated with the lignin, inhibiting their activity [39, 40].
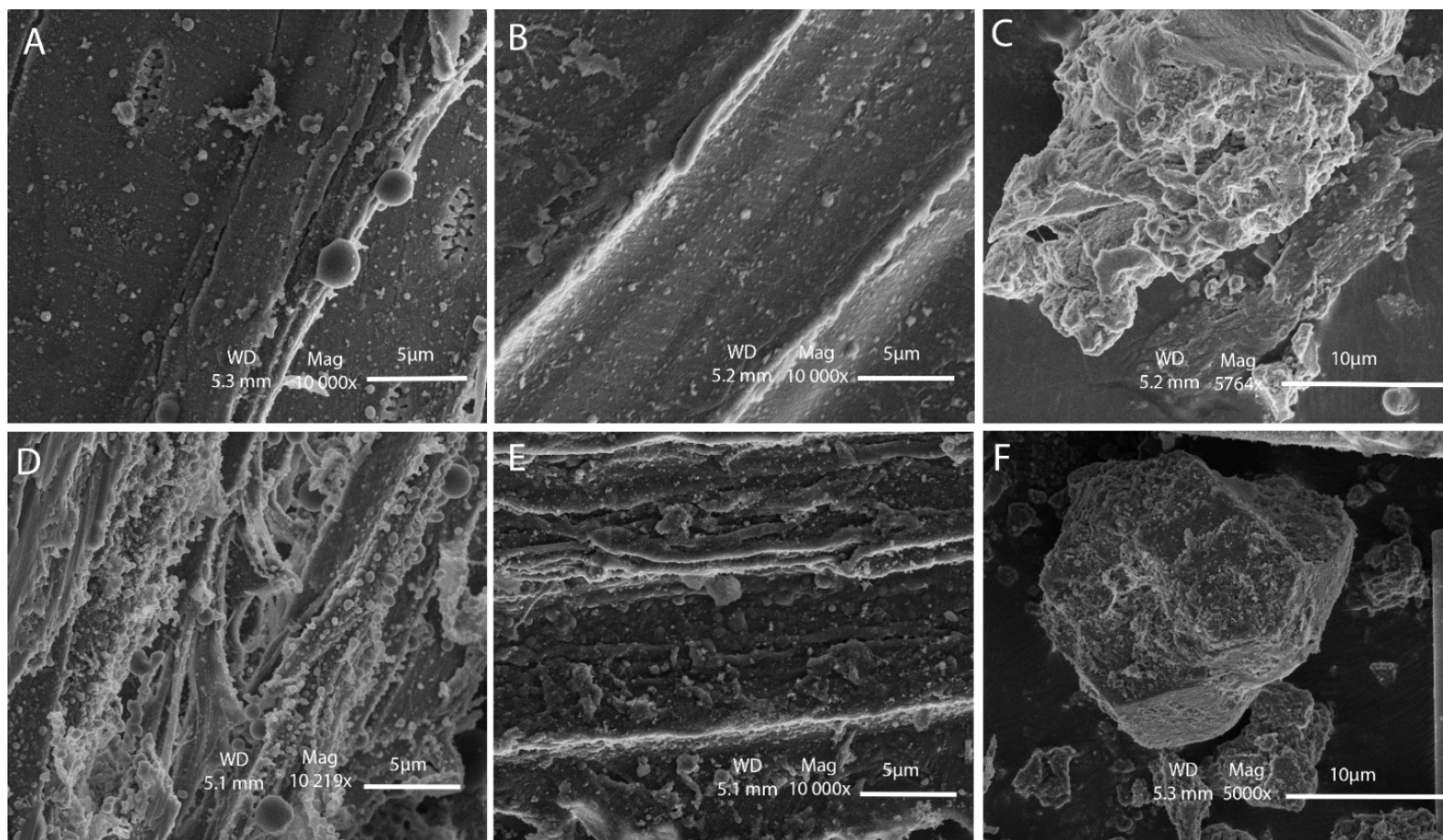
Figure 3-5. Representative SEM micrographs of pretreated, delignified eucalyptus sawdust and lignin-carbohydrate complexes removed from biomass using compressed hot water at 120°C for 2 h. A. Microwave treated biomass 15 min 180°C. B. delignified, microwave treated biomass C. recovered lignin droplets D. Liquid Hot Water pretreated biomass 15 min, 180° E. delignified, LHW treated biomass F. recovered lignin droplets

The wash-water obtained from the delignification step was also light red in color. Dynamic Light Scattering analysis showed that the wash-water contained colloidal particles of average 351±42.8 nm in size which may have been further lignin droplets too small to be filtered from the solution (ESI). Total Organic Carbon analysis showed all water samples contained carbon with the highest value being ~630 mg/L for the delignified 15 min microwave-treated biomass sample. HPLC analyses also showed trace amounts of sugar in all the samples plus a significant peak of an unknown compound. LCMS analysis of the unknown peak suggested a dimeric chemical generated by the interaction of water and a polar aromatic component of the wood resin (ESI), this suggests that the compound could be of lignin or extractives origin.

The ATR spectrum of the free lignin droplets shows several peaks common to functional groups present in lignin and carbohydrates (Figure 3-6). The peak at 1327 cm^{-1} is C=O bond stretching of syringyl groups known to be found in hardwoods and the peak at 1267 cm^{-1} represents C-O bond stretching present in guaiacyl unit [41]. The intense peak at 829 cm^{-1} is associated with the out of plane deformation band for 1,3,5-tri substituted benzene rings and the peak at 1086 cm^{-1} represents C-O bond functional groups of ring alcohols in the carbohydrate moieties [36]. Intense alkyl aromatic ethers (Ar-O) stretch is found at 1236 cm^{-1} and the carbon to carbon (C-C) stretching for the ring is represented by the peak at 1554cm^{-1}.
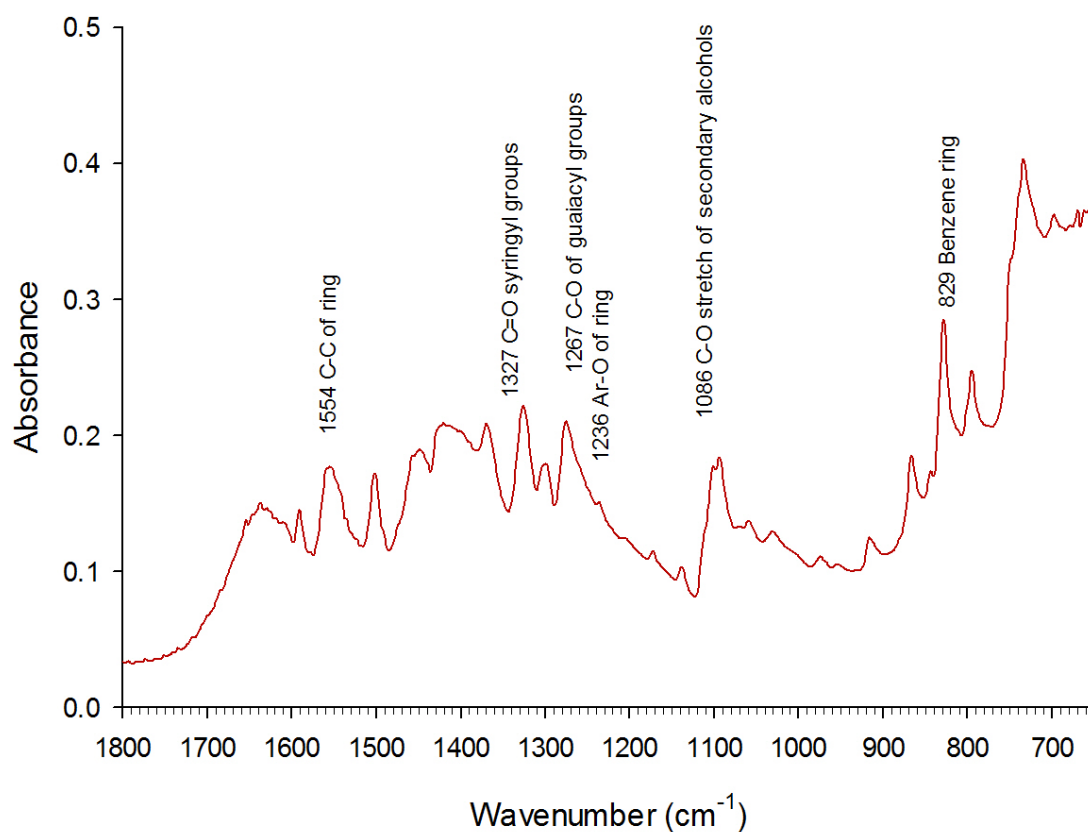
Figure 3-6. FTIR Spectrum of the recovered solid free lignin from Eucalyptus sawdust pretreated at 15 min at 180°C in water, then delignified with flowing compressed hot water at 120°C for 2 h

3.3.4 Enzymatic Hydrolysis of Microwave Pretreated Samples Yielded Complete Sugar Release

The absence of significant glucose in supernatants from microwave and LHW-pretreated samples and the low levels of 5-HMF suggests that cellulose hydrolysis was minor under these conditions. The pretreated samples were washed with Milli-Q water prior to enzyme hydrolysis as to remove residual furans, phenols and acids as their presence can have an inhibitory effect on the enzymes. Cellulases were applied to the pretreated wood samples at high enzyme loading which released 100% of the cellulose-derived sugars in microwave-treated samples (Table 2). Overall, out of the total sugars in biomass, c.a. 13% was released during the pretreatment step and the remainder during the enzymatic treatment. In comparison, without the pretreatment step, the eucalyptus sample released only 4% of the total sugars during the enzymatic hydrolysis.

Table 3-2 shows enzymatic digestion of the pretreated only and pretreated and delignified samples at a high enzyme loading (40 FPU/g). Excess enzyme loading can increase the hydrolysis rate to a degree, but in turn increase the process cost [42]. The typical amount of cellulase used in laboratory is 10 FPU/g as it gives a reasonable profile within a practical timeframe [43]. Table 3 shows enzymatic digestion results for the same pretreated biomass samples using a low enzyme loading (10 FPU/g).

During the enzymatic hydrolysis step, LHW pretreated samples released one-fifth of the sugars compared to the microwave-pretreated samples (Table 3-2). Furthermore, the total sugar yield (pretreatment + enzymatic hydrolysis) from LHW-treated samples was approximately one quarter of the equivalent release from microwave pretreated samples. The presence of residual xylan and acetyl groups bound to the xylan polymer are likely to have contributed to the slower enzymatic hydrolysis of LHW samples [26, 44].

Table 3-2. Effectiveness of microwave pretreatment with or without a further delignification step with flowing hot water on release of sugar from *Eucalyptus regnans* biomass treated with cellulases at high loading.

Sample	Pretreatment Mass Yield[#] (mg/g$_{db}$)	Enzymatic Hydrolysis Mass Yield[#] (mg/g$_{db}$)	Total Sugars Mass Yield[$] (mg/g$_{db}$)	Total Sugars % Yield[§]
	Xylose + Glucose	Reducing Sugars		
Raw	-	22.2 ± 0.8	22.2 ± 0.8	4 ± 0.1
Pretreated only / min				
MW 15	78.1 ± 1.0	523.2 ± 4.8	601.3 ± 2.1	102 ± 0.4
LHW 15	25.1 ± 8.1	102.1 ± 21.5	127.2 ± 0.6	22 ± 2.8
MW 30	112.1 ± 0.9	496.8 ± 103.7	608.9 ± 105.6	104 ± 18.0
LHW 30	32.3 ± 0.5	112.6 ± 20.0	144.9 ± 34.0	25 ± 5.8
Pretreated and Delignified /				
MW / 15	78.1 ± 1.0	497.3 ± 20.1	575.4 ± 17.4	98 ± 3.0
LHW / 15	25.1 ± 8.1	96.5 ± 29.4	121.6 ± 51.5	21 ± 8.8
MW / 30	112.1 ± 0.9	430.9 ± 120.4	543.9 ± 118.5	93 ± 20.2
LHW / 30	32.3 ± 0.5	148.9 ± 55.3	181.2 ± 69.3	31 ± 11.8

Mass yield of sugars after pretreatment and enzymatic hydrolysis are expressed as mass of the product obtained per unit mass dry biomass (mg/g$_{db}$). Sugars yield from pretreatment include xylose and glucose.

$Total Sugars Mass Yield include C_5 and C_6 Sugar yield from pretreatment and reducing sugars yield from enzymatic hydrolysis.

§Total Sugars % Yield is the percentage of available sugars produced on dry biomass mass basis.

Table 3-3. **Effectiveness of microwave pretreatment with or without a further delignification step with flowing hot water on release of sugar from *Eucalyptus regnans* biomass treated with cellulases at low loading.**

Sample	Pretreatment Mass Yield[#] (mg/g$_{db}$)	Enzymatic Hydrolysis Mass Yield[#] (mg/g$_{db}$)	Total Sugars Mass Yield[$] (mg/g$_{db}$)	Total Sugars % Yield[§]
	Xylose + Glucose	Reducing Sugars		
Raw	-	10.1 ± 2.0	10.1 ± 2.0	2 ± 0.3
Pretreated only / min				
15	78.1 ± 1.0	13.4 ± 1.8	91.5 ± 1.8	16 ± 0.3
30	112.1 ± 0.9	64.3 ± 6.7	176.5 ± 6.7	30 ± 1.1
Pretreated and delignified / min				
15	78.1 ± 1.0	14.7 ± 1.1	92.8 ± 1.1	16 ± 0.2
30	112.1 ± 0.9	104.4 ± 6.3	216.5 ± 6.3	37 ± 1.1

[#] Mass yield of sugars after pretreatment and enzymatic hydrolysis are expressed as mass of the product obtained per unit mass dry biomass (mg/g$_{db}$). Sugars yield from pretreatment include xylose and glucose.

[$]Total Sugars Mass Yield include C_5 and C_6 Sugar yield from pretreatment and reducing sugars yield from enzymatic hydrolysis.

[§]Total Sugars % Yield is the percentage of available sugars produced on dry biomass mass basis.

Overall, the microwave pretreated biomass yielded >90% xylose release from the pretreatment step and complete sugar release from cellulose digestion, resulting in a far greater sugar release compared with the LHW pretreatment. Complete release of sugars has been previously been reported with *E. grandis* wood chips in water but it required two sequential LHW steps at 180 and 200°C with vigorous stirring, followed by enzyme digestion of the biomass to achieve this [13]. The direct heating of microwave irradiation excites the water and polar species in the dissociated biomass causing friction which dissipates as heat [14]. In addition, the depolymerized monosaccharides contribute to the polar state over time and assist in the rapid heating. Therefore, microwave treatment on its own was sufficient to open up access to the cellulose and improve enzymatic sugar yield.

The cellulosic sugar release figures in Table 3-2 were obtained using excess enzyme and thus it was not possible to determine if enzyme adsorption onto the "lignin droplets" was occurring. As shown in Table 3-3, removal of lignin droplets with flowing hot water followed by hydrolysis with a low enzyme loading led to a small improvement in sugar yield for the 30 min pretreated biomass but no difference for the shorter pretreatment. A study by Yang and Wyman compared batch and flow-through pretreatment of corn stover using water and 0.1wt% sulfuric acid. It was reported that the catalyzed flow through pretreatment removes the lignin either through forming insoluble species with the acid or the lignin dissolving and flowing through the system, which increases the enzymatic digestibility [45]. In addition, they reported that the enzymatic digestibility of the corn stover increased with increasing severity of these parameters [45]. Our results indicate that longer exposure to microwave irradiation increases xylose release and lignin droplet deposition which opens up access to the cellulose fibers. Removal of the lignin droplets uncovers the cellulose fibers still further, allowing access to the enzymes. As such, enzyme activity may be reduced due to steric hindrance only in the presence of lignin and so the presence of lignin droplets do not seem to inhibit the enzyme activity. Therefore, lignin droplet removal may not be crucial for a high saccharification result as much as the disruption in the biomass matrix and opening the access to the cellulose fibers.

3.4   Conclusions

Microwave pretreatment resulted in coalesced lignin droplets on the biomass surface and hydrolyzed up to 88 mol% yield of xylose monosaccharides which was 3.5 times higher than LHW pretreatment. Greater access for enzymatic hydrolysis was achieved after microwave pretreatment of woody biomass, resulting in 100% saccharification compared to only 31% after LHW pretreated fibers from the two-step method. Removal of the lignin droplets, which accounted for over half of the lignin, prior to saccharification did not improve enzyme release of sugars compared with biomass without lignin droplet removal, which also gave 100% sugar release after enzymatic hydrolysis. These results suggest that lignin in biomass limits cellulose hydrolysis via steric hindrance rather than enzyme inhibition. Microwave pretreatment in water-only media may provide a viable option to use woody biomass as a feedstock for biofuels and chemicals production.

3.5   Acknowledgement

3.6   References

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Chapter 4A. Polar solvents facilitate efficient microwave pretreatment of woody biomass

Abstract

Microwave pretreatment of woody biomass may be the key to unlocking an energy efficient process for recalcitrant waste stream for the production of renewable chemicals and fuels. Ionic liquids have been extensively researched as they have proven to yield exceptional results via ionic conduction mechanism. However, limited research has been reported on the intentional exploitation of polar solvents for the pretreatment of recalcitrant biomass. Here we report microwave irradiation of *Eucalyptus Regnans* and *Pinus Radiata* sawdust (~0.5 mm) in the presence of formalin solution containing 1.8, 3.7 and 10 wt% formaldehyde (FA) and water as the control at 180°C for 10 minutes. With the addition of FA in the pretreatment, more than 75% of the hemicellulose sugars were released under mild microwave treatment conditions for both wood species. In water only pretreatment 32% and 55% of the hemicellulose was released from E. regnans and P. radiata, respectively. Enzyme hydrolysis of the 10 min pretreated E.regnans sample in FA released 25% more total sugars compared with the sample pretreated in water-only.

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4A.1 Introduction

Development of efficient pretreatment processes for the release of sugars and other useful fractions from renewable biomass sources is necessary for a sustainable future. Sugars derived from non-food sources such as woody biomass have the potential to be converted into a wide range of chemicals. However, woody biomass requires pretreatment to open the structure to access cellulose. Steam explosion, organosolv and sulphite pretreatment are currently the most effective approaches to remove lignin from wood [1]. However, these methods require substantial energy to be effective [1]. A more efficient alternative pretreatment method is necessary for releasing sugars from woody biomass.

Microwaves are an alternative heating mechanism to conventional heating [2], initially the radiation directly heats the sample, followed by traditional heat transfer, which leads to shorter processing time [3]. Dedicated frequencies at 2.45 GHz and 915 MHz are used for domestic and industrial microwave applications, respectively [4]. At these frequencies molecules are excited by means of ionic conduction or dipolar polarisation mechanism [4, 5]. Other frequencies within that range are mainly used for telecommunications but could be selected for microwave radiation; on the basis they operate using custom built reactors as well as licence acquired for operation on the chosen frequency. An ongoing debate surrounding non-thermal effects instigated by microwave heating has not yet been settled [4]. Early reports by Azuma et al demonstrated promising results for microwave pretreatment of wood in water as microwave irradiation led to more efficient enzymatic saccharification [6]. Recent studies in water only found the optimum conditions (30 min, 180 °C) which gave a yield 3.5 times higher than the comparative LHW pretreatment and complete sugar release after the enzyme treatment, moreover no significant effect in sugar release was observed with post treatment delignification to give complete enzymatic release [7].

While the significant potential for woody biomass pretreatment using microwaves with ionic liquids is clear [8], additional technical challenges and costs associated with recovery of the ionic liquid are drawbacks for their use. Acids and alkali catalysts have been used with microwave pretreatment of lignocellulose to solubilise hemicellulose and/or lignin [9], but their application requires corrosion-resistant process equipment and neutralization of the slurry post-pretreatment. Moreover, the use of mineral acids in particular increases the

production of inhibitory products which may affect the downstream processes such as fermentation [9, 10]. The effect of polar solvents which increase microwave absorption is yet to be explored for biomass pretreatment. Microwave absorption can vary significantly depending upon the ratio of effective dielectric loss factor (ε'') to the dielectric constant (ε). This ratio is known as the loss tangent (tan δ) and the higher the value of tan δ, the greater the efficiency of microwaves, that is, conversion of microwave power into heat. In this research, we investigated the use of solvents with high tan δ value to increase the efficiency of microwave-assisted solvolysis of woody biomass through an increase in the heating rates, and hence reduction of the energy consumption.

A recent paper published by Shuai et al. has reported that formaldehyde stabilization facilitates the hydrogenolysis to lignin monomers in lignin depolymerisation [11]. This study enables production of chemicals from the lignin monomers and also encourages further research and exploration in this area. To the best of our knowledge formaldehyde pretreatment of wood has not yet been reported under microwave irradiation. In this study it was found that 3.7 wt% formaldehyde (FA) solution is more efficient than water only for the pretreatment of wood sawdust at 180 °C and 10 min microwave exposure. The presence of formaldehyde in the solution accelerated the heating rate by 50% in the microwave. Moreover, the hemicellulose yield from FA pretreatment was almost double of that obtained with water only. Analysis of the FA microwave pretreated fibres detected the presence of stabilised lignin monomers.

4A.2 Materials and Methods

4A.2.1 Chemicals and Materials

Eucalyptus regnans sawdust (<750 μm) and ground *Pinus radiata* (<500 μm) were purchased from Pollards Sawdust, Australia and their composition was determined using the NREL method [12]. The compositions are shown in Figure 4A-1.
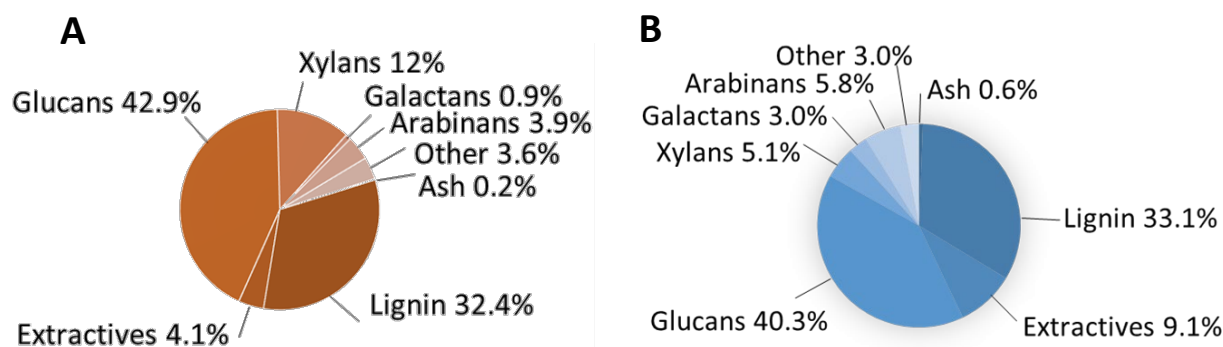
Figure 4A- 1. **Chemical compositions of (A)** *Eucalyptus regnans* **and (B)** *Pinus radiata* **determined by NREL method**

Formalin solution containing 37wt% formaldehyde stabilised with 10 - 15% methanol in water, ethylene glycol (EG), methanol and isopropyl alcohol (IPA) (all from Sigma Aldrich) were diluted in Milli-Q water, to be used as solvent.

4A.2.2 Wood pretreatment using a microwave reactor

Eucalyptus regnans or *Pinus radiata* sawdust (0.4 g) was combined with 10 ml water or solvent/water mixture. EG, methanol and IPA were added at 10 wt% loading in water, whereas formaldehyde loading was varied from 1.8 wt% to 10 wt%. A QLab Pro Microwave Digester (Questron Technology Corp, Canada) was used to heat the samples to 180°C with preteratment time ranging from 5 to 60 min. After pretreatment, the vessel was cooled to room temperature and the contents were filtered using Filtech filter paper CAT NO 1830-055, and remaining solids washed with Milli-Q water then air dried.

4A.2.3 Enzymatic hydrolysis of pre-treated biomass

Pretreated and untreated sawdust was incubated with a cellulase mixture from *Trichoderma reesei* ATCC 26921 (Sigma-Aldrich, >=700 UI/g) supplemented with cellobiase from *Aspergillus niger* (Sigma-Aldrich, ≥250 UI/g). Various enzyme loadings were used (10, 25 and 40 FPU/g) to determine the effectiveness of the pretreatment step. The samples were prepared by mixing 30 mg of air dried pre-treated or untreated solids, 1 ml 50 mM sodium citrate buffer (pH = 4.7) and the requisite volume of enzyme solution to achieve the required loading, followed by incubation for 48 h at 50°C.

4A.2.4 HPLC analysis of liquid samples

All hydrolysates from the pretreatment step and the enzyme digestions were filtered through 0.2 μm filter and diluted 5-fold in Milli-Q water. 10 μl sample was inject in a REZEX RPM Monosaccharide column (Phenomenex) using 0.5 ml/min Milli-Q water as the mobile phase at column temperature of 80 °C and refractive index (RI) detector temperature of 45 °C. Four wavelengths (195 to 285 nm) were selected on the diode array (DA) detector.

4A.2.5 2-Dimensional HSQC NMR Analysis

Approximately 1 g of the 3.7 wt% FA or water-only pretreated wood samples were suspended overnight in deuterated DMSO solvent to extract lignin. The extracted sample was centrifuged at 12,000 rpm for 10 min and the supernatant was filtered using 0.2 μm filter syringe and transferred to an NMR tube. The NMR experiments were collected on Bruker Avance 400 (9.4 Tesla magnet) with a 5 mm broadband auto-tunable probe with Z-gradients at 30°C. The spectra were collected and processed using MestReNova 12.0 software.

4A.2.6 Fourier Transform- Infrared (FT-IR) via Attenuated Reflectance (ATR) Spectrophotometry Analysis

Perkin Elmer Spectrum FTIR fitted with an ATR accessory was used to analyse solutions containing 640 mg of xylan in 10 ml water and 3.7 wt% FA. The xylan loading was equivalent to the amount of hemicellulose in 4 wt% of wood used in pretreatment experiments. Prior to analysis the solutions were heated to 120°C in the microwave and the filtered hydrolysate were analysed on the FTIR-ATR in a flow-through sample cell. The hydrolysate sample was pulled through the cell using a plastic syringe until no bubbles were observed. PolyTemp

software was first used to set the required temperature for analysis (25°C). On the FTIR Spectrum software 650 to 4000 cm^{-1} wavelength range was selected. The absorption spectra were obtained and the first derivative of the spectra was produced using the Spectrum Software.

4A.2.7 Molecular dynamic simulation method to derive dielectric constant[1]

Classical molecular dynamics simulation using GROMACS 5.1 was performed for the dielectric behaviour of 1.8, 3.7 and 10 wt% formaldehyde at 180 °C. Formaldehyde in water and methanol was simulated with the assumption that formaldehyde is present as methanediol in the aqueous phase. Polymerisation and alkoxylation of formaldehyde was avoided due to the minimal amount of methanol present and to minimise the number of variables for simplicity of the simulation. OPLS-AA force field was used for the force-field parameters and water was modelled with the SPC/E model. A cut of radius of 1 nm was used for electrostatic and Van der Waals forces to reduce computational efforts. Energy was minimised using the steepest descent method. The system was equilibrated for 100 ns to generate the temperature using velocity rescale thermostat and keeping the volume constant. Thereafter, density was optimised using NPT ensemble for 250 ps where pressure was kept constant using Berendsen thermostat. Lastly, an NVT ensemble was used for the molecular dynamic run of 10 ns where coordinates and velocities were saved every 100 ps with a 1 fs timestep. The dielectric constant was calculated using the dipole autocorrelation function of molecular dipoles by means of a first order Legendre polynomial for the angle of the dipole vector.

4A.2.8 Product yield calculations

The mass yield of the products was calculated by dividing the experimental mass of respective product by the mass of wood used in the experiments on a dry basis, as shown in equation 1.

$$mass\ yield\ (\tfrac{mg}{g}) = \frac{experimental\ mass\ of\ the\ product}{mass\ of\ original\ dry\ wood} \qquad (\ 4\ )$$

4A.3 Results and Discussion

4A.3.1 Selection of solvents and microwave absorption capacity of solutions

Table 4A- 1 provides the dielectric properties of the polar solvents examined in this study at 20 and 80°C using 2.45 GHz radiation. The dielectric constants decrease as the temperature of the solution increases. The slope of the dielectric constant per unit degrees Celsius was calculated and found that the rate for of change was lower for ethylene glycol. Pure ethylene glycol is a well-known solvent which has the highest reported tanδ, indicating that it has a high ability to convert EM waves into heat. However, reactivity/chemistry of solvents vary significantly which means tan δ value may not be the sole factor in determining a good solvent for microwave pretreatment of wood. Furthermore, wood and wood based materials have dielectric properties which are influenced by the presence of OH and CH_2OH groups in the material [13] and as such, ability to be directly heated by microwaves. Parameters such as frequency, temperature and the solvent will affect the dielectric constant [5, 14].

Formalin is a relatively inexpensive material and very widely used in industry. Formalin solution contains 37 wt% FA in water and 10-15 wt% methanol. FA is hydrated to methanediol in water and methoxylated to methoxymethanol in methanol [15, 16]. Methanediol has a geminal diol moiety which is highly reactive and polar in nature – indicating it would be suitable for microwave heating. Despite this, its dielectric properties have not been experimentally verified in literature and thus not given in Table 4A- . Formaldehyde is electrophilic and reacts readily with other functional groups [15]. Ethylene glycol has hydroxyl groups situated at either end of the 2 carbon chain, making it more stabilised than methanediol. Although water has the highest affinity for excitation by EM waves as indicated by its dielectric constant, the loss tangent shows the other solvents are capable of more efficient microwave energy absorption as they can sufficiently convert the EM waves to convert to heat.

Table 4A- 1. Reported dielectric properties of polar solvents at relevant water-solvent ratio used in this study

Solution	Dielectric Constant (ε'), 20 °C	Dielectric Constant (ε'), 80 °C	Slope ($\Delta\varepsilon'/\Delta T$)	Loss Tangent (tan δ)[*]	Ref
Pure water	80.1	61.0	0.3	0.123	[14, 17]
10wt% EG	62.9	52.9	0.1	1.35	[18]
10wt% IPA	73.1	54.8	0.3	0.79	[14, 18]

[*]Values for pure solvent at 20 °C, 2.45GHz

4A.3.2 Dielectric heating of Eucalyptus biomass in water: effect of polar solvents

Eucalyptus regnans sawdust was heated by microwave (MW) radiation for 5 to 60 min period in either water-only or in selected polar solvents, as shown in Figure 4A-2. All solvent reactions, except for ethylene glycol, reached peak xylose release and reduced thereafter due to the formation of furfural. 10wt% isopropanol only marginally increased the sugar release at 10 min pretreatment time compared with water-only (Figure 4A-2 A&B), and 10wt% ethylene glycol lowered the yield of sugars and dehydration products (Figure 4A-2 C).

3.7 wt% FA solution showed the fastest release of xylose and reached its peak at 10 min (Figure 4A-2 D). Xylose yields for FA and water-only pretreatment were 108 and 68 mg, respectively after 10 min, while both samples had minimal dehydration product formation (<5%) at this point. This corresponds to percentage xylose release of 77% and 48%, respectively. *Eucalyptus* sawdust pretreated in 10wt% methanol for 10 min at 180°C showed similar sugar yields as water-only, demonstrating that the higher yields in 3.7wt% FA solution is likely due to the catalytic action of FA and not methanol which is added as stabiliser (data not shown).

Eucalyptus regnans contain predominantly xylan backbone in the hemicellulose fraction in the form of β-1,4-linked D-xylopyranose with uronic acid residues on one of every 6 to 12 xylose monomer units [19]. However, *Pinus radiata* contain a mix of xylose, arabinose and galactose units in the hemicellulose. This may result in different yields in the pretreatment. However, our observation showed that 3.7wt% FA is still just as effective compared with *Eucalyptus* (Appendix B, Fig B-1). FA pretreated samples showed 3.5-fold increase in sugar yield than water-only after 10 min (76 mg total hemicellulosic sugars vs 19 mg per gram dry *Pinus radiata*). The total hemicellulosic sugar yield from *Pinus radiata* was however, lower than with *Eucalyptus*, for both water-only and FA, which is consistent with other reports [6]. Arabinose and galactose branches in *Pinus radiata* were fully hydrolysed during FA pretreatment whereas the mannose backbone was only partially hydrolysed.
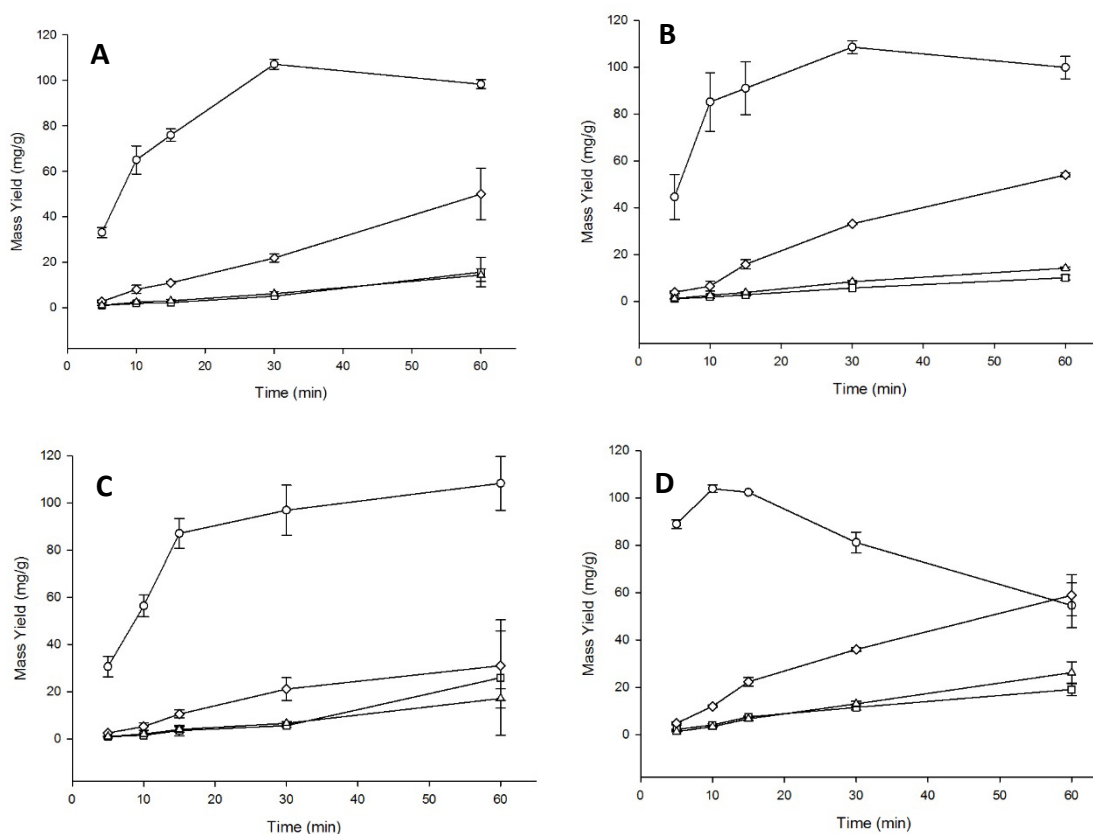

Figure 4A-2. Product yields from microwave pretreated *Eucalyptus regnans* sawdust (4 wt%) in (A) Water (B) 10wt% Isopropyl alcohol (C) 10wt% Ethylene glycol and (D) 3.7wt% formaldehyde over 5 – 60 min exposure times, T=180°C. (o) Xylose, (□) Glucose, (◊) Furfural and (Δ) 5-HMF.

4A.3.3 Systematic investigation of formalin as a catalyst for hemicellulose depolymerization

Figure 4A- shows that the highest sugar yield was achieved with 3.7wt% FA loading. Increasing the FA loading further lead to dehydration of xylose into furfural.
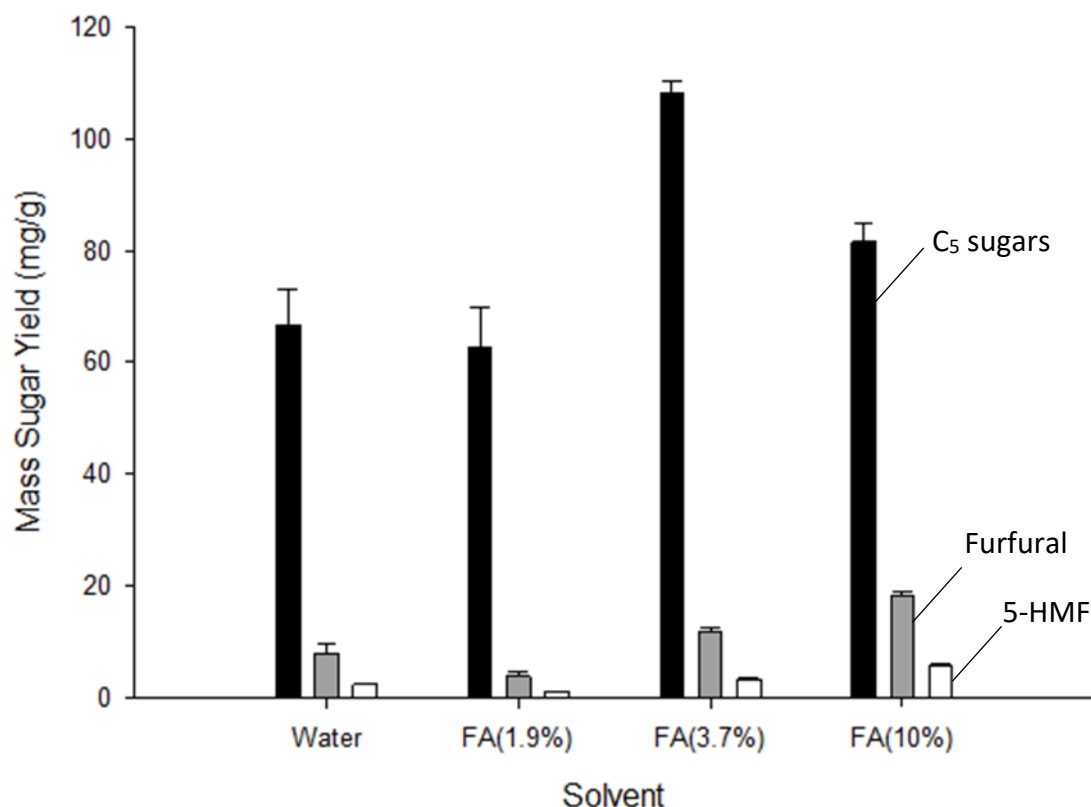

Figure 4A-3. **Effect of FA loading on the mass yield of products from pretreatment of *Eucalyptus regnans* sawdust (4wt%). Conditions: T = 180°C, t = 10 min, FA = 0, 1.8, 3.7 and 10wt%.**

The dielectric constant (ε') is an accurate indicator of electromagnetic (EM) energy conversion into thermal energy [14, 20]. However, ε'_{FA} is not available in literature. Therefore, we did power-controlled experiments in the microwave to study the heating rate of formaldehyde solutions in water. Table 4A-2 shows that pure water required the longest time to reach 180°C. Increasing the FA concentration reduced the heating time. The 3.7 wt% FA solution yielded 2-fold increase in hemicellulose sugar and more efficient heating. The calculated ε' (Table 4A-2) shows a steady increase with increasing FA concentration, which support our finding of faster heating rate.

The penetration depth of the solvent is inversely proportional to the loss tangent [21]. Therefore, solvents with a high loss tangent will have a low penetration depth. Volumetric heating in the microwave is limited to this penetration depth, beyond which the heating will be through convection and conduction [21]. Although the volume of aqueous mixtures was kept constant in this study (10 ml), changing the volume may affect the heating efficiency and hence the product yields, which must be studied in future.

Table 4A-2. Effect of FA concentration of heating time to reach T = 180°C, heating efficiency and calculated dielectric constants at 180 °C.

FA concentration (wt%)*	Time to reach 180°C (s)	Heating efficiency compared to water (%)	Dielectric Constant at 180 °C (ε')
0	430 ± 42.4	-	38.2 [22]
1.85	386 ± 4.2	110	50.9
3.7	357 ± 2.1	117	52.8
10.0	205 ± 25	152	65.0

*Obtained from dilution of 37wt% formalin into water at 5, 10 and 27 wt%, respectively and hence contains small fraction of methanol.

A proposed mechanism for formaldehyde-mediated deconstruction of hemicellulose is shown in Figure 4A-4. The hydronium ion is most likely the initial nucleophile causing cleavage of the hemicellulose. The FA initially converts to methoxymethanol in the presence of hydronium ions. Methoxymethanol will induce formation of adducts on the sugars [11]. Release of free sugar monomers from hemicellulose increase the EM waves absorption to heat, and the uronic acids contribute to the acidic environment to further catalyse depolymerisation. Uronic acids and sugar dehydration products lower the pH leading to methoxymethanol conversion into methanediol. The methoxy group attaches onto the benzyl cation on the lignin, which is discussed further in section 3.4.
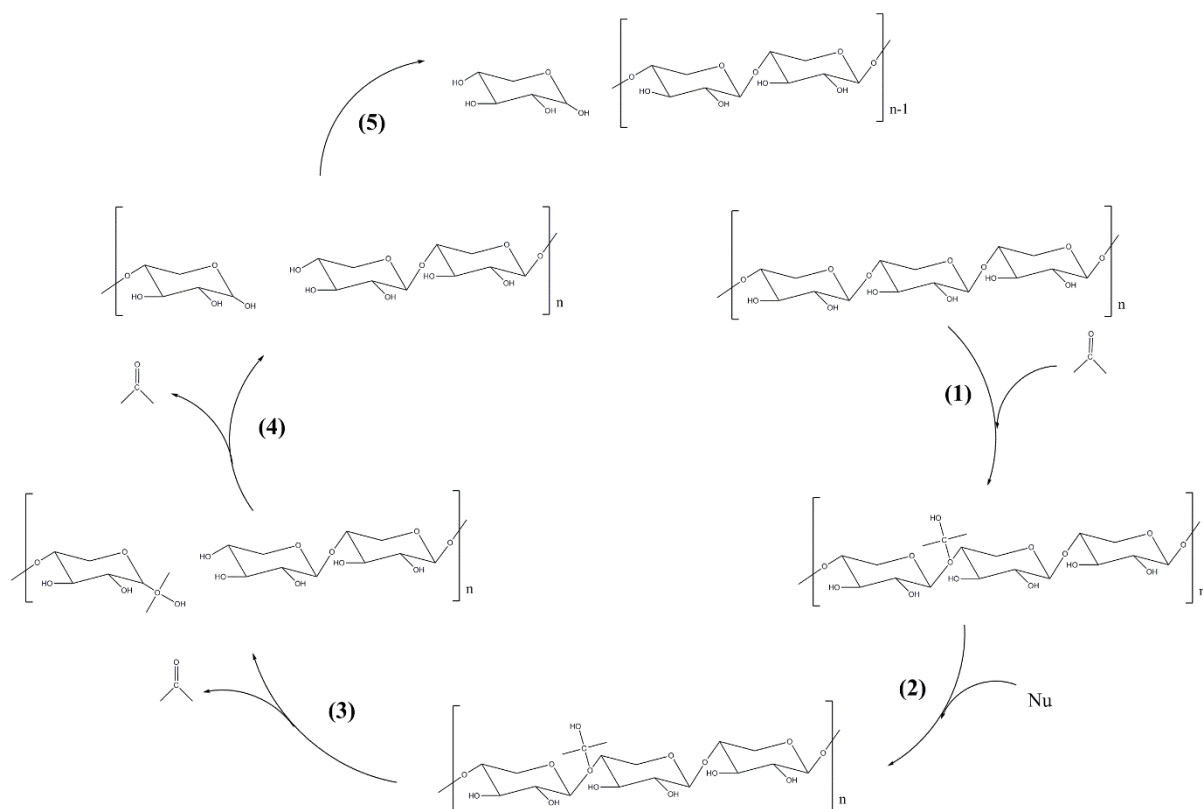
Figure 4A-4. Reaction mechanism of formaldehyde with hemicellulose **(1) The alcohol group on the oligomer attack the primary carbon on the formaldehyde to form an adduct (2) The sugar adduct draws electrons closer to itself making the carbons on either side more susceptible to nucleophilic attack (3) After partial cleavage of the oligomer to monosaccharide the adduct splits off in the form of diformyl xylose which converts back to xylose under acidic conditions (4) Complete cleavage of the oligomer (5) Free xylose saccharide and remaining oligomer**

4A.3.4 Analysis of formalin treated wood components

The supernatant from pretreatment of *Eucalyptus regnans* and *Pinus radiata* were analysed using HPLC and the remaining solids analysed using FTIR. The HPLC retention times of sugar monomers from FA pretreated samples were unchanged compared with water-only pretreatment suggesting no formyl adducts remained attached to these monomers (Appendix B, Fig B-2 and B-3). FTIR-ATR of 3.7 wt% FA, with and without xylose, were analysed over the temperature range of 25 to 60 °C. The first derivative of the results was taken to note if there was a significant variance in functional groups and reduce the noise. The wavebands present in the region 650 to 1000 cm^{-1} correspond to functional groups associated with monosaccharides. It was found that by increasing the temperature the bands slightly shift up or down in wavenumber and change shape (Appendix B, Fig B-4). A model solution of xylan in water and 3.7wt% FA was briefly heated in the microwave at 120 °C and the hydrolysate analysed at room temperature where the same spectral alterations were observed (Appendix B, Fig B-5). It has been reported in literature that the spectral shape of the IR spectra are influenced by the position of substituents on different positions of the oligomers [23]. This information supports the proposed mechanism that formyl adducts continuously and temporarily detach and reattach from the hemicellulosic oligomers.

The lignin in the remaining sawdust from the FA pretreatment was analysed by 2D NMR and compared with water-only pretreatment. Comparison of the 2D NMR analysis in Figure 4A-5 showed that in the presence of FA, 1,3 dioxane structures are formed, matching those previously reported [11]. Whereas, with water-only pretreatment this peak did not appear. FA prevents the formation of C-C bonds in lignin by stabilizing these sites to electrophilic attack [24]. As shown in Figure 4A-5, the 1,3 diols on the lignin side chain were reacted with FA to form a stable ring structure. This stabilised platform has opened up a more advanced continuous process which allows near theoretical lignin monomer yields through the subsequent hydorgenolysis [11]. Previous conventional strategies utilise solid metal catalysts in batch processes [25, 26] which achieve lignin monomer yields 3 to 7 times lower than the before mentioned stabilised platform [11].
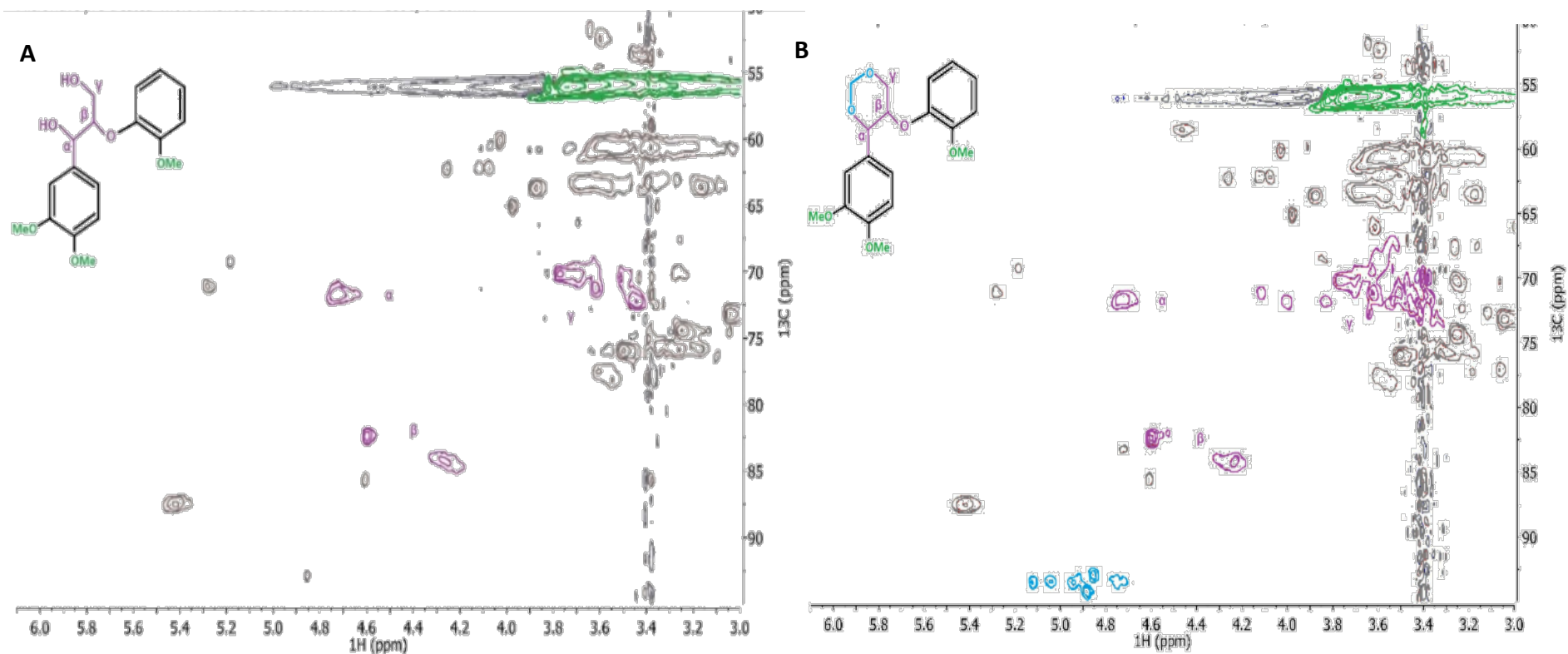
Figure 4A-5. **2D NMR analysis of DMSO-solvated lignin samples obtained from *Eucalyptus regnans* sawdust pre-treated by microwave heating at 180°C for 10 min followed by cellulase digestion to remove cellulose. Pretreatment solution (A) water (B) 3.7% FA. Contour colour representations: green= methoxy groups, purple= α, β and γ carbons on the connecting link between lignin monomers and blue= 1,3 dioxane ring formed on the lignin**

4A.3.5 Enzymatic hydrolysis of pre-treated wood samples

From the enzyme digestion results, it was evident that both water only and 3.7wt% FA pretreated sawdust are compatible with downstream processing using Trichoderma reesei. The high enzyme loading (40 FPU/g) achieved complete sugar release for pre-treated fibres in both solvents, water and FA. Table 4A-3 shows the enzyme hydrolysis using a moderate enzyme loading (25 FPU/g). It is demonstrated that FA pretreated fibres yield 40% higher sugar compared with water-only.

Table 4A-3. **Total sugar yield after enzymatic digestion of *Eucalyptus regnans* MW pre-treated (t=10 min, T=180°C) and untreated fibres using 25 FPU/g Trichoderma reesei cellulase (T=50°C, t=48 hr)**

Eucalyptus regnans sawdust	Pretreatment (mg/g)	Enzymatic Hydrolysis (mg/g)	Total Sugar Yield (%)
Raw	-	4.8 ± 1.4	1 ± 0.2
Water	66.8 ± 6.4	167.6 ± 20.1	43 ± 26
3.7wt% FA	108.0 ± 2.3	262.8 ± 13.2	68 ± 11

4A.4 Conclusions

Formalin solution was successfully used to pre-treat woody sawdust to intentionally exploit the dipolar polarisation mechanism and augment the electromagnetic conversion to heat. By increasing the concentration of formaldehyde, less time was required in the microwave to reach the desired temperature. A solution containing 10 wt% formaldehyde took 52% less time to heat to 180 °C when compared with water-only solution. Furthermore, solution containing 3.7 wt% formaldehyde released double the hemicellulosic sugar yield compared with water only at a short microwave exposure of 10 min. 2-D NMR investigation found that the effect of formalin on the pretreated wood macromolecules stabilised the lignin monomers, preventing the condensation of lignin monomers.

4A.5 Acknowledgements

4A.6 References

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Chapter 4B. Comparison against conventional liquid hot water pretreatment

Abstract

While the presence of formaldehyde (FA) was more effective than water-only for microwave pretreatment of wood, it needs to be established if FA provides any non-thermal effects in the microwave. Therefore, in this chapter a comparison of FA pretreatment in microwave (MW) is made against FA in conventional autoclave heating (LHW). The results confirm that MW pretreatment of Eucalyptus sawdust is superior with both water and FA, as the total hemicellulosic saccharide yield was 7% and 24%, respectively, for LHW pretreatment. Furthermore, the Pinus radiata hemicellulosic saccharide yields were superior with MW pretreatment. The effect of stirring was investigated to identify whether MW pretreatment was still dominant, and it was found that a mass transfer limitation exists beyond 200 rpm for both solvents and both wood types.

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4B.1 Introduction

Microwave irradiation has been debated to comprise of both thermal and non-thermal effects, whereas, only thermal effects have been associated with conventional heating [1]. The dipolarisation mechanism heats samples containing a dipole [2]. The heat is produced due the friction caused by the thermal lag in the rotational alignment of the polar material with the electromagnetic waves [3]. In contrast, the conventional heating mechanism encompasses of only convection and conduction, a slower process which begins from the outside of the vessel working its way towards the sample. Furthermore, stirring capabilities are more commonly used with conventional heating rather than microwave heating, an important factor which could influence the saccharide yield. Thus, it is important to directly compare MW pretreatment of wood in 3.7wt% formaldehyde and water-only to conventional LHW pretreatment.

4B.2 Method

4B.2.1 Wood pretreatment using an autoclave reactor

Eucalyptus regnans or *Pinus radiata* sawdust (1.6 g) were placed inside an autoclave vessel with 40 ml of water or 3.7wt% formaldehyde (FA) solution. The vessel was tightly secured and heated to 180°C with a hold time of 10 min. The heating ramp time varied between 40-50 min in the autoclave. In order to make direct comparison with the microwave pretreatment, the autoclave run was conducted without stirring. At the end of hold time, the autoclave vessel was allowed to cool in air. The handling and analysis of the reaction products were carried out using the procedures identical to microwave pretreated products described in Chapter 4A.

4B.2.2 Enzymatic hydrolysis of pretreated biomass

Pretreated and untreated sawdust was incubated with a cellulase mixture from *Trichoderma reesei* ATCC 26921 (Sigma-Aldrich, >=700 UI/g) supplemented with cellobiase from *Aspergillus niger* (Sigma-Aldrich, ≥250 UI/g). A high enzyme load of 40 FPU/g was intentionally selected to investigate any differences between microwave and autoclave pretreatment of the biomass that may improve enzyme access to cellulose. The samples were prepared by mixing 30 mg of air dried, pretreated or untreated wood, 1 ml 50 mM sodium citrate buffer pH 4.7 and the requisite volume of enzyme solution to achieve the required loading, followed by incubation for 48 h at 50°C.

4B.3 Results and Discussion

4B.3.1 Comparison with conventional heating in presence of formaldehyde

A comparison of *Eucalyptus Regnans* pretreatment in autoclave (LHW) and microwave (MW) using water and 3.7 wt% FA solution is presented in Figure 4B-1. It was found that LHW released only 7% and 24% of the total hemicellulosc saccharides in water and FA, respectively. These results are 6 and 3 times lower than the respective MW pretreatment results. It is even more remarkable difference considering the fact that LHW heating rate was much slower than microwave (50 min vs 15 min) which meant that the samples were at high temperature for longer in the LHW method. Comparison of *Pinus radiata* sawdust pretreatment with LHW and MW pretreatment is shown in Figure 4B-2. The total hemicellulosic sugar yield with water and FA were 23% and 54%, respectively with LHW. Softwood was found to be difficult to pretreat with MW, however, better hemicellulosic saccharide yields were obtained with conventional LHW pretreatment. The higher sugar yield obtained for softwood may be due to its particle size as it is ground, opposed to the hardwood sawdust.

Figure 4B-3 illustrates the anatomy of softwoods and hardwoods, clearly showing the different transportation routes of water and nutrients during the life for both wood types [1]. It has been reported in literature that softwood species, such as *Pinus radiata,* are tougher to pre-treat for two reasons (1) the higher lignin content and (2) non-porous microstructure [2]. Thus, during microwave pretreatment the microstructure could play a role in terms of solvent contact within the wood matrix. As hardwoods contain porous membranes [2, 3], the anatomy provides the solvent better access within the microstructure, and ultimately providing more surface area, during pretreatment.
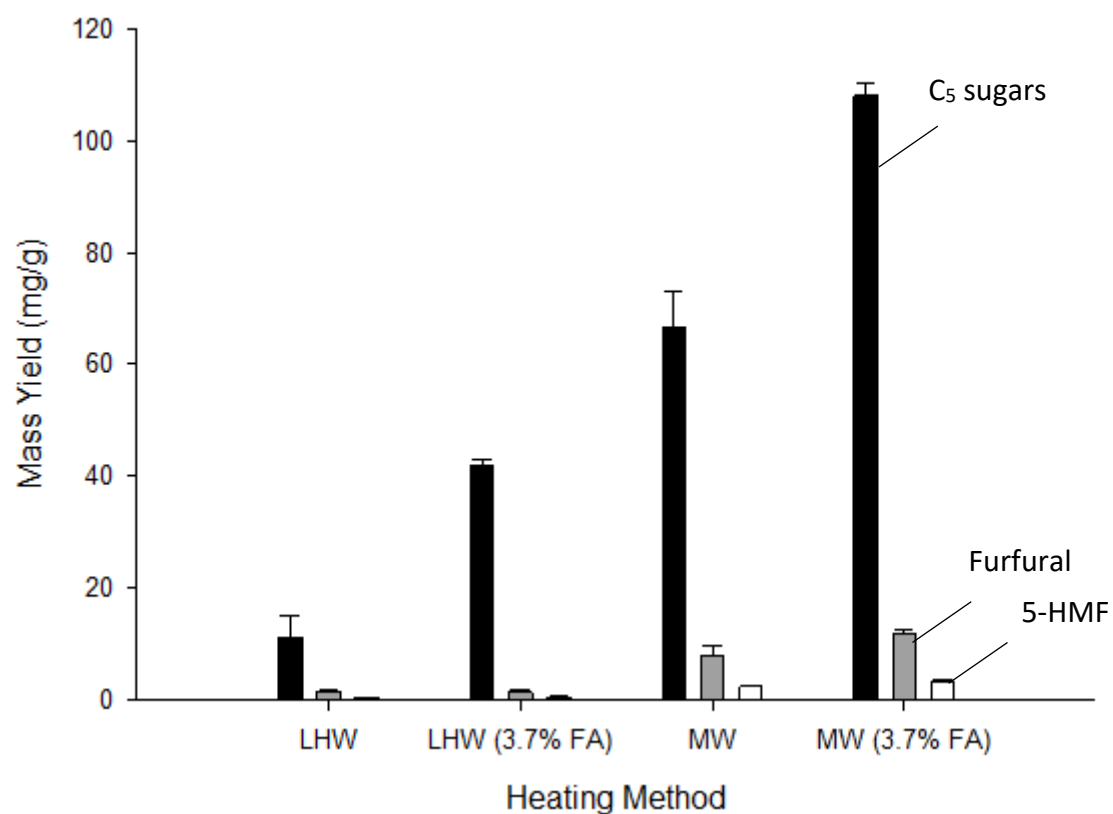
Figure 4B- 1. Release of sugars from *Eucalyptus regnans* sawdust (4% wt/v) heated at 180°C and 10 min exposure in conventional and microwave pretreatments in the presence of water only and 3.7 wt% formaldehyde
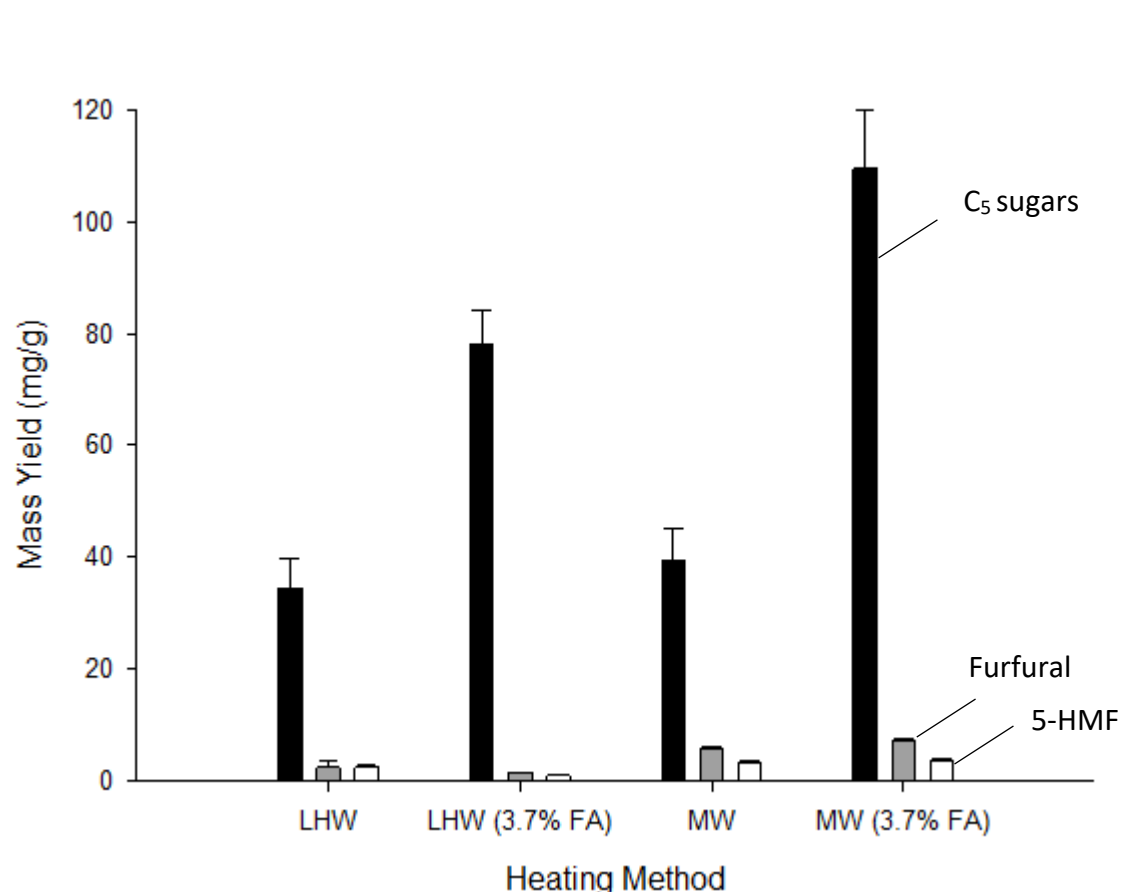
Figure 4B- 2. Mass yield of pretreatment products released from *Pinus radiata* sawdust (4% wt/v) heated at 180°C and 10 min exposure in an autoclave in the presence of Water or 3.7 wt% formaldehyde
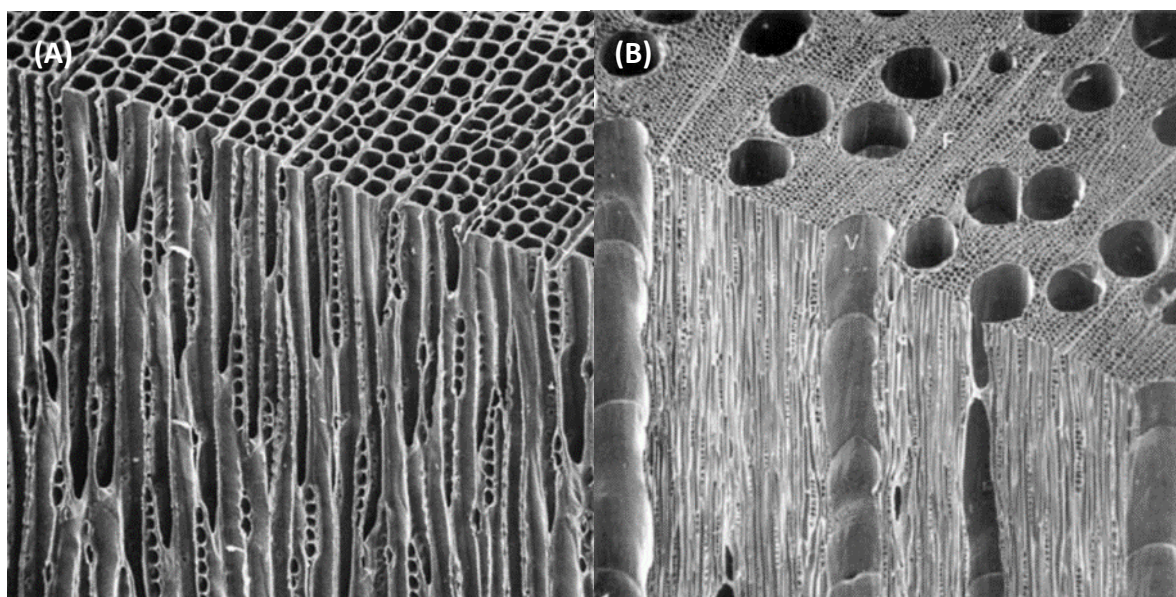

Figure 4B- 3. SEM image of (A) softwood containing axial tracheids and (B) hardwood containing porous membranes (Republished with permission of Barnett, from [4])

The comparative non-stirred LHW pretreatment yielded lower hemicellulosic saccharides compared with (non-stirred) MW pretreatment. It was found that a mass transfer limitation exists with LHW, which is overcome by the addition of stirring (Appendix C, Fig C-1). The samples were stirred up to 400 rpm and the sugar yields eventually reached MW pretreatment sugar yields and plateaued. As stirring requires additional energy input, it will add to the total pretreatment cost. Non-thermal microwave effects cannot be confirmed until comparative stirred-MW pretreatment is also analysed. From the MW pretreatment of *Eucalyptus regnans* results provided, it is a clear indication that FA accelerates the release of sugars, ultimately requiring less pretreatment time and less energy due to the exclusion of stirring.

The remaining Eucalyptus solid residue yields of the non-stirred MW and comparative LHW pretreated samples were compared and found to be consistent with respect to the saccharide yields obtained during pretreatment. Samples containing FA consumed 35% and 11% of the wood for MW and LHW pretreatments, respectively. Whereas, water only pretreated samples consumed only 20% and 6% of the wood for MW and LHW, respectively. The efficiency of LHW and MW pretreatments were further tested by carrying out enzymatic hydrolysis of the untreated and pretreated fibres. As reported in chapter 4A, a high enzyme load (40 FPU/g) released all sugars in water and FA MW pretreated woody sawdust. The same enzyme load was used for direct comparison for LHW pretreated woody sawdust. Table 4B-1 shows FA to be marginally superior at releasing sugar compared with water in LHW pretreatment also. Furthermore, the results again indicate, that LHW pretreatment releases significantly lower sugar compared with MW pretreatment (17 % for LHW Vs 68 % for MW method).

Table 4B- 1. Total sugar release after enzymatic digestion of *Eucalyptus regnans* conventionally pre-treated (t=10 min, T=180°C) and untreated fibres using 40 FPU/g Trichoderma reesei cellulase (T=50°C, t=48 hr)

Eucalyptus regnans sawdust	Pretreatment (mg/g)	Enzymatic Hydrolysis (mg/g)	Total Sugar Release (%)
Raw	-	4.8 ± 1.4	1 ± 0.2
Water	11.1 ± 3.8	64.2 ± 4.8	14 ± 8.7
3.7wt% FA	42.0 ± 0.4	52.5 ± 0.8	17 ± 0.4

4B.4 Conclusions

Conventional pretreatment of *Eucalyptus regnans* sawdust using water and 3.7% FA was not as effective as microwave pretreatment, the total hemicellulose saccharides released were 6 and 3 times lower for the solvents, respectively. Enzyme digestion of the pretreated fibres demonstrates that, microwave pretreated fibres are more susceptible to enzyme digestion regardless of the solvent used. Furthermore, FA does not appear to inhibit the downstream conversion as the sugar yield is higher than the water pretreated fibres. Non-thermal effects could not be confirmed with non-stirred MW pretreatment as LHW exhibited a mass transfer effect from the addition of stirring. Thus, comparative stirred-MW pretreatment against stirred-LHW for woody biomass could confirm the existence of non-thermal effects.

4B.5 References

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Chapter 5.   Impact of acid catalysts and wood chip surface area on pretreatment

Abstract

Pretreatment of woody biomass having high surface area i.e. sawdust, was investigated in the previous chapters. This chapter reports the effect of biomass surface area on the microwave pretreatment of wood with acid catalysts of varying strengths. The general aim of this research is to identify an effective microwave-based pretreatment that minimises the production of degradation products but reduces opex (energy required for biomass milling) and capex (acid-resistant process equipment). Microwave pretreatment at 180 °C in the absence of acid or presence of 0.05 M Sulphuric acid (SA) or 0.1 M formic acid (FoA) or 0.4 M levulinic acid (LA) with pK_a values ranging from -3 to 4.2, was investigated on *Eucalyptus nitens* wood chips with various surface areas (~1,000 to 15,000 mm^2). SA was selected for comparison against the other homogenous acid catalysts as it is widely used for acid-catalysed steam explosion of lignocellulose. It was found that in the absence of catalyst, short microwave exposure (10 min) was insufficient for complete depolymerisation of hemicellulose regardless of its surface area. With the aid of catalyst, higher surface area woody sawdust (greater than ~7,000 mm^2) yielded near complete hemicellulosic sugars compared with wood chips of lower surface area (lower than ~3,000 mm^2). FoA outperformed SA in pretreatment of ~1000 mm^2 wood chip. We have shown that FoA has potential to be used as an alternative acid catalyst for the pretreatment of wood chips to alleviate the high economics otherwise associated with the process.

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5.1   Introduction

The use of renewable feedstock such as forestry residues for the production of biofuels and chemicals can promote sustainable development as concerns for climate change are increasing. Forestry products can be composed of up to 35% lignin [1] which restricts access to the valuable monosaccharides. As such the most common method of pre-treatment to overcome the recalcitrance is the addition of acid catalysts, most commonly sulphuric acid (SA), to pretreatments such as steam explosion [2-4]. Typically, 2-5 wt% or 10-30 wt% of acid in water are used for dilute or concentrated acid catalysed steam explosion, respectively. This is followed by the application of high pressure steam to wood chips (160 – 260 °C, 0.69 – 4.83 MPa), with some of the steam rapidly vented [3, 5, 6]. The described pretreatment can be costly due to the expensive corrosive-resistant stainless-steel under pressure required for the process equipment and the additional energy input required for the grinding of the wood [7, 8]. In addition, there may be formation of salts or neutralisation required in the downstream processing [6, 9].

Reported studies have focused on conventional heating in the presence of acid catalysts for the hydrolysis of woody biomass, particularly for bioethanol production [6, 10] but few studies have investigated the effect of wood surface area on the efficiency of pretreatment. Milling of wood chips and pellets is a highly energy consuming process, the energy required is dependent on the moisture content, particle size difference between the feed and the final product and type of the material [11]. The energy consumption has been reported to increase dramatically when the size reduction of the wood falls below 5.00 mm mesh diameter [11]. If larger size wood chips could be used for pretreatments, it could significantly bring down the energy required, and subsequently the overall pretreatment cost.

Another factor which contributes to the high capital cost of woody biomass pretreatment is the need for corrosion-resistant process equipment material. Alloy 20 is a nickel-iron-chromium based super alloy which exhibits excellent resistance to chemical attack for all concentrations of SA, however, it costs 12 – 18 times more than the cheaper grade 316 SS, for which there have been reports that indicate it is less resilient [12, 13]. Dilute SA can be more harmful than concentrated as it immediately attacks the metal forming hydrogen and ferrous ions via anodic and cathodic reactions [7, 14, 15]. To eliminate the need for expensive

materials and unnecessary side reactions, weaker catalysts that are still as effective as SA in terms of pretreatments that provide high sugar release, could be used in the pretreatment.

There have been studies on the pretreatment of non-woody biomass with FoA which was shown to improve the glucan recovery. A study by Xu et al performed pretreatment of corn stover with low concentration FoA (40g FoA /kg raw corn stover) for bioethanol production. Their results showed that after simultaneous saccharification and fermentation, 76.5% of the theoretical ethanol yield was obtained from corn stover pretreated in FoA for 15 min at 195 °C [16]. This yield was 7% greater than corn stover pretreated in the absence of the acid and 51% greater than untreated corn stover. There have been no reports in the literature on using LA as a catalyst for woody biomass pretreatment to obtain a sugar platform. A study by Seemala et al did report treatment of Eucalyptus sawdust for the one pot production of 5-HMF and Furfural [17]. Various biphasic solvent mixture ratios of water/MTHF were used with 0.1 g LA, where the partition coefficient of LA allowed it to be equally miscible in both solvents. Depolymerisation of the sugars and their dehydration into furfural and 5-hydroxymethyl furfural occurred in the aqueous phase where the presence of LA prevented the rehydration of 5-HMF to LA [17].

The drivers for this research are to propose industrially relevant pre-treatment for low surface area woody biomass using microwave irradiation which has advantages over conventional heating [18]. Electromagnetic radiation heats materials containing a dipole with the penetration depth as a limiting factor, and the dissipated heat travels outwards via convection and conduction. Whereas conventional heating works its way from the outside towards the material, which is a slower process. Furthermore, acid attack on the woody biomass could behave differently under microwave irradiation compared to the reported steam explosion pretreatments. To the best of our knowledge, the bio-based acid catalysts FA and LA have not been reported for microwave pretreatment of woody biomass and thus are investigated in this study. The wood surface area was analyzed to determine how much milling of biomass is really necessary to attain effective pretreatment. Finally, enzyme hydrolysis was carried out on the pretreated 'whole' wood chips and the sawdust to determine the effectiveness between the catalysts.

5.2   Materials and Methods

5.2.1   Materials and Reagents

Eucalyptus nitens wood samples (dry mass, 1.2 ± 0.3 g) provided by HRL Technology Group Pty Ltd from Clayton were used. The compositional analysis of the feedstock was performed by Ryan Ness at National Renewable Energy Laboratory (NREL) in the United States. A swing mill was used to reduce the size of the wood chips and these were grouped into 4 sample types on the basis of length, width and thickness measurements undertaken with 6-inch stainless steel calliper on 10 representative pieces (summary shown in Table 5-1). Water used throughout the experiments was filtered to Milli-Q standards and a biomass loading of 4wt% was used for all experiments.
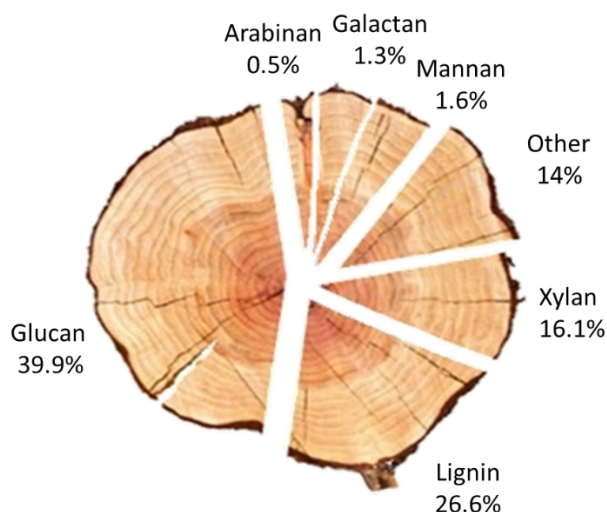
Figure 5-1. NREL chemical composition anlaysis of *Eucalyptus nitens* (Melbourne, Australia)

The surface area was estimated for a given wood particle, for each of the 4 samples, by assuming a rectangular prism shape. From the measurement of samples #1 and #2, the average dimensions were used to calculate the volume of one particle and using the density value of Eucalyptus wood, 550 kg/m^3 [19], the mass of one particle was calculated. The density was divided by the mass of wood used in the pretreatment to calculate the number of particles present. Thus, the total surface area of the sample of wood used in the pretreatment was derived by multiplying the number of particles and the surface area of one particle. For samples #3 and #4 the exact number of chips used was multiplied by the surface area estimated for one chip to derive the total surface area. The summary table of the calculations are shown in Table 5-1.

Table 5-1. **Characterisation of wood samples based on 10 particles per sample.**

Sample Type	4	3	2	1
Visual Image				
Sketch				
Average dimensions of raw wood				
Thickness, mm	4.8 ± 1.0	3.1 ± 0.9	1.5 ± 0.4	0.5 ± 0.2
Width, mm	15.6 ± 2.5	8.5 ± 1.7	0.8 ± 0.2	0.5 ± 0.2
Length, mm	36.4 ± 2.8	30.7 ± 4.7	15.6 ± 2.5	5.6 ± 1.6
Surface Area, mm^2	1,096	2,859	7,365	14,157

5.2.2 Catalyst preparation

Levulinic acid (LA, 98%), sulphuric acid (SA, ACS reagent, 95 – 98%) and formic acid (FoA, >95%) were purchased from Sigma Aldrich and Table 5-2 summarises their chemical properties. According to Torget et al. 0.5 to 5 wt% of catalysts are typically used for dilute acid pre-treatment [6] and therefore 0.5 wt% SA was used in this study. FoA and LA loading was varied between 0.5 and 5 wt% to determine the loading required for FoA and LA to achieve pentosan monosaccharide yield comparable to that of 0.5 wt% SA. The equivalent loading of FoA and LA were found to be 0.5 and 4.5 wt%, respectively.

Table 5-2. **Chemical properties of homogenous acid catalysts assessed for wood pre-treatment**

Properties		SA	FoA	LA	Water
Chemical Formula	-	H_2SO_4	HCO_2H	$C_5H_8O_3$	H_2O
Molecular Weight	g/mol	98	46	116	18
Density	g/cm^3	1.8	1.2	1.1	1
Proton Dissociation Constant	pK_a	-3, 2	3.7	4.2	14
Mass	g	0.1	0.1	0.9	20
Weight	%	0.5	0.5	4.5	100
Molarity	M	0.05	0.1	0.4	-
Normality	N	0.1	0.1	0.4	-

5.2.3 Microwave pre-treatment of wood samples

Approximately 0.8 g of each Eucalyptus samples were weighed on an analytical balance and placed in the microwave vessel with 20 ml of water-only or acid solution. The biomass loading used was restricted by the microwave vessel limitations. The vessel was tightly closed and placed inside the microwave for a temperature-controlled run. A QLab Pro Microwave Digester (Questron Technology Corp, Canada) was used to heat the samples between 160-200°C for 10 min microwave exposure. On completion of the microwave irradiation, the vessel

was cooled to room temperature and the contents filtered and washed with Milli-Q water using Filtech filter paper CAT NO 1830-055.

5.2.4 Enzymatic hydrolysis of pretreated wood samples

Pre-treated wood samples were washed with Milli-Q water to remove residual acid and degradation products and air-dried. Enzymatic hydrolysis was carried out using cellulase mixtures from *Trichoderma reesei* ATCC 26921 (Sigma-Aldrich, >=700 UI/g) supplemented with cellobiase from *Aspergillus niger* (Sigma-Aldrich, ≥250 UI/g). A high enzyme load of 40 FPU/g (Filter paper units per gram of biomass), which reflects industry practice to release maximum sugars, was used. For the smaller particle size pretreated wood, a sample (30 mg) was weighed and mixed with 1 ml 50 mM sodium citrate buffer pH 4.7. For the large chips (Samples #3 and #4) the entire chip remaining after pretreatment was used for enzyme hydrolysis in 10 ml of above mentioned buffer. The appropriate enzyme solution was added to achieve the required loading based on the mass of pretreated wood remaining, followed by incubation for 48 h at 50°C. The sugar containing supernatant was filtered using 0.2 µm syringe filter, diluted 1:5 and analyzed according the HPLC procedure below. All experiments were carried out in duplicate and the results presented as the average values.

5.2.5 Microstructure analysis of wood chip surface

The pre-treated wood chips of sample #4 were air dried. The samples were prepared by placing on a carbon tape attached to stubs and coated with approximately 1 nm of iridium. SEM images were taken using a Phenom XL SEM at 1700 magnification.

5.2.6 HPLC analysis of microwave pretreated hydrolysates

The microwave pre-treated and enzymatic digested hydrolysates were analyzed by using an Agilent Technologies 1220 Infinity HPLC. The instrument was equipped with diode array (DAD) and refractive index (RID) detectors. REZEX monosaccharide RHM column was used for analysis of the hydrolysates with 0.5 ml/min of water as the mobile phase and sample injection volume of 10 µl. The column temperature was set at 65°C and RID temperature at 45°C. Standard curves with 4 data points were created for all sugars, sugar derivatives and dilute acids for quantification.

5.2.7 Chemical yield and catalyst characterisation calculations

The mass yield of sugars or value-added chemicals, molarity and normality of the acids were calculated and summarized in Table 5-2. The catalyst turnover number (TON) was calculated by dividing the number of moles of sugar produced in the pre-treatment by the number of moles of catalyst, as shown in equation 1. The TON was calculated for each wood fraction.

$$TON = \frac{mol \; of \; sugar \; released}{mol \; of \; catalyst} \qquad (1)$$

5.3 Results and Discussion

5.3.1 Effect of wood chip size using hydrothermal microwave pretreatment

Hydrothermal microwave pre-treatment of 4wt% *Eucalyptus nitens* (samples #1 - #4) were carried out under microwave irradiation at 180°C for 10 min exposure without acid and the amount of released hemicellulose monosaccharides is shown in Figure 5-2. As sample #1 has roughly 14 times more surface area than the sample #4, it was expected that the hemicellulose monosaccharide yield would be significantly higher for the former sample. Only 22% of the total hemicellulosic monosaccharides were released in the pretreatment of #1 and #4. Our results suggest that surface area does not have an effect on the pretreatment monosaccharide yield obtained in water-only microwave treatments but could differ when acid catalysts are used. The absence of catalyst in pretreatment contributed to the overall low sugar yields. Furthermore, the microwave exposure time was intentionally short in order to minimise the formation of degradation products. By extending the exposure time there may be a more significant difference exhibited in the sugar yields for #1 and #4.

The effect of wood particle size on sugar release has not been investigated with microwave pretreatment. The most relevant study was by Brownell et al who looked at the steam explosion pretreatment of wood and considered the effect of chip size and moisture content amongst other parameters. It was found that small chips heat rapidly regardless of moisture content i.e. in dry or green state. Larger chips with a low moisture content or air dried, heat rapidly via steam penetration and condensation which occurs inside the chip. However, high moisture content chips heat unevenly and slowly via conduction, consequently giving a lower sugar yield [20]. Since our method of microwave pretreatment does not incorporate a pressure drop, the mechanism which results in the porous matrix is the direct irradiation of the sample and solvent [21]. Our results contradict the Brownell study, since irrespective of the size of the chip the total hemicellulosic sugar yield stays the same. Therefore, parameters such as extended microwave exposure time or incorporation of a catalyst, could play a role to increase the hemicellulosic sugar yield. Extending the exposure time will lead to further depolymerisation of the hemicellulose polymers to saccharides followed by dehydration of the saccharides to other value-added products. The optimum exposure time would display a balance between the sugar yield and degradation products so a judgement can be made on the effect of surface area. The incorporation of a catalyst will drop the pH of the sample

mixture, creating an environment which promotes the cleavage of the lignin-hemicellulose bonds and subsequently depolymerisation of the hemicellulose polymers.
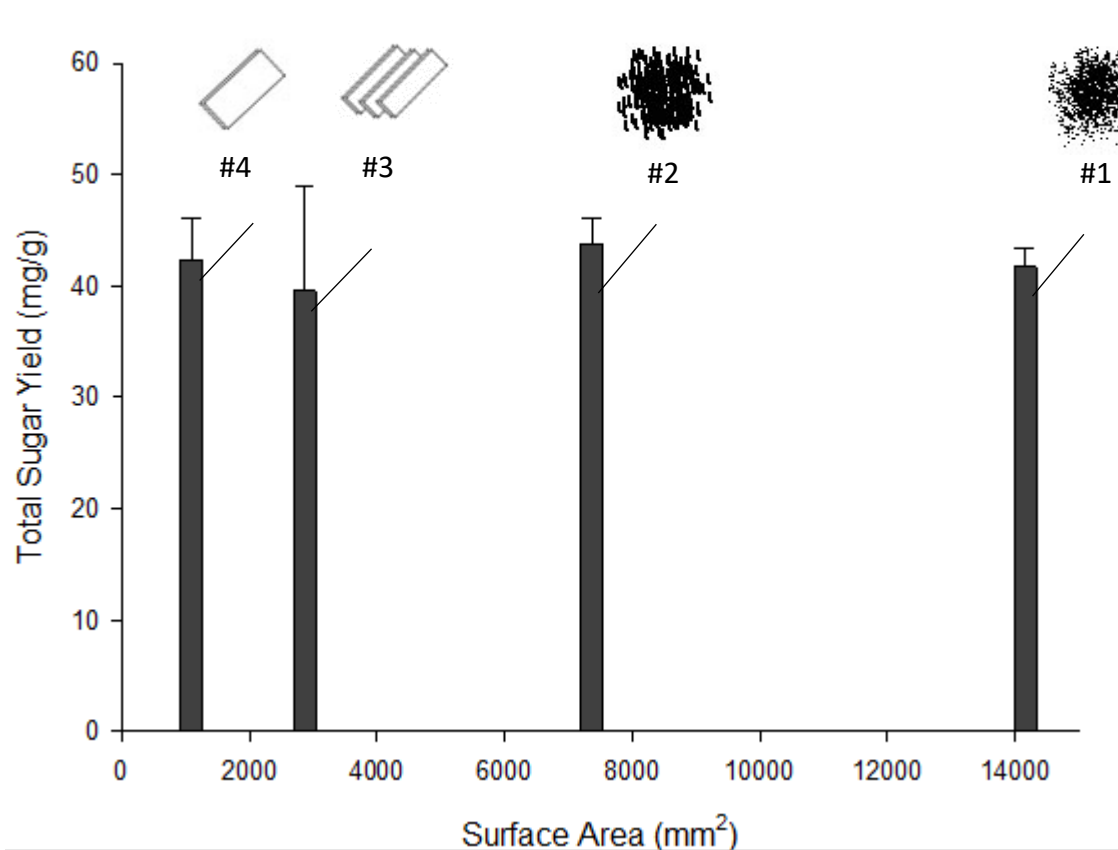

Figure 5-2. Hemicellulose-derived sugar release following hydrothermal microwave pre-treatment of 4wt% _Eucalyptus nitens_ biomass loading over a range of surface areas, T=180°C, t=10 min

5.3.2 Microwave assisted homogenous acid pre-treatment of woody biomass

The effect of chip size was investigated further using alternative catalysts in the pretreatment of woody biomass. SA is a well-known strong acid as indicated by the its low pK_a values (pK_a value shown in Table 5-2) [22, 23] while acids such as LA or FoA have higher pK_a value than SA, signifying weaker acid properties. These acids could be favourable alternatives for woody biomass pretreatment as they could eliminate the need for high grade stainless steel, reducing the capital cost. The pH of the hydrolysates for FoA, LA and water-only were similar (~3.2) while SA had a much more acidic pH of 1.5.

Optimisation of LA and FoA loadings was required to determine the loading which provides equivalent depolymerisation to that of SA. While FoA loading was similar to SA, LA loading of 16 times more than SA was necessary to achieve this purpose. The release of hemicellulose-derived sugars from *Eucalyptus nitens* sample #1 - #4 in the presence of acid catalysts is illustrated in Figure 5-3. Samples #1 and #2 had the highest amount of released hemicellulosic monosaccharides. The total hemi-monosaccharides released from sample #1 with SA, FoA and LA were 89%, 72% and 56%, respectively. Increasing the particle surface area and acid strength provided higher sugar release. It is important to note that in addition to the added acid catalysts, a mixture of acetyl groups, uronic acids and their conjugate anions, commonly found in hardwood species, also contribute towards acid hydrolysis[1]. Surface area becomes a limiting factor for sugar release when low surface area (less than 3,000 mm^2) chips are used (samples #3 and #4). Sample #4 pretreated with SA, FoA ad LA released 43% 59% and 36% of the hemi-monosaccharides, respectively (Figure 5-3).

The yield of furfural and 5-hydroxymethyl furfural produced during the pretreatments are shown in Figure 5-4. Twice the amount of combined dehydration products was observed for all SA pretreated samples (except type #1) which reduced the sugar yield. SA is a strong acid (pKa values = -3 and 2), which increases the rate of hemicellulose depolymerisation, but also increases the rate of sugar dehydration.

Although SA was the most effective catalyst for the majority of samples, FoA was superior for sample #4 and this shows potential for FoA as an alternate catalyst for wood chips. Compared with SA, FoA is a smaller compound which could diffuse inside the chip and hence provide relatively better results as the particle size increased. In a study by Sun et al, FoA behaved in

a similar manner in the pretreatment of cotton fibre cellulose. It was reported that formic acid penetrates into the crystalline framework of the cellulose making it swell and ultimately more susceptible to being crushed, as such both the amorphous and crystalline regions of the cellulose are hydrolysed [24].

LA was the least effective catalyst, however, at significantly higher loading. Since LA contains both carboxyl and ketone functional groups, it behaves better than water-only. However, as it did not release as much hemicellulosic monosaccharides as FoA and produced more dehydration products for sample with low surface area, it was not considered for further investigation with enzyme hydrolysis.
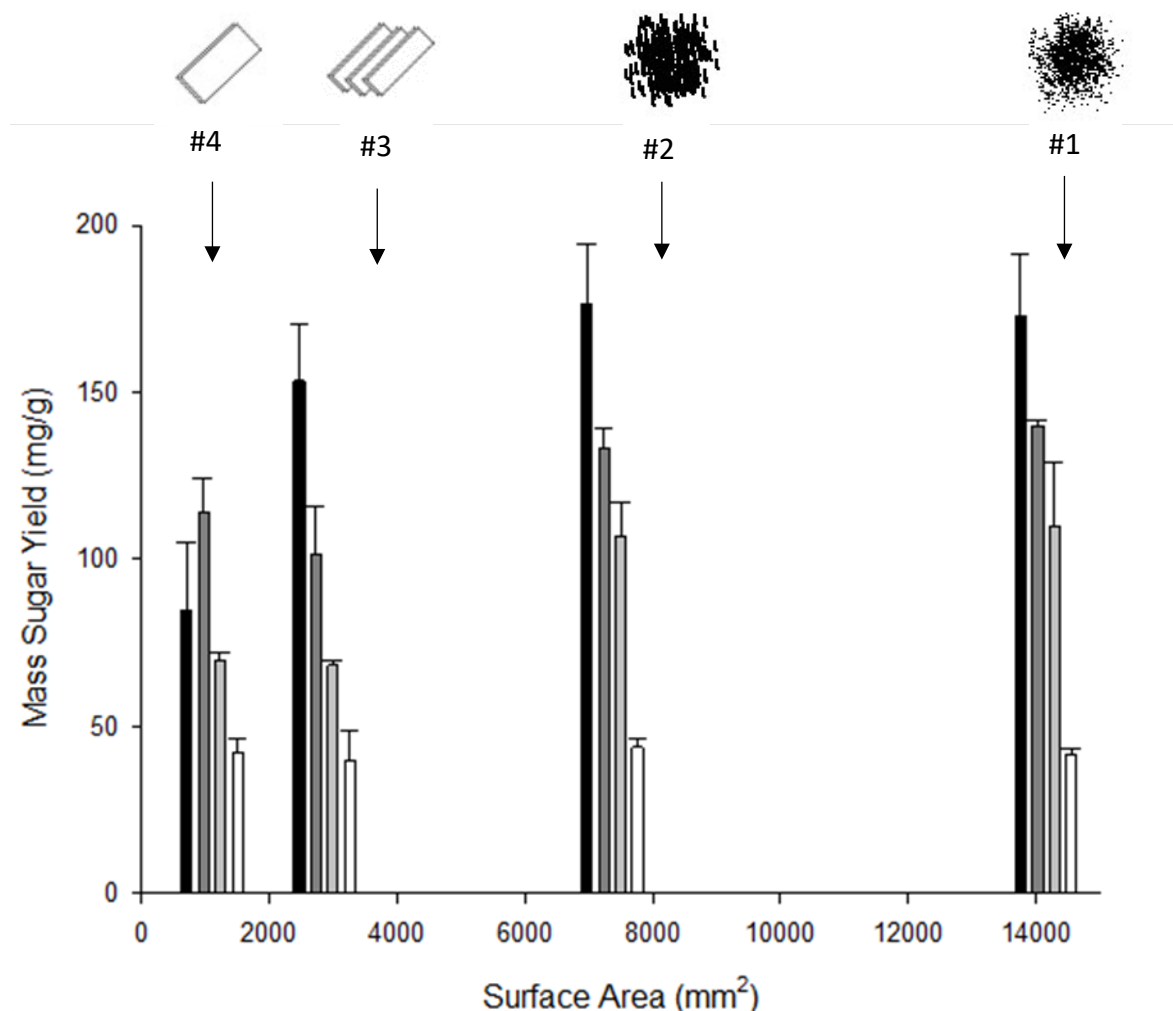
Figure 5-3. Hemicellulose-derived monosaccharide release from Eucalyptus nitens wood samples of various surface areas after microwave pre-treatment at 4wt% biomass in the presence of acid catalyst 0.05 M sulphuric acid (black bars), 0.1 M formic acid (dark grey bars), 0.4 M levulinic acid (light grey bars) and in the absence of catalyst (white bars), at T=180 °C, t=10min
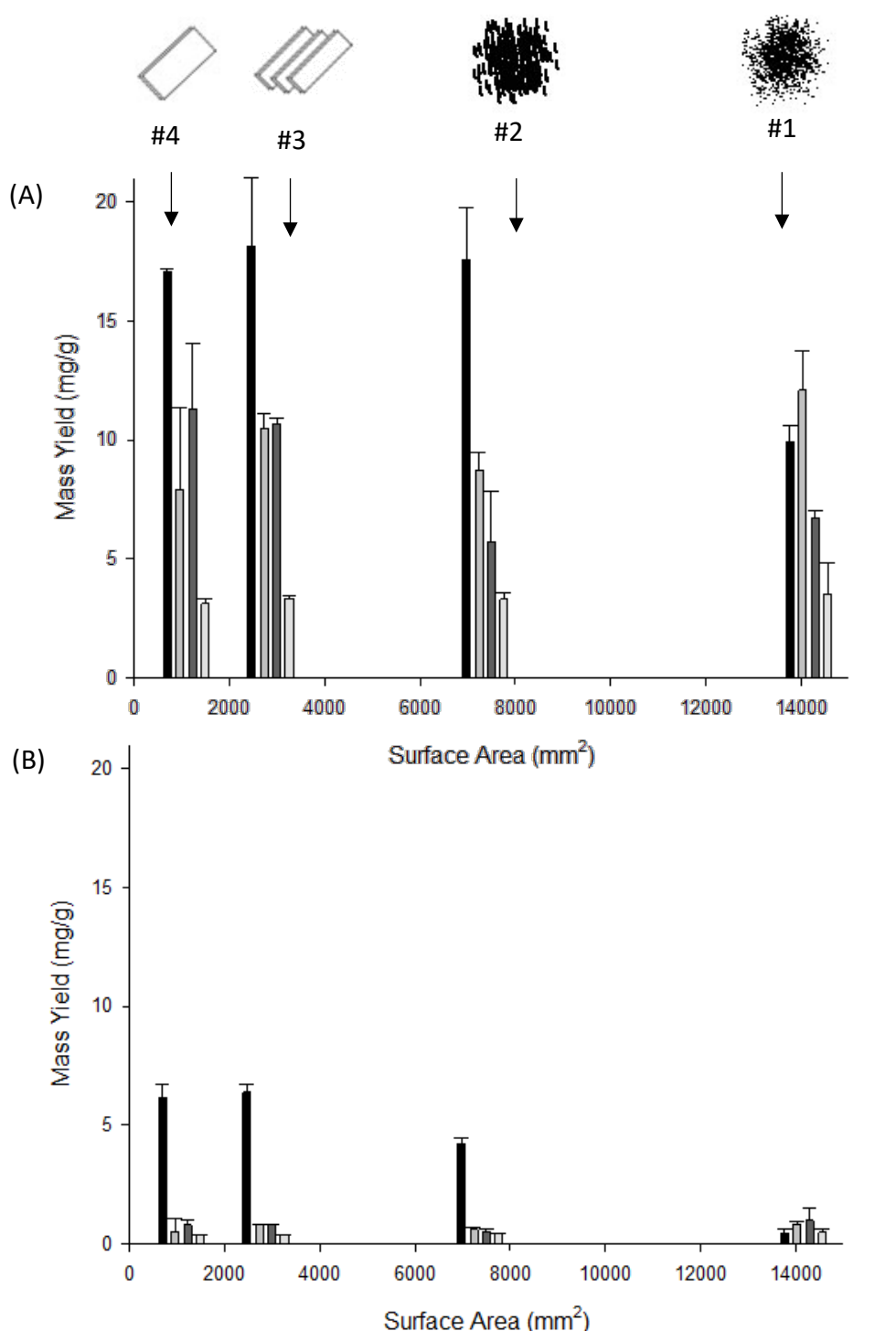
Figure 5-4. Dehydration products (A) Furfural and (B) 5-Hydroxymethyl Furfural derived from the hemicellulosic monosaccharides released from the microwave pretreatment of 4wt% wood sample types in the presence of acid catalyst 0.05 M SA (black bars), 0.01 M FoA, (dark grey bars), 0.4 M LA (light grey bars) and in the absence of catalyst (white bars) at T=180 °C, t=10min

Table 5-3 summarises the turnover number (TON) calculation for the catalysts, this gives an indication of the effectiveness of amount of the catalyst used in the pre-treatment and the total released monosaccharide yield. LA was the least effective catalyst as the TON was less than 0.1 for all the samples. FoA performs better (TON = 0.29 – 0.39) and followed by SA (TON = 0.85 – 0.94). The TON increased with increasing surface area of wood chips for all the acids. However, the change is only marginal which suggests that the catalyst loading is in excess.

Table 5-3. Turnover numbers (TON) for catalysts for respective wood fractions, (moles of sugar released in microwave pre-treatment divided by moles of catalyst used in pre-treatment)

Catalyst Type	TON for Different Samples			
	#4	#3	#2	#1
SA	0.87	0.85	0.94	0.92
FoA	0.32	0.29	0.37	0.39
LA	0.07	0.05	0.08	0.09

5.3.3 Pre-treated wood surface topography analysis

The surface of the SA and FoA pretreated wood particles appear to be more deconstructed, exposing the cellulose fibres more distinctly as shown by SEM (Figure 5-5). In contrast, the water-only and LA pretreated chips appear to have a relatively smooth surface. This is consistent with the monosaccharide yields measured in the hydrolysate and suggests that the higher strength acids were better at opening up access within the wood matrix. In addition to the deconstruction of the biomass matrix, the short duration microwave exposure showed formation of spherical shaped assemblies in the absence and presence of all acid catalysts.

Similar assemblies have been reported for non-woody biomass and referred to as lignin-carbohydrate complexes (LCC) [25]. We have previously reported formation of LCC on the surface of woody biomass at 30 min microwave exposure in water-only [18]. It was concluded that, with and without delignification of the pretreated woody sawdust, the presence of LCC did not inhibit the enzyme activity in the enzyme digestion.

There have been reports of 'pseudo-lignin' formation on the pretreated biomass surface due to low pH in acid-catalysed pretreatment conditions [26]. A study by Sievers et al investigated SA pretreatment of loblolly pinewood and reported formation of pseudo-lignin and aliphatic carbon species [27]. The harsh pretreatment conditions using strong acids, including SA, lead to accelerated degradation reactions. Analysis of the washed residues using ^{13}C CP MAS NMR found formation of 'pseudo-lignin' which has material properties similar to lignin but is derived from monosaccharides. They contain unsaturated carbons formed by acid catalysed dehydration or reforming reactions [27]. The formation of pseudo-lignin has been reported to be detrimental to enzyme activity, and as such milder conditions are recommended to avoid this [26, 28].
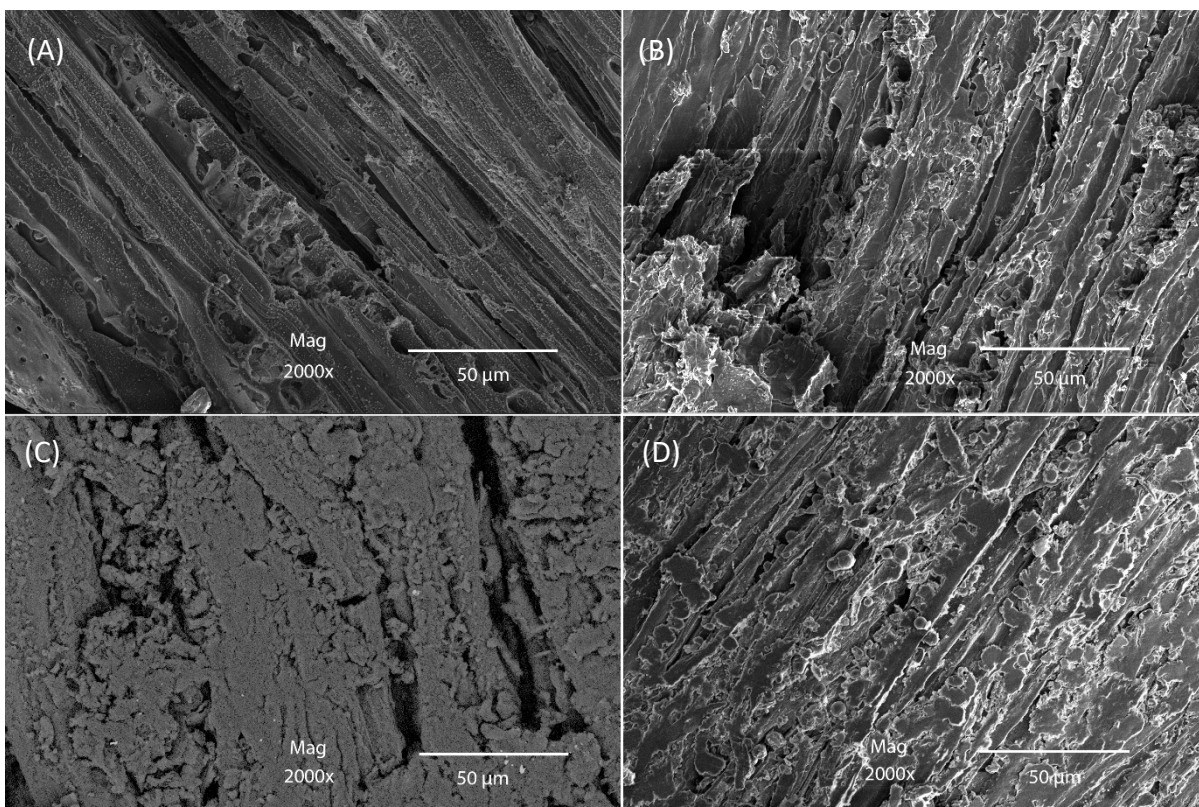
Figure 5-5. SEM images of microwave pre-treated *Eucalyptus nitens* wood chip in (A) Water-only, or catalysed with (B) Sulphuric acid (C) Levulinic acid and (D) Formic acid, T= 180 °C t=10 min, 4wt% biomass loading

5.3.4 Enzymatic hydrolysis of microwave pre-treated wood chips

The monosaccharide yields following pretreatment and enzyme digestion, and the combined percentage sugar release are compared for the acid catalysts and no added acid treatments in Table 5-4. Our results show that for sawdust sample #1, SA is by far the best catalyst choice as it yields 81% of the total sugars which is almost double that obtained by FoA. Enzyme digestion of untreated wood sample #1 yields only 4% of total sugars. It was found that acids with a low pK_a provide a better hemicellulose sugar release which leads to better cellulose digestion.

However, the results for sample #4 showed that the total sugar yield obtained from FoA is ~20%, which is higher than SA (~15%). Enzyme digestion of untreated wood sample type #4 yields only 2% of total sugars. In addition to the formation of pseudo-lignin caused by strong acids in pretreatment, Mackie et al have reported that sulphur from SA forms moieties with the lignin, referred to as ligno-sulphonate complexes (LSC) [29]. It is possible that the pseudo-lignin or LSC on the surface of the SA pretreated chips have led to this lower yield by inhibiting enzyme activity as there have been reports they can inhibit the activity of a number of biological systems, including enzymes [26, 30]. LSC have been known to increase the calorific value of biomass and as such are better suited for thermochemical conversion rather than biochemical or chemical conversion [31]. Furthermore, FoA is a much smaller chemical with respect to size and weight compared with SA. This characteristic enables it to travel within the wood matrix more easily compared to the larger catalysts. It was reported by Sun et al that FoA can penetrate within the crystalline region of cellulose, forming intermolecular hydrogen bonds which swell the structure and render it more susceptible to being crushed [24]. The above mentioned favourable characteristics of FoA make it a good catalyst for wood pretreatment.

This study confirmed that the low surface coverage of wood chips makes it difficult to be efficiently pretreated solely with water-only. As such, acid catalysts have readily available protons to accelerate the hydrolysis of hemicellulose. Optimisation of the process using FoA could lead to wood pretreatment process which yields sugar platform similar to SA pretreatment. With the added benefit of not requiring additional energy for grinding the wood and use of process equipment which reduce the overall capital cost and will not require frequent maintenance caused by corrosion.

Table 5-4. **Total sugars released after enzymatic hydrolysis of untreated and microwave pre-treated Eucalyptus niten wood samples in the presence of acid catalysts (180 °C, 10 min) fibres using a high cellulse loading (40 FPU/g), t=48 hr, T=50°C**

Pretreatment conditions	Sketch of wood sample type	Pre-treatment hydrolysate sugar release (mg/g)	Enzymatic digestion of pre-treated wood chip sugar release (mg/g)	Total sugar release (%)
Untreated	*#4	-	15.0 ± 1.0	2 ± 0.2
Microwave pretreated				
Water-only		42.3 ± 3.7	2.4 ± 0.2	7 ± 0.5
0.05 M SA		84.5 ± 20.4	9.8 ± 1.5	16 ± 1.4
0.1 M FoA		114.6 ± 5.7	7.7 ± 2.2	19 ± 0.6
Untreated	*#3	-	25.7 ± 14.6	4 ± 2.4
Microwave pretreated				
Water-only		39.6 ± 9.3	3.2 ± 0.9	7 ± 1.4
0.05 M SA		153.4 ± 16.8	21.7 ± 4.8	29 ± 2.4
0.1 M FoA		101.6 ± 14.4	6.7 ± 1.8	17 ± 0.8
Untreated	#1	-	21.9 ± 4.1	4 ± 0.7
Microwave pretreated				
Water-only		41.7 ± 1.7	82.0 ± 1.3	20 ± 0.1
0.05 M SA		172.6 ± 18.8	320.8 ± 26.0	81 ± 1.2
0.1 M FoA		140.1 ± 1.6	99.5 ± 0.2	37 ± 0.2

*Whole biomass used for enzyme hydrolysis

5.4 Conclusions

Hydrothermal microwave pretreatment of large wood chips in water-only was found to be insufficient for optimal hemicellulosic sugar release. The effect of chip size, for wood surface area ranging between 1,000 to 15,000 mm^2, was found to be an important variable for acid catalysed microwave pretreatment. SA pretreated sawdust (sample #1) gave the highest sugar yield 172.6 mg/g, and in general SA was better in terms of sugar release for all samples except the largest particle size #4. FoA released the most sugars with the least degradation products compared with the other acids for the pretreated wood chip sample #4 which has the lowest surface area. The smaller size of FoA possibly allows it to penetrate further into the wood matrix. Although the overall sugar release after enzyme digestion was low (~20%), the results indicate the potential for FoA to be further investigated for wood pretreatment. Elimination of milling of chips to finer sawdust and the milder hydrolysate pH alleviating the need for expensive high grade stainless steel can lead to lower cost for woody biomass pretreatment.

5.5 Acknowledgement

5.6 References

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Chapter 6.   Conclusions, Future Work and Recommendations

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6.1 Conclusions

This thesis successfully addressed a number of gaps identified in literature for the pretreatment of recalcitrant woody biomass. The major outcome of this research project was the development of an alternative microwave pretreatment method to acquire a sugar platform from woody biomass that incorporated mild conditions, sustainable solvents, organic acids and larger particle sizes of wood. Mostly, the effectiveness of the method was compared against conventional hot water pretreatment. The superiority of microwave pretreatment was verified, providing a faster and more efficient depolymerisation to achieve significantly higher sugar yields. Below are the specific conclusions of the thesis.

6.1.1 Necessity of delignification for optimal enzyme saccharification following hydrothermal microwave pretreatment

Direct comparison of hydrothermal pretreatment under the same conditions was carried out via microwave and conventional heating. It was found that microwave irradiation was more effective at opening up access to the cellulose fibres, yielding complete sugar release for the combined pretreatment and enzyme hydrolysis and alleviating the need for delignification. Whereas, the pretreatment sugar yield obtained for liquid hot water 3.5 times less and delignification was found to only marginally increase the sugar yield after enzyme hydrolysis.

6.1.2 Sustainable solvents for a high microwave absorption capacity to efficiently depolymerise wood

Polar solvents of different chemistries have different capacities to convert electromagnetic radiation into heat. Water is well-known to efficiently heat at 2.45GHz microwave frequency, however, the results from this thesis demonstrated that formalin is more efficient in being heated and retaining the energy under microwave radiation at higher temperatures. In addition, it is more powerful at depolymerising the recalcitrant woody biomass due to its electrophilic nature. Moreover, the lignin by-product from this process is stabilised by the formaldehyde. This result was achieved in dilute formalin which has not been reported before.

The potential of further upgrading of lignin into monomers and fuels can also be explored from this process.

6.1.3 Importance of wood chip surface area for industrially relevant pretreatment

It was found that hydrothermal pretreatment is not sufficient for efficient depolymerisation of wood chips with low surface area, which was contrary to our hypothesis. We found that the addition of an acid catalyst was necessary to open the structure and accelerate the rate of depolymerisation of the wood chips by enzymatic treatment. Formic acid demonstrated potential for further development as an ideal catalyst for this purpose and alternative to highly corrosive sulphuric acid.

The overarching objective for the thesis was to propose a sustainable pretreatment method for lignocellulosic biomass. From the investigations carried out in this thesis, it can be concluded that, although hypothetically the most sustainable approach, it is not sufficient to utilise water as the sole media for wood pretreatment. The addition of formalin assisted in enhancing the microwave heating rate by manipulation of the dipolar polarisation mechanism. However, inherent limitations for the upscaling of this method exist with respect to the production of toxic formaldehyde gases. As such, further investigation into other reactive polyols and acids that do not pose health risks but at the same time produce high sugar yields would be the future direction for a sustainable method.

6.2 Future Work and Recommendations

Further research continuing on from this thesis has a promising outlook that can lead to commercialisation of a mild, energy-efficient pretreatment of woody biomass. This chapter highlights the potential future work and recommendations of this research.

6.2.1 Alternative Catalysts

Various organic and inorganic acid catalysts were assessed for the pretreatment of wood chips to assist in the acceleration of hemicellulose depolymerisation and enhancement of

their enzymatic digestibility. Further research can be carried out on the recyclability of the organic acid in the hydrolysate back into the pretreatment process. Also, the downstream processing of the hydrolysate can be carried out to confirm compatibility of remanent organic acid with fermentation organisms such as yeast that majorly carry out the production of cellulosic ethanol. Furthermore, heterogenous catalysis can be considered for microwave-assisted pretreatment of wood. Organometallic nano-catalysts may provide an advantage when used in combination with microwave heating. This method would be suitable for smaller particle size wood particles to enhance the solid substrate to solid catalyst interaction. Furthermore, this method could be used for selective conversion of the monosaccharides to a value-added chemical such as succinic acid.

6.2.2   Lignin Regeneration

In chapter 3 it was found that the lignin coalesces together with some carbohydrates to form lignin droplets which are condensed back onto the pre-treated biomass surface. Characterisation of the lignin droplets could assist us to understand whether a trend exists with respect to the ratio of lignin and carbohydrates.

In chapter 4 it was found that weak formalin solution in water assists in the preservation of lignin by hindering condensation of the lignin to other carbons. Hydrogenolysis of the stabilised lignin would yield lignin monomers. Qualitative and quantitative investigation could be carried out on the extracted lignin. Comparison of the extracted lignin from microwave and conventional pretreatment in the presence and absence of water could assist in further understanding of the role of formalin.

6.2.3   Feedstock

Pretreatment of both hardwood and softwood was investigated in this research using woods from common forestry species of relevance to Australia and globally. Other biomass with extensively high lignin content may be explored by microwave pretreatment, such as coconut shells or walnut shells. As these are currently waste streams, by converting them into useful products provides a sustainable approach. Furthermore, by optimising the microwave process,

it may be possible to pre-treat a heterogeneous sample of biomass to a sugar platform that can be converted to value-added products.

6.2.4 Continuous Flow Microwave Reactor

The microwave instrument used for experiments in this thesis is bench scale and operates in batch mode. It would be important to know if the experiments conducted in this thesis are reproducible in a continuous flow microwave reactor i.e. whether the same sugar yields are achieved under the same conditions. Continuous flow reactors involve rotational movement which mimics a stirring effect. Thus, further investigation into the kinetics of microwave heating and direct comparison with stirred conventional pretreatment can be explored to make a strong judgement on non-thermal microwave effects.

6.2.5 Dielectric Properties of Materials

Many materials are yet to have a comprehensive dielectric properties database. One example of such materials is formalin solution which contains methanol, formaldehyde and water. The dielectric properties of materials at various temperatures, frequencies and concentrations will allow users to fast track their experimental screening process by knowingly selecting materials which are likely to provide efficient conversion of microwave energy into heat.

6.2.6 Screening of Enzyme for Downstream Conversion

A comprehensive screening analysis of the enzyme used for the downstream conversion of wood to monosaccharides could assist in determining the optimum conditions necessary to reduce the overall pretreatment cost. Conditions which could be further analysed are the incubation time from 0 to 72 hours, enzyme to substrate loading and addition of supplement enzymes to enhance the final sugar yield.

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Appendices

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Appendix A: Published Journal Article Front Page

Microwave assisted pretreatment of eucalyptus sawdust enhances enzymatic saccharification and maximizes fermentable sugar yield

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ABSTRACT

There is an urgent need of sustainable and efficient methods for the production of biofuels and chemicals from lignocellulosic feedstock. The purpose of this study was to develop a mild, cost-effective and environmentally benign pretreatment for woody lignocellulose to maximize sugar yield via enzymatic saccharification. Microwave irradiation (MW) of *Eucalyptus regnans* sawdust in water was investigated and compared directly against conventional liquid hot water (LHW) pretreatment. Following 30 min microwave irradiation at 180 °C, the sugar yield was 3.5 times higher using MW than LHW pretreatment under the same conditions. Complete release of C_5 and C_6 sugars was achieved after the two-step method of MW pretreatment followed by enzymatic hydrolysis, compared with only 4% without pretreatment and 31% after LHW. Removal of 'lignin droplets' formed on the surface of the pretreated fibers via flowing hot water showed only minor improvement in the yield of enzymatic saccharification. Our results support the hypothesis that lignin prevents access of enzymes rather than inhibits their activity. MW accelerated depolymerization of hemicellulose, opening the structure more than LHW pretreatment.

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1. Introduction

The production of fuels and fine chemicals from biomass may help mitigate anthropogenic greenhouse gas emissions and fulfil our demand for energy and materials [1]. Sustainably sourced lignocellulosic biomass, composed of the aromatic polymer lignin, and polymers of C_5 and C_6 sugars, hemicellulose and cellulose, is a widely available and relatively inexpensive feedstock at $50 per ton [2–4]. Thus, the processing of lignocellulosic biomass is of benefit across multiple industries and a green alternative to greenhouse gas emitting sources. Pretreatment is essential to open up the complex lignocellulose structure and allow access to the sugar polymers for cellulolytic enzymes [5]. An impeding factor for lignocellulose pretreatment is its recalcitrance demands a high energy input. One such example is in the lignocellulosic biofuels process, where sugar release, including pretreatment and enzyme digestion, can contribute up to 45% of the total costs [6] and is one of the main factors hindering its full commercialization [7]. Agricultural residues such as corn stover, wheat straw and bagasse have been widely investigated and some are currently used as feedstocks for lignocellulosic ethanol production [8–10]. Forestry species, in particular, eucalypts, are cultivated world-wide but have not been widely considered, due in part to their recalcitrance.

While many pretreatment approaches including hydrothermal/chemical, physical and biological have been studied for biomass, a step change in energy-efficiency and green approach is needed to improve the attractiveness of woody lignocellulose as a feedstock. The more common pretreatment technologies for lignocellulosic biomass include steam explosion (acid catalyzed/uncatalyzed) and liquid hot water (LHW) [11]. While effective, these processes generally need large energy input for heating. Water is an ideal medium for lignocellulose pretreatment due to its low cost, relative simplicity of product and by-product formation and its use avoids the need for corrective action downstream. However, when water alone is used for pretreatment, as in the LHW process, it is not as effective in comparison to acid-catalyzed steam explosion [12]. Variations of the standard LHW pretreatment method have been reported to be more effective at releasing both hemicellulose and cellulose-derived sugars following enzyme treatment of woody feedstocks but these have required two sequential LHW steps [13], increasing the energy requirement.

Microwave irradiation is energy efficient as it directly heats substances via dipole polarization and ionic conduction, as opposed

Figure A-1. Chapter 3 – Microwave Assisted Pretreatment of eucalyptus sawdust enhances enzymatic saccharification and maximizes fermentable sugar yield
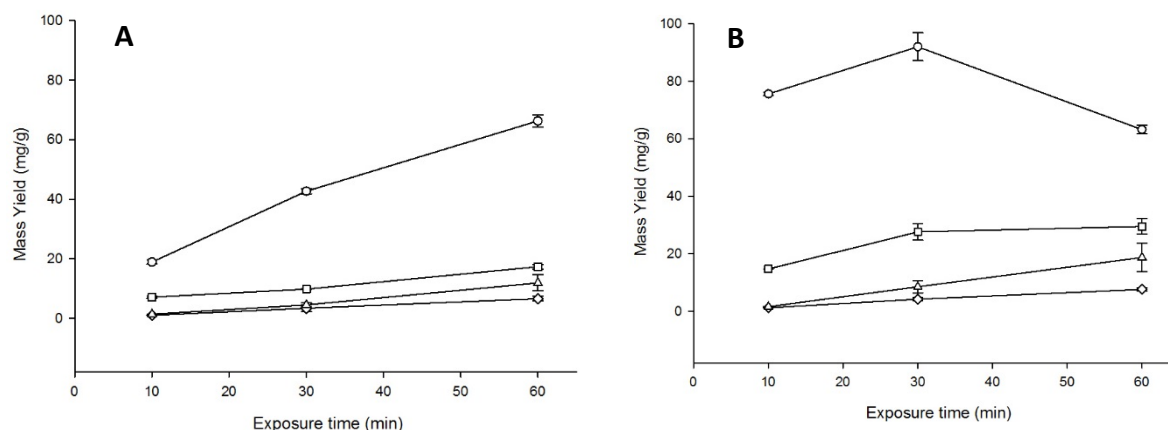
Figure B-1. **Pretreatment products released following microwave pretreatment of 4wt%** *Pinus radiata* **sawdust in (A) Water or (B) 3.7wt% FA over 5 – 60 min exposure times, T=180°C. (o) Mannose-Xylose-Galactose-Arabinose, (□) Glucose, (◊) Furfural and (Δ) 5-HMF.**
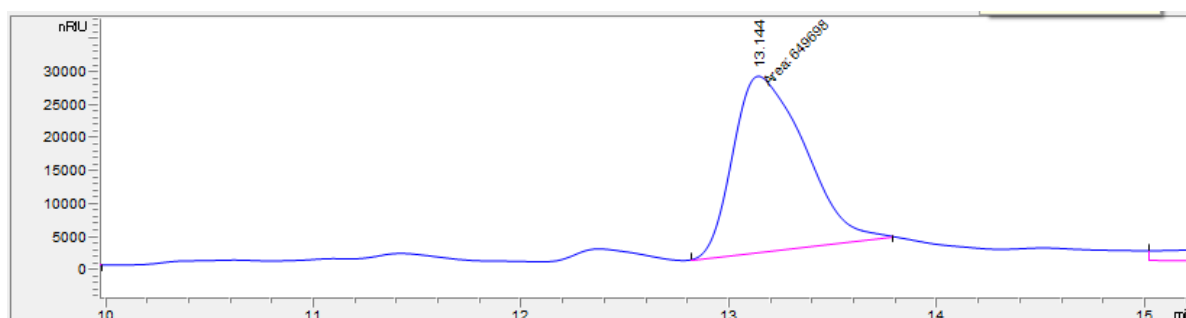

Figure B-2. **HPLC chromatgoram - Microwave irradiation of Eucalyptus regnans in 3.7% FA for 10 min at 180 °C, xylose retention time 13.144 min**
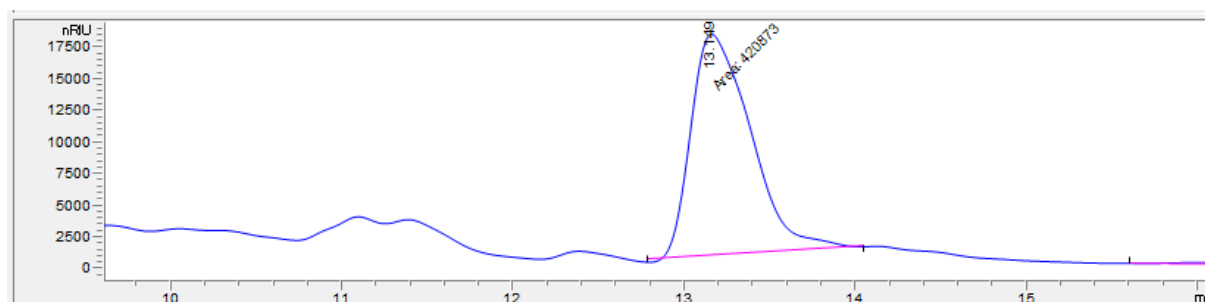

Figure B-3. HPLC chromatgoram - Microwave irradiation of Eucalyptus regnans in water for 10 min at 180 °C, xylose retention time 13.149 min
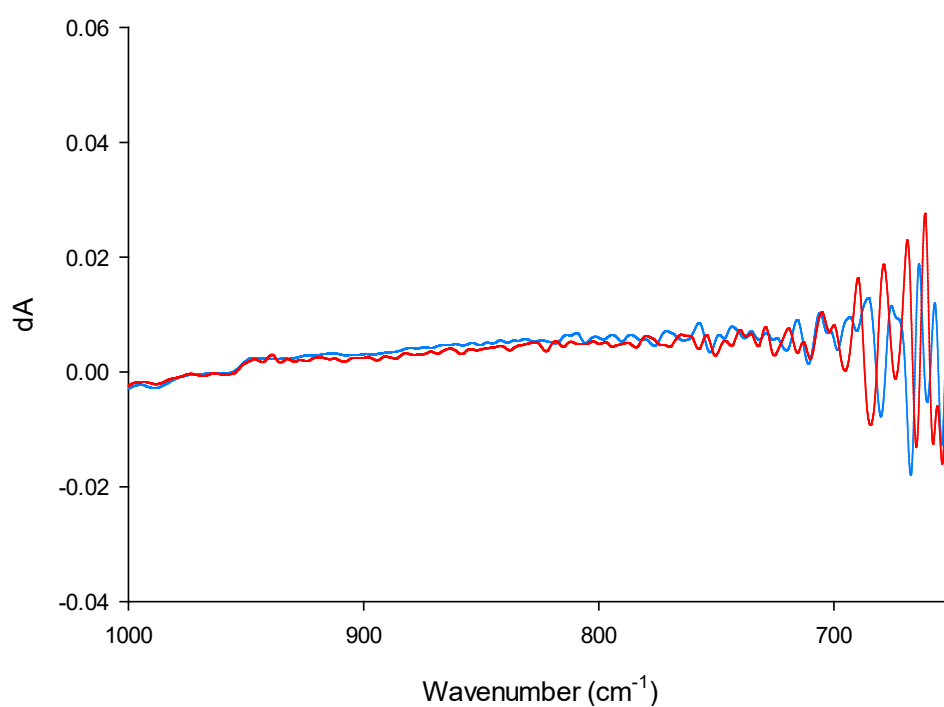
Figure B-4. FTIR-ATR first derivative of xylose in 3.7wt% FA at 25°C (blue line) then heated to 60 °C (red line).
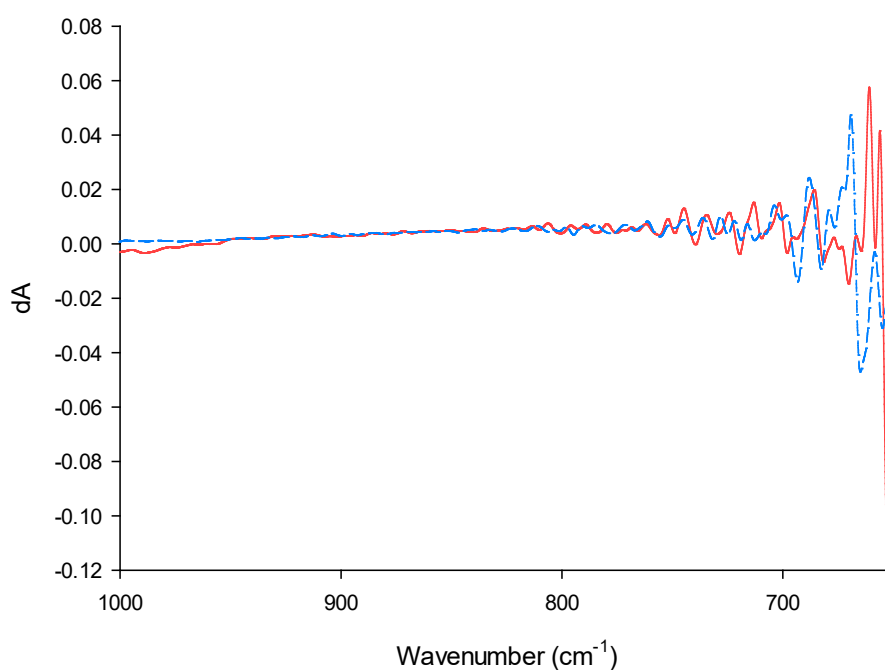

Figure B-5. FTIR-ATR first derivative of microwave treated xylan in water at 180 °C (red line) and 3.7wt% FA (blue line) at 25 °C.

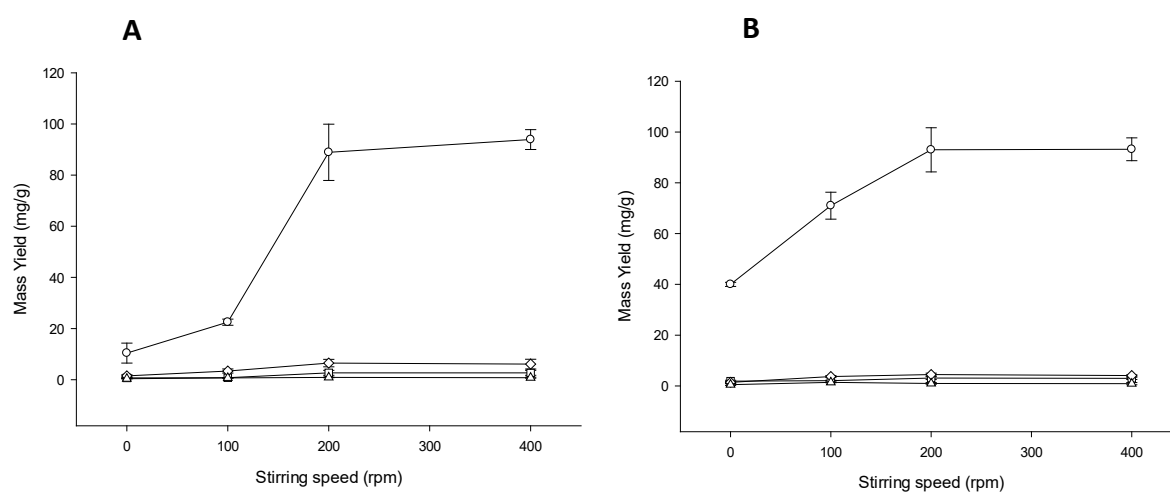**Figure C-1. Release of sugars from Eucalyptus regnans sawdust (4% wt/v) heated at 180°C and 10 min exposure in an autoclave in the presence of (A) Water or (B) 3.7 wt% formaldehyde over various stirring speeds (ο) Xylose, (□) Glucose (Δ), (◊) Furfural and 5-HMF.**