Enhancement of lignocellulosic feedstocks and biorefinery byproducts for composite and polymer applications

by

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Abstract

The volatility of the petroleum-based markets, increasing ecological concerns, and the accumulation of waste from the plastic industry have motivated a paradigm shift in research. Researchers are searching for sustainable, renewable, and competitive platforms ideally suited for replacement of conventional petroleum-based chemicals and fuels. The purpose of this research is to study the macrostructure of biomass and byproducts from the different bio-based industries for preparation and characterization of bio-based composite and polymer materials. Lignocellulosics have been identified as a key resource for this space. Natural fibres are composed primarily of cellulose, hemicellulose, lignin, and other minor components including pectic material and waxes. As determined by their native composition, they are characterized with limited thermal stability, poor water resistance, and incompatibility with most commercial resin systems. Researchers in the past have studied the effect of chemical, physical, and to some extent, biological treatment of natural fibres. Unfortunately, chemical methods in most cases are associated with large volume of organic waste and high energy requirement. Likewise, physical methods have been shown to be limited because of high capital requirements and less than desired improvement in properties. If these uncertainties can be addressed with new chemistries that are green, and technologies that are easily scalable, that would be an achievement in this area.

In the first approach investigated in this thesis, hemicellulases, pectinases and an oxidoreductase were used to enhance hemp and flax fibres. Treatment with xylanase and pectinase (polygalacturonase and pectinmethylesterase) improved the thermal properties for both fibre types. X-ray photoelectron spectroscopy (XPS) measurements confirmed reduction of the hemicellulosic content of both fibres for xylanase and pectinases. Removal of amorphous hemicellulosic material from the fibre surface and consequent exposure of the crystalline cellulose network resulted in a lower contact angle for all the treated samples. After initial property determination, enzymatically enhanced fibres were then tested for their ability to reinforce polypropylene. The removal of hemicellulose and pectic components resulted in improved thermal properties and greater water resistance whereas the mechanical properties were unaffected in most cases.

In the second approach investigated, hemp fibres were enhanced using two sulfonic acid derivative systems. An aqueous reaction medium was used with little or no organic solvent at relatively low temperatures (below 50 °C). Successful modification was subsequently confirmed through the use of FTIR. The degree of substitution for the treated fibres peaked at 30 °C and XPS data of the treated fibres were characterized by reduction of the O/C ratio and an increase in abundance of the C-C-O, attributed to the ester linkages. The “green index” of the sulfonic systems was then evaluated and confirmed using Life Cycle Analysis (LCA). The studied methods had measurable benefits in regards to impact on the environment and society when compared to mercerization, alkylation, and the production of glass fibre.

Subsequently, a novel polymer system was developed using epoxy resin and a renewable, waste stream – tall oil rosin acids, which were derived from the pulp and paper industry. Replacement up to 15 % (w/w) with TORAs resulted in no change in mechanical properties of the plastics. This study demonstrated the successful integration of waste streams from the pulp and paper industry validate a multi-product bio refinery concept.

In summary, this thesis has demonstrated that a better understanding of the structural and compositional properties of natural fibres can result in technologies tailored for specific applications. Additionally, it has been demonstrated that chemical methods and/or enzymes can be used to enhance natural fibres for composite applications. The thesis also demonstrates that there is potential to use waste streams, such as TORAs to produce plastics with a renewable and sustainable index. Finally, this work demonstrates that these technology platforms are more environmentally sustainable than the conventional petroleum-based platforms and established methods in the literature.

Dedication

I dedicate this thesis to my caring and supporting parents, Morris and Devi George. Also, to my wife, Youmattie George (Monica), I dedicate this work as a testimony for the trying times we faced during my tenure here at the University of Alberta. Love you all.

I would like to begin this work with one of my all-time favorite quotes, hope someone reading this, is truly inspired!

"We should be taught not to wait for inspiration to start a thing. Action always generates inspiration. Inspiration seldom generates action."

-Frank Tibolt

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Lastly, I would like to thank my immediate and extended family for their abundant encouragement, support, and love over the journey of my PhD dissertation. My beautiful and understanding wife, Youmattie George (Monica), I cannot express how much your love and caring has guided me in these challenging years. Thank you for waking me each day, encouraging me during the darkest days and celebrating the many great times. I love you.

Table of Contents

List of Tables

List of Figures

Abbreviations and Symbols

XPS	X-ray photoelectron spectroscopy
ToF SIMS	Time of Flight Secondary Ion Mass Spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
TGA	Thermal gravimetric analysis
AFM	Atomic force microscopy
SEM	Scanning electron microscopy
DSC	Differential scanning calorimetry
NF	Natural fibres
NFC	Natural fibre composites
Θ	Angle
A2S	Aniline-2-sulfonic acid
AT3S	4-aminotoulene-3-sulfonic acid
$SOCl_2$	Thionyl chloride
PP	Polypropylene
PHA	Polyhydroxyalkonates
PLA	Polylactic acid
RTM	Resin transfer molding
LCA	Life cycle analysis
ρ	Density
γ	Surface tension
η	Viscosity

CHAPTER 1.

Introduction and Objectives

Western Canada's economy is driven by the mining and the agricultural sectors. The agricultural sector produces lignocellulosic material for high value products such as pulp, paper, value added products such as biofuels and bio-based materials. The availability of agricultural rich land and developed methodologies for growing lignocellulosic materials such as natural fibres and trees needed for the pulping industry are the main driving forces for this dissertation. Also, the current environmental and economic times have called for technologies that are built on a renewable platform that are capable of ideally substituting fossil fuel derived materials [1-2]

One key market forecasted by many researchers will be the bio based materials sector. Specifically, the formulation of bio composites, production of bio resins, and bio plastics will be very advantageous and rewarding [3-4]. The use of natural fibres, pulps, and waste materials from the mining and pulping industries will create value from materials that are sourced from a renewable source and aid in effectively closing bio refinery gaps. These renewable feedstocks such as natural fibres and pulps were characterized and used for bio composite applications. A partnership with Novozymes and Alberta Innovates Bio-Solutions allowed for the study of different enzyme classes on the macromolecular properties of natural fibres and pulp samples. The systems studied were hemicellulases, pectinases, and a laccase. The effect of the different enzymes on hemp, flax, and different types of pulps were investigated. Also, swelling of hemp fibres using caustic prior to enzymatic treatment was investigated. The aim here was to determine whether disrupting the macrostructure would result in fibres better suited for enzymatic treatment. The surface and thermal properties of the treated samples were studied using X-ray photoelectron (XPS), Force tensiometry (contact angles), Thermal gravimetric analysis (TGA), and Scanning electron microscopy (SEM).

Specific objectives of the thesis were:

- Study the effect of enzymatic treatment on surface and thermal properties of natural fibres
- Chemical enhancement of lignocellulosic material for composite application, a chemical mechanistic insight coupled with influence on surface and thermal properties

- Investigate the effect of chemical and enzymatic methods on the nano properties of hemp fibres as determined by Atomic force microscopy and spectroscopy
- Development, property evaluation, and improvement of bio-based materials made out of enhanced fibres
- Life cycle analysis and environmental comparison of the developed methods versus accepted methods for chemical methods of lignocellulosic materials
- Development and property evaluation of plastics from tall oil rosin acids and epoxy resin

In Chapter 2, the use of lignocellulosic materials, specifically bast fibres, for composite applications is reviewed. The aim was to do a thorough investigation into the use of bio-based materials as reinforcements, types of polymers available for compounding, and the different processing methods. Also, of interest, was how competitive were the bio composites studied over the past decade. To this end, key life cycle assessment and techno economic analyses were reported. In the end, a better understanding as to which fibre types, polymers, and techniques are best suited to be competitive with the petroleum based benchmarks were realized.

In Chapter 3, the effect of different classes of enzymes on natural fibre macrostructure were investigated. Hemicellulases, pectinases, and an oxidoreductase were supplied by Novozymes via a grant to determine whether the enzymes were candidates for enhancement of natural fibres for composite applications. The effect of the enzymatic treatments on the surface and thermal properties of hemp and flax fibres were reported. X-ray photoelectron spectroscopy and force tensiometry were used to study the effect of the enzymes on the surface properties. Thermal gravimetric analysis was used to determine changes in thermal stability of the different systems.

Chapter 4 outlined a chemical method for the enhancement of hemp fibres. Specifically, hemp fibres were enhanced using two sulfonic acid derivative systems. A suite of surface characterization techniques including X-ray photoelectron spectroscopy, Secondary ion mass spectroscopy, Force tensiometry and Atomic force microscopy and spectroscopy were used to characterize the changes in surface properties. Morphological and thermal properties were studied using Scanning electron microscopy and Thermal gravimetric analysis, respectively. The studies reported in Chapter 3 and 4 demonstrates the technical feasibility of using these enhanced fibres for reinforcing polymers.

In Chapter 5, Atomic force microscopy and spectroscopy was used to investigate the changes in surface roughness and adhesion force of the chemically and enzymatically treated hemp fibres. The scanning of the surfaces of the fibres at the nano scale enabled a better insight into the changes of the microstructure of the hemp fibres. This study is one of the first to specifically monitor changes in surface forces and how this may influence the properties of compatibility with resins.

The development of biocomposites from enzymatically treated flax and hemp fibres is presented in Chapter 6. The mechanical, morphological, water resistance, and thermal properties of the composite were reported. Improvements in thermal and water resistance properties were found as a result of the degradation of the thermally labile and highly polar hemicellulosic material. Also, Scanning electron micrographs showed better dispersion between the treated fibres and polypropylene.

In Chapter 7, the development of a plastic system from epoxy and tall oil rosin acids (TORA) was reported. Rosin acids are a waste stream from the pulping industry. The reaction chemistry and properties of the manufactured plastics were reported. Partial replacement of epoxy with this abundant and renewable agent can be used to produce plastics with a variety of properties, depending on the applications.

In Chapter 8, the environmental impact of the methods developed versus widely accepted chemical methods of modification are presented. Mercerization and alkylation of hemp fibres were used as the benchmark. Sulfonic acid derivative treated fibres were compared to the established chemical methods. Life cycle assessment allows researchers to pin point the processes within a system which have the largest influence on the environment and humans. These studies are becoming more relevant as the bio based space gets more recognized and competitive with established petroleum based platforms.

The last chapter, Chapter 9, provides concluding discussion that presents the overview of the big picture of enhancement of lignocellulosic material using the different methods. Also, a link of how a total bio refinery concept can be adopted is discussed.

As an important point, all numbers out of this thesis are quoted as mean ± standard deviation, in cases of exception, this will be stated.

As a side note, the numbers expressed in this thesis are reported with statistical significance related to the error of the measurements and protocols used and not corrected to account for standard deviation uncertainly. They are reported in this manner to allow for future manipulation and calculation.

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CHAPTER 2.

Literature Review: Prospects of using bast fibres in composite materials: fibre and polymer characteristics, economic, environmental, and technical properties[1]

2.1 INTRODUCTION

The industrial age has brought technologies that are efficient and involves enormous scale of non-renewable hydrocarbon feedstock and products. As of the past few decades, a better understanding of the environment and how anthropogenic factors affect it has been documented. As a result, humans have been searching for technologies that are economically competitive and environmentally friendly. Hence, research undertakings have been explored to develop technology platforms that mimic fossil petroleum based technology with the hope the new technologies can supplement or replace the obsolete ones. One area of great interest has been the production of bio-based materials from lignocellulosic material [1]. In fact, significant progress has been made in material science, where we have seen replacement of conventional materials in various applications.

Natural fibres for composite applications has received widespread attention of late. These fibres are low-cost if sourced locally, are characterized with low density, and have high specific mechanical properties [2]. Also, these fibres are nonabrasive and biodegradable. Nevertheless, limitations such as poor thermal stability, moisture absorption, and incompatibility with petroleum-based resins have reduced the outlook and potential of these fibres.

Many researchers have published reviews in this area because of the depth of research done and the many discoveries made. In this chapter I hope to comprehensively cover the research areas discussed from 2005 – 2015, including what’s relevant to bast fibres, polymers, testing of bio composites, and evaluation of these materials via techno economic and life cycle analyses. This thesis will not address natural fibres from animals (*e.g.* silk or wool) or those resulting from wood, seeds (*e.g*. coir or cotton), leaves (*e.g.* abaca, pineapple, or sisal) or grasses (bamboo, miscanthus, or wheat). A comprehensive review was done on cellulosic pulps from wood samples by Eichhorn *et al.* (2001) [98]. The chapter would cover the structural and composition of bast fibres, the different methods used for modification,

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[1] A version of this chapter has been prepared for peer review.

and the different types of polymers available for composite applications. Also, with the economic and environmental concerns, a comprehensive section of Life cycle assessment and Techno economic analysis will be presented.

2.2 NATURAL FIBRES: STRUCTURE AND PROPERTIES

Natural fibres are continuous filaments that can be spun into thread or rope. Natural fibres can be used as a reinforcement of composite materials or compressed into sheets to produce paper. According to Ahmad *et al.* (2015) [3], natural fibres are of two main types: natural or synthetic fibres. Also, fibres with origin from nature are obtained from three main subcategories, animals, vegetative parts of plants, and minerals. Natural fibres are obtained from vegetative parts of the plants such coir, flax, and bamboo etc. while animals fibre includes wool and silk, and cellulose fibres such as cotton, and mineral fibres such as asbestos [1]. Plants fibres can be subcategorized as bast, seed, or hard fibres. In this chapter, a bit of bast fibres will be expounded on. To get an idea of the classification of the different type of fibres, a summary is presented in Figure 2.1.

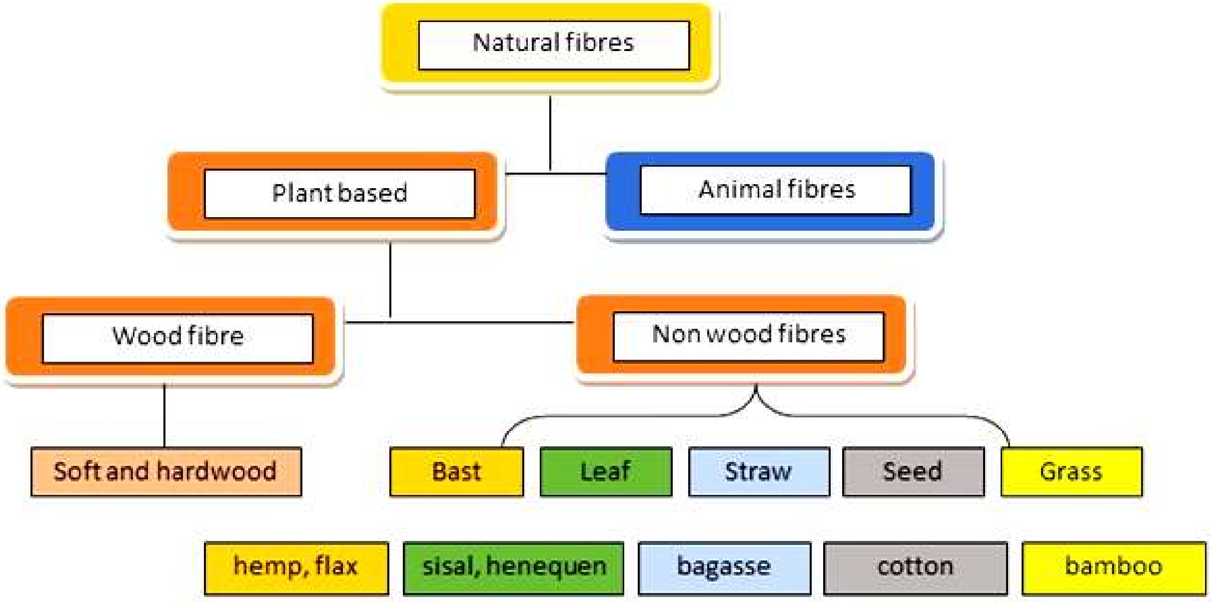

Figure 2.1 A basic classification of some of the main fibre type. The color-coding illustrates each class with examples [4].

Cannabis, family *Cannabaceae*; species: *Cannabis indica, Cannabis ruderalia, and Cannabis sativa L.*, has been found on every continent in this hemisphere. *In every society where people discovered Cannabis hemp, they often discovered the five uses for hemp namely; hemp fibres, oil from the seeds, the seeds for food, a medicine, and for its narcotic properties. Cannabis use has existed for over ten thousand years and is one of the oldest crops used for cultivation. It was cultivated in China as early as 4000 BC (Website: Global Hemp: http://www.globalhemp.com/2001/01/hemp-history.html May 30, 2015). Hemp* does not require fertilizer, herbicides, or pesticides to grow well and this renders it very attractive for composite applications. In suitable warm conditions, it can grow to 4 m under 12 weeks. Hemp is a fine, light-colored, lustrous, and strong bast fibre obtained by processing. The main process involves degradation of the outer stalk by microbial degradation via a process called retting. The color and cleanliness vary considerably according to the method of preparation of the fibre. The fibre ranges in length from 1.0 - 2.5 m [5]. The lower grades are dark cream and contain mainly non-fibrous matter. In North America, the revitalization of the renewable space has seen extensive research in hemp and its applications. In context, here in Canada, hemp has been grown since 1998. The main motivation has been to grow hemp for the production of healthy food and environmentally friendly products such as biocomposite and textiles. The establishment of the Canadian Hemp Trade Alliance enabled farmers to have a voice in the trading of their products (Canadian Hemp Trade Alliance – January 30, 2015). *Linum usitatissimum*, flax, has been grown in Canada primarily for its seed oil and flax seeds. According to the Flax Council of Canada, flax has 12 growth stages. Flax fibres were one of the first fibre type used for reinforcement in automobile applications. In 1941, Henry Ford pioneered the first car from a hemp and flax body [6, 7]. A few other bast fibres, such as kenaf, jute, and ramie have also found widespread use, but are not so popular in Canada. In the next few sections, a detailed explanation into the structural arrangement of bast fibres will be given, and how these features affect the properties.

2.2.1 Structural features of bast fibres

Fibres are glued together by a pectic and or lignin rich middle lamellae to form specialized tissues that fulfill specific applications for the plant. Stiff cellulosic fibrils are embedded in the plant cell walls as the major load carrying component. These fibrils are randomly distributed within an amorphous layer of lignin and hemicellulose. Cellulose is a linear polymer composed of β- D-glucose units linked via β-1, 4-glycosidic bonds. The cellulose chains aggregate into well packed crystalline regions and random amorphous pockets. Hemicellulosic units bond between cellulose and lignin via hydrogen bonding and covalent bonding, respectively. Hemicelluloses are heteropolymers built from neutral sugars (glucose, mannose, galactose etc.) and some uronic acids. On the other hand, lignin is a macromolecule of phenylpropane units and it is incorporated in the cell wall after cellulose and hemicelluloses have built up during cell wall assembly [8].

The cell wall of plants constitutes several layers, with different thickness and chemical composition. The cell wall of fibres consist of a primary cell wall and a secondary cell wall which consists of three layers, S1, S2, and S3 [4]. Within the secondary cell wall, cellulosic fibrils are organized in S or Z-helix parallel to each other. The angle of the parallel cellulose micro fibrils to the longitudinal cell axis is called the cellulose micro fibril angle. Matured fibres normally have a micro fibrilliar angle of 0 to 20^{o}. In Figures 2.2 and 2.3, the arrangement of each level within the secondary cell wall, and the major components are illustrated.

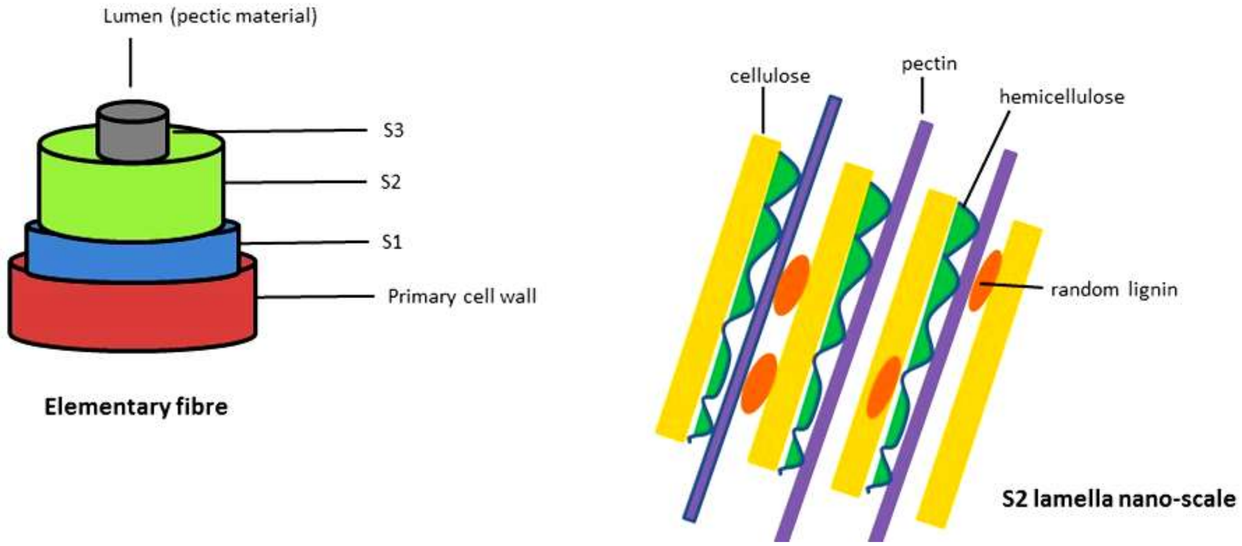

Figure 2.2 A schematic of the elementary fibre and the important S2 sections are highlighted in the above illustration.

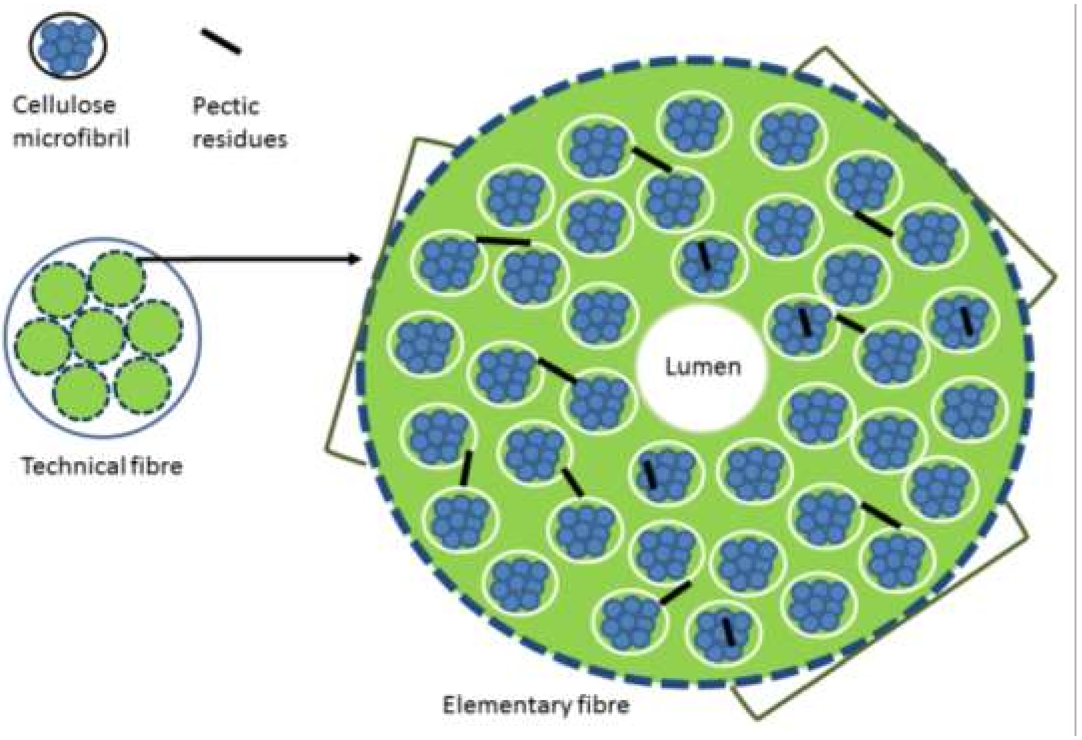

Figure 2.3 Cross-sectional presentation of technical and elementary fibre distribution of bast bundles

Within the cell wall, cellulose is arranged helically in micro fibrils in the middle lamellae. The angle of arrangement of the micro fibrils within the lamellae strongly influences the axial tensile properties of the fibres. The S2 subsection of the secondary cell wall has been of much interest to scientist because of the inherent size relative to the other sections, which significantly contributes to high strength. The S1 layer functions in controlling fibre stability in compression via limiting excess lateral cell expansion. On the other hand, the S3 layer contributes to cell stability by resisting hydrostatic pressure within the cell. In summary, the combined laminate structure of the cell wall is considered to be of importance in maintaining the functioning integrity of the plant [9].

2.2.2 Chemical composition and physical properties

Bast fibres are comprised primarily of cellulose, hemicellulose, and lignin with minor fractions of pectin and wax. Pectin consists of heteropolysaccharides and provide flexibility to the plant stems. On the other hand, wax coating of the fibres serve to protect the internal structure form water penetration and insects [6]. The physical properties and chemical composition of natural fibres and conventional man-made fibres are presented in Table 2.1 and 2.2.

Table 2.1 Physical and mechanical properties of natural and man-made fibres

Fibre type	Physical properties		Mechanical properties				Refs.
	Density (g/cm^3)	Diameter (µm)	Tensile strength (MPa)	Young's Modulus (GPa)	Elongation at break (%)	Specific modulus	
Hemp	1.48	-	389-900	35	2-4	32-48	[2]
Flax	1.5	-	344-1000	27	1.2-3.2	50	[2]
Jute	1.46	40-350	393-800	55	1.5-1.8	38	[2, 3]
Kenaf	1.45	70-250	930	53	1.6	7-21	[3]
Carbon	1.82	8.2	2550	200	1.3	87	[3]
E-glass	2.55	< 17	3400	72	3.4	28	[2, 3]
S-glass	2.5	-	4600	85	4.6	34	[3]

The physical properties of natural fibres are very important because these properties can influence the eventual applications. Fibre width and length can assist with calculating the aspect ratio (length/width) of the fibre samples. The aspect ratio significantly influences the strength of the composites after reinforcement.

As provided in Table 2.1, natural fibres have comparable specific mechanical modulus with man-made fibres. In other words, natural fibres' property per density is equal or better than glass fibre when compared. This may have implications in weight reduction of key components for the automotive and the airline industries. For the bast fibres considered, it can be noted that the mechanical properties increase as the cellulose content increases in Table 2.2.

Table 2.2 Chemical composition of different fibre type [2, 4]

Fibre type	Chemical composition (%)					Micro fibrilliar angle
	Cellulose	Hemicellulose	Lignin	Pectin	Moisture	
Hemp	70-74	18-22	4-6	0.9	6-12	2-6.2
Flax	64-72	20-24	2	1.8-2.3	8-12	5-10
Jute	61-72	18-22	12-13	0.2	12-14	8
Kenaf	45-57	8-13	21.5	0.6	6-12	2-6

In Table 2.2, the micro-fibrilliar angle is presented for a few of the more popular bast fibres. The angle determines the stiffness of the natural fibres. According to Ahmad *et al.* (2015) [2], the micro-fibrils tend to have a spiral orientation to the fibre axis, explaining the ductility of the fibres. Also, if the micro fibrils are parallel to the fibre axis, the fibres are characterized as rigid and have high tensile strength. As illustrated in this section, natural fibres have properties comparable to man-made fibres and can be used to replace or supplement them in composite applications. Nevertheless, natural fibres are characterized with a few key limitations. These include limited thermal stability, moisture absorption, and poor compatibility with petroleum resins. As a result, the next section of this chapter will highlight the research progress made in the past decade addressing these limitations.

2.3 MODIFICATION OF NATURAL FIBRES FOR COMPOSITE APPLICATIONS

Pretreatment of the fibres can result in clean surfaces and chemical changes that address the concerns mentioned in section 2.2.2. In this section, the main chemical, physical, and biological methods for enhancing natural fibres will be discussed, specifically applied to bast fibres.

2.3.1 Chemical modification

The many hydroxyl groups from cellulose, hemicellulose, and lignin renders natural fibres amenable to chemical changes. Chemical modifications may activate these groups or can introduce new chemical moieties that can lead to better mechanical and chemical interlock with polymeric matrices [5, 10]. In a comprehensive review on pretreatment of natural fibres, Kalia *et al.* (2013) [10], cited that the most popular methods of chemical modification are mercerization, acetylation, silane treatment, and peroxide treatment via various coupling agents. A review of the latest advancements for each method is highlighted in subsections to follow.

2.3.1.1 Mercerization

Zhang *et al.* (2014) [11] utilized an orthogonal experimental design to study the effects of different experimental factors on the physical properties of hemp fibres. They found that a fibre to solution ratio of 1:10, a reaction time of 5 hours, and alkali concentration of 10 g/L were the best experimental conditions. They also were of the opinion that mercerization leads to removal of non-cellulosic components. It was also important to highlight that alkali concentration had a greater effect than treatment time. Alkali concentration significantly affected fibre fineness. Caustic treatment of kenaf fibres has shown to increase tensile properties as a result of increased intermolecular interaction and increased crystallinity of cellulose. Also, mercerization can lead to a reduction in hydroxyl groups after longer treatment times, rendering the fibres better for compatabilization with organic matrices [12]. In a similar study, Hashim *et al.* (2013) [13] investigated the effect of temperature and alkali concentration on kenaf fibre bundles. Interestingly, they found an interaction between the two variables and how this affects the fibre bundles. Also, the fibre bundle diameters reduced by approximately 30.12 % and 42.92 % due to alkali concentration and temperature, respectively. Mercerized jute fibres were used at different fibre ratios to reinforce polypropylene and the effect on mechanical properties reported. It was found, mercerized fibres had better adhesion within the matrices when compared to untreated fibres, and a loading of 20 % fibre was ideal [14]. In summary, mercerization of natural fibres lead to specific non-cellulosic component removal and disruption of hydrogen bonding. Ionization of hydroxyl groups to the alkoxide ion as shown in equation 1.

$$Fibre - OH + NaOH \rightarrow Fibre - O - Na + H_2O + impurities$$

2.3.1.2 Acetylation

Kabir *et al.* (2011) [15] used a number of methods for modifying hemp fibres for sandwich composite structures. These methods included alkalization, silane, and acetylation treatments. They observed that the acetylated fibres were characterized with several cracks and very rough surfaces based on the SEM micrographs. In their opinion, this may be as result of the reaction of the acetyl groups with the hydroxyl groups thereby changing the molecular orientation of the cellulose network. It was also reported that acetylation of the fibres resulted in improved initial degradation temperatures and steady improvement of strength properties for the treated fibres and composite sandwiches, respectively. Bledzki *et al.* (2008) [16] demonstrated that acetylation of flax fibres using acetic anhydride with a perchloric acid (60 %) catalyst, resulted in fibres with superior properties when compared to the untreated fibres. They reported increased moisture resistance of the treated fibres and a decrease in both degree of polymerization and crystallinity as the percentage of acetylation agent increased from 1 to 30 %. They were of the opinion, the deposition of acetylated amorphous components on cellulosic surface accounted for this observation. Finally, the enhanced fibres when used to reinforce polypropylene were characterized with significant increases in strength and flexural properties. These two studies demonstrates the different strategies and feedstocks that have been used for acetylation and the improved properties obtained.

A key observation was reported by Tserki *et al.* (2005) [17] when they found for the first time, that the ratio of lignin and hemicellulose significantly influences the degree of acetylation of any feedstock. In that study, they found that when fibres were acetylated or propionylated, there was removal of non-cellulosic components such as waxes and this in turn changes the surface morphology. Finally, the reaction was confirmed using FTIR. The appearance of a peak about 1735 – 1737 cm^{-1} was due to esterification of the hydroxyl groups, resulting in increased stretching vibration of the carbonyl functionality. In the past, acetylation has been used to produce cellulose acetates from fibres for applications in the paper and textile industry. In one such application, Liu *et al.* (2006) [18] investigated the optimum conditions for preparation of cellulose acetate from ramie fibres using acetic anhydride. Under conditions of 80 °C for 1 hour and at a 1:6 ratio of ramie to acetic anhydride, the researchers were able to prepare a new absorbent material for organic waste in water. In summary, these studies highlighted the success achieved with surface acetylation using a number of reagents and different types of fibre in the past.

2.3.1.3 Silane treatments

Silanes are recognized as efficient coupling agents that can find applications in composites and adhesive applications. To achieve binding between the fibres and polymers, the silane agent should have functional groups as shown below in a generic formula:

$$R_{(4-n)} \text{------} Si \text{------} (R^1X)_n$$

Where n = 1, 2, R is an alkoxy group, X represents an organofunctionality and R^1 is an alkyl bridge that connects the silicon atom and the organofunctionality [19]. In this generic scheme, the organofunctionality interacts with the polymer matrices. Aminosilanes such as y-aminopropyltriethyoxysilane (APS) has been reported mostly extensively in the literature as a coupling agent for natural fibre composites [19]. On the other hand, researchers have also investigated vinyl- and acryl- silanes because they can form covalent bonds between fibres and the matrix. These silanes require the presence of a peroxide initiator [20]. Hajiha *et al.* (2014) [21] studied hemp fibres when impregnated in solutions containing different functionalities such as acetylation, alkalization, and silanation to improve compatibility between the fibres and matrix. In their communication, the hydrolysable alkoxy group of the silane reacts to form silanol, in presence of moisture. The silanol then reacted with the fibre surfaces, forming covalent bonds to the fibre surface. It should be noted, very low concentrations, (1 wt. %) of the silane were used in their experiments. X-ray photoelectron spectroscopy confirmed the presence of Si in hemp fibres, indicating the presence of these residues on the surface of the fibres. Also, silanation significantly improved the percentage degradation of the hemp fibres based on thermal gravimetric analysis done.

As mentioned in section 2.3.1.1, mercerization is one of the most common methods for treating fibres. In 2011, Lu *et al.* [22] reported a comparison between treating jute fibres with caustic and a silanating agent (triethoxyvinylsilane) and how these treatments can affect the thermal properties. It was interesting, in that 24 hours of mercerization and 3 hours of silanation (both solutions of 5 wt. %) were needed to enhance the thermal properties of the hemp fibres. Despite the long hours, mercerization produced better fibres based on physical observation using Scanning electron microscopy. In the end, to utilize chemical methods, the question has to be asked, how long and at what operating parameters can we achieve the best fibres suited for the given applications. Also, one should carefully consider the waste that are produced and what precautions need to be taken when working with these hazardous chemicals.

2.3.2 Physical modification

Physical methods have been used to enhance fibres for composite applications in the past. These methods are on a gradual decline because of the expensive initial investments and poor properties of the fibres after treatment when compared to glass fibre, in terms of compatibility with matrices. In this section, a survey of a couple of the most promising and understood methods of physical modification will be explained. These methods are cold plasma and corona discharge.

2.3.2.1 Plasma treatment of natural fibres

Plasma can be defined as a gaseous environment composed of charged and neutral species with an overall zero charge density. Plasma has been regarded as a clean and dry method for fibre treatments. Plasma can result in primarily two types of surface changes: 1) surface etching as a result of chain scission which is obtained by non-polymerizing gases such as He, O_2, and 2) polymerization or grafting using polymerizing gases such as fluorocarbons or hydrocarbons [10]. Marais *et al.* (2005) [23] utilized cold plasma and autoclave treatment to solve the problems of water permeation and poor mechanical properties of flax reinforced composites. The authors noted, cold plasma modified the surfaces of the flax fibres without affecting the bulk properties and operated under a solvent free system with very short treatment times (5 minutes). The technique involved using helium. The plasma treatment involved placing the flax fibres into a reactor, pumping the final pressure of 10^{-4} Pa and then introducing the gas in a controlled manner. The radicals produced reacted with oxygen from the atmosphere. On the other hand, the autoclave treatment was as done as per normal, conditions of temperature of 130 °C for 30 minutes in an autoclave were used. Cold plasma treated fibres were characterized with radicals on the surface which reacted with the unsaturated polyester chains and this better adhesion led to a reduction of water permeability of the composites and improved mechanical properties. But, autoclave treatment was found to have a more pronounced effect on moisture resistance. In summary, plasma treatment gave better mechanical properties while autoclave treatment resulted in composite with improved moisture resistance.

Plasma treatment has also shown to impart rough surfaces and degradation of fibres due to etching mechanism [24]. Treated jute fibres were characterized with a hydrophobic surface which in turn translated to better fibre and matrix adhesion. Hence, composites made from jute fibre and unsaturated polyester resins exhibited improved tensile and flexural properties. In essence, better fibre adhesion with matrix, as evident from micrographs containing less fibre pull out, resulted in better composite properties.

2.3.2.2 Corona discharge

Corona treatment changes the surface energy of fibres. Corona discharge is an electrical discharge brought on by ionization of a fluid surrounding a conductor that is electrically energized [25]. Ragoubi *et al.* (2009) [26] demonstrated that hemp fibres treated with corona discharge can be used to reinforce polypropylene resins and this resulted in improved tensile properties. They found that treatment of the polypropylene does not significantly affects the mechanical properties. The fibres were treated in a cathode discharge tube (CDT) which consisted of a horizontal cylindrically shaped glass reactor 10 cm in diameter and 30 cm in length. Two planar rectangular electrodes were placed in the center of the reaction chamber. A low voltage generator (15 kV) facilitated corona discharge. In an interesting work, Pizzi *et al.* (2009) [27] produced composites from corona discharge treated non-woven mats of flax and hemp and two natural resin systems. They reported that the treated fibres significantly improved the tensile and flexural properties of the biocomposites. Also, the resins used, commercial mimosa flavonoid tannin extract and a mix of mimosa tannin + hexamine glyoxalated organosolv lignin, aided the compatibility with the natural fibres when compared to petroleum based resins. The studies outlined here presents the usage of corona discharge, a green technique for modification of fibres and the subsequent reinforcement into composites. These composite materials tend to show significant improvements in mechanical properties.

2.3.3 Biological enhancement of natural fibres

The use of biological agents to enhance the properties of natural fibres for composite applications is becoming increasingly popular and understood. Researchers are embracing these technologies because chemical methods tend to produce a large amount of waste and can be hazardous to handle. On the other hand, physical methods require large capital investment. Hence, the idea of an option which can be recyclable, require mild operating parameters, and well-studied can be employed for treating natural fibres. Fungi and enzymatic treatment of fibres has been receiving increasing attention of material scientists of late because of the advantages outlined above [4].

2.3.3.1 Enzymatic treatment

Saleem *et al.* (2008) [28] studied the effect of pectinase treated hemp as a reinforcement for polypropylene composites. Hemp fibres were incubated in 8 % (v/v) SIHA-Panzym® DF in 1 M sodium hydrogen carbonate. Samples were removed from the enzyme broth after 4 hours and rinsed with tap water and air dried. One key finding from this study was that the enzymatic treatment of the fibres resulted in decreased cross sectional area, which resulted in significant decrease of the tensile strength of the fibres. Despite this, the tensile strength and modulus increased significantly for the pectinase treated reinforced composites, regardless if the compatabilizer, maleic anhydride was present. Also, treated fibres led to composites with improved flexural properties. The researchers were of the view that the loosening of the pectic lamellae accounted for the decreased tensile properties of the fibres and the mechanical characteristics of the composites were independent of the compatabilizer maleic anhydride. Finally, there was an improvement in mechanical properties for the composites despite a decrease in tensile behavior of the fibres because refining the fibres using pectinase allowed for larger surface to mass ratio of the fibres resulting in a better interfacial area between fibres and matrix.

During the processing of bast fibres, retting is a very important microbial process which separates industrially useful fibres from the non-fibre tissues. Several methods have been used to ret fibres in the past, including water and dew retting. The problem with the two mentioned methods is the dependence on weather and production of large pools of waste, usually in the fields. As a result, researchers of late have employed enzymatic retting to circumvent these problems. In one such study, Akin *et al.* (2006) [4, 29] investigated the effect of alkaline pectate lyase and ethylene diamine tetra acetic acid (EDTA- calcium chelator) at various formulations on the retting of flax fibres. Pectate lyase at levels of about 2 % of the commercial product for 1 hour at 55 °C followed by treatment with 18 mM EDTA for 23 - 24 hour at 55 °C provided fibres with the best properties. The authors concluded that the enzyme and chelator worked synergistically to produce the best retted flax fibres.

The studies outlined in this section discuss the advances that have been made in enzyme technology for treatment of natural fibres. These fibres may be efficiently retted or prepared for composite applications using enzymes.

2.3.3.2 Fungal and bacterial enhancement of fibres

Li and Pickering (2008) [30] used a combination of alkali and fungal treatments to improve the hemp fibres so as to make them more compatible with resins. They used *Basidomycetes* (white rot fungi) and *Zygomycetes* to treat hemp fibres at 27 °C for 2 weeks. A 10 % NaOH solution was also carried out at a processing temperature of 160 °C and a time of 45 minutes. Their main finding was an improvement in tensile properties of fibre reinforced polypropylene composites. They were of the opinion that mechanical interlocking between the fibre and resin was the major mechanism that facilitated better properties. In fact, fungi in combination with alkali treatment led to extensive de-fibrillation of the hemp fibres, creating more pits as evident from the SEM micrographs allowing for better mechanical interlock.

Similarly, hemp fibres were treated with the fungus *Ophiostoma ulmi* obtained from the elm tree infected with Dutch elm disease [31]. Treated fibres were characterized with reduced moisture absorption and improved acid-base characteristics. Indirectly, improved acid-base properties allowed for better fibre and matrix adhesion, as evident from the improved mechanical properties for hemp reinforced unsaturated polyester resin. The studies outlined here, explore a vastly untapped area of research, the use of fungal and other biological agents for enhancing of natural fibres. The area has many possible benefits, given that most enzymes of interest in this area are obtained from fungi.

2.4 ADVANCES IN THE POLYMER/RESIN RESEARCH

The fibres within a composite carry most of the structural load. The matrix can be thought of doing everything else. The matrices contribute to shape, surface appearance, environmental tolerance, and durability of the composites. Currently, the polymer market is dominated by non-renewable petroleum based polymers such polypropylene, polyethylene, and epoxy [25]. As a result of climate sensitivity, volatility with supply of petroleum, and limited fossil fuel reserves, governments are encouraging the adoption of new technologies that look into renewable and sustainable polymers. Hence, companies such as DuPont and Cargill - Dow have embarked on elaborate and scientifically challenging tasks for producing bio-based polymers that are comparable to their petroleum counterparts on the economic and property scales.

2.4.1 Petroleum based systems

A survey of the literature returned numerous hits for polypropylene (PP), polyethylene (PE), epoxy, and polyester as the most commonly selected petroleum based matrix because of the available literature and easy workability.

2.4.1.1 Thermoplastic systems

2.4.1.1.1 Polyethylene (PE)

Several mechanical properties (deformation, fracture, thermal diffusivity, thermal conductivity, and specific heat) of flax fiber/PE biocomposites were evaluated in a study done by Li *et al.* (2008) [32]. There were improvements in strength and stiffness combined with high toughness by varying the fiber volume fraction and controlling the bonding between layers of the composite. It was noted that the thermal conductivity, thermal diffusivity, and specific heat of the flax/PE composites decreased with increasing fibre content, but the thermal conductivity and thermal diffusivity did not change significantly at temperature range (170 – 200 °C) studied. The specific heat of the biocomposites increased gradually with temperature.

Lu and Oza (2012) [33] investigated the effect of reinforcing virgin (vHD/PE) and recycled high density PE (RHD/PE) matrix with hemp fibres. In that study, to improve the adhesion with the matrix, the fibres were treated with 5 % (wt.) NaOH. The authors reported that the recycled PE composites were characterized with better mechanical and thermomechanical properties when compared to the virgin PE (at 40 % hemp reinforcement) composites. The main reasons for this difference may be because of difference in density (0.98 g/cm^3 for rHD/PE and 0.95 g/cm^3 for vHD/PE), which suggested that the rHD/PE had less branching, therefore having stronger intermolecular forces and tensile strength. Secondly, during recycling, additive compounds such as polyalkylene imine could improve the compatibility of the hemp fibre with the rHD/PE.

Researchers have also used low density polyethylene (LD/PE) for composite applications using fibres as the reinforcing agents. One interesting study was done by Arrakhiz *et al.* (2012) [34], where they investigated the effect of reinforcing LD/PE using doum-fibres. The authors reported an improvement of 145 % in tensile strength of the composite when compared to neat LD/PE. The high percentage of fibre in the composites (30 and 40 %) led to decreased thermal stability of the composite materials as the fibre percentage increased. A co-rotating twin screw extruder was utilized for producing the composite materials after mixing the fibres with the polymer.

2.4.1.1.2 Polypropylene (PP)

Polypropylene is one of the more established resins for composite materials. Malkapuram *et al.* (2008) [35] comprehensively reviewed the use of natural fibre PP composites prior to 2007. They concluded based on their assessment, that flax and jute are the most important bast fibres for reinforcing PP. Hemp fibres enhanced by grafting glycidyl methacrylate (GMA) were used to reinforce isotactic PP [36]. Fibre-matrix interactions were increased with the use of the compatabilizers resulting in improved tensile and thermal properties. Also, higher ratios of fibres led to better stiffness of the composites as a result of better interfacial adhesions and higher reinforcing content. Arbelaiz *et al.* (2006) [37] investigated the crystallization of flax and PP composites. Kinetic parameters were calculated using the established Kissinger method. Flax fibres were mercerized and then used to produce the composites. Fibres (30 % wt.) were compounded with PP and pelletized and kept in a vacuum oven until injection molding. The main motivation for this study was to determine how the addition of filler (flax) affected the crystallization of the matrix because the mechanical properties are dependent on the crystalline structure of the composite materials. It was found that the addition of flax fibres increased the crystallization rate, and this depended on the crystallization temperature and degree of modification of flax fibres. The studies outlined the versatility of using PE and PP as matrix in bio composites. Additionally, thermoplastics offers the avenue of recyclability after product end life.

2.4.1.2 Thermoset systems

2.4.1.2.1 Epoxy systems

Epoxy resins are a class of reactive prepolymers and polymers which contain epoxide groups. These polymers can either crosslink with themselves through catalytic polymerization or with a range of co-reactants such as hardeners or curing agents. In this section, a few studies highlighting the use of epoxy as a matrix in the natural fibre composite research will be documented.

Jute fibres treated with caustic in combination with organosilane coupling agents was used to reinforce epoxy resin [38]. The treated fibres exhibited higher contact angles, indicating a decrease in hydrophilicity of the fibres. To this end, treated fibres were characterized with increased interfacial bonding with the epoxy resin, resulting in an increase in apparent interfacial shear strength. It should be noted, interface characterization using the single fibre pull-out test was used to determine the shear strength. Also, the authors reported shear strength of the epoxy composites to be a factor of 4 times higher than when PP was used a matrix. This can plausibly be as a result of covalent bond formation, specifically epoxy ring opening at the interface.

The reinforcement of epoxy with mercerized kenaf fibres resulted in increased flexural strength as reported by Yousif *et al.* (2012) [39]. The increase in adhesion force between the matrix and fillers as a result of fibre treatment was the main contributory factor for the improved properties. In effect, better adhesion results in less debonding, detachment, or pull out of fibres. In fact, treated fibres exhibited only breakage at the end of the fibre upon failure when used to reinforce the epoxy resin. The authors were very optimistic as a result of their findings, claiming that kenaf fibres exhibited superior properties as a reinforcement for epoxy when compared to other natural fibres, especially when compared using the flexural loading.

Mekonnen *et al.* (2013) [40] investigated the thermosetting properties of specified risk material obtained from the rendering industry. SRM was hydrolyzed and the fragments obtained were cross linked using chemical methods to produce a polymer mimicking the properties of epoxy. In fact, the hydrolyzed protein was used as a curing agent for epoxy resin. Protein fractions of 20, 30, 40, and 50 % were used to supplement the epoxy resins. There were significant improvements in water resistance and mechanical properties of the studied systems. It was found that 20 % protein and epoxy had the highest mechanical strength, solvent, and water resistance. This work demonstrated that otherwise waste materials can be used to produce polymers or plastics (depending on the applications) and have comparable or better properties than their petroleum based counterparts.

2.4.1.2.2 Polyester systems

Polyester resin systems have been used extensively in the past. Polyester is a category of polymers that contain the ester functional groups in their main chain. Baley *et al.* (2006) [41] reported a study where chemical treatment of flax fibres improved the adhesion with unsaturated polyester. In fact, they noticed an increase in adhesion between the polymer and the fibres for both the acetic anhydride and formic acid treatments. They measured the adhesive forces via wetting and thermodynamic work of adhesion as outlined by the Young- Dupré equation. The authors choose to rely on this compromise instead of manufacturing composite materials because a strong correlation was established by Nardin and Schultz (1996) [42], having investigated a number of polymer-fibre pairs and the work of adhesion. Hence, the work of adhesion can be used as a litmus test to determine whether treatments are effective, instead of investing in production of composites, just to realize the adhesion was poor.

A novel chemical grafting method was used to enhance hemp fibre for reinforcing unsaturated polyester [43]. Hemp fibres were treated with N-methyl acrylamide (NMA) with sulfuric acid as a catalyst. Compression molding was used to produce composites from the polyester and the enhanced fibres. The composites were characterized with increased tensile strength, flexural modulus of rupture, and flexural modulus of elasticity, and improved water resistance. FTIR confirmed covalent bonding between NMA and the surfaces of the hemp fibres. Bodros *et al.* (2006) [44] evaluated the properties of flax fibre reinforced polyesters. They studied polylactide acid (PLA), L-polylactide acid (PLLA), polyhydroxybutyrate (PHB), polycaprolactone, and polybutylene succinate (PBS) systems. The main aim of this study was to determine whether bio-based materials are possible replacements for glass fibre reinforced materials in structural applications. The authors used film stacking to produce the composite materials needed for testing. They reported increases in tensile properties with increased fibre volume loading. It was reported that the flax reinforced PLA and PLLA had higher tensile strength and Young's modulus than the established flax/PP counterpart.

A relative new area of study has been the mixing of natural fibre – glass fibre for reinforcement of different polymer matrices. Atiqah *et al.* (2013) [45] studied the effect of using kenaf-glass (KG) fibres to reinforce polyester matrices. Kenaf fibres were initially treated with 6 % sodium hydroxide and then pressed into a mat. Kenaf (7.5 % wt.) and glass fibres (22.5 % wt.) were used for all composite material. Two thick metal plates were used for sheet molding of the material. Three layers of glass and kenaf fibres were used in each composite material. Fibre treatment had a significant effect on the flexural strength, tensile strength, tensile modulus, and impact strength. The mercerization process enhanced the adhesion between the surface of fibre and the matrix which plays an important role in improving the mechanical properties of the KG-UPE hybrid composites. Composites made from kenaf fibres alone had significantly lower impact strength. The authors were of the opinion that the developed KG-UPE hybrid composite material suitable for structural applications.

The few studies outlined in this review just touches the surface of the potential of using polyester matrices, either petroleum or renewable based, for composite applications. Nevertheless, a picture of the wide range of usage as well applications were highlighted.

In summary, the selection of an appropriate matrix for composite applications depends on several factors. The end application will determine whether we can recycle the composites, making thermoplastics a better option. Also, the use of different types of fibre results in different composite properties. Depending on the best criteria, we may be able to select the best filler and the corresponding matrix for that scenario.

2.4.2 Emergence of bio based polymers

It was forecasted by Babu *et al.* (2013) [46] that by 2015, bio based polymers would account for approximately 1 % of polymers worldwide. Based on numbers published in 2014 by the U.S. Department of Energy, the globalization of technologies should make this number a realization. First generation bio-based polymers are derived from agricultural feedstocks such as corn and potatoes. However, the food debate resulted in a shift in recent times to bio-based polymers via biotechnology pathways. Researchers have studied the conventional petroleum based pathways and tried to mimic these systems using fermentations to produce building blocks from renewable resources not limited to food based feedstocks. Lignocellulosic material, fatty acids, and organic waste have been used as starting materials for these applications [46]. There are three main pathways for the production of bio based polymers. The first stream uses natural bio-based polymers such as starch with modification. The other two paths, will be of more focus in this review. Fermentation or the use of chemistry followed by polymerization to produce polymers such as polylactic acid and polyethylene will be focused on. Thirdly, the production of polymers such as polyhydroxyalkanoates via direct bacterial synthesis will be highlighted. These different pathways are summarized in Figure 2.4. Given the limited space and the essence of the message, the production [46] and properties [47] of these polymers will not be discussed, rather the applications of using as matrices for natural fibres will be touched on. A summary of the different technology platform for the production of bio-based polymers is highlighted in Figure 2.4.

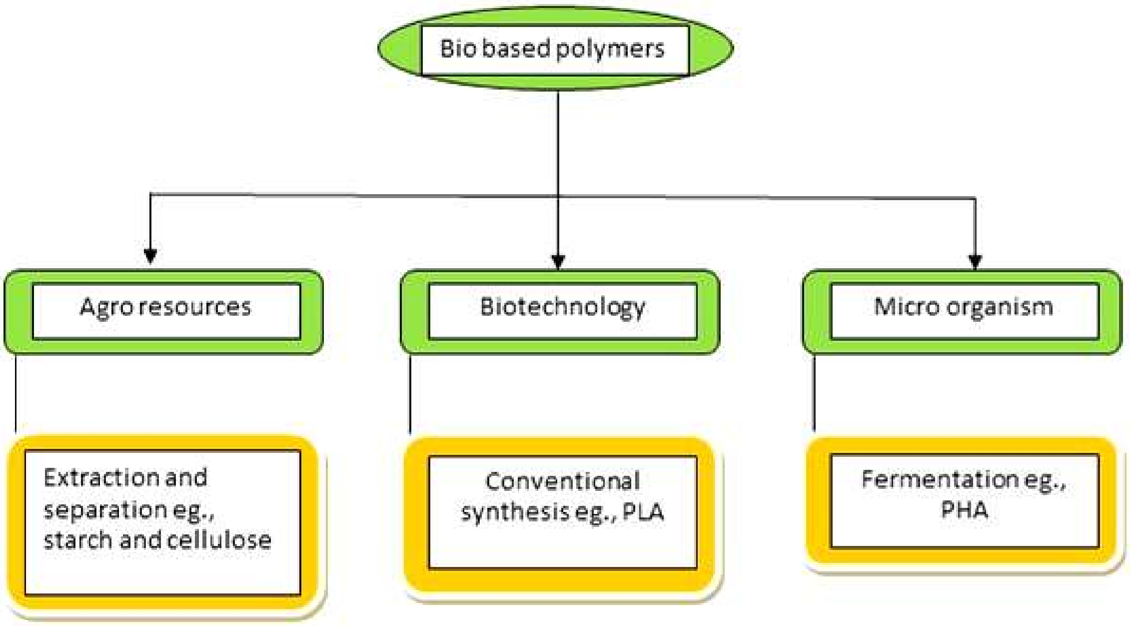

Figure 2.4 Production pathways for bio based polymers and the most popular examples.

2.4.2.1 Polylactic acid (PLA)

PLA was commercialized in early 1990. PLA belongs to the aliphatic polyester family with the basic monomer being lactic acid. PLA can either be synthesized by direct poly-condensation reaction or ring-opening polymerization of the lactide monomer. NatureWorks LLC has developed a low-cost continuous process for the production of PLA via a low molecular prepolymer lactide dimers condensation [48]. PLA has properties such as good transparency, glossy appearance, high rigidity, and applicable to a variety of processing equipment. It should be noted, PLA behaves as a thermoplastic polymer which has great potential to replace conventional polymers such as polystyrene and polyethylene terephthalate (PET) [49].

Taib *et al.* (2014) [50] investigated the mechanical and morphological properties of kenaf reinforced PLA composites. In that study, the authors tried another path for improving the impact performance of the composite materials. Instead of optimizing the fibre-matrix interface or filler property, they increased the matrix toughness by adding an impact modifier. The PLA used in that study was sourced from NatureWorks in pellet form, with a glass transition temperature of 63 °C and melting temperature of 160 °C. Ethylene acrylate copolymer was used as the impact modifier (IM). They reported formation of a ductile and flexible interface between the fibre and PLA. Also, there were improvements in the impact strength and elongation at break, but a decrease in tensile strength. The decrease in tensile strength can be as a result of the rubbery behavior of the IM and its low mechanical properties, hence reducing the crystallinity of the PLA used. The thermal behavior of PLA and kenaf composites was investigated [51]. Triacetin and glycerol were used as plasticizing agents. Composites were made from mercerized kenaf fibres at 30 %. The use of the compatabilizers improved the thermal properties of the biodegradable composite materials.

In a very different model study, Jia *et al.* (2014) [52] developed fully biodegradable PLA fibre reinforced PLA and poly (butylene succinate) (PBS) matrix composites. PBS is a biodegradable thermoplastic polymer produced from condensation of glycol and succinic acid obtained from renewable resources [53]. The composite material produced in that study are termed self-reinforced composites (SRC). The use of PLA as both the filler and matrix, gives rise to this category of bio composite material. It should be noted, for these types of composites to be produced, there needs to be significant difference in melting points to allow for sufficient melting of the polymer and to maintain the mechanical properties of oriented fibre. As a result, highly oriented PLA fibres (semi-crystalline) was used as the reinforcement while amorphous PLA film as the matrix. PBS, which has lower melting temperature, was also employed as the matrix of the PLA fibre reinforced PBS composite to allow for comparison with the PLA self-reinforced composite. The main finding reported was significantly better mechanical properties for the PLA-SRC than in PLA-PBS composite. The author believed the better interfacial adhesion between the semi-crystalline PLA and amorphous PLA was the main contributory factor. The studies elaborated on in this section highlighted the versatility of using PLA as a starting material for totally biodegradable composite materials.

2.4.2.2 Polyhydroxyalkanoates (PHA)

PHAs are composed primarily of 3-hydroxy fatty acid monomers which form polyester. These monomers are synthesized by both gram-positive and gram-negative bacteria without any serious hazardous effects on the host organism [54]. PHA accumulations occurs when the cells of the host organism experience a nutrient imbalance such as excess carbon with limited nitrogen or oxygen [54]. PHAs extracted from bacterial cells have properties mirroring those of conventional plastics especially thermoplastics. An advantage, depending on the application, is that PHA can be degraded within 6-9 months by numerous microbes producing carbon dioxide and water [55].

Gunning *et al.* (2013) [56] evaluated polyhydroxybutyrate (PHB) based natural fibres composites. The interest was to determine whether the composite comprising of hemp and jute fibres would have comparable properties to natural fibre or glass fibre reinforced petroleum based resins. A twin screw extruder was used to produce the pellets, which were then injection molded into tensile samples for testing. The researchers tried to replicate the processing conditions similar to those employed when polypropylene was used as a resin. The adhesion between natural fibres and PHB is very poor [58] and the authors reported significant decrease in tensile properties for all fibre types. On the other hand, jute and hemp reinforced PHB exhibited significant increase in flexural modulus. The main reason for this plausibly could have been the high level of hydrogen bonding between the fibres and the polymer. Also, as the percentage of fibres increased, the biodegradability of the composites increased exponentially.

Bacterial poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) was reinforced with acetylated flax fibres [58]. The bacterial copolyester had an average 3-hydroxyhexanoate content of 12 % while flax fibres were treated using acetic anhydride using sulfuric acid as a catalyst. Composites were prepared using two different processes namely, 1) compression molding and 2) suspending fibre mats in water prior to compounding into a sandwich (three polymer layers and two fibre mats). Surface esterification of the fibres resulted in better tensile strength when compared to the untreated fibres when used to reinforce the polymer. Another key finding was the acceleration of crystallization kinetics in the presence of the fibres, hence reducing processing time.

The emergence of worldwide concerns surrounding the environment and economics has resulted in expansion of research areas in the composite area. As a result, researchers have looked for new materials that are both renewable and sustainable. The mimicking of conventional technology platforms to produce bio based polymers that are renewable and biodegradable (interest depending on application) has been of great interest. A few of the most promising polymers include PLA and PHA and in the future the market will be determined by which technologies are competitive and green.

2.4.3 Petroleum vs renewable based polymers reinforced natural fibre composites

As mentioned before, the use of bio based polymers is a very attractive idea, but in order to be deployed for large scale production, these polymers need to be competitive with established petroleum based polymers. Biocomposites should exhibit properties that are comparable and at the same time be economically competitive. In this section, a few studies will be detailed where bio-based polymers were compared with the petroleum based matrices, in terms of properties and applications. Bajpai *et al.* (2012) [59] compared the performance of natural fibre reinforced PLA and PP composites. The polymers were reinforced with a number of fibres, including sisal fibres. PLA composites were prepared by hot pressing through a film stacking procedure. Four layers of woven fibres were incorporated in each of the composite fabricated. Natural fibre reinforced PLA exhibited better tensile strength and moduli than the PP counterparts. The authors were of the opinion that the PLA and fibres had a better interfacial adhesion leading to improved tensile properties. This was evident from the SEM micrographs they presented, showing clearly that the main mechanism of failure was clean fractured surfaces. In fact, the fibres were encapsulated in the PLA, thereby resisting the applied forces. As a result, this report proved that using untreated fibres, PLA can be used in applications where PP is predominant and have very comparable or even better properties. Table 2.3 summarizes the key mechanical properties of fiber reinforced PLA when compared to those corresponding fibre reinforced PP and PE.

Table 2.3 Influence of fibre type and matrix type on the mechanical properties of composites

Fibre type	Mechanical properties- Tensile strength (MPa)			Reference
	PLA	PP	PE	
Hemp	30 ± 0.8	29.0 ± 1.2	24.8 ± 0.2	33, 92, 95
Flax	40-45	71 ± 0.9	60 ± 0.6	37, 93, 96
Jute	73	142	27.2 ± 0.8	60, 61
Kenaf	130	23	52	94, 97

It should noted, although the idea was to try and represent a variety of different systems, because processing and fibre volume affects the tensile properties, a few benchmarks were kept constant. Firstly, studies where fibre volumes of 20-30 % were used are presented. Also, compression and injection molding were the main techniques used for manufacturing the composites. As can be seen, fibre reinforced PLA can be competitive with petroleum based fibre reinforced thermoplastics.

From Table 2.3, it can be observed that jute reinforced PLA and PP outperformed the other systems [60]. In this particular study, the researchers were not just interested in comparing PLA vs PP composites, but also investigated the effect of a novel processing technique. Specifically, the authors investigated the effect of long-fibre reinforced unidirectional composites. A tubular braiding technique was used to produce microbraid yarn from jute fibres. In essence, they carefully aligned the fibres in one line using a metallic frame. By doing this, the fibres were parallel and uniform. The microbraid yarns were then compression molded to fabricate the specimens. Improved mechanical properties were reported for microbraid yarn reinforced PLA and PP composites. According to the authors, improvements in the mechanical properties are related to various factors including the wettability of resin, interfacial adhesion, orientation, and uniform distribution of matrix-fibres and the lack of fibre attrition and attenuation during tubular braiding process.

Another key observation from Table 2.3 is the superior tensile properties for kenaf reinforced polymers. The main contributory factor to high tensile strength is the adhesion or the interface between the two components [61]. Kenaf fibres are unique because of the low cellulosic and relatively high lignin content when compared to the other fibre types discussed in this chapter. As a result, the surface of kenaf fibres would be more suited for adhering to the non-polar matrix because of the phenyl propane residues of lignin.

In summary, bio-based polymers can be competitive or even better property wise than the petroleum based counterparts. The key determinant would be to reduce the cost of manufacture and the deployment of these composite materials would be a reality. In the following sections, a few key processing parameters will be reviewed and the competitiveness of bio-based materials will be assessed as reported by Life cycle analysis and Techno economic analyses.

2.5 PROCESSING TECHNIQUES FOR NATURAL FIBRE COMPOSITES

2.5.1 Factors to consider before processing

A few very important parameters should be considered prior to processing of natural fibres into composites. These factors should include moisture content, fibre type, and the fibre content required. The moisture content has been shown to significantly affect the properties of natural fibre composites if not carefully monitored. Faruk *et al.* (2012) [25] highlighted this in their review. Also, if fibres are stored prior to processing, high moisture contents can facilitate biological degradation. Moisture absorption is directly related to the humidity and accumulates with humidity. Chemical treatments previously highlighted have shown to reduce the water absorption of the fibres via either coating the hydrophilic surfaces or removal of specific moisture attractive components [1].

The fibre content and type have been shown to significantly affect the processing of natural fibres [25]. Specifically, fibre length, aspect ratio (length / diameter of fibres), and chemical composition can all influence the processing. For higher fibre content, compounding with a thermoplastic becomes a bit more tricky and cumbersome. Hu and Lim (2007) [62] reinforced PLA with different fibre volumes of hemp (30, 40, and 50 %). They reported best performance with a fibre loading of 40 %. Also, compounding leads to thermal deterioration of the fibre samples, depending on the components. Lignin has the best thermal stability, followed by cellulose and hemicellulose. As a result, fibres high in lignin, such as kenaf, would exhibit better thermal properties during processing.

Higher fibre content improves the impact strength. Unfortunately, increased fibre content increases the composite's odor and water uptake. Also the composite's ductility can be adversely affected. Usually, most mechanical properties of a fibre can be enhanced by increasing the aspect ratio [25]. In summary, careful thought needs to be placed into determining which fibre type, the fibre loading, and moisture content is best for processing into composites. In the next section, a few of the more popular processing techniques are summarized.

2.5.2 Processing techniques for natural fibre reinforced thermoplastics

The most common methods for producing composites using thermoplastics will be highlighted in this section. The techniques for the manufacture of natural fibre reinforced polymer matrix composites have been looked at previously by Summerscale *et al.* (2010) [7] and Ku *et al.* (2011) [63]. The selection of a suitable process to fabricate composites, have resulted in engineers focusing mainly on a few key criteria. These factors would include the desired properties, size, and shape of the resulting composites. Also important are the manufacturing cost and production speed. The size of required composite would be dictated by the manufacturing process used. For example, injection and compression molding are preferred for small sized composites, while for larger pieces, open molding and autoclave processes are most applicable. The mixing of a hydrophobic (thermoplastic) and hydrophilic (fibres) material leads to aggregates within the composites and this creates the possibility of flaws within the composite materials [64]. As a result, researchers have constantly tried to improve on the adhesion between the two components, as outlined in previous sections using a number of different modification methods.

2.5.2.1 Extrusion

Extrusion can be used to create objects with fixed cross-sectional profiles. This is achieved by pushing or pulling a material through a die with a given cross-section. Oksman *et al.* (2009) [65] studied the influence of fibre microstructure on the mechanical properties of extruded composite materials. They used jute and enzyme treated flax fibres to reinforce PP. The composite materials were manufactured using a long fibre thermoplastic (LFT) processing method whereby rovings are continuously fed into an extruder. A twin screw extruder was utilized to compound the samples and PP plastic. A temperature profile of 180-200 °C between the feeding zone and the die was maintained to ensure complete and homogenous mixing. They observed increases in flexural stiffness with increased fibre loading. Flax composites were characterized with the highest flexural stiffness. It was noted that the use of 2 % maleated PP significantly improved the composite properties, because of the improvement in mixing as a result of better compatibility.

Ma *et al.* (2005) [66] investigated natural fibre reinforced thermoplastic starch composites. Corn starch and fibres were mixed and stored overnight and then continuously fed into a single screw Plastic Extruder SJ025 operating at 20 rpm. The temperature profile of the extruder barrel was maintained with heating zones of 120, 130, 130, and 100 °C from the feeder to the die. There was good mixing of the fibres and resin as evident from SEM micrographs they presented and increased tensile strength with increasing fibre loading. Conversely, there was a constant decrease in elongation and energy break with increasing fibre content. These observations were influenced by the water content because water plausibly separately formed hydrogen bonds with the starch and fibre, hence substituting the original fibre-starch interaction. It was observed that at high water contents, the fibre contents had no effect on the tensile strength.

The studies outlined here expounded on the importance of direct extrusion. The process is simple and can be used specifically for renewable polymers such as starch and PLA. The next processing types, are more adopted for medium scale products.

2.5.2.2 Injection molding

Injection molding can be thought of as a process which forces a measured quantity of mixture (molten polymer and fibre) into a mold cavity. Pellets (made from extrusion) are fed individually into a hopper and then into a heated compression barrel consisting of a rotating screw. The barrel is heated to facilitate transformation of the pellets into a viscous liquid which can be injected into the mold cavity. The composite is then cooled and subsequently removed. Some of the main functions of the screw includes generating heat by viscous shearing to melt the polymer, applying the shear force to mix the polymer and fibre, and acting as a piston to force the mixture of fibres and molten polymer through nozzle into a matched-metal closed mold. It should be noted, fibres tend to align based on the applied shearing force and as a result, there are layers that form during the process. The composite aligned with the wall of the molds are characterized with uniformly aligned and known as the skin [67].

Not many studies have been reported using this technique to produce composite materials from renewable polymers and bast fibres. Serizawa *et al*. (2006) [68] investigated the reinforcing PLA with kenaf fibres using injection molding to produce electronic parts. A twin-screw extruder was used to combine the PLA and kenaf fibres with varying volume of fibres (5-30 %) at 184 °C. Test samples of the required dimensions were obtained via injection molding, with injection temperature of 180 °C and a mold temperature of 25 °C. The key results included an increase in heat resistance and tensile modulus as the volume of fibre increased. In fact, the crystallization rate of PLA was improved with the addition of kenaf fibres, thereby improving the mechanical properties (crystalline PLA is stronger than amorphous PLA). Carus and Gahle (2008) [69] reported the possibility of injection molding using natural fibres to produce reinforced plastics that they believe will have a niche market in the future. They reported that the high market growth driven by an increased awareness of the environmental concerns and the multitude of biomaterials, will propel this specific technology in the future. They forecasted two digit growth of injection molded produced plastics in the coming years.

An interesting turn of research has been the use of hybrid reinforcement agents. In essence, researchers are interested in using glass fibres in conjunction with natural fibres to reinforce polymers. The main motivation for this move has been the excellent properties exhibited by glass fibre composites when compared to the natural fibre composites. As a result, combining the two would add a green index to the process while conserving on resources that can be used in other processes.

Panthapulakkal and Sain (2006) [70] investigated using short hemp fibres with glass fibre to reinforce PP. A Brabender Plasticorder was used to melt blend the fibres and polymers (170 °C and 60 rpm for 5 min). The cooled mixed samples were then granulated, before being injection molded (205 °C). Glass fibre (wt. %) was varied from 0-15 and hemp fibre (wt. %) was varied from 25-40, with the aim of always maintaining 40 % reinforcing agent in the composite. The results indicated that hybridization with glass fibres produce composites characterized with enhanced performance. Also, there were significant improvements in thermal and water resistance of the hybrid composites.

The studies outlined explained the versatility of using injection molding. The technique can be readily used with renewable polymers such as PLA or petroleum based resins such PP. The composite material formed can be crafted into a specific shape, depending on the application of the materials.

2.5.2.3 Compression molding

Compression molding is a combination of hot press and autoclave processes. Hot press consists of stacking mats together inside a preheated mold cavity while autoclave process can be thought of laying up thermoplastic prepregs in a specific sequence. A biodegradable composite material was produced from hemp fibres and PLA polymer [71]. Hemp fibres were mercerized, then used at different volumes to reinforce the PLA polymer. The fibre and polymer were stacked layer by layer (tried to ensure uniform weight per layer), then the mold with the layers was placed onto a heating panel of a hot press machine. The temperature was set at 170 °C and a pressure of 1.3 MPa was applied and maintained for 10 min. The mold was removed from the hot press and the pressure maintained. Results indicated that fibre treatment was important, because removal of non-cellulosic material resulted in better adhesion with polymer. Composites were characterized with improved mechanical properties when compared to the virgin polymer.

Adekunle and Akesson (2010) [72] studied the properties of a novel soybean oil thermoset polymer and flax fibre composite. Compression molding was used to produce samples for testing. Fibre mats were initially treated with NaOH solution for 1 hour and then used to prepare the composite materials. Hand layup was used to produce laminates with eight sheets of fibre mat. Compression molding was done at 170 °C for 5 min at 40 bar on a hot press. The composite materials were characterized with impact properties similar to glass fibre mats. This was attributed to the good adhesion between the flax fibres and the renewable polymer. There were corresponding improvements in flexural properties as well.

Jute fibres were used to reinforce potato, sweet potato, and corn starch polymers [73]. A thermo regulated compression molding press was used to prepare all samples after the starch powders were mixed with varying amounts of plasticizers and fibres (5-12.5 %). Temperatures of 130, 150, and 175 °C were used with processing times varying between 25 and 45 min. Composites produced using a processing time and temperature of 45 min and 175 °C, respectively gave the best ultimate tensile strength. There was approximately 100 % increase in tensile strength when the virgin polymer was reinforced with the fibres. Glycerol and ethylene glycol were the best plasticizers. These studies outlined the importance of compression molding as researchers are moving from petroleum based to renewable polymers.

2.5.3 Processing techniques for natural fibre reinforced thermosets

2.5.3.1 Resin transfer molding (RTM)

RTM can be used to produce component parts made from fibres and polymers. RTM can be used to mold large surface areas and complex shapes. The chemical reaction of the resins are thermally activated from the fibre mat or preform, and the mold wall. Hemp fibres and an unsaturated polyester resin were used to produce composite materials via RTM [74]. The aluminum mold was closed manually by screws distributed around the cavity. The inlet ports were located at the bottom of the mold, at opposite sides. Hot water was circulated at the bottom of the mold to maintain the required temperature. Fibre mats having dimensions of the mold were placed in the cavity and heated to 55 °C. The resin was then injected under pressure. The composite was cured for a hour. It was found that the mechanical properties of the composites increase significantly when the fibre content was increased. Laminates were produced from kenaf and polyester by optimizing the moisture content, mold temperature, and mold pressure [75]. In the lab built resin transfer molder, resin and catalyst were injected separately by two pistons connected to an arm. It was found that varying the molding parameters had little effect on the properties of the composites produced. Tensile and flexural strength increased with low fibre volume fractions. Water resistance also improved with less fibre in the composite.

Rassman *et al.* 2010 [75] investigated the effect of processing conditions on the mechanical and water absorption of resin transfer molded kenaf polyester laminates. Rectangular fibre mats were placed lengthwise, aligned parallel to the mold, at fibre volumes of 15, 22.5, and 30 %. Resin was injected from a single injection port located at the lower part of the mold. Pressure of 250 kPA was used to compress the 15, 22.5, and 35 % fibre composites. All composites were post cured for 3 hour at 80 °C. Polyester reinforced with 30 % had higher tensile and flexural strength. Not surprising, the higher fraction fibre reinforced polymer led to more water absorption in the composite materials. Also, water absorption caused all three dimensions of the laminates to increase. However, the increase in length and width was small comparable to the increase in thickness. Resin transfer molding can be used for large composite parts, where a fixed or defined mold is available.

2.5.3.2 Pultrusion

Pultrusion can be thought of as a continuous process for producing composite materials. Reinforcing fibres are saturated with the liquid resin, pulled through a heated die to form a part of specific shape and dimensions. Angelov *et al.* (2006) [76] were one of the first to pultrude bast fibres into a fixed profile. They varied the preheating and die temperatures while pulling flax fibres and PP yarns. After passing through the preheating zone (approximately 600 mm at temperatures of 155-166 °C), the profile was pulled through the die. The temperature of the hot die was tested at 200 and 210 °C while the cold die (after the hot die) was maintained at room temperature. The pulling speed was varied from 8-38 cm/min. The conditions which gave the best tensile strength (comparable to samples compression molded) are given in Table 2.4.

Table 2.4 Influence of processing parameters on the main mechanical properties of flax and PP composites

Mechanical Test	Value	Processing parameter			
		Preheating temp. (°C)	**Hot die temp. (°C)**	**Pulling speed (cm/min)**	**Cool die temp. (°C)**
Flexural modulus (GPa)	7.1-7.9	160	230	24	25
Flexural strength (MPa)	82-95	160	230	24	25
Impact strength (kJ/m^2)	80-10	166	240	36	25

Values adopted from Angelov *et al.* (2006) [76]

Based on the values reported in Table 2.4, it can be appreciated that depending on the application of the composite piece, researchers can produce varying specimens depending on whether tensile or impact properties are important.

As often mentioned, water absorption is one of key barriers for natural fibre composites. The effect of pultruding jute fibres with unsaturated polyester on the water absorption of composite materials were reported [77]. The researchers submerged samples in distilled, sea, and acidic water at room temperature for up to 3 weeks. Jute fibres (roving) were submerged in a resin bath. The roving guider was used to ensure fibres were not accumulating and result in weak points in the composites. Fibres were impregnated with the resin in a tank, which was then pulled through a steel die to obtain the desired shape. The authors reported the composites absorbed water according to a pseudo-Fickian profile, hence the water absorption can be predicted at any time. As expected, the composites material were characterized with poor mechanical properties after soaking when compared to the samples before soaking.

Singh and Gupta (2005) [78] studied the performance of jute fibre reinforced phenolic resins (polyurethane) as a possible building material for door frames. The jute fibre mats were impregnated with the resin and passed through a hot die at an approximate speed of 0.4-1 m/min. Within the die, the phenolic resin cured to produce the defined shape of the composite. Jute profiles were subjected to 43, 62, 83, and 95 % relative humidity and immersion in distilled water for 60 days. Composites exposed to 83, 62, and 43 % relative humidity had less than 5 % water absorption over a 60 day period. On the other hand, samples immersed in distilled water and exposed to 95 % relative humidity had absorptions greater than 10 %. For all samples, except the immersed composite samples, there seem to be saturation after 20 days of exposure. The exposed samples were characterized with poor tensile strength. Nevertheless, the authors were of the opinion that the composites exhibited good properties and can replace the wooden door frames currently in use.

In this section, the versatility of pultrusion was demonstrated. The technique can be used to craft composites of different shapes and size. Also, there is little limitation with the types of thermosets it can accommodate. In general, pultrusion and resin transfer molding are two of the more common processes used to set composite materials.

In summary, the type of processing technique adopted for a given system would depend on the size, shape, and polymer to be used in the composite. Although, thermoplastics offers recyclability, thermosets tend to produce composites with better impact properties. Hence, depending on the application, the right fibres and polymers can be selected to give the desired composite material.

2.6 ASSESSMENT OF NATURAL FIBRES COMPOSITES

Bio composites produced from natural fibres (NF) and polymers need to be competitive with glass fibres reinforced counterparts. Also, composites made entirely of biodegradable material such as PHA and natural fibres need to be comparable to those made from established petroleum resins. In this section, studies that highlight the key aspects of these different composites will be detailed.

2.6.1 Life cycle assessments (LCA) of biocomposites

LCA can be used to weigh the environmental impacts of producing a specific material for a given application. Researchers in the field of Materials Science have recently showed an interest in determining the environmental burden of their different methods for fibre enhancement and the possibly of using these fibres for polymer reinforcement. The results obtained can be used to optimize the environmental performance of a single product via identifying the key factors contributing to the whole. Categories reported under LCA include climate change, global warming, acidification, ozone depletion, eutrophication, among other factors. Typically, a life cycle analysis consists of four key phases. Firstly, definition of the goal and scope of the research should be done. Included in this section should be the functional unit (the object of assessment) and the aim of the study. Life cycle inventory, is seen as the most critical step of the process. At this stage, researchers have to list and organize the pollutant emissions and consumption of resources. The next phase is the actual impact assessment which classifies and evaluates the environmental impact of the pollutants emitted during the process. Lastly, life cycle interpretation allows the investigator to analyze and interpret results and estimate the uncertainties of the process [79]. In the end, a complete LCA can be used to study a specific product/process and determine whether it is feasible as a product/process.

The main aim of this review is to focus on natural fibre reinforced polymers (petroleum and bio based), hence a close study at environmental impacts of producing these fibres would be advantageous. Le Duigou *et al.* (2011) [80] investigated the feasibility of producing flax fibres for composite applications. Their main findings included favorable environmental indicators such as climate change, acidification, and non-renewable energy consumption for flax production. On the other hand, eutrophication due to the use and production of fertilizers has been the biggest challenge for the process. It was recommended that fibres that require less fertilizers or more resistant to local condition can be grown to counter this limitation.

La Rosa *et al.* (2014) [81] conducted comparative LCA for a bio based versus petroleum polymer composites. An eco-sandwich made from natural fibres and bio based epoxy resin was compared against a traditional sandwich made from epoxy and glass fibre. It should be noted, this study was a cradle to manufacture, hence environmental impacts that resulted from production of hemp, bio based epoxy, petroleum based epoxy, and glass fibre were considered. The functional unit used for this study was an eco-sandwich panel (0.4 × 0.4 × 0.02 m). Waste scenarios were discussed. Life cycle assessments of the bio-based polymers showed favorable results in terms of environmental impacts and energy use compared to petroleum-based products. When comparing the environmental impact of producing 1 kg of hemp and glass fibres, the impact categories were higher for glass fibre production (exception being land occupation because of the large amounts land needed for production of hemp fibres). The researcher's model included organic hemp, hence the effects of ecotoxicity and eutrophication were diminished as a result of not using pesticides and fertilizers. For the traditional epoxy composite, the main contributor to environment effects were the production of epoxy resin (~ 66.9 %) and glass fibre (~27.9 %). On the contrary, the production of hemp (~7.29 %) and epoxy (~88 %)

for the eco-sandwich were comparatively lower than the previously mentioned. Further, comparing the petroleum based epoxy with eco-epoxy it was revealed that there were significant reductions in abiotic depletion and greenhouse gas emission when producing the eco-epoxy resin. Finally, comparing the composites revealed significant lowered impacts on human health and ecosystems for the eco-epoxy system. From the results, the major contributory factor to the impact (in both cases, eco-sandwich and traditional sandwich) is due to the use of epoxy resin.

A comparative LCA was done to evaluate the possibility of replacing conventional petrochemical plastics with poly (3-hydroxylbutyrate) (PHB) based composites [82]. Unlike many studies where a few well known indicators are discussed (as in previous study), for this study the environmental impact was evaluated using nonrenewable energy use (NREU) and global warming potential over a 100 years horizon (GWP100). The general lack of data regarding the PHB production process forced this compromise. PHB composites were environmentally superior to conventional polymers. Also, natural fibres were seen as the most promising filler in order to further boost the environmental performance of the composite material.

One of the main applications for natural fibre composite is the automotive industry. Comparing the environmental burdens of hemp reinforced plant based epoxy versus its petroleum based counterpart can assist in deciphering whether natural fibre composites are feasible [81]. In this scenario, the functional unit was a composite panel made from the respective systems. As highlighted in a previous publication from the authors [81], the hemp bio based epoxy system has less environmental impacts when compared to the petroleum based composite. Importantly, the life cycle analysis also considered end of life scenarios. The authors concluded that despite the difficulties with recycling epoxy, one scenario may be coprocessing in cement kilns and subsequent use in cement manufacturing. This end of life option would make the bio based application even more attractive for the mentioned applications.

Shen and Patel (2008) [83] reviewed the life cycle assessment of polysaccharide based materials. One section of their report detailed the feasibility of replacing glass fibre with flax and hemp fibres for automotive applications. When compared to glass fibres, natural fibres required less energy for production and emitted less greenhouse gases. They also mentioned that currently in Germany, car manufacturers are replacing a portion of glass fibre components (up to 3.5 kg per car) in interior panels and this correlates to better fuel efficiency as a result of weight reduction. Specifically, there were 45 and 16 % saving in nonrenewable energy (NREU - MJ per piece) for hemp and flax interior panels, respectively. In addition, hemp used for interior side panel and front sub frame resulted in 13 and 81 % greenhouse gas savings, respectively (kg CO_2 eq. per piece).

Alves *et al.* (2010) [84] reported a unique study of its own, where they compared the environmental impacts of using jute fibres versus glass fibres to reinforce unsaturated polyester to produce a structural frontal bonnet for a buggy. The authors were interested in jute fibres because of its ease of cultivation, high yearly production, and high specific mechanical properties. The functional unit used for comparison was a bonnet for an off road buggy, made from six layers of bi-axial jute or glass fibres reinforced unsaturated polyester. The differences in mechanical properties of the two bonnets were obvious, but the jute bonnet still achieved a useful mechanical behavior. The study was a complete cradle to grave analysis, with four phases namely, raw materials to production, use phase (fuel consumption), final disposal, and the total life cycle. The case study demonstrated that jute fibre composite presents the best solution for enhancing the environmental performance of the buggy's enclosures, hence improving the environmental performance of the whole vehicle. Despite the fuel consumption becoming lower using jute fibers, due to the weight reduction of the vehicle, LCA pointed out some unknown impacts in production and disposal phases of the bonnets, specifically related to the logistic transports of the jute fibers and the recycling scenario of the bonnets. Despite being an assessment of environmental impacts, the authors also included social, economic, and technical opinions. Socially, jute cultivation will result in an income source for locals, resulting in sustainability of the region, and during production, there are fewer health risks. In terms of economic properties, jute fibres cost significantly less than glass fibre and jute has approximately the same capital cost for technology deployment. Technically, as seen before in this report, treatment of the fibres results in better composite properties. In summary, based on the findings of this comprehensive study, better logistics, the production of organic jute (no fertilizer or pesticide), and social acceptance, can make natural fibre automotive parts a reality rather than a case study.

Joshi *et al.* (2004) [99] reviewed comparative life cycle assessments of natural fibre and glass fibre composites with the main aim to identify the main drivers and impact factors. They concluded that natural fibre composites are superior because of lower environmental impacts (in absence of fertilizer and pesticides) when compared to glass fibre composites, the weight reduction in component has the potential to improve fuel efficiency and reduce emissions in the use phase (for automotive industry), and the end of life incineration of natural fibre composites can result in recovered energy and carbon credits.

Natural fibre composites are an attractive alternative for replacing glass fibre reinforced petroleum based polymers at the moment. Based on the findings presented, depending on the applications, NFC are comparable and in some cases better. In addition, natural fibre reinforced renewable polymers has been studied widely, but the potential for large scale deployment isn't yet realized because of few key bottlenecks such as poor yields, high capital cost, and poor mechanical properties of resulting composites.

2.6.2 Techno economic analysis

Investment in research and innovation is at the heart of sustainability and the battle against environmental changes. Techno-economic analysis can be used to establish a bottom up perspective, detailing whether technologies and products are comparable to conventional petroleum based materials. Techno-economic modeling when developed in harmony with the technology ensures market driven prices. Applying techno-economic modeling at the onset of a research venture with all factors considered can help with averting misspent efforts and investments. These models can provide cost and performance boundaries that can ensure that the materials are allocated to the best process at a given time within a given cycle.

In the area of natural fibre composites or bio based polymers, studies of these nature are rear. Given the infancy of many of the processes and the many factors that influence them, few technologies have been commercialized. In fact, many companies have these data and it’s seldom accessible to the wide public. In one case, Wageningen Ur Food and Biobased Research publicly disclosed their key findings on an UltraFibre project initiated in 2010 [85]. In their study, the effect of atmospheric pressure plasma (APP) was used to process hemp and flax fibres and used to subsequently reinforce PLA, PP, and unsaturated polyester (PE). In context, it was reported that if the processing speed can be increased by a factor of 2-10 for each of different composite studies, then the process may be economically feasible based on preliminary techno-economic analysis.

In a report detailing the potential of bio based polymers in Europe, done by the European Commission in 2005, a few key findings influencing the feasibility of these technologies included: agricultural crop protection and harvest, industrial production and processing of polymers, structure of industry use phase, and waste management [86]. Also, a few recommendations were given to better stimulate the sector. These included non-food industrial use of agricultural products, integration of policies, and public financing. In terms of specific bio based polymers mentioned in this report, a few obstacles for deployment of PLA were given. Cost (fermentation cost), manufacturing (process energy requirements are high), and environmental concerns (lack of waste management and composting infrastructure) were mentioned as the main bottlenecks in the production of PLA on a competitive large scale. On the flip side, the advantages of the production also revolved around those three same factors, specifically, the raw material required is in oversupply and cheap. During processing, PLA can be implemented with minor investment cost because it has compatibility with thermoplastic processing equipment. With respect to environmental factors, Cargill Dow produced PLA has seen widespread acceptance by consumers because they are willing to pay more for environmentally friendly products.

Procter and Gamble (P&G) in the same reported were quoted for their work with PHA and the markets. Based on their assessment, market potential depends on production cost decreasing to USD 1.50 per kg, composting infrastructure, and applications where disposal and degradation are important [86].

Yan *et al.* (2013) [87] studied the technical and economic factors affecting fibre reinforced polymer (FRP) substation framework. Despite the use of glass fibre instead of a natural fibre or bio based material, this study can serve as a guide in terms of what factors can potentially affect the deployment of natural fibre structure materials of these nature. E-glass and epoxy composites made by pultrusion process was used as the main component of the substation framework. E-glass was selected because of excellent mechanical properties, electrical insulation, and it is readily accepted in the structural engineering field. In this respect, natural fibres that mimic these properties can be tested for such applications. Key technical observation included the traditional substation frame usually adopts steel structure, reinforced concrete or mixed structure. Also it is mainly composed of truss-column, steel structure, or a mixed structure, mainly composed of truss-column, truss-beam, and ground wire column. Based on the flexural rigidity and compressive stiffness equivalence principle, FRP members can be used to replace the steel members and be comparable. Economically, the use of FRP tube can reduce the expense of 10 % by replacing steel gantry with FRP gantry. This study indirectly correlates that if natural fibres can be cheaper than glass fibres and have comparable flexural properties, then it can be used as a possible replacement in such applications. Key would be insulating the fibres, so they are not exposed to moisture.

Koutinas *et al.* (2014) [88] reviewed the valorization of industrial waste via fermentation for the production of chemicals and biopolymers. They included a section on the techno economic analysis of the few important pathways. They cited that a complete techno -economic analysis can only be done after the development of a provisional process flow sheet that highlights the main processes. Importantly, they highlighted that one of the main factors limiting the fermentation pathway for production of PLA is the recovery of lactic acid from the broth and the expensive nutrient sources such as yeast extract. In terms of the dollar line, they cited that PHA at an annual production of 4500 tonne will cost \$6.08 kg^{-1} and require a fixed capital investment of \$28.6 M. As can be appreciated, those numbers are high when compared to the cost of producing petroleum based polymers because these conventional systems have been optimized and well-studied. The authors concluded that the factors that need to be improved to ensure bio based processes are competitive include but not limited to: 1) better final product concentration (kg product per m^3 fermentation broth), 2) better productivity, and 3) better yield (kg product relative to kg of substrate).

In the end, few studies on techno economic analysis are documented in the literature that are focused on natural fibre, natural fibre composites, and biopolymers. As a result, little is known about the main bottlenecks and what's preventing large scale deployment of these technologies. In effect, a careful study of each step, comparing it with conventional systems, will allow researchers to re-invent and make these process better technically and economically. This is definitely one of the areas for research challenge and explorations.

2.6.3 Challenges and future research perspective

The challenges for deploying natural fibres based materials and biopolymers have been touched periodically in this chapter. In this section, the aim would be to highlight these collectively and what can be done to address these shortcomings. To ensure these bio based technologies are deployed, the main ideas need to lobbied and used to persuade policy makers. Points such as lower use phase emissions due to weight reduction, energy, and carbon credit after incineration and possibility of recycling components need to be reinforced.

Specifically, for natural fibre composites, new methods need to be used that integrates a total bio refinery concept, whereby the byproduct or waste from one stream can be used to improve the properties of these fibres for composite applications. By doing this, we rely on a cheap, easily available material that can possibly have adverse environmental effects if not used. Another key aspect would be to try to model synthetic pathways against conventional technologies. The development of similar catalyst, specific to the materials of interest and less affected by system parameters are needed.

With respect to natural fibre composites, some of the major constraints inhibiting the large scale deployment of the technology include:

- Inconsistency among batches in terms of fibre qualities
- Performance bottlenecks, especially poor tensile and impact properties
- Lack of good moisture resistance
- Uncertainty with supplies and hence price fluctuations

The main limitations listed above can be addressed in the future, with careful planning at the governmental and international levels. Also, new pioneering research needs to be done to enable better adhesion between fibres and polymers, hence improving on the poor water resistance and mechanical properties.

2.6.4 Current trends and applications

The composite market is forecasted to reach 210,000 metric tonnes by 2020 (CW Composites World). North American market will account for at least half of the world wide composites, followed by Europe and Asia [6, 7]. The applications of composite materials are far reaching, including, mechanical structures and electrical insulators. More specifically, Cheung *et al.* (2009) [89] reviewed the possible applications of natural fibre composites (NFC) for bioengineering and environmental applications. They cited that the main industries that NFC can tap into are the aerospace, leisure, automotive, construction, and sporting industries. Although automotive and aerospace applications account for a substantial portion of the products, there is great potential in the sporting and medical fields. In fact, in Europe, natural fibres are used in automotive interior components such as door panels, bonnets, boot liners, and parcel shelves. Interestingly, every Mercedes and BMW models feature these renewable components. Also, Fiat and Ford uses up to 10 kg natural fibre per car [6]. Advantages to the automotive industry includes good mechanical properties, less health risk when working with natural fibres, and lack of splintering of parts during any accident. Importantly, weight in most parts (20-30 %) and lower cost of natural fibres, will lead to less fuel consumption over a given distance and better economies of scales, respectively.

The applications of natural fibre composite are greatly influenced by the properties of the given composites. Example, hemp and kenaf fibre reinforced PLA are characterized with high tensile strength, but low impact strength, hence these should be used for parts that are subjected to low impact stress such as furniture and boarding. On the contrary, other fibre types such as cotton and flax exhibit better impact properties with favorable tensile properties and can be used in interior car parts and helmets [90]. Holbery and Houston (2006) [91] critically reviewed the use of natural fibre reinforced polymer composites in the automotive industry. They mentioned the rise in using natural fibres in cars especially in Europe, for door panels, seat backs, headliners, dashboards and interior parts. Natural fibres such as hemp, flax, and jute resulted in benefits as a result of weight reduction and less carbon emission during manufacturing. The use of renewable materials also contributed to less of a reliance on foreign oil and the volatility. Despite these innovative successes, they were of the opinion the major challenges inhibiting the large scale acceptance has to do with homogenization of the fibre properties and a better understating of the adhesion between the matrix and fibres. In the end, the application of natural fibre composites would depend on the specifics of the application purpose and the properties of the natural fibres.

CONCLUSIONS

A few conclusory remarks based on the extensive survey of the literature for bast fibre based composite materials are outlined below.

1) The use of bast fibres has increased with the years, mainly as a result of increased environmental concerns and the low cost of these fibres. The range of application of these fibres is vast, but their utilization as reinforcement for polymeric composites with petroleum and renewable based thermoplastic and thermoset matrices is very promising.

2) Bast fibres such as hemp, kenaf, and flax are attractive because of their superior mechanical properties when compared to other lignocellulosic fibres. These fibres are primarily composed of cellulose, hemicellulose, lignin, and other minor components such as pectic material and extractives. The distribution, morphology, and relative amount of each component within the macrostructure is fibre specific.
3) Based on previous remark, the study of bast fibres as possible reinforcements will require a better understanding of the properties of the fibres, the interface with polymeric matrices, and the properties of the manufactured composite materials.
4) Bast fibres and lignocellulosic material in general are characterized with a few limitations that render them incompatible with most polymeric systems. Researchers have focused on mitigating these short comings by studying different chemical, physical, and biological methods to improve the fibre surfaces and bulk material.
5) Selection of the right polymeric matrices depends on the property of each phase, the interface, and the end application of the material. In fact, depending on the type of polymeric matrix, thermoplastic or thermoset and petroleum based or renewable, fibre may have a different effect on the final properties of the composites.
6) Future research on fibres and their reinforcement potential has to be done. Specifically, studies focused on examining the interface of these materials, factors affecting the water absorption, and eventual degradation in properties need to be better understood.

In summary, the potential of using bast fibres in composite materials is promising, but requires specific attention with a few key aspect. As highlighted in this report, researchers need to focus their attention to the right applications, based on the properties of the manufactured materials. Also, in the end, more assessment studies need to be done to convey the message that bast fibre reinforced composites are competitive environmentally and economically with established petrochemical platforms.

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CHAPTER 3.

Surface and thermal characterization of enzyme treated natural fibres: an insight into the effect of fibre geometry and macrostructure[2]

3.0 RATIONALE

Enzymes have been widely studied with respect to lignocellulosic degradation for biofuel production. In this research chapter, the main aim was to study the effect of a couple xylanase, pectinases, and an oxidoreductase on the macrostructure of hemp and flax fibres. Also, the key objective was to investigate whether removal of specific fractions affected the surface and thermal properties. A macro structural perspective was investigated and the effect of the fibers' geometry was found to significantly affect the enzymatic process.

3.1 INTRODUCTION

High-performance composite materials require a dispersed phase with elevated surface wettability and elevated cohesion with the polymeric matrix in order to attain efficient transfer of stress within the structure. While natural fibres constitute an attractive alternative to glass fibres owing, their limited thermal stability and incompatibility with synthetic polymer matrices constitute a significant challenge toward industrial implementation of these materials platforms [1-3]. Several stategies have been investigated to address these deficiencies. For examples, mercerization [4], acetylation [5] and silane treatment [6] are known either to reduce hydrogen bonding within the complex structure or by reacting with the many hydroxyl groups on the surface [7. 8]. Physical methods have also been studied and a comprehensive review was compiled by Mukhopadhyay and Fangueiro (2009) [9]. Methods such as steam explosion [10], plasma treatment [11] and corona discharge [12] promote separation of fibre bundles into more homogenous structures that can be evenly deposited in one direction in the matrix materials.

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An alternative to chemical and physical methods is represented by the rapidly expanding use of biological agents such as fungi [13, 14] and enzymes [15-17]. Biological modifications offer several advantages over chemical and physical methods. They can selectively remove hydrophilic pectic and hemicellulosic material and require lower energy input. Enzymatic systems, in addition, can be recycled after each use [18]. Li and Pickering (2008) [18] used chelators and enzymes to separate hemp fibres into individual bundles. They found that the crystallinity (x-ray diffraction) and thermal properties (thermal gravimetric analysis) improved after separating the bundles. In another study, Pietak *et al*. (2007) [17] investigated the surface wettability of natural fibres using atomic force microscopy and contact angle measurements. They measured an increase in adhesion force for treated samples because enzymatic and chemical treatments (steam explosion) removed the primary cell wall exposing the secondary wall. The same study provides clear evidence of hemicellulose removal from hemp fibres.

Enzymatic treatments have also been used to modify the physical network of natural fibres. Kardas *et al.* (2009) [19] investigated the effect of four enzyme treatments (Lipase A, Lipase AK, Lipozyme and Esterase) on the micro-topography of polyester fabric. They found that the esterase preparation was the most effective method for producing a more uniform and homogenous texture of the fabric material. Saleem *et al.* (2007) [20] treated bast fibres with pectinase and then reported the mechanical characteristics of the reinforced thermoplastic composites. According to their study, hemp fibres treated with 8 % of the enzyme in maleic anhydride medium, were characterized by increased tensile strength, flexural strength and moduli of elasticity. These improvements were attributed to higher aspect ratio of the fibre bundles which resulted in better dispersion within the polymeric matrix (polypropylene). A recent studied conducted on bamboo fibres using a number of enzymes (xylanase, cellulase, pectin lyase and laccase) revealed that the different systems were effective in improving fibre fineness (Liu *et al*. 2012) [21], presumably as a result of the removal of the more polar hemicellulosic fraction.

The scope of this study was to investigate the effect of five commercially available enzyme systems (xylanase, pectinmethylesterase, polygalacturonase, laccase and a xylanase with cellulase background) on the surface of two different sources of bast fibres, hemp and flax. It should be noted that each of these enzyme system may have accessory enzymes such α-glycosidase and arabinosidase. Fundamental surface chemistry and thermal information collected in this work provided the basis for better understanding the properties of natural fibres and how these may influence the fibre-matrix interface. Enzymes enable the modification of the fibre surface properties with limited or no effect on the bulk properties because, owing their size percolation into the inner structure is impeded. For example, Boisset *et al.* (1996) [22] while studying the action of a recombinant cellulase on the cross section of cottons fibre for applications in the textile industry found that there was no indication of enzyme penetration or damage to the interior of the fibres.

To the best of our knowledge, this study is one of the first that examines the influence of solely enzymes in the absence of any mediator (chemicals) on thermal properties of natural fibres specifically for composites applications. Removal of pectic and hemicellulosic materials resulted in improved thermal properties based on thermal gravimetric analysis. X-ray photoelectron spectroscopy and contact angles indicated the removal of the hygroscopic primary cell wall. The findings reported serves to link enzymes as a pretreatment method for natural fibres to be used in composite applications.

3.2 EXPERIMENTAL

3.2.1 Materials

Mechanically processed hemp and flax samples were provided by the Alberta Biomaterials Development Centre located in Vegreville, Alberta. The samples were placed in air tight bags and stored at 4 °C. All enzymes were provided by Novozymes (Bagsvaerd, Denmark) and stored at 4 °C. Sodium acetate (99%, mol wt. 82.03 g/mol), glacial acetic acid (99.7%, mol wt. 60.05 g/mol), sodium phosphate dibasic (99%, mol wt. 141.96 g /mol) and sodium hydroxide (99%, mol wt. 40.00 g/mol) were obtained from Fisher Scientific. Sodium citrate monohydrate (99%, mol wt. 214.11 g/mol) was purchased from Sigma- Aldrich. Sodium phosphate monobasic (99%, mol wt. 119.98 g/mol) was obtained from Acros Organics. Citric acid (99%, mol wt. 192.13 g/mol) was sourced from EMD Chemicals. Sulfuric acid (98%, mol wt. 98.075 g/mol) and calcium carbonate (99%, mol wt. 100.09 g/mol) were also sourced from Fisher Scientific. D-(+) - Glucose (99.5%, mol wt. 180.16 g/mol), D-(+) - Galactose (99.5%, mol wt. 180.16 g/mol), D-(+) - xylose (99%, mol wt. 150.1 g/mol) were obtained from Sigma. Distilled water was used for all analyses.

3.2.2 Washing of natural fibres

Fibres were washed using 2 % (w/v) Sparkleen (No. 1) industrial detergent for 1 hour at 70 °C to ensure removal of dirt and any surface contaminant that may affect the enzymes activity. Samples were filtered and washed several times with distilled water. Fibres were dried in a convection oven at 80 °C for 5 hours and stored in a desiccator [22].

3.2.3 Natural fibre composition

The methods outlined by Ramadevi *et al.* (2012) [23] were adopted to estimate the lignin, hemicellulose, and cellulose content. Briefly, the lignin content was measured by placing 2 g of sample in a 500 mL beaker and 15 mL of 72 % sulfuric acid was added and agitated at 100 rpm for three hours at 25 °C. 200 ml of water was then added to the mixture and boiled for two hours. Once cooled, after 24 hours, lignin was transferred to a crucible and washed with hot water. The crucible with its content was dried at 105 °C and weighed every hour until a constant weight was reached.

The holocellulose content was measured by placing 160 mL of water, 0.5 mL of acetic acid, 1.5 g of sodium chloride and 3 g of fibre in a beaker. The beaker was placed in a water bath maintained at 75 °C for an hour, after which 0.5 mL of acetic acid was added with 2.5 g of sodium chloride. This was repeated for another two hours. Once completed, the beaker was then placed in an ice bath and cooled. The isolated holocellulose was sequentially washed with acetone, ethanol and water. The sample was finally dried at 105 °C until constant weight was reached.

The content of α-cellulose was measured by adding 10 mL of 18 % (w/v) sodium hydroxide to 2 g of the isolated holocellulose. The fibres were continuously agitated at 120 rpm and kept at 20 °C. Every 5 minutes, 10 mL of the sodium hydroxide solution was added for another half hour. 35 ml of water was added to the beaker and kept for another hour. The holocellulose residue was isolated by washing with 100 mL of 8.5% sodium hydroxide, 200 mL of water, 15 mL of 10 % (w/v) acetic acid and water. The contents of the crucible were dried at 105 °C until constant weight was reached.

The hemicellulose content was calculated by subtracting the hollocelulose from the α-cellulose content [10].

ASTM Method D4442- 07 was followed to determine the moisture content of the flax and hemp samples. Approximately 0.5 g (done in triplicate) of sample was weighed in a pre-weighed and dried tin pan. The pan was placed in a convection oven at 105 °C ± 5 °C for 5 hours. The sample pan was removed, cooled in a desiccator and then weighed. The sample was heated, cooled and re-weighed until constant mass. The moisture content was calculated based on percentage mass lost.

ASTM Method E1755-01 was used to estimate the ash content of the different fibre samples. The method involved weighting approximately 0.5 g (done in triplicate) of fibre sample into a pre-weighed crucible. The crucibles were heated to 575 °C ± 25 °C for 3 hours or until all the carbon was eliminated. To avoid causing flames, the sample was heated to 250 °C and gradually increased to 575 °C. The residue left after 3 hours was cooled in a desiccator and weighed. The contents were heated in a convection oven at 105 °C until constant weight. The ash content was calculated as a mass percentage.

The Dumas method or Combustion Nitrogen Analysis (CNA) method was used to determine the total organic and inorganic nitrogen content of the different samples. A LECO TruSpec CN Instrument was used for all analyses. The nitrogen content of the samples was used to estimate the protein concentration with a constant factor approved by the Canadian Grain Commission [24]. In all cases, approximately 0.1 g (done in triplicate) finely chopped fibre was used. The protein content was estimated by multiplying a protein factor of 6.25 by the total nitrogen content [24].

3.2.4 Enzymatic treatment of hemp and flax fibres

Approximately 1.0 g of fibre was weighed into a 125 mL Erlenmeyer flask. Optimal conditions for each enzymatic reaction were provided by Novozymes (Table 3.1). Concentrations of 2, 6 and 10 % (% w/v) enzyme stock with corresponding enzyme activity as specified in Table 3.1 were used to treat each fibre type. For all experiments, the liquid (mL) to fibre (g) ratio was maintained at 50:1 to facilitate complete wetting of fibres. Enzymatic treatments were conducted for 90 minutes under constant agitation (80 rpm) at the optimum temperature in a standard water bath. Enzymes were deactivated by heating at 90 °C for 10 minutes. Fibres were washed with excess warm water to remove traces of enzyme and buffer reagents [25]. All samples were dried at 80 °C for 5 hours and stored in polyethylene bags for subsequent analysis. All experiments were carried out in triplicate.

Table 3.1 Characteristics of each enzyme system with corresponding activity at optimum conditions. For each system, pH was maintained using buffers.

Enzyme	Optimum conditions		Activity
	pH	Temperature (°C)	
Xylanase	7	70	1000 AXU/g
Xylanase (10% cellulase)	6	50	2500 FXU-S/g
Polygalacturonase	4	45	3800 PGNU/ml
Laccase	7	50	1000 LAMU/g
Pectinmethylesterase	5	45	5 PEU/ml

3.2.5 Characterization of fibres

Prior to all analyses, samples were pre-conditioned at 80 °C for 5 hours to maintain constant moisture content for all treated and untreated samples.

3.2.5.1 Morphological characterization

Micrographs of fibre surfaces of untreated and enzyme treated fibres were taken using Hitachi S-2700 Scanning Electron Microscope (SEM) equipped with a Princeton Gamma Tech (PGT) IMIX digital imaging system and a Prism Intrinsic germanium (IG) detector. A gold putter coater was used to induce conductivity for all samples. A resolution of 4 nm was used for all samples. 0.1 ± 0.005 g of fibre was mounted on conductive adhesive tape, sputtered coated with gold palladium and observed using a voltage of 15 – 20 kV [26].

3.2.5.2 X-ray photoelectron spectroscopy (XPS)

XPS was used to study the elemental composition and the O/C ratio of the surface of the different fibres following treatment. Samples were subjected to high and low resolution scans. Approximately 0.2 ± 0.005 g of fibre was pressed into a disc with diameter of about 0.5 mm for analysis. A Kratos Ultra 165 X-ray Photoelectron Spectrometer with energy spectra ranging from 0 – 1000 eV was used to study all samples. O/C ratios for the different samples were compared to the 0.83 for cellulose, hemicellulose, pectin, and 0.35 for lignin. Samples were scanned for C1s (284 eV) and O1s (530 eV) [27]. To maximize the analysis incident area, a spot size of 30 μm of the fibre surface was used to record each spectrum. Survey spectra were acquired using pass energy of 50 eV for low resolution and 20 eV for high resolution. The fibres were mounted on aluminum foil and then compressed using nickel plates. The sample assembly was placed in the sample processing chamber of the spectrometer for analysis. An eight (8) channel multi-detector was employed for analysis [28].

Data treatment was performed using CasaXPS program (Casa Software Ltd., UK). Mole fractions were calculated using normalized peaks based on acquisition parameters after a linear background subtraction and consideration of experimental sensitivity factors. C1s spectra were analyzed with a Gaussian product function, by maintaining the FWHMs of all components to within the range of 1.200 to 1.600 [28].

3.2.5.3 Force tensiometry/Contact angle measurement

A 700 Sigma One Attension tensiometer (Biolin Scientific) was used to measure the contact angle. All samples were pre-conditioned at 80 °C for 3 hours prior to analysis. The Washburn capillary rise method was used to characterize the fibre bundles [29, 30]. For each experiment, triplicate analysis was done for each sample with three cycles of immersion (three measurements taken on a single fibre). The first 0.05 mm of each fibre was ignored because the edges of the fibres are not spherical and can influence the result. An immersion speed of 5 mm/min was used for both advancing and receding measurements. Given the nature of the technique, both advancing (penetration of the fibre into liquid) and receding (recession of the fibre from liquid) contact angles were calculated for each cycle. Equation 3.1 was used to estimate the contact angle for each sample.

$$Wetting\ force = Liquid\ vapor\ surface\ tension * perimeter\ fibres\ (P) * cos\theta$$

Equation 3.1

Where θ is the unknown contact angle and *P*, the perimeter of the natural fibres tested (the perimeter was assumed to be constant among fibres - fibres fitted into a probe provided by the instrument supplier). The contact angle for each sample was calculated using the measured wetting force.

3.2.5.4 Thermogravimetric Analysis (TGA)

Experiments were conducted using a Thermal Analysis Instruments TGA Q50 (TA Instruments) apparatus under a flow of nitrogen to study the effects of heating on stability of the different treated natural fibres. Platinum pans were used given the high temperatures and the ease of cleaning. The temperature range selected was from room temperature to 600 °C at a rate of 10 °C per minute. For each sample, triplicate runs were done. All results were reproduced to 5 % error or better.

3.2.6 Statistical Analysis

All experiments were replicated at least three times and results were expressed as mean value ± standard deviation. The statistical analyses of the data were conducted using the statistical software package SAS Version 9.4. It is important to note, that each system was compared to the control and not among each other given the difference in activity for the enzymes and the different mode of substrate attack. To identify significant differences between mean values for control and a given enzyme system, Kruskal Wallis Test was applied to the data populations involved, with a 95 % confidence level ($P < 0.05$).

3.3 RESULTS AND DISCUSSION

3.3.1 Characterization of flax and hemp

The chemical analysis of the major components of flax and hemp is presented in Table 3.2. With the exception of the cellulose content (measured at 74 and 81 % on a dry weight basis for hemp and flax respectively) the data collected was in line with the values reported by Mwaikambo and Ansell (2002) [4].

Table 3.2 Chemical compositions for flax and hemp samples analysed relative to values from the literature (given in mass percentage).

Component (%)	Experimental samples		Literature values[a]	
	Hemp	Flax	Hemp	Flax
Moisture	7.34 ± 0.19	8.12 ± 0.18	10	8
Protein	6.34 ± 0.30	6.18 ± 0.28	2.1	3
Ash	2.31 ± 0.13	1.53 ± 0.19	-	-
Cellulose	67.8 ± 2.25	64.1 ± 3.09	67	65
Lignin	7.24 ± 0.64	4.14 ± 0.42	3.3	2.5
Hemicellulose	12.1 ± 3.51	16.1 ± 3.02	16	16

[a] Bismarck, A. *et al*. 2001 [31].

In another study, Tserki *et al.* (2005) [8] found that the relative amount of hemicellulose to lignin influences the kinetics of chemical modification of wood, flax and hemp fibres. Interestingly, their characterization showed 7 and 15 % (weight %) for minerals and proteins respectively. This fraction constitutes a large portion of dissolved and colloidal matter which is known to affect the activity of enzymes and fungi. The moisture content for the samples studied was comparable with the results of Tserki *et al*. (2005) [8] of approximately 9.1 % (weight %). The experimental samples had a high amount of protein when compared to the values reported by Bismarck *et al*. (2002) [31] in Table 3.2. The values available in the literature do not specify the nature (organic or otherwise) of the nitrogen source. The test utilized in this study (DUMAS combustion method) estimates the total nitrogen instead of solely organic nitrogen. Apart from low cellulose content, the composition of the experimental fibres was comparable to literature values.

Characterization of the treated samples based on the three main components namely cellulose, hemicellulose and lignin are presented in Table 3.3. This set of data, integrated with XPS and contact angle measurements enables a correlation between surface composition and polarity. Xylanase + cellulase treated hemp were characterized by a significant ($p<0.05$) reduction in the cellulosic content while hemp treated with pectinases (polygalacturonase and pectinmethylesterase) showed reduction in the hemicellulosic content. One plausible reason for the latter observation may be the removal of pectic matter (minor component ≈ 0.5-2% dry weight) bonded to hemicellulosic content which leads to disintegration and rupture of the network. The same trend was observed for these enzymes when applied to flax fibres. In addition, laccase treated flax fibres were characterized with a significant reduction ($p<0.05$) in the lignin content. The flax fibres were more susceptible to enzyme attack based on the characterization of the treated fibres."

Table 3.3 Chemical compositions of the treated samples with respect to cellulose, hemicellulose and lignin.

		Component (% of dry weight)		
Fibre	System	Cellulose	Hemicellulose	Lignin
Hemp	Untreated	67.8 ± 2.25	12.1 ± 3.51	7.24 ± 0.64
	Xylanase	67.2 ± 1.46	7.89 ± 0.81	7.04 ± 0.61
	Xylanase + cellulase	57.4 ± 2.12	11.8 ± 1.02	6.48 ± 1.35
	Laccase	68.0 ± 2.48	10.9 ± 0.86	6.88 ± 0.05
	Polygalacturonase	68.6 ± 4.58	6.40 ± 2.21	5.09 ± 1.10
	Pectinmethylesterase	68.3± 1.31	7.45 ± 1.81	6.14 ± 1.17
Flax	Untreated	64.1 ± 3.09	16.1 ± 3.02	4.14 ± 0.42
	Xylanase	63.6 ± 2.65	11.4 ± 1.25	3.53 ± 0.09
	Xylanase + cellulase	54.5 ± 1.31	8.69 ± 1.96	4.18 ± 0.89
	Laccase	63.1 ± 2.09	13.4 ± 2.64	1.45 ± 0.12
	Polygalacturonase	61.3 ± 1.99	2.55 ± 0.84	1.43 ± 0.28
	Pectinmethylesterase	61.1 ± 1.72	4.15 ± 1.82	2.81 ± 0.06

The fibre yield for each component was calculated using the equation below.

$$\frac{100\ \% \times \%\ mass\ of\ fibres\ after\ treatment}{\%\ component\ after\ treatment}$$

E.g. % cellulose (Xyl + Cel treated hemp) = (100 / 57.42) × 91.63 = 64.3 %

Table 3.4 Composition after treatment, recalculated to reflect changes in each component

Fibre	System	Chemical component after treatment (% dry weight)			% Mass Loss after treatment
		Cellulose	Hemicellulose	Lignin	
Hemp	Untreated	67.77	12.14	7.24	-
	Xyl + Cel	64.29	13.21	7.25	10.58
	PG	75.12	6.98	5.55	8.17
	Xyl	70.07	8.23	7.34	4.12
	Lac	70.42	11.36	7.12	3.22
Flax	Untreated	64.08	16.10	4.14	-
	Xyl + Cell	68.07	10.85	5.22	19.44
	PG	80.26	3.34	1.87	23.21
	Xyl	69.40	12.41	3.85	8.31
	Lac	70.04	14.83	1.61	9.91

As expected, removal of the hemicellulosic content, will result in concentrating the cellulose component. This is very advantageous for composite applications because cellulose contributes the tensile strength needed to reinforce the matrix. PG and Xyl+Cel were the most promising systems.

3.3.2 Morphological characterization

Five enzyme systems were used to treat hemp and flax samples. The fibre sections were randomly selected. The possible influence of changes in stem morphology (fibres from different sections of stem) was eliminated via processing by standard methods approved by the Pulp and Paper Technical Association of Canada and in accordance with industrial standard methods in use at Alberta Innovates Technology Futures (Vegreville, Alberta). The morphological changes for the different treated samples showed similar pattern as represented in Figure 3.1. In Figure 3.1, the micrographs for an untreated (a), laccase (b), xylanase + cellulase (c), and polygalacturonase (d) treated samples. It is clear from the images when compared to the control (untreated), the fibre bundles become more visible. Gutierrez *et al.* (2012) [32] demonstrated that laccase in the presence of mediators (HBT) removed lignin, including aliphatic derivatives from wood feedstocks. The aim of our work was to use the enzyme solely (without any chemicals) and study whether there were any surface changes due to the removal or rearrangement of the lignin fragments. Researchers have studied the macromolecular arrangement of the different components of wood based and non-wood based feedstocks. The widely accepted theory is that lignin forms a random wrap-like structure around the cellulosic bundles [1].

It is plausible that the removal of lignin exposes the bundle as seen in both hemp and flax systems. On the other hand, a combination of xylanase and cellulase (c) and polygalacturonase (d) treated samples exhibited an increase in surface roughness and bundle exposure. Xylanase is known to degrade hemicellulosic based material which is found around the cellulosic fibre bundles as pockets of different branched and linear polysaccharides [33]. Xylanase attacks these pockets of polymers breaking β-1, 4 bonds found in xylan releasing monomers resulting in the formation of cracks on the fibre surfaces. Hemp samples treated with polygalacturonase showed clear evidence of removal of material from the fibre surface possibly as a result of the degradation of polygalacturonan, a major component of pectin found predominantly in the primary cell wall [34]. Baracat *et al.* (1989) [35] showed that this family of enzymes disrupts the pectic middle lamella and primary cell walls exposing the cellulosic network during the degumming of fruits for food and fermentation applications. Using this base of knowledge, one can remove the hygroscopic pectin content of natural fibres and this should translate into an improvement in thermal resistance. The work reported for the hemp samples are in agreement with the literature. Reddy and Yang (2005) [26] suggested in a review that the use of enzymes such as pectinases and hemicellulases for dry retting will be an important area of focus in the coming future. Although the ultimate aim during retting and enhancing of fibres is similar, the dosage, time and type of enzyme will impart different features. In fact, when enhancing the fibres, the aim is to modify the surface only by selective removal of components but for retting the aim is to remove the components that hold the network together producing fibrils that can be modified. In retting, the enzymes removed the encrusting substances such as pectin and hemicellulose resulting in fibres with greater surface roughness. It was their opinion that these fibres can then be treated to render them more hydrophobic so as to improve the adhesion with polymeric matrices.

Flax fibres treated with xylanase + cellulase showed the same pattern of surface roughness as did the hemp sample. The fibre bundles were more exposed in the case of flax samples, possibly as a result of an initially higher amount of hemicellulose (Table 3.2) and less cellulose. Flax fibres treated with were also characterized by bundle fibrillation and increased in surface roughness. In summary, it is evident from the SEM micrographs that enzyme treatment of hemp and flax led to individual bundle exposure and a clean surface.

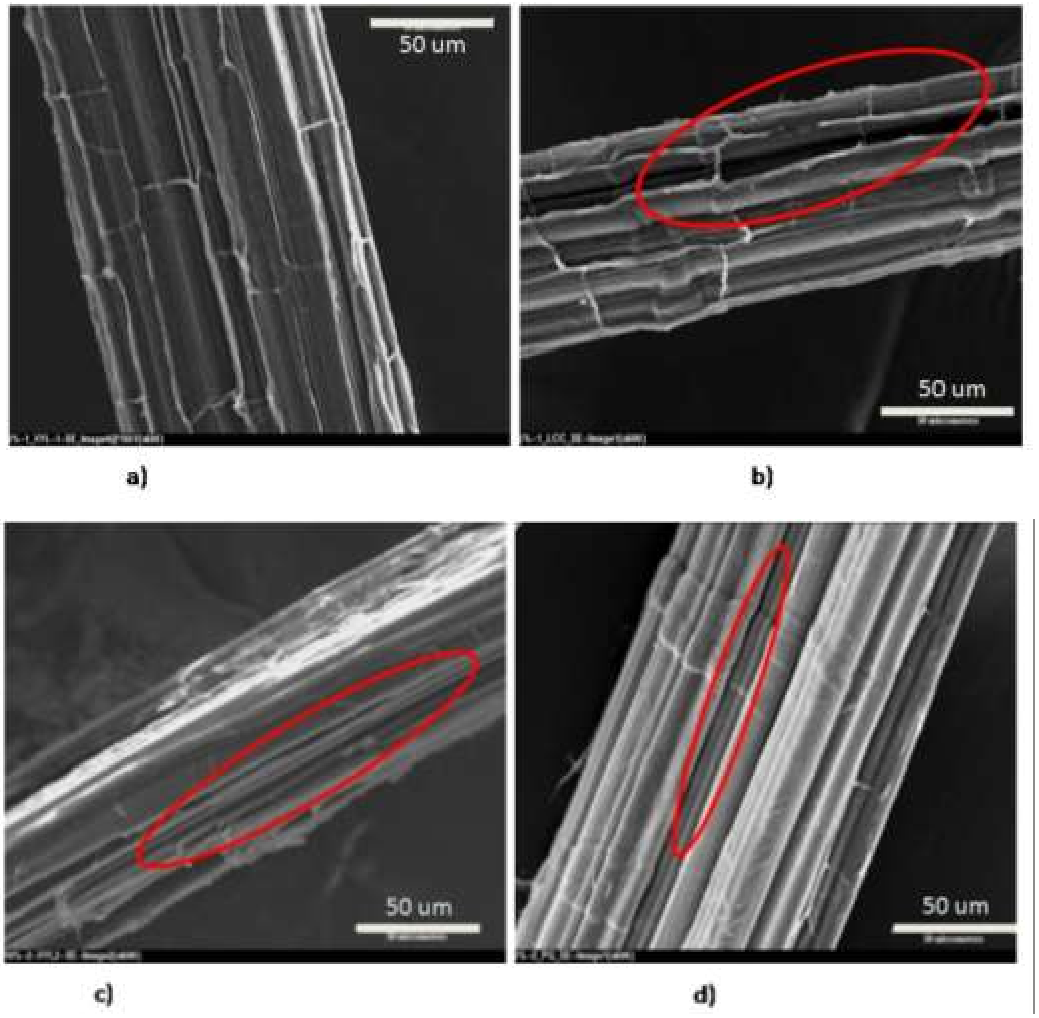

Figure 3.1 Micrographs of treated and untreated hemp; a) control, b) laccase, c) xylanase+cellulase, and d) polygalacturonase. The red inserts show the defibrillation in the laccase and xylanase + cellulase treatments and the individual bundle exposure in the hemp treated with polygalacturonase.

3.3.3 X-ray photoelectron spectroscopy

Most of the characterization studies of natural fibres using XPS have been carried out on chemically treated samples [24, 28, 36]. In addition to modifying the surface of the fibres, chemical pretreatments may also alter the inner structure adversely affecting the mechanical properties [37]. On the other hand, enzymatic reactions are limited to the surface of the fibres because access to the inner structure is precluded. An example of the high resolution carbon C1s spectra for hemp fibres showing the different fractions associated with each carbon is shown in Figure 3.2. The parent peak was partitioned into its different carbon signatures, depending on the binding energy. The three major signatures studied were C-C due to carbon backbone, C-O and O-C-O at 285, 286.2-286.6 and 287.3-288.1 eV respectively.

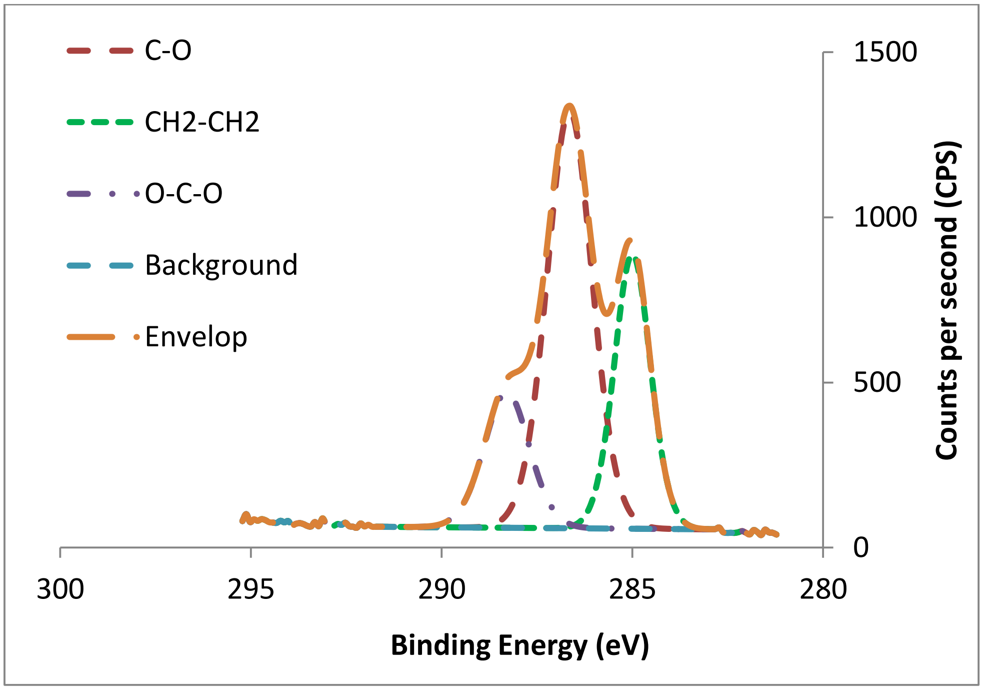

Figure 3.2 High Resolution deconvoluted Carbon C1 XPS Spectra for untreated hemp fibres illustrating the different carbon abundance.

Table 3.5 summarizes the data obtained using XPS and shows the changes in the atomic percentage of carbon involved in different bonds and the O/C ratio for each treatment. The data was obtained by fitting the carbon peak at 285 eV using Gaussian or Lorentzian functions (depending on the fitting necessary). Analysis of the data was done by measuring the areas under the curves (O and C) and computing the O/C ratios for each enzyme system. The C peaks were partitioned into its signatures using CasaXPS software. These components correspond to C-C, C-O and O-C-O as outlined by Zafeiropoulos *et al.* (2003) [24].

Table 3.5 Abundance of the different C1s signatures for the different enzyme treated fibre samples and the corresponding O/C ratio.

Sample	Treatment	Signature (eV)- Atomic %			O/C
		C-C 285	C-O 286.2-286.7	O-C-O 287.3-288.1	
Hemp	Control	35.4 ± 4.62	42.9 ± 1.34	20.1 ± 7.71	0.63 ± 0.09^{a}
	Xylanase + cellulase	36.8 ± 2.24	37.7 ± 2.92	25.2 ± 4.70	0.52 ± 0.04^{a}
	Xylanase	26.0 ± 1.26	41.5 ± 9.16	18.4 ± 2.82	0.65 ± 0.02^{a}
	Polygalacturonase	25.0 ± 2.70	55.0 ± 2.31	20.1 ± 2.23	0.71 ± 0.05^{a}
	Pectinmethylesterase	32.1 ± 5.89	37.9 ± 2.02	29.4 ± 6.17	0.65 ± 0.06^{a}
	Laccase	25.0 ± 3.87	49.8 ± 5.34	22.0 ± 3.08	0.45 ± 0.09^{b}
Flax	Control	24.6 ± 0.60	54.6 ± 0.89	20.9 ± 1.49	0.67 ± 0.01^{a}
	Xylanase +cellulase	28.0 ± 0.94	47.9 ± 2.88	22.2 ± 0.98	0.52 ± 0.04^{c}
	Xylanase	29.3 ± 0.69	49.4 ± 2.53	21.3 ± 2.80	0.67 ± 0.03^{a}
	Polygalacturonase	46.2 ± 5.64	38.4 ± 4.32	15.4 ± 1.61	0.18 ± 0.01^{d}
	Pectinmethylesterase	43.4 ± 1.96	40.5 ± 2.09	16.2 ± 0.80	0.18 ± 0.00^{b}
	Laccase	23.7 ± 1.84	52.7 ± 2.57	23.65 ±2.65	0.35 ± 0.16^{e}

^{a,b,c,d,e} treatments with the different letters are statistically different at a confidence level of 0.05 (n=3) when compared to the control (not among enzymes). Kruskal Wallis Non -Parametric statistical analysis was used to analyze all samples. This test was used given the small sample size and the non-parametric pattern of the data.

The abundance of the C-C bonds, which are attributed to waxy substances and carbon backbone [37], was reduced after every enzymatic treatment in the case of hemp. It should be noted that all enzyme treatments were compared to a control and not among treatments given that each enzyme system is distinct both in the relative amounts used and the mode of action. The distribution of heterogeneous layers of waxes on the fibre surfaces was also observed by the SEM micrographs, in good agreement with literature results [28]. There was no significant ($p<0.05$) change in the C-O signature for most treatments.

The primary component of hemp and flax is cellulose while hemicellulose amounts to approximately 15 % hemicellulose. According to Zafeiropoulos *et al*. (2003) [28], these two major components with a range of monomers have an O/C ≈ 0.83. For the hemp treated samples, when compared to the control, polygalacturonase treated fibres had an O/C of ≈ 0.71, suggesting that the degradation of the middle lamella and primary cell wall may have exposed the less hygroscopic cellulosic network. This is also supported by the increase in the C-O functionality. In the case of hemp treated with xylanase, there was a decrease in C-OH functionality, possibly as a result of hemicellulose removal which left the cellulosic bundles covered with lignin. No significant change in the O/C ratio was measured, indicating that the cellulose structure probably remained intact.

Pectinmethylesterase decreased the O/C ratio of flax sample as a result of pectin de-esterification into pectic acid and methanol [34]. These constituents are found primarily in the cell wall and degradation exposes aromatic rich lignin and its derivatives which cover the cellulose, hence the decrease in O/C. On the other hand, a combination of xylanase and 10% cellulase decreased the C-O while increasing the C-OH functionality indicating that the hemicellulosic fraction was degraded exposing the more crystalline cellulose. Pectinmethylesterase and xylanase in the presence of cellulase background had no significant effect ($p< 0.05$) on the surface chemical composition when compared to the control experiment.

Flax treated with xylanase and laccase showed no difference from the control for the surface chemical composition and will not be discussed further. However, flax treated with polygalacturonase, an enzyme highly specific in attacking pectin derivatives in the primary cell wall and middle lamella of the fibres [34], exhibited a significant reduction in the O/C ratio. Bateman and Basham (1976) [34] did a comprehensive review on microbial enzymes capable of degrading plant cell wall components of different material and found that polygalacturonase was one of the most effective when it comes to pectin degradation agents during fruit ripening. A plausible explanation for the reduction of the O/C can be exposure of lignin components after the polygalacturonan removal. This observation is also supported by the reduction in C-OH signature of hemicellulose on the fibre surfaces and a corresponding increase in aliphatic (C-C) signature (due to waxes and derivatives of lignin). The flax system was more susceptible to the enzyme treatments used. Both hemicellulases (pectinmethylesterase and polygalacturonase) reduced the O/C ratio as a result of hemicellulose removal and exposure of lignin on the surface. The O/C ratio for lignin was 0.35 while for hemicellulose material it was approximately 0.85, hence the removal of the latter will result in concentration of the former on the surface, reducing the O/C ratio. The geometrical orientation of the individual fibres in a bundle provides a plausible explanation for the reactivity differences of these fibres [38]. A smaller lumen and 7-sided nature of the fibre cross section with sharp peaks makes flax fibres more susceptible to enzymatic attack. The folds in the cross section allow greater surface access for the enzymes, resulting in greater hemicellulose removal [38]. In summary, the activity of the different enzymes depends on geometrical orientation of the individual fibres within a bundle and chemical composition of the natural fibres.

3.3.4 Force tensiometry

Table 3.6 shows the effect of each enzymatic treatment on the contact angle of the fibres studied. The advancing contact angle for each system was presented because there was no difference between the advancing and receding contact angles ($p<0.05$). Typically, differences between the advancing and receding angles may be due to geometric heterogeneity or small scale roughness and chemical heterogeneity. Despite the known heterogeneity of the natural fibres, the absence of hysteresis in almost all cases indicated the enzymatic treatment did remove the specific components resulting in a clean fibre.

All enzyme treatments of hemp fibres resulted in significant ($p<0.05$) reductions of contact angle which are correlated to a greater surface hydrophilicity [13]. It must be emphasized that the structural arrangement of the different chemical component of a single fibre strand is complex. According to

Biogiotti *et al.* (2004) [39] the fibre network consists of basically four distinct layers namely the secondary wall, primary cell wall, middle lamella and the layers (S1, S2) in between the primary and secondary walls. The outermost layer consists primarily of hemicellulose covered cellulose. Each section of the network because of the varying chemical components that are abundant, have their unique characteristic polarity [39].

Table 3.6 Contact angle for the different enzyme treated hemp and flax.

		Contact angle (°)	
System	**Enzyme**	**Control**	**Sample**
Hemp	Xylanase + 10% Cellulase[a]	89.1 ± 0.07	88.7 ± 0.03
	Xylanase [a]	89.1 ± 0.07	88.7 ± 0.04
	Polygalacturonase[a]	89.1 ± 0.16	88.5 ± 0.03
	Pectinmethylesterase	89.0 ± 0.16	88.8 ± 0.17
	Laccase[a]	89.1 ± 0.02	88.7 ± 0.08
Flax	Xylanase[a]	89.0 ± 0.18	88.5 ± 0.04
	Xylanase + 10% Cellulase[a]	89.0 ± 0.04	88.6 ± 0.02
	Polygalacturonase	89.0 ± 0.13	88.8 ± 0.22
	Pectinmethylesterase	88.9 ± 0.17	88.6 ± 0.29
	Laccase	88.8 ± 0.43	88.9 ± 0.20

[a] Treated samples that were significantly different from the control ($p<0.05$).

Specifically, xylanase in the absence of cellulase has been used in the pulp and paper industry to facilitate lignin extraction and degradation of xylan, a major fraction of the hemicellulose [40]. The removal of the xylan is known to expose the cellulose hydroxyl and carboxyl groups that were otherwise covered. Similarly, xylanase treatment in the presence of the cellulase background removed the hemicellulose content from the primary cell walls of the fibre bundles and causes some degradation of the cellulosic network as evident in the SEM micrograph. Polygalacturonase was used to depolymerize pectin/pectic acid found in the middle lamellae by cleaving the glycosidic bonds via hydrolytic reactions. This leads to either crystalline cellulose or other hemicellulosic content within the primary wall [41]. Laccase treated hemp resulted in a decrease in contact angle because the removal of lignin exposes the cellulosic backbone (as supported by increase in [C-OH] from XPS study) [42]. Pectinmethylesterase had no effect on the surface polarity for the hemp fibres. The enzyme systems studied resulted in an increased surface hydrophilicity plausibly because of the removal of lignin and hemicellulose, exposing the cellulosic backbone.

Laccase and the pectinases had no significant effect ($P<0.05$) on the flax fibres when compared to the control. However, the flax system when treated with both hemicellulases showed similar values when compared to the hemp samples. Interestingly, the contact angles decreased more than when compared to the hemp fibres treated with the same enzymes. As mentioned before, one plausible reason for this may be the geometrical orientation differences between the two fibre types. Wiener *et al*. (2003) identified a simple and robust method to differentiate between flax and hemp. Flax fibres have a larger surface area because of the 7-sided peak conformation of the individual fibres in a bundle while hemp fibres are characterized by a polygonal shape with rounded peaks. This may be the reason for the greater susceptibility of the flax fibres to enzymes because the structure allows greater accessibility for the enzymes [38].

The prevailing view, based on studies conducted using model systems such as hexenuronic acids [42], sugars [33] and fruit cell walls [41] is that the rate of degradation of these components depended on the composition of the material. The data presented in this research clearly demonstrate that the modification of natural fibres using enzymes depends both on the chemical composition and geometrical orientation of the individual fibres within a bundle.

Flax fibres are characterized by many contours (roughness) within each bundle and hence a larger surface area compared to the hemp fibres. The size of the enzymes is at least four orders of magnitude smaller than that of an individual bundle and at least ten times smaller than a fibril. It is reasonable to expect unencumbered access to the crevices present on the flax fibre surface, resulting in greater reactivity. Based on this observation, flax fibres may be considered a better option for enzymatic treatments to produce natural fibres suitable for composite materials.

At this moment, there is no clear consensus about the potential of improved hydrophobicity correlating to better adhesion with polyolefin resins. It is hypothesized that by increasing the contact angle of treated fibres, there is a better compatibility between the resin and fibre. In the end, this should correspond to improved stress transfer within the composite material and better properties.

3.3.5 Thermogravimetric Analysis

Figure 3.3 shows a typical set of thermographs expressed as the derivative weight lost function for xylanase + cellulase treated hemp. Two regions of significance are observed. The first region of degradation (200 to 265 °C) corresponds to hemicellulose degradation and the second region (265 to 400 °C) corresponds to cellulose degradation [43]. Tajvidi and Takemura (2008) [44] reported that lignin is the most thermally resistant, cellulose being the intermediate and hemicellulose the least stable.

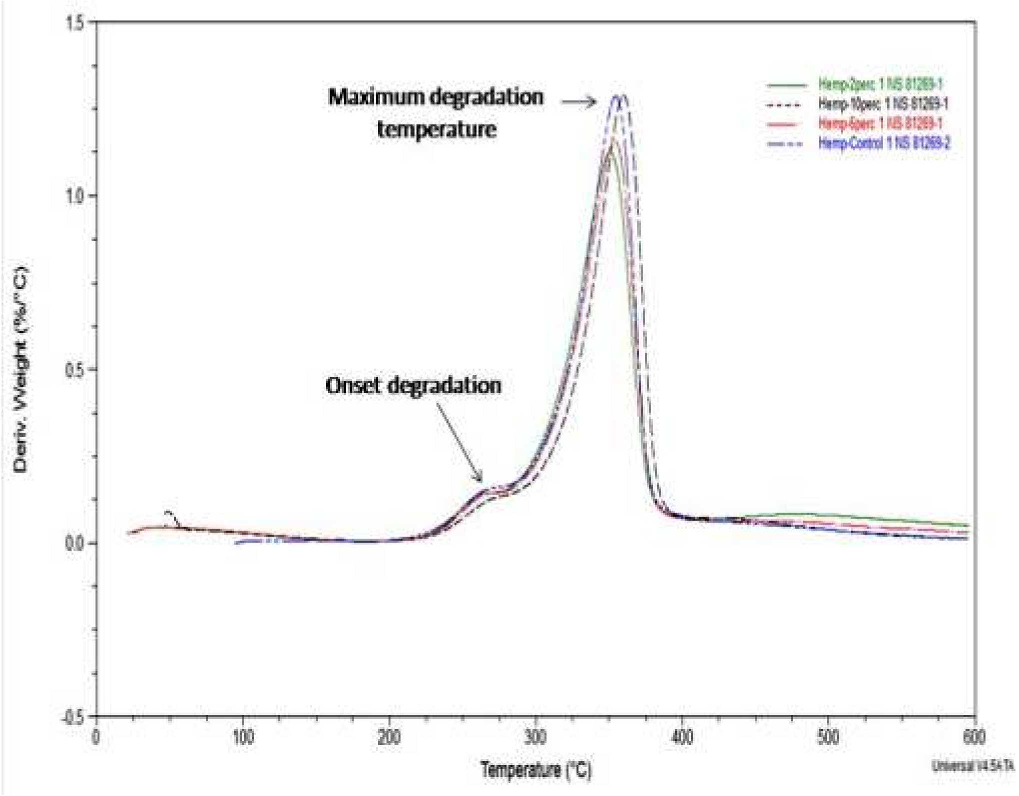

Figure 3.3 Derivative weight loss of xylanase + cellulase treated hemp at three different concentrations depicting the different parameters measured.

Results for the different enzymatic treatments are represented in Table 3.7. The parameters presented included the percentage degradation (% weight), which corresponds to the weight lost from the onset degradation temperature (≈ 260 °C) to 400 °C. The max degradation temperature is the maximum of the derivative weight curve as illustrated in Figure 3.3.

Table 3.7 Thermal properties for different treated samples (n=3, $p<0.05$)

		Parameter		
Fibre	System	% Degradation [a] (% wt.)	Onset degradation [b] (°C)	Max. degradation temperature [c] (°C)
Hemp	Control	62.4 ± 0.08	275 ± 0.16	355 ± 0.52
	Xylanase + cellulase	58.3 ± 0.64	277 ± 0.52	351 ± 0.31
	Xylanase	54.8 ± 1.34	275 ± 1.05	346 ± 1.04
	Polygalacturonase (PG)	68.9 ± 1.36	270 ± 0.06	365 ± 0.50
	Pectinmethylesterase(PME)	60.9 ± 0.21	275 ± 0.00	355 ± 0.53
	Laccase	59.8 ± 1.26	275 ± 1.24	348 ± 1.24
Flax	Control	65.9 ± 0.78	266 ± 0.00	364 ± 0.97
	Xylanase + cellulase	68.8 ± 0.71	272 ± 0.48	366 ± 0.48
	Xylanase	70.6 ± 2.39	268 ± 1.05	369 ± 0.28
	Polygalacturonase	67.0 ± 4.29	267 ± 6.58	362 ± 0.91
	Pectinmethylesterase	64.6 ± 5.47	267 ± 3.37	362 ± 2.88
	Laccase	66.5 ± 0.62	264 ± 0.40	362 ± 0.40

[a] When compared to the control, all systems were significantly different at ($p<0.05$), except flax treated with PG and PME.

[b] For enzyme treated hemp, only xylanase + cellulase and PG were significantly different at ($p<0.05$)

[c] Laccase, xylanase, xylanase + cellulase treated hemp and laccase, xylanase treated flax were significantly different from the control at ($p<0.05$).

Hemp fibres treated with laccase were characterized by a decrease in percentage degradation while no changes in the onset of degradation were observed. The maximum degradation temperature was lowered because the removal of lignin bonded cellulose. Laccase is responsible for removing the lignin fraction of the fibres, thereby exposing the less stable hemicellulose and cellulose. This explains the increase in degradation of the flax treated samples, but was contrary to the hemp treated samples for the same enzyme. This may be possible because hemp has a greater amount of cellulose and less hemicellulose than the flax fibre. Removal of the lignin exposed the cellulosic network which is more resistant than hemicellulose. Hemp treated with pectinmethylesterase (PME), xylanase and xylanase + cellulase exhibited an improvement in thermal degradation. This is expected given that these enzymes removed the hemicellulosic fraction of the fibre. Specifically, PME removed the pectic content while xylanase degraded the xylan backbone of the hemicellulose structure. There was a decrease in the maximum degradation temperature for the hemp samples when compared to the control because the components removed resulted in a weaker network because off disruptions in bonding within the network. Hemp fibres treated with polygalacturonase were characterized by lower thermal stability as indicated by the increase in percentage degradation. This can be attributed to the removal of polygalacturonan which weakens the primary cell wall, ultimately leading to a weaker macrostructure [34].

Flax fibres treated with xylanase + cellulase were characterized by lower thermal resistance when compared to the control experiments. Flax fibres typically have more hemicellulose than hemp fibres and therefore the removal of xylan resulted in a weaker structure (Table 3.2). Further evidence in support to this explanation was the increased maximum degradation temperature which could be attributed to the removal of the least stable hemicellulosic fraction. Flax fibres treated with laccase were characterized by a lower maximum degradation and onset of degradation temperatures. The removal of lignin located in the interstitial spaces of cellulose and hemicellulose is known to act as an adhesive holding the fibrils network together the network together [45]. Laccase removes lignin, which correlated to a decrease in these properties because the structure is less rigid. In summary, enzymes can be used to selectively degrade components that limit thermal stability. The pectinases had no significant ($p<0.05$) effect on the thermal properties of the flax fibres and will not be discussed further.

To our best knowledge, this is the first report detailing the use of enzymatic treatments to improve the thermal properties of natural fibres for composite applications.

3.4 CONCLUSION

Enzymes can be effectively used to remove the hygroscopic pectic and hemicellulose material producing a more homogenous fibre surfaces with improved thermal properties. From a structural standpoint, the removal of these components resulted in an increase in individual bundle exposure, as observed from the SEM micrographs. The ultrastruture of flax fibres allows for more efficient enzymatic treatment compared to the hemp fibres making it a superior feedstock option for reinforcing polymeric matrices. Enzymes can be used to address the limited thermal stability of natural fibres by selectively removing the least stable pectic and hemicellulosic content. In summary, enzymatic treatment of natural fibres such as hemp and flax can be used to improve both surface and thermal properties for subsequent modifications and incorporation in polymeric matrices.

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CHAPTER 4.

Chemical enhancement of hemp fibres using sulfonic acid derivatives: reaction chemistry, surface and thermal characterization[3]

4.0 RATIONALE

The main object of this study was to investigate the effect of two sulfonic acid derivative systems on the thermal and surface properties of hemp fibres. The end goal is to improve these properties to render these fibres water resistant and thermally stable for composite applications. In the literature many chemical methods have been investigated, but in most cases large amounts of waste and hazardous conditions are used. In this research chapter, we present a system where organic solvents were avoided when possible, the reaction temperature was kept below 50 °C, and the chemical reagent was used in an aqueous phase.

4.1 INTRODUCTION

The uncertainty surrounding oils prices, its dwindling reserves, along with increased environmental concerns have resulted in renewed attention to the development of new eco-friendly products and materials. The use of lignocellulosic natural fibres as fillers for matrices offers an opportunity to transition from glass fibre reinforced composites. Natural fibres are advantageous due to their biodegradability, low density, low abrasion on processing equipment, and comparable specific mechanical properties to glass fibre reinforced composites [1]. Despite these properties, incompatibility with non-polar matrices, moisture absorption, and vulnerability to thermal degradation are the main bottlenecks for the use of natural fibres in composites. The incompatibility and moisture absorption defects results from the highly polar surface of the fibres. This in turn leads to poor composite properties because of poor stress transfer and swelling of composite macrostructure. The modification of natural fibres to circumvent these problems, enabling the utilization of these abundant and biodegradable feedstocks.

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[3] A version of this chapter has been previously published: **Michael George**, Paolo Mussone and David Bressler, Carbohydrate Polymers 134 (2015), 230-239

The properties of composites are influenced by the fibre architecture (aspect ratio and orientation of fibres), fibre-matrix interface, fibre modification, and the processing technique used [2, 3]. Lei *et al*. (2012) [4] using a 3D tension - shear chain illustrated that aside from the volume fraction, shape, and orientation of the reinforcements, the distribution of fibers also played a significant role in the mechanical properties of unidirectional composites. It was their opinion that the stair wise model (fibre assembly in nature) is more applicable for composites formulation compared to random staggering. Their work may provide useful guidelines and insights for the design of high performance short fibre reinforced composites in the near future. The effect of the fibre matrix interface is of utmost importance because it facilitates the transfer of stress evenly within the composite. This allows the structure of the composites to withstand the effect of the load without failing. In order to induce better compatibility, researchers have focused on modifying natural fibres to render them less hydrophilic by using physical [5], chemical [6], or biological methods [7]. The processing technique used to combine the fibres with matrices depend on the nature of the polymer (thermoplastic or thermoset), the end application of the composites, and the desired relative volume of fibre to be used. Resin transfer molding [8, 9] and injection molding [10] are the popular methods for processing thermosets and thermoplastics, respectively. With those thoughts in mind, one can appreciate the intricacies associated with improving the properties of natural fibre composites.

The chemical composition and structural assembly of natural fibres dictates the extent of their potential modification. Plant fibres are composed mainly of cellulose, hemicellulose, and lignin. Minor components such as waxes and pectin are regarded as surface impurities. The chemical composition, including moisture content, can help to determine properties and end uses of the fibres. For instant, higher cellulose content correlates to high strength and stiffness and is ideal for polymer reinforcement. Cellulose is considered the most important component of plant fibres with relevance to the composite world [11, 12]. The chemical structure of cellulose contains three hydroxyl groups, which in the macromolecular cellulose network forms hydrogen bonds. Two of these hydroxyl groups form intermolecular bonds, while the third forms intramolecular hydrogen bonds [13]. The ultimate aim is to modify these hydroxyl groups so as to reduce the surface polarity without affecting the strength of the fibre network.

Researchers in the past have focused on grafting different chemicals onto the surface of plant fibres. The main aim was to reduce surface polarity by chemically modifying the hydroxyl groups. Bledzki *et al*. (2008) [14] studied acetylation of flax fibres and its polypropylene composites. It was found that there were improvements in the water absorption properties along with the tensile and flexural strength of the composites. In another study, the effect of acetylation and propionylation on the surface properties of hemp and flax were studied [15]. Acetic and propionic anhydride were used to esterify the surface of the fibres. Their findings included a reduction in moisture absorption of the fibres, a smoother surface, and importantly, they found that the amount of hemicellulose relative to lignin dictated the extent of esterification. These studies served to show that chemical modifications have been successful in the past in addressing the key limitations of fibres for composite applications.

As documented in the previous paragraph, significant work has been done on understanding different chemical methods for fibre derivatization with focus on mechanisms and reaction kinetics. But these chemical methods in most cases require large quantities of solvents and produce hazardous chemical waste. The present study seeks to investigate the effect of derivatives of sulfonic acids on the surface and macro properties of hemp fibres. The sulfonic acids used are soluble in water, require mild reaction temperatures, and were studied at very low concentrations. Hemp fibres are obtained from the bast of sativa plants and are associated with high tensile and flexural properties. The derivative sulfonic acids (aniline-2-sulfonic acid- A2S and 4-aminotoulene-3-sulfonic acid- AT3S) were modeled using the reaction conditions outlined in the communications cited in the previous paragraphs. Specifically, the derivative sulfonic acids were used to produce acid chlorides that were subsequently used to modify the hydroxyl groups of the fibres. The reaction mechanism (as proposed in section 4.3.0) parallels an acetylation mechanism. A2S and AT3S have benzene rings and methyl additions, both of which serves to improve the surface properties for resin reinforcement.

4.2 EXPERIMENTAL

4.2.1 Materials

Aniline-2-sulfonic acid [A2S] (173.19 g/mol, 99 %) and 4-aminotoulene-3-sulfonic acid [AT3S] (187.22 g/mol, 99 %) were sourced from Sigma (Minneapolis, U.S.A). Acetone (58.08 g/mol, 99.9 %), anhydrous ethanol (Mol. wt. 46.07 g/mol) and sulfuric acid (98.08 g/mol, 99.99 %) used were obtained from Sigma

(Minneapolis, U.S.A). Hydrochloric acid (36.46 g/mol, 37 %), anhydrous sodium hydroxide pellets (40.00 g/mol, 98 %) and phenolphthalein solution (2 % in ethanol, 318.32 g/mol) were purchased from Sigma (Minneapolis, U.S.A). Distilled water was used for experiments.

4.2.2 Preparation of acid chlorides

Prior to all analyses, samples were pre-conditioned at 80 °C for 5 hours to maintain constant moisture content for all treated and untreated samples. All analyses were done in triplicate. An established and proven procedure outlined by Youngs *et al*. (1958) [28] was used to convert the sulfonic acids into the corresponding acyl chlorides. The main modification to the method was substituting phosphorus pentachloride with thionyl chloride. Approximately 4.2 g (0.035 mol) of thionyl chloride was added to each reaction flask gradually over a 30 minutes period.

4.2.3 Chemical treatment

A 250 mL round bottom flask was used for all the reactions. Approximately 1.0 g of hemp fibres (detergent washed and oven dried) was placed in a flask and 100 mL of the maximum sulfonic acid soluble in water (0.01 M) was added to the flask slowly. A few drops of pyridine were added during the course of the reaction. The flask was heated to the desired temperature and the reaction maintained for 90 minutes. Reaction temperatures of 30, 40, and 50 °C were studied.

In preliminary reactions, the treated fibres were Soxhlet extracted with ethanol for 2 hours to remove un-reacted reagents. Fibres were also washed with the solvents and the results indicated little or no difference between Soxhlet extracted and solvent rinsed fibres. Hence, fibres were washed instead of Soxhlet extracted onwards. The samples were washed sequentially with 25 mL ethanol and 25 mL acetone to remove excess reagents. The fibres were oven dried at 80 °C for 5 hours and stored for future analysis. Apart from the washing step, organic solvents were avoided as the main aim is to utilize the system in an aqueous medium.

4.2.4 Characterization of hemp fibres

4.2.4.1 Degree of substitution

The grafting of the sulfonic acid chlorides structures onto the fibre surfaces was monitored via tracking the degree of substitution of the hydroxyl groups. This method involved alkaline hydrolysis (0.5 M NaOH) of ester bonds and quantification of excess hydroxide ions using a back titration. The method used had to be optimized because the method was predominantly used for pulp samples rather than natural fibres. Natural fibres are different both in composition and surface geometry. It was found from preliminary experiments, hydrolysis at 30 °C yielded reproducible results. As a result, this temperature was used for all hydrolysis thereafter.

Approximately 0.1 g of hemp fibre was used for all analyses. The hydrolysis parameters as well as the volumetric method were adopted from the work of Freire *et al.* (2005) [16]. Following alkaline hydrolysis, the excess NaOH was back-titrated using 0.5 M HCl. Then 0.4 mL excess HCl was added to each sample and left overnight to settle. This was then titrated with 0.5 M NaOH. The degree of substitution was calculated using the formula provided by Freire *et al.* (2005) [16].

4.2.4.2 Fourier Transform Infra-Red Spectroscopy

Transmission Fourier transform infrared spectrophotometry (FTIR) was performed using a Shimadzu 8400 S, taking 100 scans for each samples with a resolution of 4 cm^{-1}. Three replicates were taken from each sample set for analysis. The fibres were pounded in a mortar then added to 150 mg of potassium bromide (KBr). Fibres and KBR were carefully dried before disk preparation and were subjected to FTIR analysis immediately. The spectra obtained were altered by smoothing by a factor of 20, to improve the sharpness of the peaks. All spectra were reproducible for each sample.

4.2.4.3 Morphological characterization

Micrographs of fibre surfaces of untreated and chemically treated fibres were taken using a Hitachi S-2700 Scanning Electron Microscope (SEM) equipped with a Princeton Gamma Tech (PGT) IMIX digital imaging system and a Prism Intrinsic Germanium (IG) detector. A gold putter coater was used to induce conductivity for all samples. A resolution of 4 nm was used for all samples. 0.01 ± 0.005 g of fibre was mounted on conductive adhesive tape, sputtered coated with gold palladium and observed using a voltage of 15 – 20 kV [17].

4.2.4.4 X-ray Photoelectron Spectroscopy (XPS)

X-ray photoelectron spectra of the fibre surfaces were obtained using a Kratos Ultra 165 X-ray Photoelectron Spectrometer with energy spectra ranging from 0 – 1000 eV. XPS was used to study the abundance of different carbon functionality and the O/C ratio of the surface of the different fibres following treatment. High and low resolution scans were needed for analysis. Approximately 0.05 ± 0.005 g of fibre was pressed into a disc with diameter of about 0.5mm for analysis. The analyzed area was 300 ×300 µm. At least 6 different spots were analyzed for each sample. Data treatment was performed using the CasaXPS program (Casa Software Ltd., UK). Mole fractions were calculated using normalized peaks based on acquisition parameters after a linear background subtraction and consideration of experimental sensitivity factors. C1s spectra were analyzed with a Gaussian product function, by maintaining the FWHMs of all components to within the range of 1.200 to 1.600 [15, 18].

4.2.4.5 Time of Flight Secondary Ion Mass Spectroscopy

Mass spectroscopy was used to study the chemical information obtained from the samples by measuring the molecular weight of the different fragments produced when the sample was sputtered with fast ions. A ToF SIMS IV (Ion ToF GmbH) instrument equipped with a reflectron ToF analyzer was used for all samples. The primary ion beam used was a 20 keV $^{69}Ga^{+}$ ion beam rastered over an analysis area of 0.5 mm by 0.5 mm at a rate of 5 kHz. The beam current used was 0.4-0.6 nA and the pulse width 25-30 nm which resulted in primary ion dose within the static SIMS regime. Operating pressure of 10^{-9} mbar was maintained during all experiments. Mass range of 0-400 m/z was recorded, but only 0-320 m/z was presented because the ions of interest fall within this range.

4.2.4.6 Force Tensiometry/Contact angle measurement

A 700 Sigma OneAttension tensiometer was used to measure surface polarity for the different systems. The instrument was operated in the contact angle mode and the surface specific properties needed for hemp and flax were taken from Aranberi *et al*. (2003) and Baley *et al*. (2006) [19, 20]. All samples were pre-conditioned at 80 °C for 3 hours prior to analysis. The method outlined here closely follows the one mentioned in an earlier publication from our group [18]. Washburn or capillary rise method was used to characterize the fibre bundles. This technique involved measuring the weight gain due to fibre penetration into the test liquid (distilled water) and the force exerted on the fibre during immersion.

For each experiment, 6 fibres were done for each sample with three cycles of immersion (three measurements taken on a single fibre). This was done so as to maintain the reproducibility given the complex and heterogeneous nature of natural fibres. A force detection of 1.0 mN/mm and an immersion depth of 1 mm were used for all analysis. The first 0.02 mm of each fibre was ignored because the edges of the fibres are not spherical and can influence the results. An immersion speed of 5mm/min was used for both advancing (penetration of fibre into liquid) and receding measurements (removal of fibre from test liquid). Given the nature of the technique, both advancing and receding contact angles were calculated for each cycle. Equation 1 was used to estimate the contact angle for each sample.

$$\text{Wetting force} = \Upsilon\text{I}\nu * \text{P} * \cos\theta \qquad \text{Equation 1.}$$

Where $\boldsymbol{\Upsilon\text{I}\nu}$ is the liquid surface tension, **P** the perimeter of the probe used to hold the fibres and $\boldsymbol{\theta}$ is the unknown contact angle. Given that the wetting force is measured per depth of immersion, the contact angle for each sample can be calculated.

4.2.4.7 Thermogravimetric Analysis (TGA)

The influence of the thermal stability of the treated fibres was evaluated using thermal gravimetric analysis. The percentage degradation (between 200–300 °C) and the initial degradation temperature were calculated from the thermo graphs obtained. All experiments were conducted using a Thermal Analysis Instruments TGA Q50 apparatus under a flow of nitrogen to study the effects of heating on stability of the different treated natural fibres. Platinum pans were used given the high temperatures and the ease of cleaning. The temperature range selected was from room temperature to 600 °C at a rate of 20 °C per minute. For each sample, triplicate runs were done. All results were reproduced to 5% error or better.

4.2.5 Statistical analysis

Experiments were replicated at least three times and results were expressed as mean value ± standard deviation. The statistical analyses of the data were conducted using the statistical software package SAS Version 9.4 and Microsoft Excel 2013 embedded T-test. T-test (one tailed, comparison of group variance) was used for data that was normally distributed (tested the variance) and for those systems that were not, to identify significant differences the Kruskal Wallis Test was applied to the data populations involved, with a 95 % confidence level ($P < 0.05$).

4.3 RESULTS AND DISCUSSION

4.3.1 Proposed Reaction Mechanism

The hypothetical reaction mechanism for both A2S and AT3S when reacted with hemp fibre surfaces plausibly follows a methylation scheme. The proposed reaction schemes are highlighted in Figures 4.1 and 4.2.

Pyridine was used as proton acceptor to drive the reaction forward [21, 22]. Also, possible side reactions between the amine ($-NH_2$) group and the chloride group (produced from acyl chloride) were minimized using pyridine to quench any radicals or reactive species formed. Also, as mentioned before, low concentrations (0.01M) of the acids were used to limit any side reactions. Soxhlet extraction was used to remove any residual unreactive species from the fibre samples. FTIR confirmed the chemical changes and will be discussed in section 4.3.3. The proposed reaction involved the reaction of an acid chloride with a hydroxyl or alkoxide ion. Hemp fibres were initially soaked in 5 % (w/v) NaOH for 30 minutes prior to reaction with the acid chloride (fibres were washed and neutralized to pH 7 prior to converting to acid chloride). An explanation of the plausible mechanism of the acid chlorides with the fibre surfaces is given in Figure 4.3.

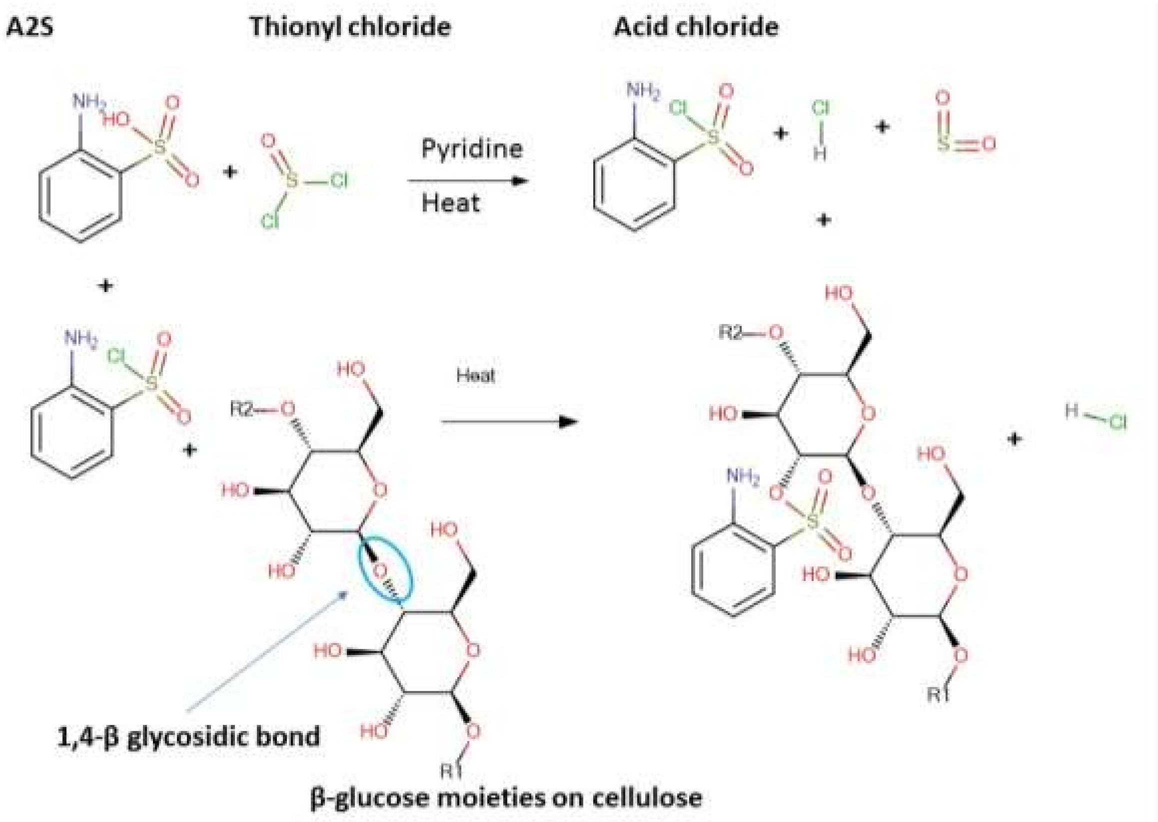

Figure 4.1 Proposed reaction scheme for aniline -2- sulfonic acid (A2S) with the hydroxyl groups on the surface of the hemp fibres.

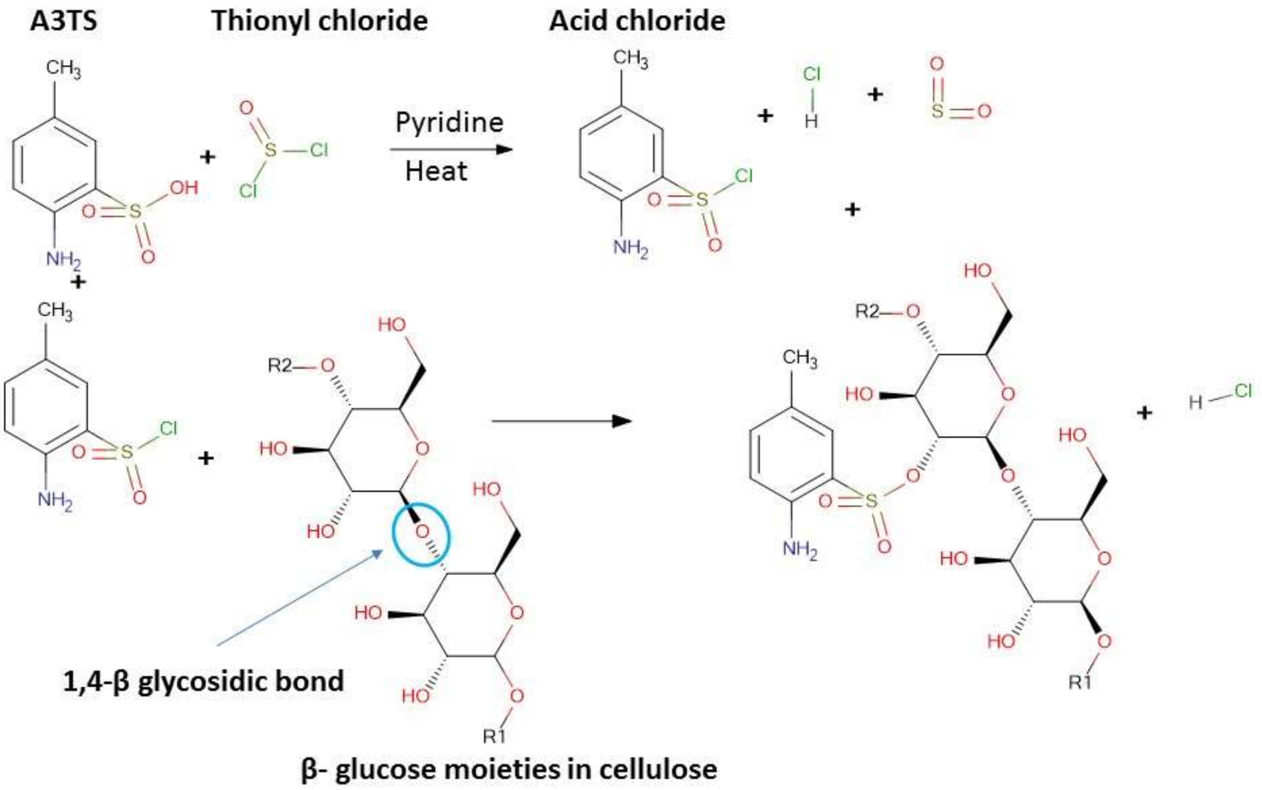

Figure 4.2 Proposed reaction for 4-aminotoulene-3-sulfonic acid and the hemp fibre hydroxyl groups.

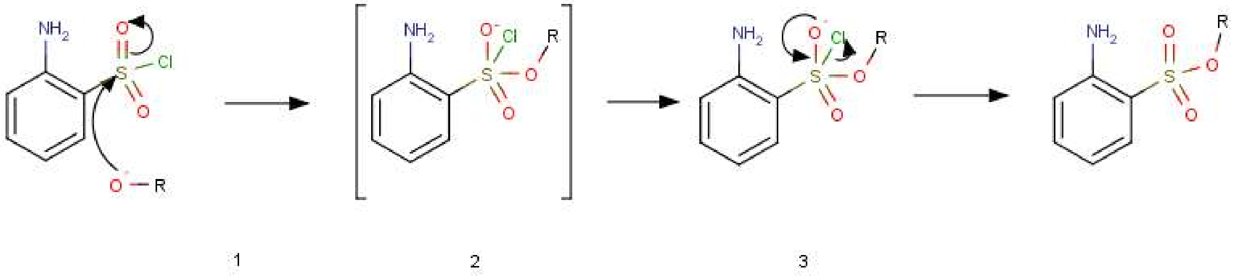

Figure 4.3 Detailed proposed reaction mechanism for the reaction of interest: reacting acid chlorides (A2S) with the hemp fibres (R-O$^-$ represents the alkoxide ion of a surface group on hemp fibres).

The hydroxyl groups that can be possibly modified on each glucose residue of the cellulosic chain are located on carbons 2, 3, and 6 [7]. In the schemes in Figure 4.1 and 4.2, the hydroxyl group on carbon 2 is reacted. These carbons are accessible because they are not involved in any bonding within the native cellulosic network of the hemp fibres. The reaction follows the fundamental steps of an esterification reaction. At the onset of the reaction, the sulfur atom in AT2 or AT3S is fairly positive as the oxygen atoms attached are more electronegative and pulls electron density away from sulfur. In step 1 of the reaction, there is a nucleophilic attack on the fairly positive sulfur atom by one of the lone electron pairs on the oxygen of a hydroxyl groups or alkoxide atom located on the hemp surface. The third stage (elimination phase) results in the collapse of the oxygen atom reforming a stable double bond and pushing off the chloride ion. The resulting product is a sulfonic derivative of the hemp fibres bonded via an esterification reaction. The main bonds contributing to product formation were evident when the samples were studied using FTIR.

4.3.2 Degree of substitution

The degree of substitution of the different acids onto the surface of the hemp fibres was characterized by a titrimetric method. Reaction temperature was varied to study the thermodynamics of the reaction. The maximum soluble acid in water was used so as to avoid using organic solvents. This was one of the main objective because organic solvents are relatively expensive, harmful to the environment, and not readily recycled. Figure 4.4 depicts the influence of temperature and the effect of the different acids on the degree of substitution.

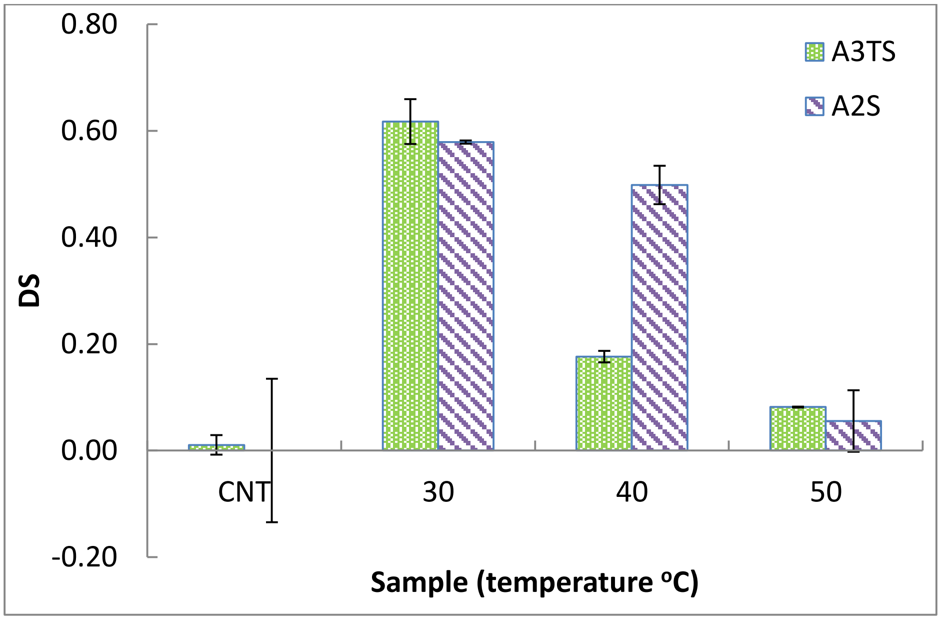

Figure 4.4 Influence of reaction temperature and nature of sulfonic acid on the degree of substitution (DS) on the hemp fibre surfaces. A2S, aniline-2-sulfonic acid and A3TS, 4- aminotoluene-3- sulfonic acid.

The significant differences ($p<0.05$) found at the different temperatures for both systems may plausibly be explained by changes that the fibre macrostructure undergoes at higher temperatures or the rearrangement of the lignin found on the surface of hemp fibres. Interestingly, with an increase in temperature, the degree of substitution or the extent of reaction diminished significantly. In fact, for both acids, 30 °C was the optimum temperature. This is a very advantageous temperature for enhancing fibres into materials suitable for composite applications. Tserki *et al.* (2005) [15] acetylated the surfaces of different natural fibres using acetic or propionic anhydride and found that the best yields were obtained with a temperature of 120 °C for at least 120 minutes, depending on the fibre type. They reported the high lignin contents in wood indirectly resulted in a higher degree of substitutions. Similarly, Rodwell (2004) [23] reviewed the literature and found that in the absence of a catalyst or cosolvent, the acetylation of lignocellulosic material with acetic anhydride would proceed at reaction temperatures between 120 and 160 °C. According to that study, a small quantity of acetic acid was needed in the reaction mixture to swell the cell wall of the material to initiate the reaction. From these studies, it is clear that the esterification required a high reaction temperature and initial macrostructure swelling, but the process outlined in this chapter proceeds at temperatures just above room temperature and with little or no swelling.

4.3.3 Fourier transform infrared spectrophotometry (FTIR)

The IR spectrum of the unmodified fibres along with the spectra of the two chemically treated hemp fibre systems is presented in Figure 4.5. Evidence for the reaction schemes outlined previously in section 4.3.2 are confirmed by the emergence of a new organic sulfonate stretch at 1110-1120 cm^{-1} and a peak at 740-770 cm^{-1} characteristic of ortho disubstitituted benzene fractions. In addition, both of the sulfonic acids used were primary amines and the appearance of a peak at approximately 1550-1560 cm^{-1} confirmed the presence of this functionality. The peaks were identified using the National Institute of Standards and Technology database. The chemistry of the method outlined here is different when compared to established methods of acetylation such as acetic anhydride or fatty acid chlorides. Frisoni *et al.* (2001) [24] showed that acetic anhydride in the presence of sulfuric acid catalyst can be used to esterify the surface of steam exploded flax fibres. The chemistry of this system involved the emergence of peaks corresponding to the carbonyl functionality (C=O) at 1745 cm^{-1} and an alkyl stretch ($C-CH_3$) at 1375 cm^{-1}. On the other hand, Freire *et al.* (2005) [15] investigated the effect of modifying cellulose using fatty acids and found that the optimum systems were characterized with the emergence of an ester carbonyl band at 1735-1750 cm^{-1} and an increase in the intensity of the C-H band at 2800-2950 cm^{-1}. The changes in chemical functionality as a result of the type of reagent used in the previous studies were confirmed using infra-red spectroscopy. Similarly, in this communication, the changes were clearly highlighted and explained.

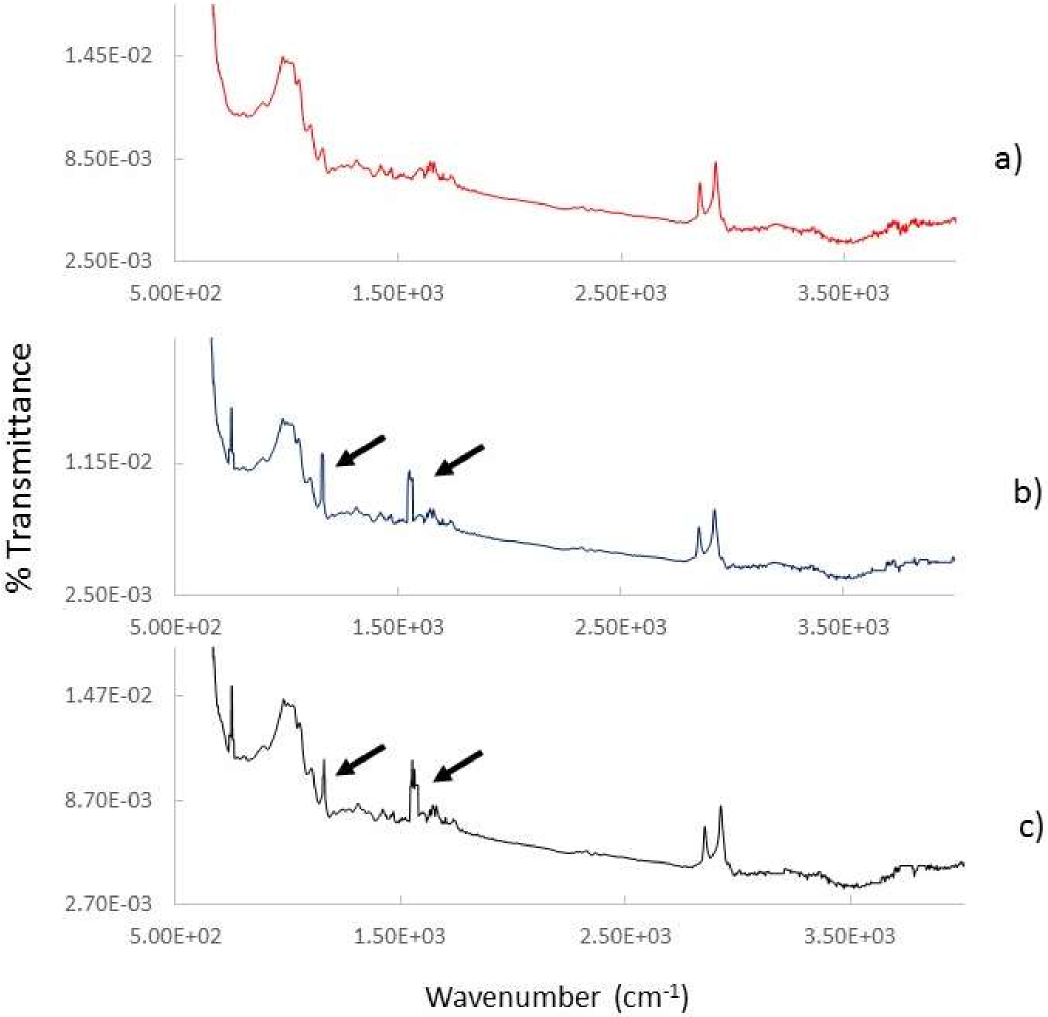

Figure 4.5 IR spectra for unmodified (a), A2S (b), and AT3S (c) treated hemp fibres.

4.3.3 Morphological characterization

Scanning electron micrographs of unmodified and sulfonic acid treated fibres are shown in Figure 4.6. The surfaces of the treated fibre seemed to be void of overlying material which resulted in a smoother looking surface. On the unmodified fibre an unevenly distributed layer is observed along the fibre surface. Upon modification, this layer is partially or totally removed. Tserki *et al.* (2005) [15] in their study of the effect of acetylation using acetic anhydride on natural fibres mentioned that this layer may be primarily due to waxy substances on the fibre surface. The replacement of hydroxyl groups with the sulfonic acid derivatives could plausibly explain the reduction in surface roughness of the hemp fibres after treatment. Additionally, previous publications from our team has shown the action of caustic (NaOH) can lead to partial removal of waxy compounds and hemicellulosic material [18]. Also, the action of the acid chlorides plausibly also contributed to "cleaning" the surfaces of the hemp fibres.

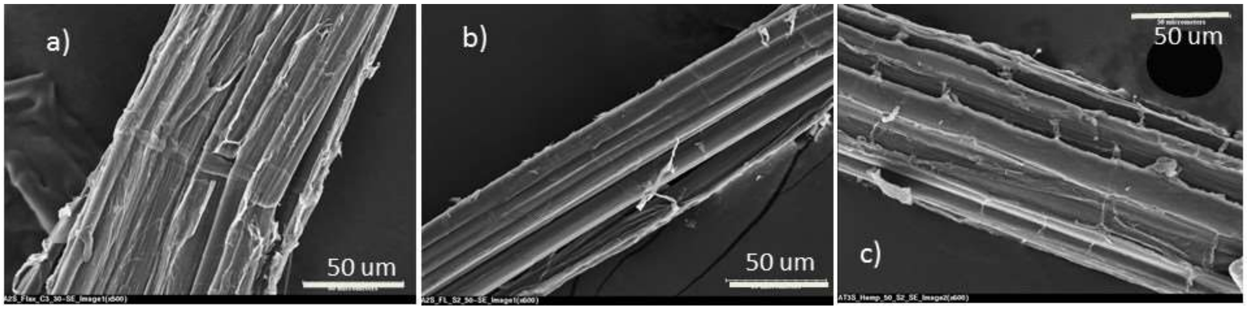

Figure 4.6 Scanning electron micrographs for unmodified (a), A2S (b), and AT3S (c) treated hemp fibres.

4.3.4 X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy was used to determine the abundance of the different carbon signatures for each of the samples. The parent peak was partitioned into its different carbon signatures, depending on the binding energy. The binding energies quoted by Zafeiropoulos *et al.* (2003) [25] in their study that investigated the influence of surface treatments on flax were used. The major signatures studied were C-C to alkyl stretches (285 eV), C-O (286.2-286.6 eV), C-C-O (286.7-287.2 eV), and O-C-O (287.3-288.1 eV). Table 4.1 summarizes the data obtained using XPS and shows the changes in atomic percentage of carbon involved in different bonds and the corresponding O/C ratio for each sample. All analyses involved fitting the carbon peak at 285 eV using Gaussian/Lorentzian functions. Data analysis was done by measuring the areas under the curves (O and C) and computing the O/C ratios for each system.

Table 4.1 Influence of chemical treatment on the abundance of the different carbon species and the corresponding O/C ratio.

Sample	Relative abundance of different signatures (%)				O/C[1]
	C-C	C-O	C-C-O	O-C-O	
A2S treated hemp					
Untreated Control	34.5 ± 2.36	43.9 ± 1.61	17.8 ± 0.24	3.84 ± 0.51	0.198 ± 0.001^a
30 °C	34.4 ± 2.37	45.5 ± 0.92	14.6 ± 0.87	5.54 ± 1.68	0.174 ± 0.004^b
40 °C	38.6 ± 2.14	38.4 ± 1.73	17.0 ± 0.78	6.06 ± 1.00	0.181 ± 0.012^b
50 °C	32.5 ± 1.03	42.4 ± 0.41	18.3 ± 1.70	6.75 ± 0.25	0.215 ± 0.010^a
AT3S treated hemp					
Untreated Control	37.71 ± 1.91	30.8 ± 3.71	25.3 ± 5.63	5.98 ± 0.41	0.201 ± 0.021^a
30 °C	36.50 ± 3.37	40.4 ± 2.21	16.1 ± 1.91	6.96 ± 0.40	0.179 ± 0.006^b
40 °C	36.31 ± 2.32	33.6 ± 3.08	23.0 ± 2.56	7.08 ± 0.70	0.193 ± 0.011^b
50 °C	35.22 ± 3.13	45.5 ± 0.55	16.0 ± 0.57	3.35 ± 1.99	0.215 ± 0.000^a

[1] Values with the same letters are not significantly different at $p<0.05$. The two acid systems were not compared.

Hemp fibres treated with A2S were characterized with a significant ($p<0.05$) decrease in the O/C ratio for treatments done at 30 and 40 °C. The grafting of the hydrophobic benzene ring associated with the reagent coupled with the action of the base plausibly contributed to this reduction in oxygen moiety on the surface. Also, the removal of waxy component from the surface resulted in the exposure of the cellulose network. Hemp fibres treated at 30 °C using A2S were characterized with a significant ($p<0.05$) increase in O-C-O but a reduction in C-C-O signature. This observation could have resulted from the synergistic effect of caustic couple with the acids used. The removal of waxy and hemicellulosic content plausibly resulted in lignin exposure. This is supported by the corresponding reduction in O/C ratio. Contact angle measurements discussed in section 4.3.4 confirmed the reduction in oxygen species on the surface resulted in an increase in hydrophobicity. Also, the introduction of the polar sulfur atoms onto the surface has shown to not affect the polarity of the fibre surfaces. In fact, we found an improvement for this property. One plausible reason for this may be the random, and amorphous distribution of hemicellulose relative to lignin, and heterogeneity of these components on the surface. Also, partial removal of hemicellulose of the fibres may have exposed the hydrophobic lignin content.

Hemp fibres treated with AT3S were characterized by a significant reduction in C-C-O signature. The reasoning for this could be the same as given above for the A2S system. XPS confirmed the changes in chemical carbon signatures for each of the system studied and the effect on the O/C ratio which is a direct measure of polarity.

4.3.5 Time of flight Secondary Ion Mass Spectroscopy

ToF SIMS fragmentation patterns were used to specifically obtain information pertaining to the uppermost 2 nm of the sample surface. SIMS is very sensitive to any minute alteration or contamination on the surface. Hence to detect whether any chemical modification had occurred, this technique was very applicable. The positive ToF SIMS spectra for unmodified and treated hemp samples is given in Figure 4.7.

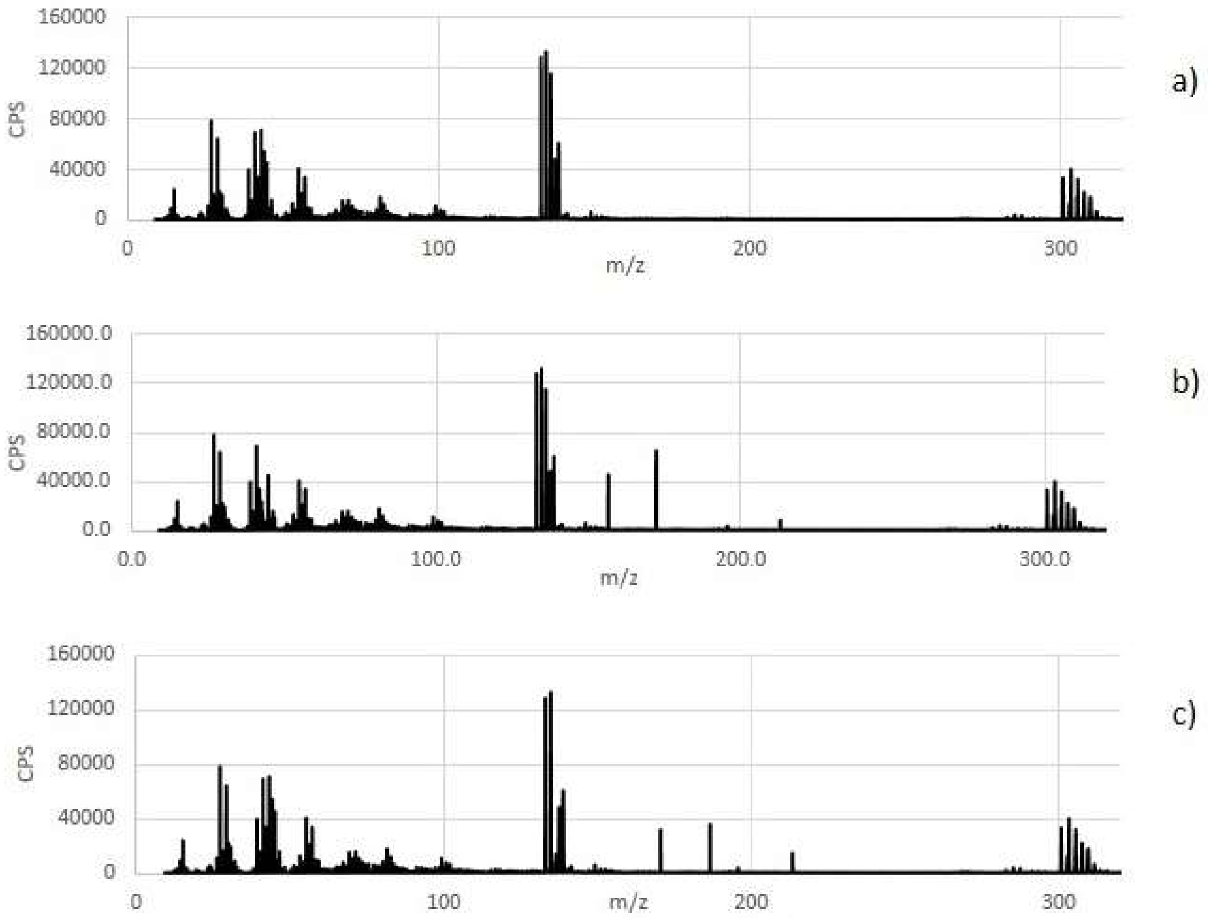

Figure 4.7 Secondary ion mass spectra for a) unmodified, b) A2S and c) AT3S treated hemp fibres

When compared to the control, there were distinguishable differences specifically between 150 – 200 m/z. For the A2S system, emergence of peaks at 156 and 172 amu could possibly be due to the fragments H_2N-(C_6H_4)- SO_2^+ and H_2N-(C_6H_4)- SO_2-O^+, respectively. Likewise for the AT3S treated fibres, peaks at 170 and 186 amu could probably arise from H_2N-C_6H_3 (CH_3)-SO_2^+ and H_2N-C_6H_3 (CH_3)-SO_2-O^+ fragments respectively. Other minor peaks occur around 225 amu, the nature and possible identity of these were not obtained. Clearly it can be seen that the fibre integrity was maintained given that the main peaks for natural fibres found between 0-100 m/z were well preserved. One reason for this was that the experiments were done at temperatures just above room temperature. In summary, the proposed method can be used to selectively target the uppermost exposed layer of the fibre surface for modification.

4.3.6 Force tensiometry/contact angle measurements

Table 4.2 shows the effect of the different chemical treatments on the contact angle of the hemp fibres studied. The advancing contact angle for each system was presented as there was little or no difference between the receding and advancing angles. All chemical systems except the AT3S at 40 °C studied showed significant ($p<0.05$) increase in contact angle. This observation corresponds to an increase in surface hydrophobicity and confirms that the chemical systems imparted changes in chemical functionality onto the surface of the hemp fibres that changed their polarity. The presence of the bulky benzene group on the surface plausibly induced this reduction in polarity. In fact, the positioning of the bulky benzene rings above the hemp fibre surface coupled with the capping of the hydroxyl groups possibly synergistically resulted in the reduction in contact angles observed. In this case, the reaction steric would predominantly result in the benzene ring above the surface instead of being embedded into the fibre network. Interestingly, despite the presence of a number of highly polar moieties inherent to the reagents such as $-NH_2$, -O, and -S, it was found that these chemical groups played a little role in contributing to the polarity. Cantero *et al.* (2003) [26] investigated the relationship between mechanical behavior of natural fibre composites and the surface properties of chemically treated flax fibres. They showed an increase in contact angle of natural flax resulted in improved mechanical properties for the composite materials. Specifically, maleic anhydride grafting resulted in an increase of compatibility of the natural fibres with polypropylene. The heterogeneity of the hemp fibre surface components could result in hydrogen and electrostatic bonding which promote a structure that is much more rigid and moisture resistance. In the next section, it is illustrated how the changes in chemical rigidity induced improvement in the thermal properties of the hemp fibres.

Table 4.2 Influence of the different chemical treatments at different temperatures on the advancing contact angle of hemp fibres.

Sulfonic acid	System	Contact angle
A2S	Untreated Control	88.8 ± 0.16^{b}
	30 °C	89.4 ± 0.14^{a}
	40 °C	89.3 ± 0.18^{a}
	50 °C	89.4 ± 0.16^{a}
A3TS	Untreated Control	88.9 ± 0.13^{b}
	30 °C	89.4 ± 0.08^{a}
	40 °C	89.3 ± 0.28^{ab}
	50 °C	89.5 ± 0.06^{a}

For A2S and AT3S, each system parameter was compared to the control and other temperature treatments within the same group (A2S systems were compared, not A2S vs. AT3S). Results with different superscript letters signify statistical differences at $p<0.05$.

4.3.7 Thermogravimetric analysis

One of the inherent limitations of natural fibres for composite applications is the poor stability below 260 °C. Mulinari *et al.* (2010) [3] alluded to this in their review on the effect of chemical modification on the mechanical and thermal properties of the fibres and composites. The two thermal parameters investigated were initial degradation temperature (temperature at which 10 % of sample degraded) and percentage degradation (degradation between 200-300 °C). The results for the different chemical systems are represented in Table 4.3 and Figure 4.8. Studying the reaction scheme outlined earlier would display a number of functionalities that are readily available for hydrogen bonding (-NH2, -O), which should result in a more rigid macrostructure.

Table 4.3 Thermal properties of the different systems studied. The temperature at which 10% of the sample was degraded (initial degradation temperature).

Reagent	Sample	Start of degradation (°C)
A2S	Untreated Control	266 ± 0.40^{c}
	30 °C	269 ± 0.01^{a}
	40 °C	268 ± 0.24^{b}
	50 °C	268 ± 1.43^{bc}
A3TS	Untreated Control	258 ± 0.30^{b}
	30 °C	258 ± 4.30^{b}
	40 °C	263 ± 1.91^{a}
	50 °C	265 ± 2.86^{a}

For A2S and AT3S, each system parameter was compared to the control and among the groups (A2S systems were compared, not A2S vs. AT3S). Results with different letters signify statistical differences at $p<0.05$.

It should be noted, the heterogeneity of natural fibre components can significantly influence chemical reactions of this sort. Specifically, observation of the controls for A2S and A3TS highlights this challenge. Hence, for each system, comparisons were made within each system and not between the two acid treatments. A2S was found to be more effective when compared to AT3S in improving the thermal properties of hemp fibres. The presence of the $–CH_3$ on the AT3S, could impart different reactivity when compared to the A2S. The general understanding is that the greater the substitution on the benzene ring, the higher chance of steric hindrance becoming a major factor to consider. Hammett (1937) [27] in one of the earliest studies that laid the foundation for understanding the reactivity of benzene rings substituted (meta or para position) and how this influenced the reaction of a group attached to one of these positions provided a few general conclusions that may be very useful to this work. It was found that the temperature and solvent had little or no influence on the reaction yields. In fact, factors such as chain length of the side chain and the dielectric constant of the medium had a significant effect on the products formed, the nature of the path taken to that product, and the yield.

He developed an equation that catered for these factors and concluded that the substituents act by internal electron displacement, hence the preference of reactivity in most cases. In summary, A2S was found to be better suited to this application because AT3S has a $–CH_3$ group that possibly prevented the approach of the sulfonyl chloride to the hydroxyl groups, to allow orbital overlap.

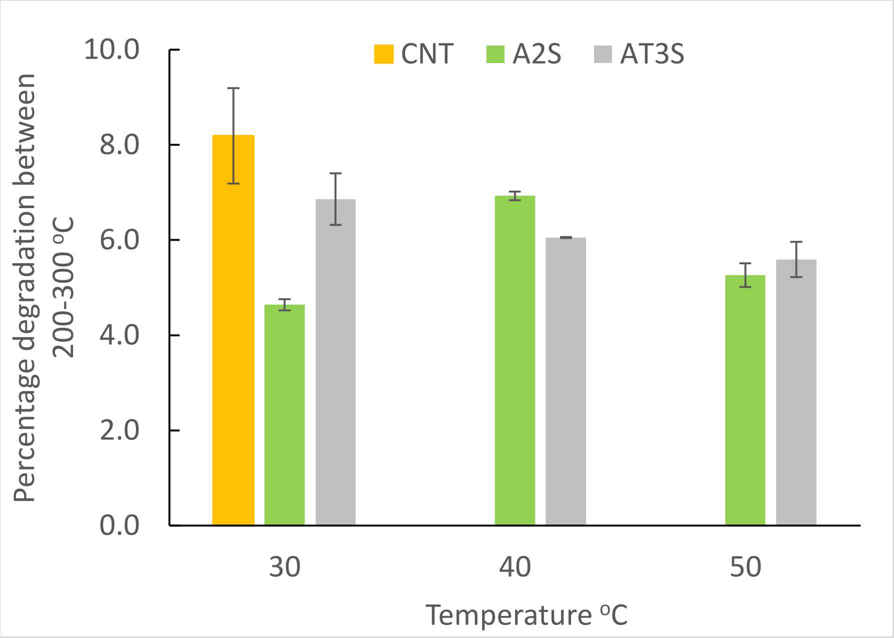

Figure 4.8 Percentage degradation for the different systems (n=3). One control was presented because there was no significant difference amongst controls at different temperatures and for different acids.

Figure 4.8 compared the percentage degradation of the different systems with a control. In terms of the specific systems, hemp fibres treated with A2S at 30 °C were characterized with the least degradation. The presence of the stable benzene rings on the surface led to these observations by rendering the macrostructure of the hemp fibres more rigid. Also, partial removal of surface impurities led to improved thermal stability. Furthermore, these improvements in thermal properties could result from the formation of good bonding between the chemical agents and the fibre surface hydroxyl groups.

4.4 CONCLUSION

Sulfonic acid derivatives can be effectively used to enhance the surface and thermal properties of hemp fibres for composite applications. From a chemistry point of view, grafting of these acid residues that contain bulky benzene rings, resulted in fibres with reduced surface polarity and improved thermal properties. The capping of the hydroxyl groups on the surface and production of a more tightly bonded rigid structure, respectively, led to these key observations. Additionally, the systems described here were optimized to work best at temperatures as low as 30 °C and in an aqueous phase. The use of organic solvents was limited during the reaction. One key finding from the study was that the substitution pattern on the benzene ring influences the effectiveness of the chemical during reaction with the fibre surface hydroxyl groups. A less sterically hindered aniline-2-sulfonic acid treated fibres displayed much more promising results when compared to 4-aminotoluene-3-sulfonic acid treated hemp fibres.

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CHAPTER 5.

Characterization of chemically and enzymatically treated hemp fibres using atomic force microscopy and spectroscopy[4]

5.0 RATIONALE

Natural fibres and other lignocellulosic material enhanced for composites applications have been extensively characterized and reported as changes in chemical composition, functionality, morphology, and structure. In this chapter, we seek to report on the physical and topographical changes associated with modifying hemp fibres. Enzymatic, chemical, and a combination of both were used to prepare the test specimens. AFM was used to investigate the effect of these different treatments on the roughness and adhesion forces of these fibres. These measurements have a direct relationship with predicting the compatibility with polymeric resins.

5.1 INTRODUCTION

Hemp fibres are characterized by low density and manufacturing costs, low abrasion on processing equipment, and comparable specific mechanical properties relative to glass fibre composites [1]. Recent demonstrated applications of hemp fibres in industrial products include applications such as reinforcement of matrices for the automotive and housing industry [1]. Despite these advances, the widespread, large scale utilization of hemp and other natural fibres is limited due to their poor compatibility with industrial petroleum based polymeric matrices [2, 3]. The underlying cause for these challenges is commonly attributed to the surface chemical characteristics of the major components of natural fibres including, cellulose, hemicellulose, and lignin. For each molecule of β- glucose in the complex linear cellulose structure, there are three free hydroxyl groups within and on the surface which contributes to a high surface polarity. This structural arrangement renders natural fibres incompatible with non-polar matrices, resulting in poor stress transfer from the matrix to the fibres and consequently challenges meeting mechanical and impact standard requirements [1]. Additionally, these hydroxyl groups lead to moisture absorption accelerating the composite property degradation

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over time [1]. These limitations can be overcome by protocols such as surface modification including alkylation, benzoylation, and silanation [4-6] and the use of compatabilizers [7]. While these methods have demonstrated in the laboratory and at the industrial scale, limited in depth information is available above the effect of these methods on the surface physical properties and topology. These are critical parameters as the uppermost layer of the fibres is in direct contact with the matrices in composite materials.

The microstructure of hemp fibres consists of the primary cell wall which surrounds the secondary cell wall (S_1, S_2 and S_3) and the middle lamella. This fibrilliar arrangement lends to the high strength and compact nature of the fibres. The primary cell wall consists of cellulose and hemicellulose and constitutes approximately 10 % of the cell's cross-sectional area [8]. A major contribution of the mechanical properties associated with natural fibres results from the high cellulosic content of the secondary cell wall. This model structure sub-divides into three sections namely S_1, S_2, and S_3, with S_2 being the major section. An understanding of how this fibrilliar assembly is affected after fibre modification is of great importance to further improve upon the previous research in this area [8].

Atomic force microscopy (AFM) can be used to acquire an image, determine the surface roughness and adhesion forces of samples. It is commonly known as a Scanning Probe Microscope (SPM). To acquire an image, the SPM raster-scans the probe over a small area of the sample, measuring the local property simultaneously. AFM operates by measuring force between a probe, which is a sharp tip (3-6 um tall pyramid with 15-40nm end radius) and the sample. The lateral resolution of AFM is low (~30 nm) due primarily to convolution, but the vertical resolution can be up to 0.1 nm. As a result, images are acquired generally by measuring the vertical and lateral deflections of the cantilever by using the optical lever. The optical lever operates by reflecting a laser beam off the cantilever. The reflected laser beam strikes a position-sensitive photo-detector consisting of four-segment photo-detector. The differences between the segments of photo-detector of signals indicate the position of the laser spot on the detector and thus the angular deflections of the cantilever. In contact mode, AFM uses a feedback mechanism to regulate the force on the sample. The AFM not only measures the force on the sample but can also regulate it, allowing acquisition of images at very low forces. In summary, AFM is a robust and technical apparatus that can be used to determine topological features of samples [18].

AFM has emerged as a very useful tool for probing interfaces to determine properties such as roughness, thickness, and morphology. Balnois *et al.* [8] investigated the effect of different chemical treatments on the microscopic and adhesion properties of flax samples. Their results indicated that the treated fibre surfaces were smoother and less heterogeneous in topology. Interestingly, none of the chemical treatments (NaOH, NaOH with acetic anhydride, and formic acid) yielded any changes in roughness. In their opinion the adhesion force between the tip and sample was due mainly to capillary forces. Han and Choi [9] studied the effect of electron beam at different intensities on the morphology of henequen fibres and AFM was one of the techniques used to study the changes. It was found that surface impurities and the pectin layer were removed resulting in striations in the surface of the natural fibres. Wang *et al.* [10] were interested in the morphological changes from the micro to nanoscale for chemically treated hemp fibres. They investigated how high pressure defibrillation and chemical treatments affected the fibre network at different size scales. Findings included from the microscopy study lead to a unique morphology of interconnected web-like structure of hemp fibres for each treatment. This paper adds a different dimension, in that different methods, chemical, enzymatic and a combination of both were used to treat hemp fibres and the effect on the surface charges were measured.

The goal of this work was to study the effect of mercerization, enzymatic, and chemical modifications, individually and in combination on the adhesion force and roughness of hemp fibres. The study was outlined based on two hypotheses. The first hypothesis involved using a green and specific method of modification via enzymes to remove the hygroscopic hemicellulosic content. This should indirectly result in a more uniform and less hygroscopic fibre system. Secondly, the use of a novel chemistry involving two sulfonic acids was studied. The main aim was to graft the aromatic based acids onto the surface of the hemp fibres via an esterification reaction so as to reduce the surface polarity. The measurements presented here identified the fundamental physical and chemical surface microstructural changes in the natural fibres that play a key-role in determining solid-solid interactions between fibres and polymeric matrices. In addition, the data collected in this paper constitutes a useful framework to establishing suitable reinforcement candidates for applications based on the availability of surface physical and chemical characterization.

5.2 EXPERIMENTAL

5.2.1 Materials

Mechanically processed hemp fibres were provided by the Alberta Biomaterials Development Centre located in Vegreville, Alberta. The samples were placed in air tight bags and stored at 4 °C. Enzymes used were provided by Novozymes (Bagsvaerd, Denmark) and stored at 4 °C. Sodium acetate (99 %, mol wt. 82.03 g/mol), glacial acetic acid (99.7 %, mol wt. 60.05 g/mol), sodium phosphate dibasic (99 %, mol wt. 141.96 g /mol) and sodium hydroxide (99 %, mol wt. 40.00 g/mol) were obtained from Fisher Scientific. Sodium citrate monohydrate (99 %, mol wt. 214.11 g/mol) was purchased from Sigma- Aldrich. Sodium phosphate monobasic (99 %, mol wt. 119.98 g/mol) was obtained from Acros Organics. Citric acid (99 %, mol wt. 192.13 g/mol) was sourced from EMD Chemicals. Aniline-2- sulfonic acid (95.5 %, mol wt. 173.19 g/mol) and 4-aminotoulene-3-sulfonic acid (95.5%, mol wt. 187.22 g/mol) were sourced from Sigma. Distilled water was used for all analyses.

5.2.2 Treatment of fibres

5.2.2.1 Enzymatic treatment

Approximately 1.0 gram of fibre was weighed into a 125 ml Erlenmeyer flask. All experiments were carried out in triplicate. Optimal conditions for each enzymatic reaction were provided by Novozymes (Table 5.1). The procedure outlined by George *et al*. (2014) [11] was used for each enzymatic treatment and is summarized in the lines to follow. For each system, 6 % (w/v) of enzyme was added to each flask. For all experiments, the liquid (mL) to fibre (g) ratio was maintained at 50:1 so as to facilitate complete wetting of fibres. Fibres were exposed to each enzyme for 90 minutes and agitated at 80 rpm at the enzymes' optimum temperature in a standard water bath. Enzymes were deactivated by heating at 90 °C for 10 minutes. Fibres were washed with excess warm water to remove traces of enzyme and buffer reagents [11]. All samples were dried at 80 °C for 5 hours and stored in polyethylene bags for subsequent analysis [12].

Table 5.1 Characteristics of each enzyme system with corresponding activity at optimum conditions.

Enzyme	Optimum conditions		Activity*
	pH	Temperature (°C)	
Xylanase	7	70	1000 AXU/g
Xylanase + cellulase	6	50	2500 FXU-S/g
Laccase	7	50	1000 LAMU/g

*AXU= Acid Xylanase Number, FXU= Farbe Xylanase Unit, LAMU= Laccase Myceliophthora Units

These enzyme systems were selected because of the degradation of hemicellulosic fraction (xylanase), producing a more crystalline and strong material. Also, the deposition of lignin fragments on the surface has been shown to improve the surface properties of the fibres, hence laccase was studied.

5.2.2.2 Mercerization

The hemp fibres were treated with 10 % (w/v) of NaOH as outlined by Kalia *et al.* [13]. Fibres were treated at 50 °C for 90 minutes. The fibres were washed with distilled water and then neutralized using 0.5 % (w/v) glacial acetic acid. Samples were dried at 80 °C in an oven for 5 hours and subsequently stored for analysis.

5.2.2.3 Sulfonic acid treatment

Two sulfonic acids, aniline-2-sulfonic acid (A2S) and 4-aminotoulene-3-sulfonic acid (AT3S) were investigated. These acids were studied because of the presence of the aromatic functionality and the acid groups which technically allow grafting onto the surface of the hemp fibres. The main goal was to reduce the surface polarity of the hemp fibres by reacting the fibres surface hydroxyl groups with the acid groups via an esterification reaction. These acids are characterized with limited solubility in water, hence all treatments were conducted at a concentration level of 0.01 M (the maximum in water). No prior work was reported on these acids; hence the parameters adopted were from the literature with similar chemical treatments such as acetylation [4, 14].The parameters included exposing fibres to the acids (fibre to solution was 1:50 w/w) at 30 to 50 °C for 90 minutes in a 250 ml round bottom flask fitted to a condenser [14]. A water bath was used to maintain the temperature during the course of the reaction. After completion of the reaction, fibres were washed with acetone and dried in at 80 °C for 5 hours.

5.2.2.4 Hybrid treatment

Hemp fibres were treated with 10 % NaOH as outlined above and treated using enzymes. The procedure for enzyme treatment was the same as outlined in section 5.2.2.1. The objective here was to determine whether initial swelling of the macrostructure would lead to better enzyme access.

5.2.3 AFM characterization

5.2.3.1 Sample preparation

At least four (4) fibre samples were randomly selected from each treatment for analysis. Double sided tape was used to attach these fibres onto 5mm AFM specimen disc. The samples were prepared in a room maintained (AFM equipment housed there) at ambient conditions. Samples were then mounted on the sample stage for subsequent analysis.

5.2.3.2 AFM Analysis

Atomic force microscopy and spectroscopy were performed using a Bruker Dimension Edge™ AFM and a JPK NanoWizard® II AFM respectively. All AFM measurements were made in ambient conditions in a room with climate control. Specifically the temperature and humidity of the room were maintained within the temperature of 22 ± 1 °C and relative humidity of 70 ± 5 %. The images and force measurements were collected using tapping and contact modes, respectively. For tapping (contact) mode, a silicon nitride cantilever with force constant 42 (0.2) N/m and resonance frequency of 320 (13 kHz) purchased from NanoWorld Point Probe NCH was used. As for the samples treated with the sulfonic acids and both caustic and enzyme, a Bruker non-conductive silicon nitride cantilever coated with reflective gold with a spring constant of 0.58 N/m and a resonance frequency between 40-75 kHz was used. A tip with a higher spring constant was used for these samples because in some cases during scanning, the higher adhesion forces resulted in the tip getting stuck to the sample. The force applied to the tip was maintained around 20 ± 5 nN for the enzyme and NaOH + enzyme treated sample. The force was varied within ± 5 because some of the sample surfaces (specifically the sulfonic acid treated fibres) were overly ‘sticky’. The scanning speed used to acquire images was varied depending on the type of sample being analyzed and the resolution obtained. Adhesion measurements were made after the contacting the surface and scanning the topography. Force curves on different areas were randomly collected for a total of 10 force curve per sample. The entire force-distance curve was collected for each sample. Given that initially the cantilever deflections and sample deformations were not known, the only distance that can be controlled was the Z distance (the displacement of the piezo). The cantilever deflection was converted into a force measurement by employing the retraction deflection curve of the contact region. As reported by Balnois *et al.* Hooke's Law $F = -k\Delta Z$ where k is the spring constant and was used to estimate the force. It should be noted, for each measurement, the zero force was when the tip was retracted from the surface. To ensure good reproducibility in the fibre preparation, for each treated fibre type, analysis was performed on four replicas and for each replica three measurements were taken [15-18].

The root mean square roughness (RMS) was determined from the tapping mode images using NanoScope Analysis (Version 1.40). The raw height images were subjected to the flatten function, which removed unwanted features such as bow, tilt, and noise, and clean image function that removed spikes and streaks. The roughness was then measured for areas from 1 to 100 µm^2 [8, 15]. The calculation done was based on the equation below, where Z_i represents the height of the $_i$th pixel, Z_m was the mean image height and n was the total pixel in the image [8].

$$RMS = \sqrt{\sum_{i=1}^{n} (Z_i - Z_m)^2 / n}$$

Force curves collected from the JPK system were analyzed using JPK DP, data processing software (version 4.0).

5.2.4 Statistical analysis

All experiments were replicated at least three times and results were expressed as mean value ± standard deviation. The statistical analyses of the data were conducted using the statistical software package SAS Version 9.4. It should be noted for the enzyme systems, each system was compared to the control and not among each other given the difference in activity for the enzymes and the different mode of substrate attack. To identify significant differences between mean values for control (raw) and a given system, the Kruskal Wallis Test was applied to the data populations involved, with a 95% confidence level ($P < 0.05$).

5.3 RESULTS AND DISCUSSION

5.3.1 Enzyme and mercerized treated hemp fibres

The enzymes investigated were xylanase, xylanase + cellulase, and laccase. One of the main aims of this research study was to investigate individually and in combination, the effect of mercerization and enzymatic treatments on the surface topography features and adhesive properties. Mercerization is known to alter the fibre macrostructure via hydrogen bonding disruption and network swelling [19]. Hence, mercerization was used to swell the macrostructure of the hemp fibres to allow greater penetration of the enzymes into the bulk material. The enzymes used in this work are known to degrade the hemicellulosic (xylanase) [20] and lignin (laccase) [21] fractions of the hemp fibres. However, to the best of our knowledge this is the first study aiming to measure surface roughness and adhesion forces as a function of these treatments individually and in combination. Figure 5.1 compares the surface roughness for different spot areas for hemp fibres treated with Xylanase.

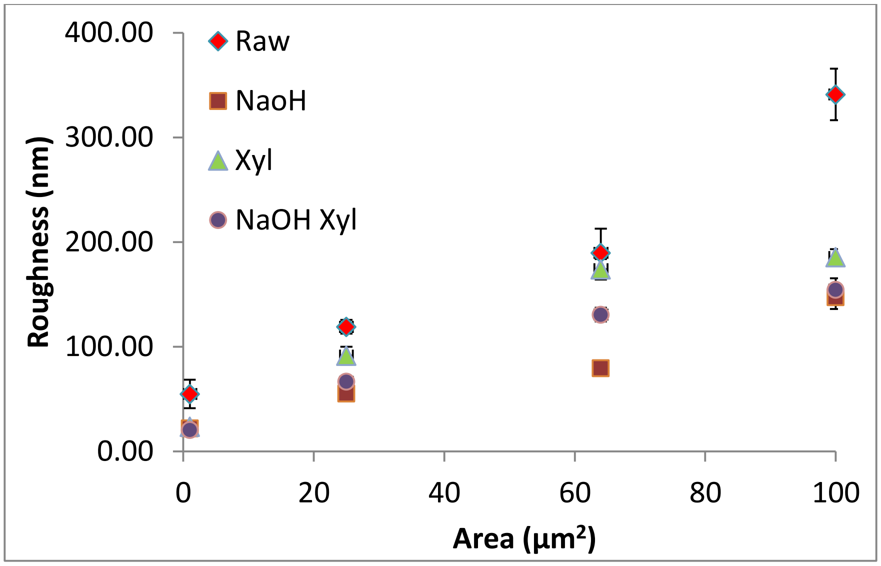

Figure 5.1 Surface roughness for NaOH and Xylanase treated hemp fibres (Xyl = Xylanase)

Hemp fibres treated with NaOH when compared to the control/raw fibres, showed a significant ($p<0.05$) reduction in surface roughness plausibly because of bundle disruption and network swelling [10, 19]. This pattern can be observed from the height images in Figure 5.2, where the hemp fibres appeared to be de-fibrillated and bundles more exposed when exposed to NaOH. Xylanase is known to degrade the xylan network found predominantly in the primary cell wall leaving exposed uniform crystalline cellulose [20, 22]. For the hemp fibres studied here, xylanase treatment resulted in a reduction of surface roughness at 1, 25, and 100 µm^2 spot areas. Both of these observations were supported by the increase in adhesion forces observed in Table 5.2, lending to an increase in surface hydrophilicity. The removal of the hemicellulosic content possibly exposed the more uniformly packed cellulosic hydroxyl groups [20] which interacted more with the polar tip. The increase adhesion forces for the NaOH treated hemp fibres plausibly was due to the swelling of the structure of the hemp fibres which could have exposed a greater surface area of predominantly cellulose while the xylanase treatment plausibly removed the hemicellulosic content exposing the cellulose backbone. When exposed to base, the hydroxyl groups of hemp fibres were converted into the alkoxide form and the structure of the cellulosic network was changed, thereby allowing for better interaction with the polar silicon nitride tip. Fibres treated with base has shown changes in the form of cellulose and the bonding within the network [4, 13]. As a result, to balance this charge and to account for the disrupted bonding, the sodium ions would be solvated by the water molecules present. Removal of hemicellulose exposes the cellulosic backbone, which is more hydrophilic. This results in more moisture retention on the surfaces of the fibres. As a result, it is plausible to conclude that the increase in adhesion forces for xylanase treated hemp fibres could be due to increased capillary and van der Waals forces [17]. It is noted that adhesion force measurements can be complimented with surface properties especially surface tension or contact angle. As reported in an earlier publication from our group, the use of the enzymes (xylanase, xylanase + cellulase and laccase) removed the specific components targeted and resulted in significant changes in contact angle. In fact, the removal of the hemicellulosic content and lignin resulted in a significant reduction in the contact angle. This supports the claims made here, where it’s reported that the systems treated with enzymes exhibited more interaction with polar tip when compared to the control [11].

Table 5.2 Effect of different treatments on the adhesion force for hemp fibres

Sample	Force (nN) mean ± SD
Raw/control	9.34 ± 0.94[b]
NaOH	32.7 ± 6.48[a]
Xylanase	29.7 ± 6.90[a]
NaOH + Xylanase	7.84 ± 0.76[b]
Xylanase + cellulase	13.4 ± 2.50[c]
NaOH + (Xylanase + cellulase)	21.2 ± 4.24[a]
Laccase	10.9 ± 1.32[c]
NaOH + Laccase	14.1 ± 2.00[b]

[a, b, c] Treatments with each enzyme type were compared to the control/ raw sample and among each other in the group. Treatments with the same denotation are not significantly different at $p<0.05$ from each other.

Force measurements did not detect any significant differences between the sequential treatment (NaOH + enzyme) and the control experiment. There was a reduction in surface roughness when compared to the xylanase treated fibres but an increase when compared to the mercerized sample. A plausible reason for this could be the initial swelling of the structure, resulted in better enzyme access (larger surface area) hence aiding removal of a larger portion of the primary cell wall exposing the cellulose and lignin secondary structure. Lignin is aromatic and much more heterogeneous in structure when compared to cellulose and would exhibit less interaction with the polar tip.

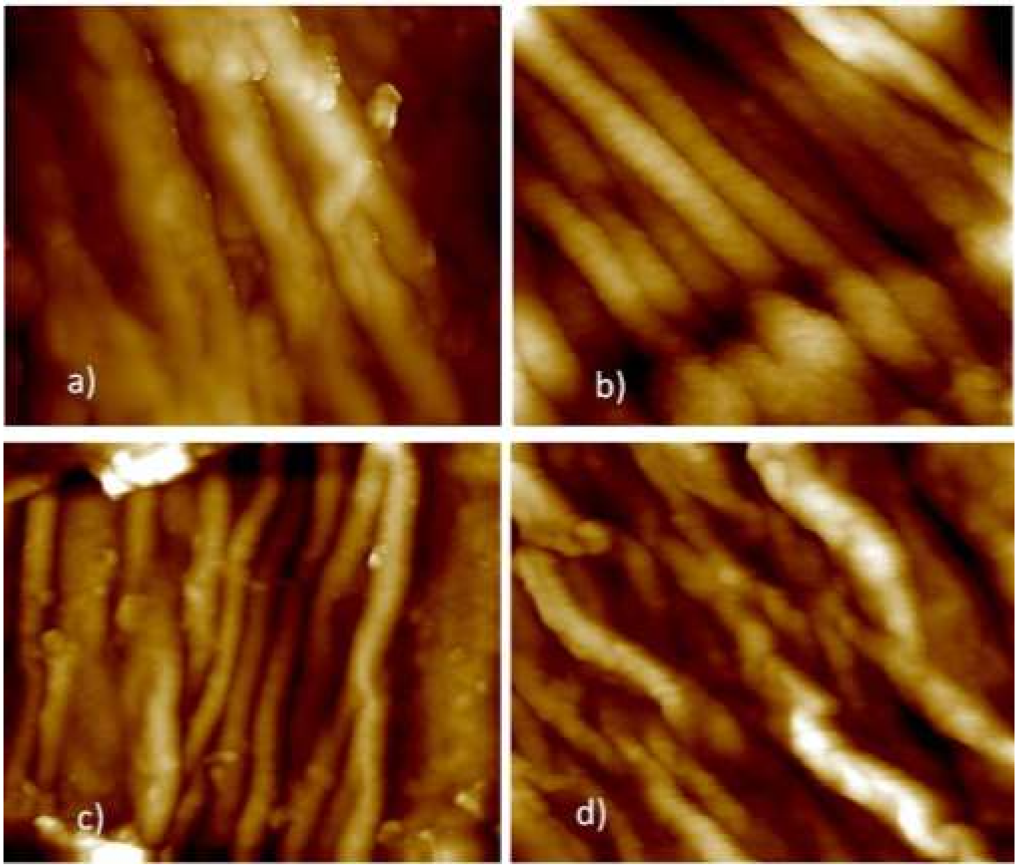

Figure 5.2 AFM height images (5 × 5 µm) of hemp fibres after different chemical and enzymatic treatments. Images for a) Raw b) 10 % NaOH c) xylanase and d) NaOH + xylanase treated hemp fibres. Dark colors signifies lower surfaces, such as pits and valleys while lighter colors are higher.

Hemp fibres treated with xylanase + cellulase and NaOH showed the same trend as the hemp fibres treated with xylanase. The only exception was when the hemp fibres were treated with enzyme (xylanase +cellulase) the roughness (Figure 5.3) increased relative to the previous enzyme system (Xylanase) and was characterized by lower adhesion forces (Table 5.2). Vries and Visser [22] outlined the simultaneous degradation of the xylan backbone and cellulose using an enzyme system of cellulases and xylanases. They concluded that the xylanases degraded the xylan network, exposing the cellulosic bundles for the cellulases. In this study, the decrease in surface roughness for the enzyme treatment relative to the raw sample may plausibly be due to removal of random amorphous framework exposing the cellulose bundles [23].

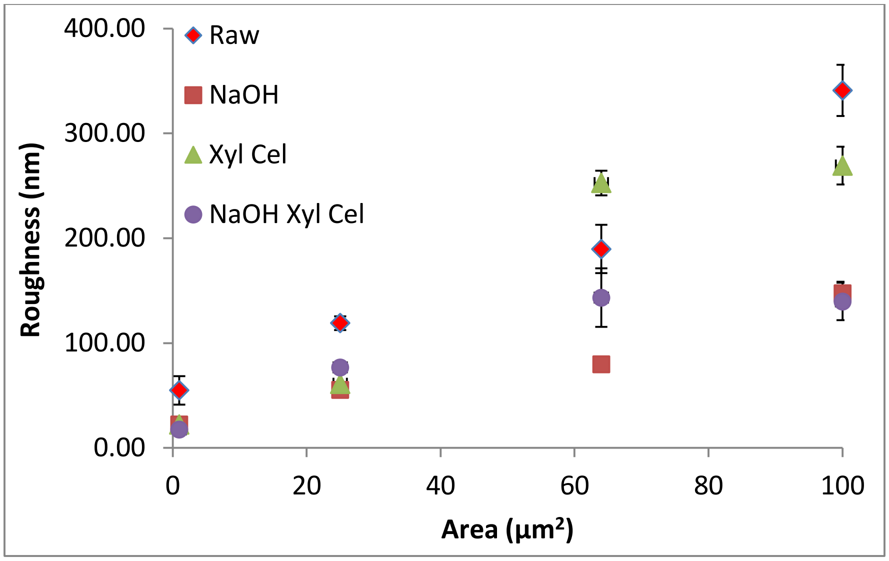

Figure 5.3 Surface roughness for NaOH and Xyl Cel treated hemp fibres (Xyl Cel = xylanase cellulase)

This observation could explain the lower force detected for the xylanase + cellulase treated hemp fibres when compared to the xylanase treated hemp system. The removal of hemicellulose by the xylanase system exposed the accessible cellulose. On the other hand, the removal of hemicellulose + accessible cellulose (xylanase + cellulase) as reported by George *et al.*, [11] exposed the least polar crystalline cellulose. This explains the reduction of the adhesion force for the xylanase + cellulase treated hemp fibres because of the reduction in interaction with the polar silicon nitride tip [24].Hemp fibres treated with NaOH and hybrid (NaOH + xylanase Cellulase) systems were characterized by the same trend as observed for the previous system. Similar mode of action (xylanase vs xylanase +cellulase) and enzyme dosage for both systems may account for this. On the other hand, hemp fibres in the presence of NaOH and the enzyme system, was characterized by a significant ($p<0.05$) increase in adhesion force relative to the raw fibres. As mentioned before, NaOH possibly improved the effectiveness of the enzyme treatments because of disruption to the fibre network and swelling which increases the surface area [10]. As a result, more of the xylan network bonded to the cellulosic bundles were significantly degraded ($p<0.05$) increasing the surface forces [24].

Finally, hemp fibres treated with NaOH + xylanase when compared to NaOH + (xylanase + cellulase) showed a significantly lower adhesion force. One plausible reason for this may be the greater penetrating action of the NaOH + (xylanase +cellulase) system which removed the hemicellulosic content and exposed the accessible cellulose. According to Mohanty *et al*. [1], accessible cellulose is much more hydrophilic than the other components. Hence this explains the increased adhesion forces. Also, the arrangement of the fibre component may have influenced the reaction as well. Specifically, removal of hemicellulose could have exposed the randomly arranged lignin fragment which will in turn have less of an interaction with the polar probe for the NaOH + xylanase treated fibres. It is worth noting that the specific attack of these enzymes are influenced by the geometrical arrangement of the components within each fibril [11] and the access of these enzymes to the targeted component [1].

The roughness data and force measurements for the hemp fibres treated with Laccase are shown in Figure 5.4 and Table 5.2 respectively. Madhavi and Lele (2009) [21] and Kunamneni *et al.* (2008) [25] studied the effect of Laccase during the degradation of recalcitrant lignin and concluded the removal of lignin exposes the cellulose backbone of the lignocellulosic material. The main aim in this study was to use the Laccase system to remove the lignin fraction from the hemp fibres and investigate how this impacted the surface roughness and bonding at the uppermost 5 nm.

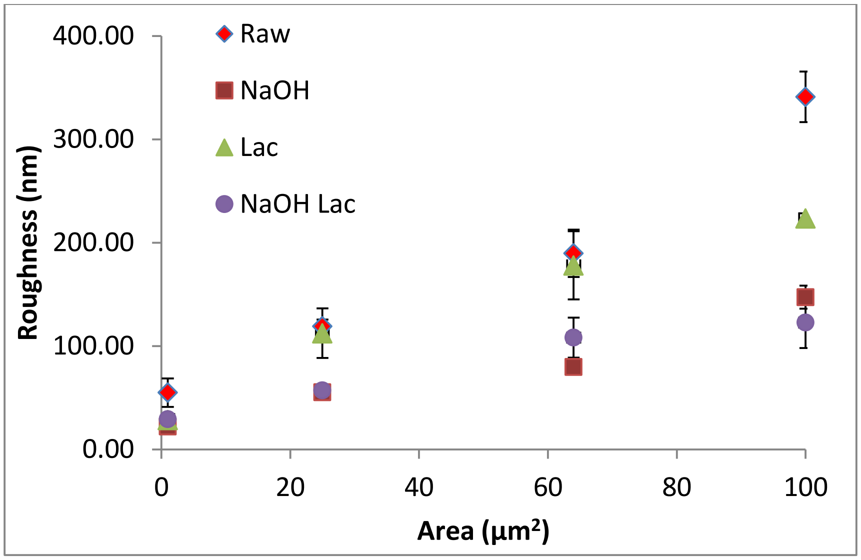

Figure 5.4 Surface roughness for NaOH and Laccase treated hemp fibres (Lac = Laccase)

Interestingly, hemp fibres treated with Laccase were characterized with no significant increase in surface forces ($p<0.05$) as shown in Table 5.4 and when compared to the raw fibres, exhibited similar surface roughness. One plausible reason for this may be the hierarchical nature of the hemp fibres, with the lignin primarily confined to the inner regions of the primary cell wall and lumen [8]. In a similar study, Balnois *et al.* (2007) [8] demonstrated that the lignin fraction of flax fibres were intact because of its geometrical arrangement in the lumen of the fibres and resistance to the chemical methods studied. On the other hand, the hybrid treatment (NaOH + Laccase) resulted an increase in the adhesion force, indicating an increase in surface polarity. The use of the caustic disrupted the fibre network allowing the Laccase to access the lignin network. The degradation of lignin, which acts as an adhesive medium for the entire macrostructure of the fibres, exposed the hemicellulosic and cellulosic content, hence increasing the surface polarity which correlates to a higher interaction with the tip. This observation was also supported by the significant ($p<0.05$) reduction in surface roughness for the hybrid treatment because the removal of the lignin found in the intercellular regions resulted in a smoother surface.

5.3.2 Hemp fibres treated with derivative of sulfonic acid

The hypothetical reactions for the two sulfonic acids with a molecule of β-D-glucose are highlighted in Figure 5.5 and explained in greater in the previous chapter (Section 4.3.1). The reaction mechanism involves the production of the acid chloride derivative of each acid, given that in this form the reaction is kinetically favored because Cl atoms are better leaving groups that the hydroxyl groups [25]. In the second step, the acid chloride reacts with one of three hydroxyl groups (C-2, C-3, C-6) found on each molecule of β-D-glucose of the cellulose exposed on the surface. The reaction may proceed either with the presence of cellulosic or hemicellulosic hydroxyl groups. Natural fibres possess 4 % of the total hydroxyl groups on the surface [25]. These are the major contributors to poor interfacial adhesion to hydrophobic based matrices, [1] hence these acids were grafted onto the surface to improve the hydrophobicity. Pyridine was used as a catalyst because Bledzki *et al.* (2008) [26] showed when flax fibres were reacted with acetic anhydride, that in the absence of a catalyst, esterification does not result in uniform product.

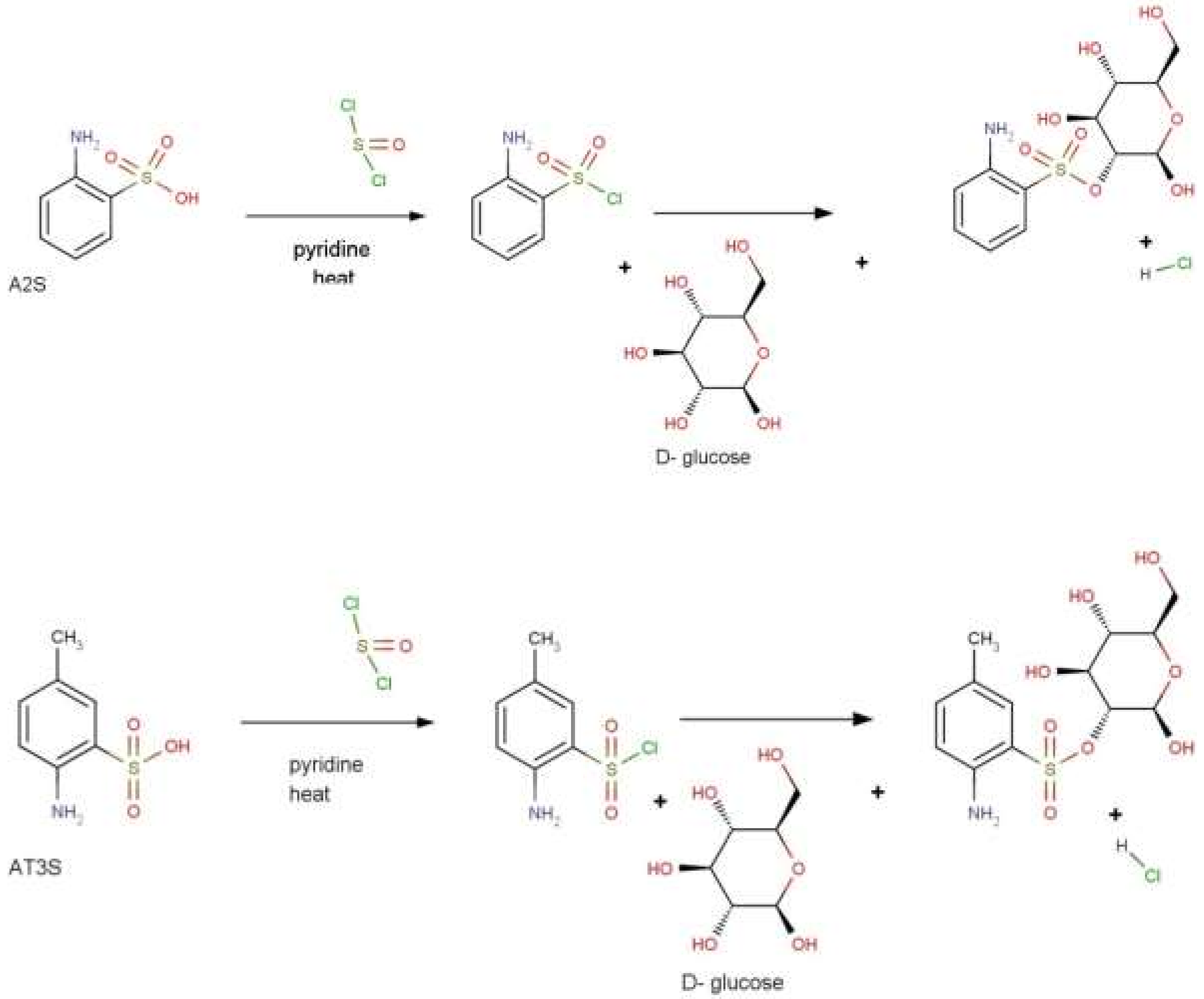

Figure 5.5 Reactions for aniline-2-sulfonic acid (A2S) and 4-aminotoulene-3-sulfonic acid (AT3S) with a β-D-glucose molecule hypothetically found on the surface of the hemp fibres. Pyridine was used to drive the reaction forward by chelating with the H-Cl as it is produced.

The influence of the two acids on the surface roughness for the treated hemp fibres is given in Figure 5.6. As expected with any acid system as demonstrated in the bioethanol area of study, exposing lignocellulosic material to acids results in degradation of the primary cell wall [26-28]. This degradation corresponds to a cleaner surface with exposed cellulosic OH groups for grafting of the sulfonic derivatives. A reduction in the forces on the surface signaled a reduction in the free OH groups on the surface. As shown in Table 5.3, AT3S significantly ($p<0.05$) reduced the surface forces plausibly due to the reaction outlined in Figure 5.

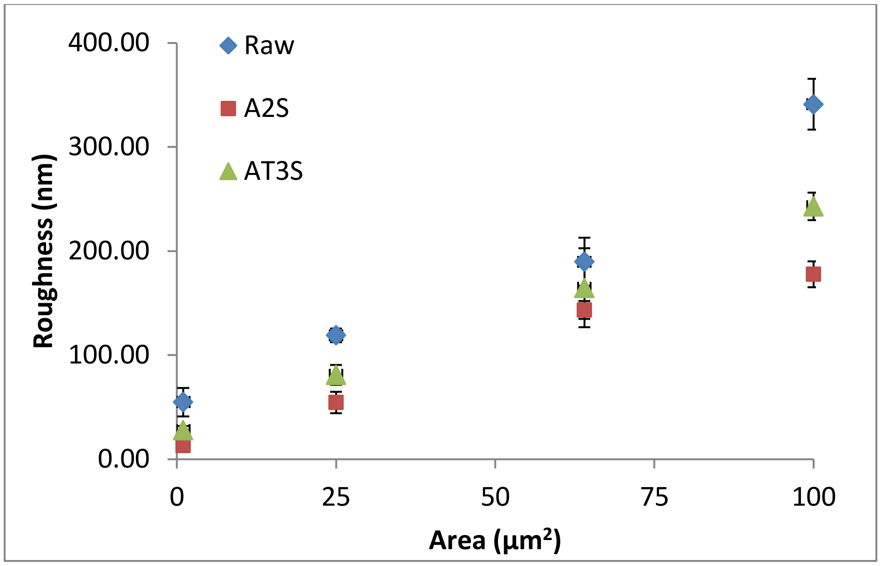

Figure 5.6 Surface roughness for hemp fibres treated with derivatives of sulfonic acids (A2S= aniline-2-sulfonic acid and AT3S= 4-aminotoulene-3- sulfonic acid)

Table 5.3 Effect of different treatments on the adhesion force for hemp fibres treated with the sulfonic acids.

Sample	Force (nN) mean ± SD
Raw	9.34 ± 0.94[a]
A2S	8.06 ± 0.77[a]
AT3S	7.38 ± 0.78[b]

[a, b] Treatments with the same denotation are not significantly different at $p<0.05$

Based on the main aim of this part of the study, it has been demonstrated that a water soluble and organic solvent free sulfonic acid system can be used to enhance hemp fibres for composite applications. In fact, it was demonstrated that the reaction of these moieties results in significant reduction in adhesion force between the polar tip and the sample. This signals that the overall surface polarity has decreased. This study will compliment and allow for a better understanding of the influence of any given chemical method on the microstructure and physical properties of hemp fibres.

5.4 CONCLUSION

This study demonstrated, through the use of a colloidal probe that hemicellulases can be used to reduce the surface roughness while increasing the adhesion forces on the surface. Mercerization treatment prior to enzymatic treatment plausibly resulted in better enzyme access to the macrostructure of the hemp fibres. This was especially noticeable for hemp fibres treated with laccase because NaOH allowed access to the inner structure that is lignin filled. Grafting of sulfonic acids onto the surfaces of the hemp fibres resulted in lower adhesion forces when compared to the raw fibres. This may be due to a reaction involving the hydroxyl groups on the surface of the fibres, exposing the hydrophobic aromatic rings of the acids to the polar silicon nitride tip. This study highlights that in order for natural fibres to be a competitive option for polymer reinforcement, the use of enzymes, a green and specific method of modification can be used to selectively remove hygroscopic hemicellulose. Similarly, the use of novel chemistry involving cheap and reactive sulfonic acids can be used to significantly reduce the surface polarity via grafting of the aromatic artefacts onto the surfaces of the fibres.

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CHAPTER 6.

Enzymatically treated natural fibres as reinforcing agents for biocomposite material: mechanical, thermal, and moisture absorption characterization[5]

6.0 RATIONALE

Enzymatic treatments were used to prepare hemp and flax fibres for reinforcing polypropylene. Selective removal of hemicellulosic and pectic material was the main focus because these components are responsible for water uptake and the poor thermal stability of the fibres. The resulting treated fibres were used to reinforce polypropylene and the properties were evaluated.

6.1 INTRODUCTION

Substantial research has been focused in the utilization of biomass feedstock as reinforcement for polymeric composites. Natural fibres have high specific strength, low cost, low specific density, and are renewable [1]. Natural fibres are characterized by lower density compared to commercial glass fibre. As such the resulting composite materials adopted for example by the automotive industry, will be lighter, resulting in superior fuel economy. Additionally, according to Franco and Gonzalez (2005) [2], glass fibres are abrasive to processing tools and can result in discomfort for workers.

Despite these advantages, natural fibres are inherently thermal labile, are highly polar, and are characterized by limited compatibility with non-polar resins. Researchers have investigated many different methods to enhance natural fibres for composite applications. For example, mercerization [3, 4] and acetylation [5, 6] have shown to improve the properties of these fibres in composite materials. However, widespread deployment of these methods is limited owing to high-energy processing and disposal cost. Because of these limitations, considerable efforts have been recently focused on developing biological methods to modify natural fibres. These technologies typically require milder reaction conditions, can be designed to incorporate enzyme recycling, and reuse schemes, allow high level of reaction control on the degree of modification of the fibre [7, 8].

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[5] A version of this chapter has been submitted for peer review: **Michael George**, et al., Composites Part B.

Pectic and hemicellulosic content of fibres are the primary components contributing to surface polarity, which leads to moisture absorption, and have low thermal stability relative to cellulose and lignin. In an earlier study reported by our research team [7], hemp and flax fibres treated individually with hemicellulases, pectinases or a laccase exhibited improvements in thermal and surface properties. Aracri *et al.* (2010) [9] used a different approach for enhancing flax and sisal fibres. They employed laccases for grafting simple phenols onto the surface of the fibres. Their main finding was an increase in kappa number, which suggested that phenol groups had been incorporated into the pulp samples. The specificity coupled with the extensive research available using enzymes for degradation of lignocellulosic materials can serve as an excellent platform for using enzymes to treat fibres for composite applications.

Mohanty *et al.* (2000) [1] reviewed the properties of the different chemical components of natural fibres. An indication of the relative moisture absorption propensity and thermal degradation are represented below.

Component	**Moisture absorption**	**Thermal degradation**
Hemicellulose /pectin	+++++++	+++++++
Accessible cellulose	+++++	++++++
Non-crystalline cellulose	+++	+++++
Lignin	++	++
Crystalline cellulose	+	+++

No. of **+** indicates propensity to absorb moisture or degrade during heating.

Yang *et al.* (2007) [10] investigated the characteristics of the main components of natural fibres using pyrolysis. They reported hemicellulose and cellulose degradation between 220-315 °C and 315-400 °C, respectively. Lignin was much more stable, but varied over a range of 160-500 °C depending on to what moieties it was bonded. Specifically, hemicellulosic bonded lignin degrades below 250 °C but regions of pure lignin degrades at temperature as high as 500 °C. With these relationships in mind, we hypothesized that removal of hemicellulose (xylanase), pectin (polygalacturonase) and accessible cellulose (xylanase + cellulase) would lead to greater moisture resistance and higher thermal stability. The enzymes being studied in this communication were previously screened against the same feedstocks and complete characterization of the substrates removed was reported [7, 10]. In these previous studies, the combination of xylanase and cellulase resulted in the removal of a major fraction of the hemicellulose content, while the pectinases, especially polygalacturonase, removed a higher mass fraction of the fibre samples. The low pectic content of these fibre types (2-3 %) could not have accounted for the removal of close to 10 % of the natural fibres. In fact, the enzymes provided were listed as having accessory enzymes and in the case of polygalacturonase, other pectinases and hemicellulases were likely present. As a result, partial removal of pectic material and hemicellulose linked to these substrates, may have accounted for the higher hydrolysis yields reported [11]. On the other hand, the laccase used in these experiments principally removed or modified the lignin component of the natural fibres. As we will report later, this has implications in the mechanical properties of composites made from treated fibres.

A major goal of this study was to investigate the compatibility and performance of enzyme treated natural fibre reinforced polypropylene composites. The primary aim was to determine whether the removal of specific components, namely pectin (limited) and hemicellulose, would influence the mechanical, thermal, and hygroscopic properties of the composite materials. It was also important to determine whether removal of up to 10 – 15 % of the fibres' mass would adversely affect the mechanical and thermal properties.

6.2 EXPERIMENTAL

6.2.1 Materials

Two types of bast natural fibres were used as reinforcements for polypropylene (PP). Decorticated hemp and flax fibres were obtained from Alberta Innovates Technology Futures, Edmonton, Alberta. The samples were placed in airtight bags and stored at 4 °C. All enzymes were provided by Novozymes (Bagsvaerd, Denmark) and stored at 4 °C. Sodium acetate (99 %, mol wt. 82.03 g/mol), glacial acetic acid (99.7 %, mol wt. 60.05 g/mol), sodium phosphate dibasic (99 %, mol wt. 141.96 g/mol), sulfuric acid (98 %, mol wt. 98.075 g/mol), calcium carbonate (99 %, mol wt. 100.09 g/mol) and sodium hydroxide (99 %, mol wt. 40.00 g/mol) were obtained from Fisher Scientific. Sodium citrate monohydrate (99 %, mol wt. 214.11 g/mol) was purchased from Sigma- Aldrich (Oakville, Ontario). Sodium phosphate monobasic (99 %, mol wt. 119.98 g/mol) was obtained from Acros Organics (Geel, Belgium). Citric acid (99 %, mol wt. 192.13 g/mol) was sourced from EMD Chemicals. Distilled water was used for all analyses. M Holland CP510 polypropylene thermoplastic resin was used for compounding with the fibre samples.

6.2.2 Methods

6.2.2.1 Enzymatic treatment of natural fibres

The enzymatic treatment of hemp and flax fibres was studied and documented in a previous publication from our research team [7] and in chapter 3. In that communication/chapter, we focused on screening 5 enzyme systems, namely: xylanase + cellulase, xylanase, polygalacturonase, pectinmethylesterase, and laccase, and their effect on the macrostructure of hemp and flax fibres. The surface and thermal properties were reported. Based on that finding, a few key systems were selected for subsequent scaling up for composite production. In the present research, the experiments had to be scaled up to obtain sufficient amounts of fibre for compounding. In each case, 1 kg of natural fibre was treated for each system. The conditions for each enzyme system was provided by Novozymes (Bagsvaerd, Denmark) and given in Table 6.1. Hemp or flax fibres were treated with xylanase, polygalacturonase, laccase, and xylanase + cellulase. Hence, there were 8 systems tested in total (2 fibre types' × 4 enzymatic treatments).

Table 6.1 Conditions for each enzyme system

Enzyme	Optimum conditions		Activity
	pH	Temperature (^{o}C)	
Xylanase	7	70	1000 AXU/g
Xylanase (10% cellulase)	6	50	2500 FXU-S/g
Polygalacturonase	4	45	3800 PGNU/ml
Laccase	7	50	1000 LAMU/g

Previously reported in a publication from our group [7]

Given the large quantities of fibres needed for compounding, 100 g batches were treated in large beakers (2 L) using 2 % (v/v) enzyme solution (enzyme + required buffer solution). The fibre to liquid ratio was maintained at 1:15. The beakers were placed in a shaking water bath (80 rpm) at the enzyme specific optimum temperature for 90 minutes. The treated samples were then heated at 90 °C for 10 minutes to facilitate enzyme deactivation. It should be noted, the control samples were treated at the respective temperatures in the buffer solutions. The fibres were finally washed using distilled water and dried in a convection oven at 80 °C for 5 hours [7]. Samples were stored in polyethylene bags until compounding.

6.2.2.2 Composite preparation

For the eight enzyme systems (four hemp and four flax) the fibres were adequately dried (80 °C for 4 hours) before compounding. Fibre samples were pelletized using a Kahl pellet press using a ½ inch thick die with ¼ inch diameter holes. A fixed mass of 20 % of the fibre samples was used to reinforce NFPP for all systems. The control for the experiments were untreated hemp or flax. The NFPP was compounded using a Thermoscientific 24/40, co-rotating twin-screw extruder with custom screw profile. The temperature profile of the extruder was adjusted for each system to compensate for any variability in viscosity. The samples were then injection molded using a 34-ton BOY 30A model. Dog bones were subsequently obtained for testing. The parameters utilized are summarized in Table 6.2.

Table 6.2 Injection molding parameters for the NFPP

Parameter	Value
Processing temperature (barrel/nozzle)	160-175-180-180 °C[1]
Cycle time	35 seconds
Injection pressure	70 bar
Clamp pressure	34 tons

[1] Temperature zones within the barrel of the injection molding equipment (varies within ± 5 °C).

6.2.2.3 Thermal analysis

Thermogravimetric analysis (TGA) was used to determine the thermal stability of the dog bones. ASTM 2250-11 (Standard test method for measuring thermal stability by TGA) was followed using a TA Instruments Q600 analyzer for all sample analyses. Approximately 5-10 mg of each sample was heated at 10 °C per minute from 20 to 600 °C under nitrogen atmosphere. All samples were analyzed in a platinum pan. Three samples were taken from the failed/tested part of the specimens (per failed dog bone). Three specimens (failed dogbone) were randomly selected to be tested for each treatment.

6.2.2.4 Mechanical property testing

The controls for the hemp and flax reinforced polymer composites were untreated hemp and flax reinforced polypropylene. Prior to testing, samples were conditioned at 23 °C and 50 % relative humidity for 48 hours. Tensile strength tests (modulus of elasticity, tensile strength, and percentage elongation) were determined according to ASTM D638-10 (Standard test method of tensile properties of plastics). The tests were performed using Instron 4302 and 3369 tensile machines with 10 kN and 5 kN load cells, respectively, at 5 mm/min crosshead speed. It should be noted that a 50 mm extensiometer was used to measure the initial (up to 10%) strain (elongation) of the different specimens.

Impact strength (Charpy toughness) of the specimens was measured according to ISO-179-02 (Determination of Charpy Impact Properties- Instrumented). A standard two foot pound (2.71 Joule) pendulum was used for the test. Un-notched specimens were used for all tests except virgin polypropylene samples.

6.2.2.4 Water absorption testing

The water resistance performance of the composite specimens was determined using virgin (untested) dog bones samples. ASTM D570-98 method (determination of water absorption in plastics) was followed for the water absorption measurements [12, 13]. The method involved immersing at least 5 samples of each system. Samples were dried in a convection oven for 6 hours at 80 °C until constant weight prior to immersion (W1). The dried specimens were immersed in approximately 100 mL distilled water at room temperature for four weeks. Upon removal from water, the extra water on the surface of the specimens was carefully wiped with a clean dry absorption cloth, and the mass of the specimens were measured immediately (W2). The percentage of water absorbed was calculated by weight difference between the samples before and after soaking using equation 1:

$$Water\ absorption(\%) = \left(\frac{W2 - W1}{W1}\right) * 100$$

6.2.2.5 Scanning electron microscopy

Micrographs of composite materials were taken using a Hitachi S-2700 Scanning Electron Microscope (SEM) equipped with a Princeton Gamma Tech (PGT) IMIX digital imaging system and a Prism Intrinsic germanium (IG) detector. A gold putter coater was used to induce conductivity for all samples. A resolution of 4 nm was used for all samples. At the point of fracture of the composites, sample specimens were mounted on conductive adhesive tape, sputtered coated with gold/palladium and observed using a voltage of 15 – 20 kV [7, 13]. At least six cuts were made from each sample specimen.

6.2.3 Statistical analysis

All experiments were replicated at least three times and results were expressed as mean value ± standard deviation. The statistical analyses of the data were conducted using the statistical software package SAS Version 9.4. To identify significant differences between mean values for a control and a given enzyme system, Kruskal Wallis Test was applied to the data populations involved, with a 95% confidence level ($P < 0.05$).

6.3 RESULTS AND DISCUSSION

6.3.1 Characterization of fibre mass

The flax and hemp fibres were detergent washed [7] before any treatment. The mass change as a result of each enzyme treatment is summarized in Table 6.3. It should be noted, this data is taken from a previous publication (reproduced with permission from Elsevier) [7] and recalculated to reflect what is the mass of each component going into the composite material. The main aim was to try and determine whether removal of specific fractions of the hemp and flax systems would affect the mechanical, thermal, and hygroscopic properties of these fibres when used in composite materials.

The recalculation was done as followed, where the % of a given component is:

$$\frac{100\ \% \times \%\ mass\ of\ fibres\ after\ treatment}{\%\ component\ after\ treatment}$$

E.g. % cellulose (Xyl + Cel treated hemp) = (100 / 57.42) × 91.63 = 64.3 %

It should be noted, the mass of fibres after treatment is equal to 100 - % mass lost or removed during treatment.

Table 6.3 Compositional analysis of hemp and flax fibres after enzymatic treatment

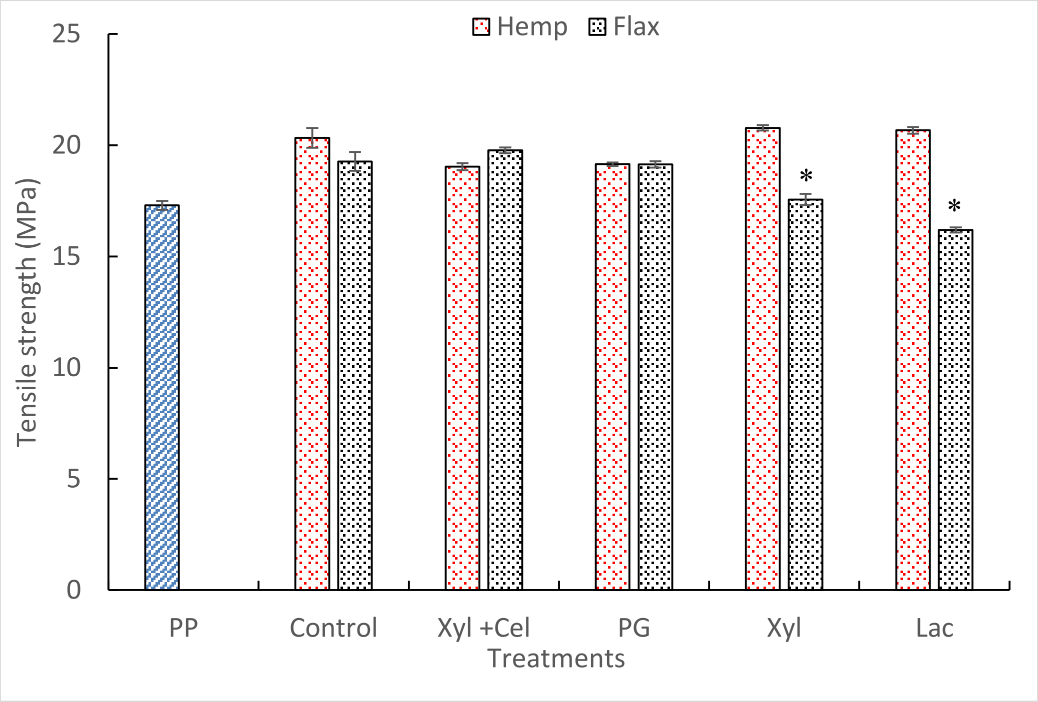
Fibre	System	Chemical component after treatment (% dry weight)^a						% mass Loss after treatment
		Cellulose	Hemicellulose	Lignin	Pectic	Protein	Ash	
Hemp	Untreated	67.77	12.14	7.24	3.95	6.32	2.31	-
	Xyl + Cel	64.29	13.21	7.25	2.36	9.42	3.47	10.58
	PG	75.12	6.98	5.55	0.53	8.09	3.72	8.17
	Xyl	70.07	8.23	7.34	1.51	9.72	3.12	4.12
	Lac	70.42	11.36	7.12	1.49	7.36	2.25	3.22
Flax	Untreated	64.08	16.10	4.14	7.18	6.18	1.53	-
	Xyl + Cell	68.07	10.85	5.22	2.99	10.13	2.74	19.44
	PG	80.26	3.34	1.87	1.36	9.92	3.25	23.21
	Xyl	69.40	12.41	3.85	3.40	8.16	2.78	8.31
	Lac	70.04	14.83	1.61	3.53	7.76	2.23	9.91

Reprinted with permission from Elsevier [7]

Hemp and flax fibres treated with xylanase, polygalacturonase, laccase, and xylanase + cellulase were characterized with significant changes in mass percentage. The most significant loss of mass after treatment was for the PG and Xyl+Cel treatments. As would be seen in the sections to follow, these systems were characterized with the best thermal and water resistance properties. PG treatment had the most significant decrease in hemicellulose (HC) and pectin for all treatments tested. As a result, the cellulosic percentage going into each composite for this treatment was significantly higher. Specifically, laccase removed a major fraction of the lignin from the flax fibres, while the hemicellulases, xylanase and xylanase + cellulase removed 10-20 % mass of fibres. The increase in protein and ash contents in some cases can be attributed to residual enzymes being trapped in the fibre network after treatment. Based on these numbers, we hypothesized that the removal of these very hygroscopic (pectic material and hemicellulose) and thermally labile (pectic material) fractions can lead to better reinforcing agents for composite applications. Nevertheless, the authors are aware that processing of these fibre materials (via either heating with buffers or during extrusion) can lead to disintegration of the individual fibre strands, making them better suited for dispersion within the matrix of interest.

6.3.2 Mechanical properties

The mechanical strength of a composite materials is a property of the reinforcement used, the type, and formulation of the polymer/matrix, and the nature of the interaction between the two phases. The tensile strengths of the different systems are illustrated in Figure 6.1.



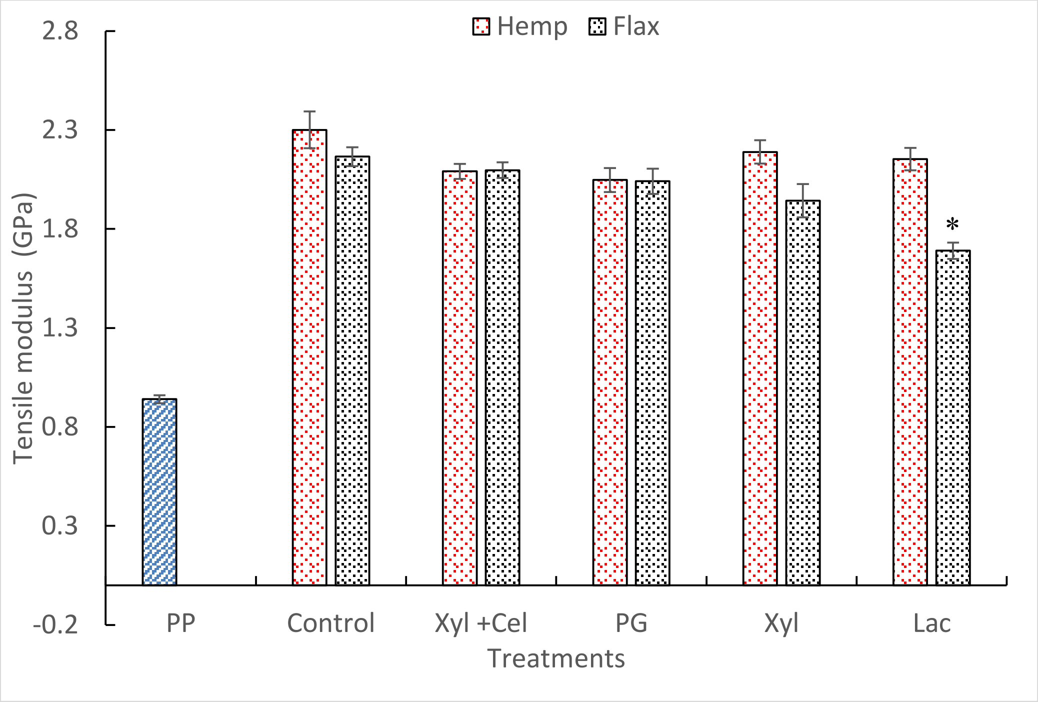
*Significantly different from control at $p<0.05$

Figure 6.1 Tensile strength of unreinforced PP and various natural fibre composites with the following enzymatic fibre treatments: unreacted (Control), xylanase + cellulase (Xyl +Cel), polygalacturonase (PG), laccase (Lac), and xylanase (Xyl) NFPPs.

Figure 6.1 indicates predominantly no change in tensile strength for the composite materials when compared to the untreated fibre reinforced matrix (controls). The Lac and Xyl treated flax fibre reinforced polypropylene were the only exceptions. The lower tensile properties observed for Lac treated flax may be due to significantly lower lignin values after treatment (although the exact mechanism for this drop in the mechanical properties is unclear at this time). It should be noted, PG treated flax also experienced a similar drop in lignin content but did not show any drop in mechanical properties. However, PG treated flax also had a significantly higher cellulose content which could compensate for any effect that reduced lignin may have caused (i.e. increased cellulose content typically correlated to increased fibre strength and modulus). Flax fibres have a higher hemicellulosic content compared to hemp fibres and the micro structural arrangement of the components led to better removal of the targeted lignin and hemicellulose fractions [7, 13, and 14]. As a result, it is possible removal of lignin, which acts as an adhesive material within the macrostructure of the fibres, plausibly led to a weaker fibre structure thus reducing overall composite strength. Similarly, removal of hemicellulose, which is randomly distributed within the macrostructure of fibres, plausibly led to a weaker fibre structure as well. As previously reported, kraft pulp (high cellulose content) is a good reinforcing agent because of the homogeneity of the filler and the good dispersion within PP as reported by Beg and Pickering (2008) [16]. Another key aspect, the heterogeneous compositional nature of these fibres can influence these characterization test significantly. The authors tried to homogenize the samples when received, and then take a random representative sample from the lot.

However, the tensile strengths of the NFPP were significantly improved when compared to virgin polypropylene (17.3 ± 0.2). Overall, the removal of targeted hemicellulose (xylanase treated) and pectic (polygalacturonase treated) material did not adversely affect the tensile strengths of the composites except in the case of the higher hemicellulosic content flax fibres. This is promising as we previously reported that approximately 4-19 % and 8 - 23 % of the fibres mass was removed when treated with Xyl (or Xyl + Cel) and PG, respectively [13]. Li and Pickering (2008) [15] studied the effect of chelators, enzymes, and chelators + enzyme treatments of hemp fibres and how this would affect the tensile properties of the composites. The tensile values of treated fibres reinforced composites they reported were significantly lower than the values in this communication. Their low tensile values may have been a result of the removal of lignin, the component of the fibre structure that holds the macrostructure together. In summary, removal of the pectic (PG targeted) and hemicellulosic content (XYL targeted) did not affect the tensile strength of the composites but as reported by Li and Pickering (2008) [15], removal of lignin through laccase treatment can adversely affect this property.

Similarly, there was no change in tensile modulus of the different composite systems with the exception of laccase treated flax (Figure 6.2). At this point, the reason for this drop in tensile and strength modulus is unclear as previously mentioned and there is scope for further investigation. As such, the stiffness of the composites was maintained despite the removal of specific portions of the reinforcing fibres. Reinforcing polypropylene with the fibres resulted in significant improvement in tensile modulus when compared to virgin polypropylene (0.94 ± 0.02).



*Significantly different from control at $p<0.05$

Figure 6.2 Tensile modulus of unreinforced PP and various natural fibre composites with the following enzymatic fibre treatments: unreacted (Control), xylanase + cellulase (Xyl +Cel), polygalacturonase (PG), laccase (Lac), and xylanase (Xyl) NFPPs.

Similarly, there was no change in the elongation at break for the enzyme treated fibre reinforced composites except for laccase treated flax fibres (Figure 6.3). It should noted, the elongation of break (%) for the virgin polypropylene was in excess of 100 % and hence was not represented in the graph. This is expected, because the addition of the filler (fibres), reduces the ductility of the polymer. The values for elongation of break for the composites reported in this study are better than those reported by Pracella *et al.* (2005) [18], when they reinforced polypropylene with hemp fibres functionalized by grafting glycidly methacrylate. In fact, the values obtained by our study is better by at least a factor of 4, when 20 % fibre mass is used.

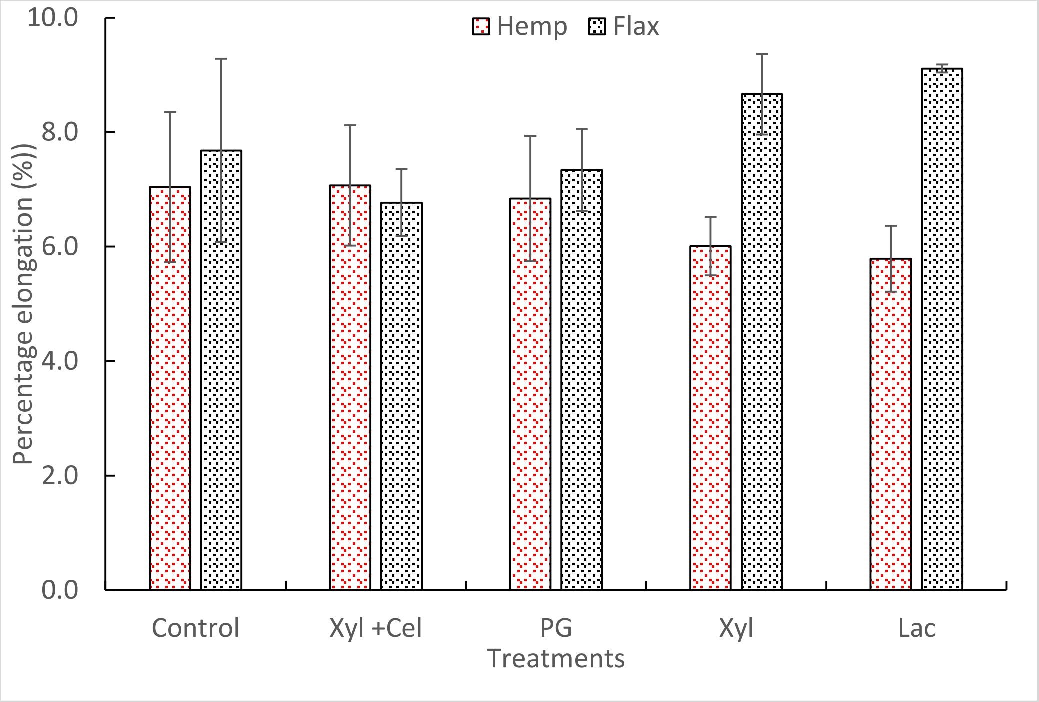


Figure 6.3 Percentage elongation for the various natural fibre composites with the following enzymatic fibre treatments: unreacted (Control), xylanase + cellulase (Xyl +Cel), polygalacturonase (PG), laccase (Lac), and xylanase (Xyl) NFPPs.

The Charpy impact strength of the different composite materials was estimated to determine the amount of energy absorbed during fracture (Figure 6.4). Once again, removal of various components of the natural fibres had no significant effect on the Charpy impact strength of the composite materials, even in the case of laccase treated flax fibres. Bledzki *et al.* (2007) [17] compared the mechanical properties of abaca, hemp, and flax reinforced polypropylene composites. In our study, the reported impact strengths are within limits of those reported by Bledzki *et al.* (2007) [17] for 20 % flax and hemp reinforced polypropylene composites.

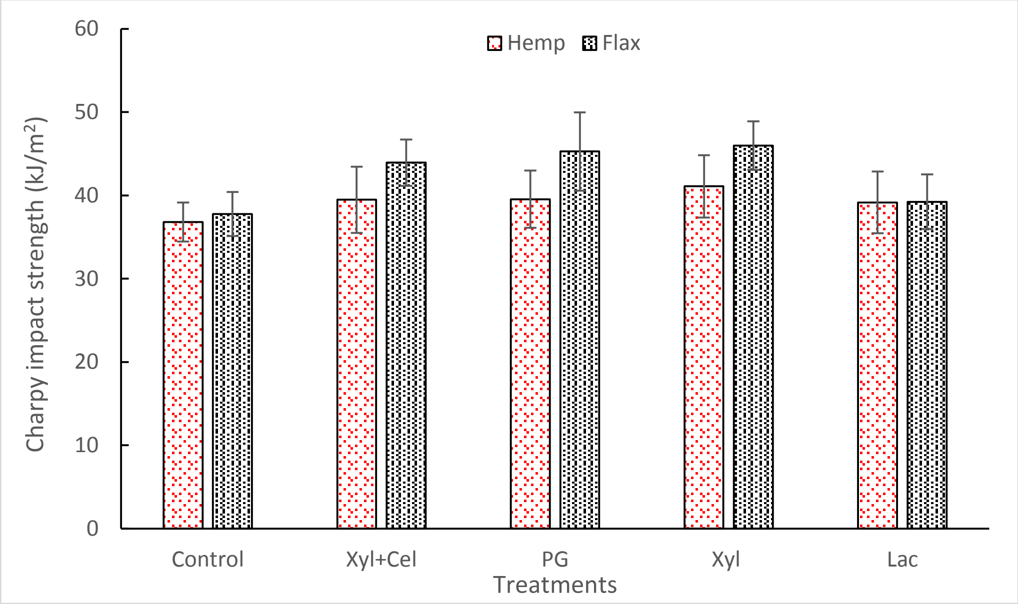


Figure 6.4 Charpy impact of the various natural fibre composites with the following enzymatic fibre treatments: unreacted (Control), xylanase + cellulase (Xyl +Cel), polygalacturonase (PG), laccase (Lac), and xylanase (Xyl) NFPPs.

6.3.3 Thermal stability

The thermal stability of composites plays a key role in both processing and determining the ultimate type of applications of the materials. The thermographs (single runs) for the different enzyme treated fibre reinforced matrices are given in Figure 6.5. Results presented for the hemp systems were very similar to the flax system and thus only the hemp is shown. Most of the composites were characterized by two stages of degradation. This can be attributed to the different thermal stabilities of cellulose, hemicellulose, and lignin components of the hemp and flax fibres. Previous studies have shown that in lignocellulosic materials, hemicellulose, and pectic materials degrades at a lower temperature than cellulose and lignin [7, 18]. Thus, we expected the removal of thermally labile hemicellulose and pectic materials through enzymatic treatment would improve the thermal properties of the resulting composites. Accordingly, polypropylene reinforced with polygalacturonase or xylanase treated hemp fibres were characterized with one stage of decomposition. It is plausible that the removal of pectic material and hemicellulose respectively led to this observation.

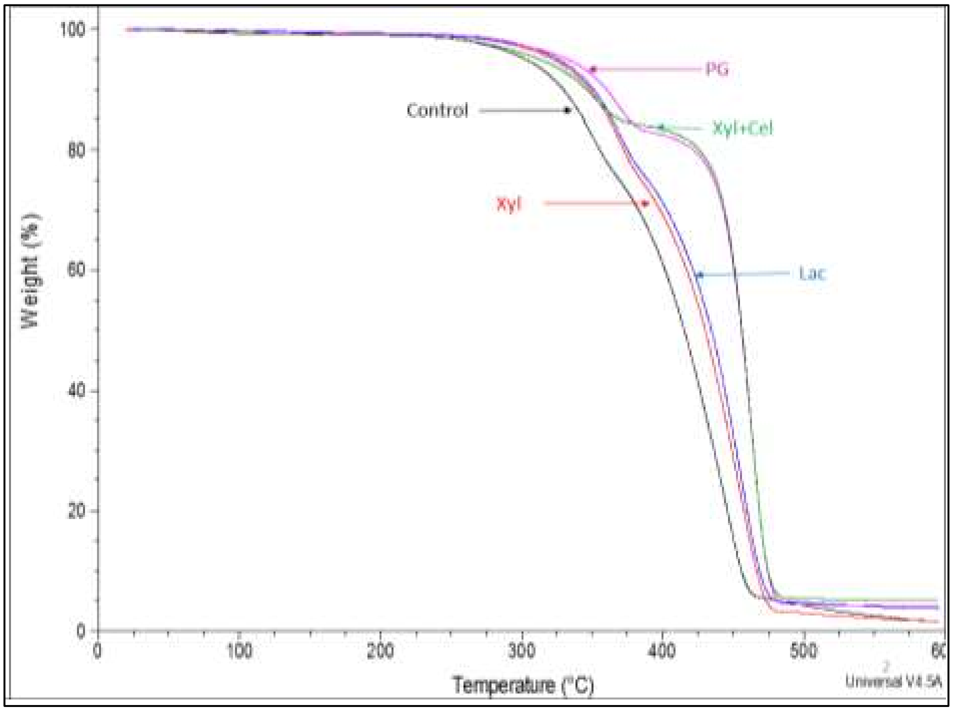
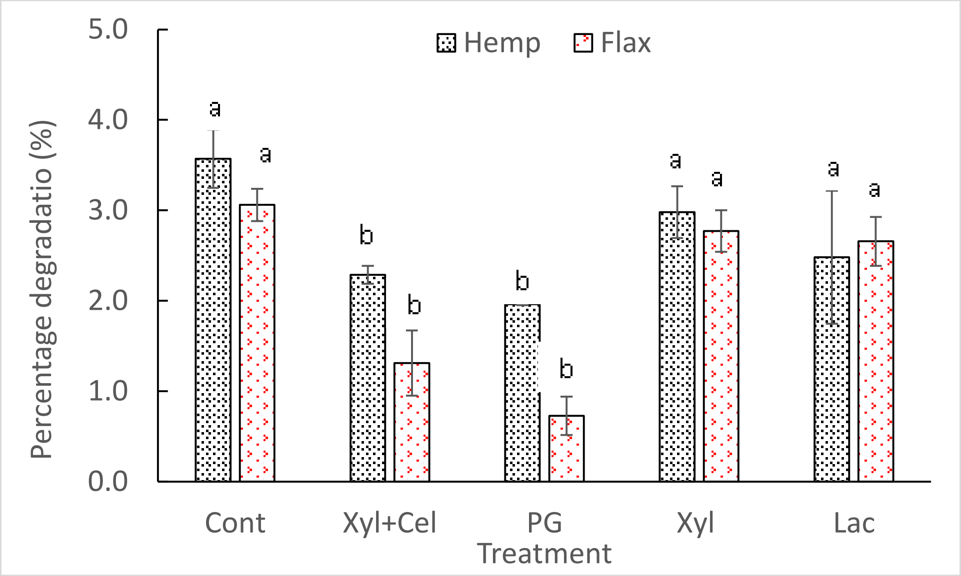


Figure 6.5 Sample TGA thermographs of CNT – control, Xyl – xylanase, Lac – laccase, PG – polygalacturonase, and Xyl + Cel – xylanase + cellulase treated hemp reinforced composites. Each experiment was replicated at least three times for each specimen and three dog bones of the same treatment were randomly selected.

The percentage degradation between 200 and 300 °C is presented in Figure 6.6. This degradation limit was selected for two main reasons. Firstly, the natural fibre samples tend to degrade exponentially after 200 °C. It can be observed that there were significant ($p<0.05$) decreases in the mass percentage of composite material that degraded over this temperature range for incorporating Xyl + Cel and PG treated hemp and flax fibre. This is in agreement with our results above that showed the removal of hemicellulosic and pectic material resulted in better thermal resistance of the composite materials.

Aside from the removal of hemicellulose and pectic materials, treatment of hemp or flax fibres with Xyl + Cel and PG also resulted in decreased roughness of the fibre surface as reported previously [14]. This can plausibly allow for improved fibre dispersion, interaction with polypropylene, and adhesion with the matrix. This cannot be seen conclusively from the micrographs presented in section 6.3.4, but it is a possibility. It should be noted, the mechanical property of the manufactured composites are dependent on the individual components, the interface, and the dispersion/orientation of the filler within the matric. But for the thermal properties, the resistance to heating is more dependent on the components of the composite material. As a result, removing the hemicellulose and pectic content can result in thermal improvements. For hemp and flax fibres treated with laccase, there was no improvement in percentage degradation due to of the removal of lignin, the most thermally stable component.



$^{a, b}$ Comparison was made among each NFPP system relative to the control for that fibre type. Different letters signify difference at $p<0.05$.

Figure 6.6 Percentage degradation (between 200-300 °C) for the controls (CNT), xylanase + cellulase (Xyl +CEl), polygalacturonase (PG), laccase (Lac), and xylanase (Xyl) NFPPs.

Also, the removal of accessible cellulose using Xyl+Cel could have triggered the changes observed. Giudicianni *et al.* (2013) [21] reported that the degradation of cellulose is influenced by the presence of lignin that seems to favor that decomposition of levoglucosan to form lower molecular weight compounds at the expense of levoglucosan polymerization. Also, higher amount of hydrogen bonding between crystalline cellulosic chains leads to more a more ordered and packed cellulose region, thus increasing the thermal decomposition temperature for Xyl + Cel treated fibre samples [22 - 24]. This explains the better performance of the Xyl+Cel over the Xyl system.

The temperatures at which twenty five percent of the composite materials are degraded are presented in Table 6.4. When compared to the controls, composites with fibres (hemp and flax) treated with Xyl + Cel (removal of hemicellulose and accessible / amorphous cellulose) or PG (pectic material) were characterized by better resistance to degradation. The increase in temperature needed to remove or degrade 5-15 % of the fibres' mass may again be explained by the partial removal of thermally labile hemicellulose and pectic material.

Table 6.4 T_{25} for different composite systems (CNT – control, Xyl+Cel – xylanase + cellulase, PG – polygalacturonase, Xyl – xylanase, and Lac – laccase treated fibre reinforced composites).

System	Temperature for 25 % degradation (°C)	
	Hemp	Flax
Control	397 ± 9.01^b	401 ± 4.16^b
Xyl + Cel	438 ± 8.45^a	419 ± 1.44^a
PG	446 ± 3.47^a	429 ± 3.71^a
Xyl	428 ± 2.99^a	417 ± 2.00^a
Lac	411 ± 3.84^b	413 ± 2.67^a

^{a,b} Samples with different letters are significantly different at $p<0.05$. Comparisons were made between each treatment and control for that fibre type.

Hemp and flax fibres treated with laccase were characterized by no changes. One reason for this may be the low lignin content of these specific fibre types. Hence, the laccase treated and controls samples are not appreciably different. It should be noted that there was a reduction in mechanical properties for the composites when laccase was used to treat the fibres, but this was not the case for the thermal properties. Thermal properties of natural fibre composites are dependent on the composition of the fibres within the matrix while mechanical property is influenced by the interface, the distribution of the fibres, and the type of natural fibres and matrix. Hence, removal of the appreciably low lignin content can impact the interface because lignin plausibly binds better with polypropylene when compared to cellulose and hemicellulose. On the other hand, the thermal properties of these materials are based on transitions and specific degradation of each component at a given temperature [19].

However, the thermal stability of all the biocomposites was within acceptable ranges. Furthermore, enzyme systems such as Xyl+Cel and PG, imparted properties that may be advantageous in the future.

6.3.4 Water resistance

The moisture absorption of composites is an important determinant that influences the effectiveness of these composite materials when used in a specific application. Many polymers and polymeric composite materials absorb moisture in humid atmospheres or when immersed in water, which results in deterioration of the fibre and or matrix interface. Water absorption and subsequent deterioration of the composites adversely affect many properties of the composites, including the mechanical properties [13, 16].

Five specimens per sample were immersed for each system. The percentage moisture absorption for the different systems are presented in Figure 6.7. From the graphs, all enzymatic treatments are shown to decrease moisture absorption relative to untreated (control) fibre composites. Xyl+Cel and PG treatments are seen to provide the greatest reduction in moisture for both hemp and flax fibre composites. Based on Table 6.3, it can be assumed that the removal of hemicellulose and pectic material may play a role in this improved moisture resistance.

According to Espert *et al.* (2004) [20], moisture absorption into composite materials is governed by three distinct mechanisms. The main phenomenon can be thought of diffusion of water molecules between the micro gaps of the polymer chains or the transport along the fibre itself via capillary and diffusion mechanisms. The other less common mechanisms are capillary transport along fibre/matrix interface. The dominant mechanism in this study can be determined looking at scanning electron micrographs presented in the next section. It can be assumed that the weak interfacial bonding between the polar fibres and the non-polar polypropylene resulted in debonding of the fibres and matrix, allowing water molecules to percolate into the composite material. Removal of polar moieties on the surface of the fibres through enzymatic treatment allows for better interaction within the NFPP.

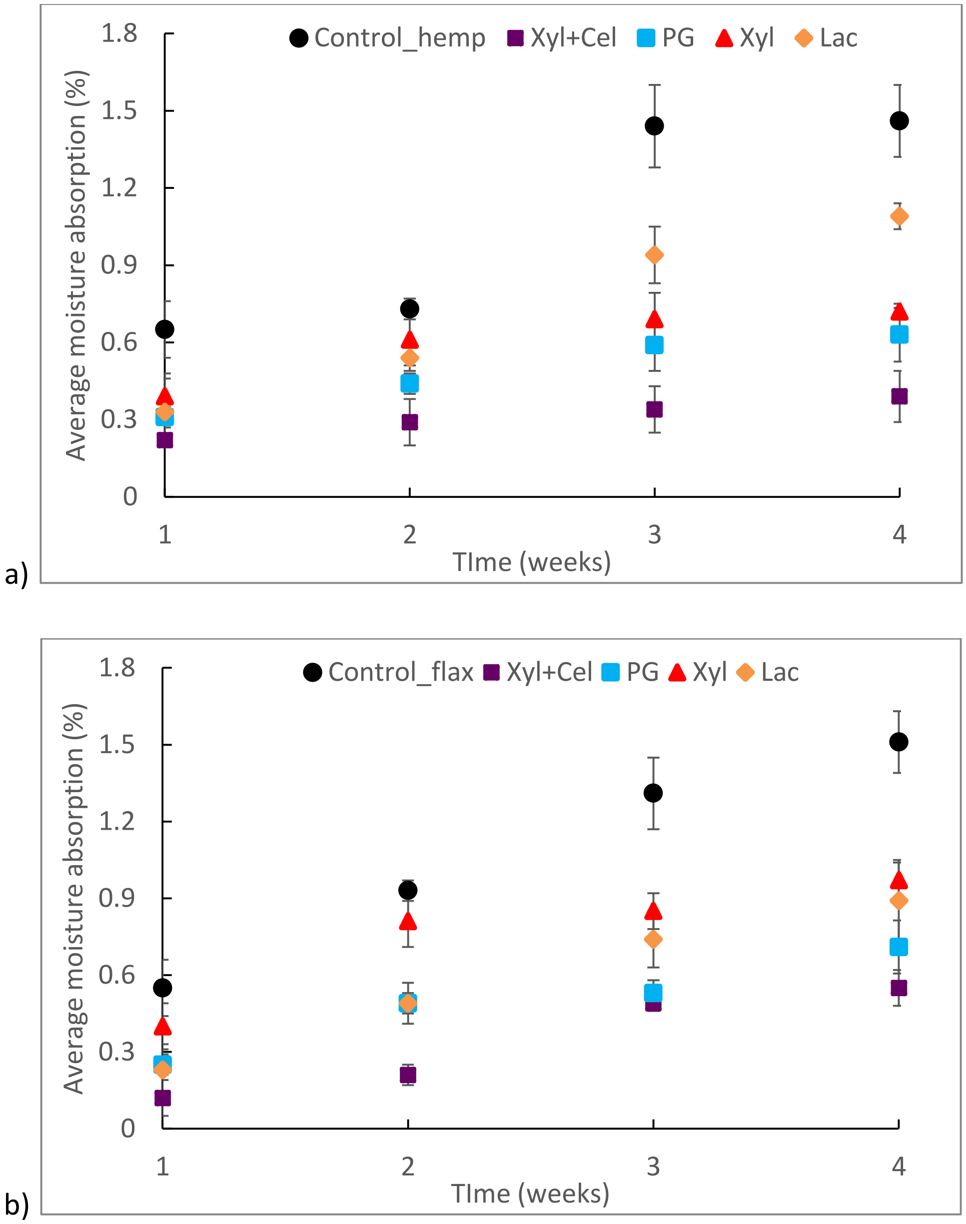


Figure 6.7 Water absorption over 4 weeks for untested hemp (a) and flax (9) composites made from enzyme-treated (control, Xyl – xylanase, Lac – laccase, PG – polygalacturonase, and Xyl + Cel – xylanase + cellulase treated fibre reinforced composites)

In summary, enzymatic treatment of hemp and flax fibres prior to compounding with polypropylene results in significant improvements in moisture resistance depending on the specific removal of components.

6.3.5 Scanning Electron Microscopy

Scanning electron microscopy has been used to study the surface morphological patterns of modified natural fibres [1, 7, 11]. It should be noted that, the micrographs presented in Figure 6.8 were taken from the site of fracture of the composite material after Charpy testing. The images obtained for both systems, hemp and flax were quite similar, and thus only those for hemp are presented. Based on observation of the micrographs, the control sample was characterized with greater damage as result of testing. One plausible reason for this may be the poor interfacial bonding or mixing exhibited by the untreated fibres because of the high surface polarity. Comparison of these treated fibres relative to the control reveals a better distribution of matrix and fibre, indicating better mixing. Also, treated samples were characterized with a uniform and finer fibre size distribution (no bulky chunks as in control) based on visual observation.

In summary, SEM micrographs indicate that the control interacts less with the matrix and fibre pull out is more evident.

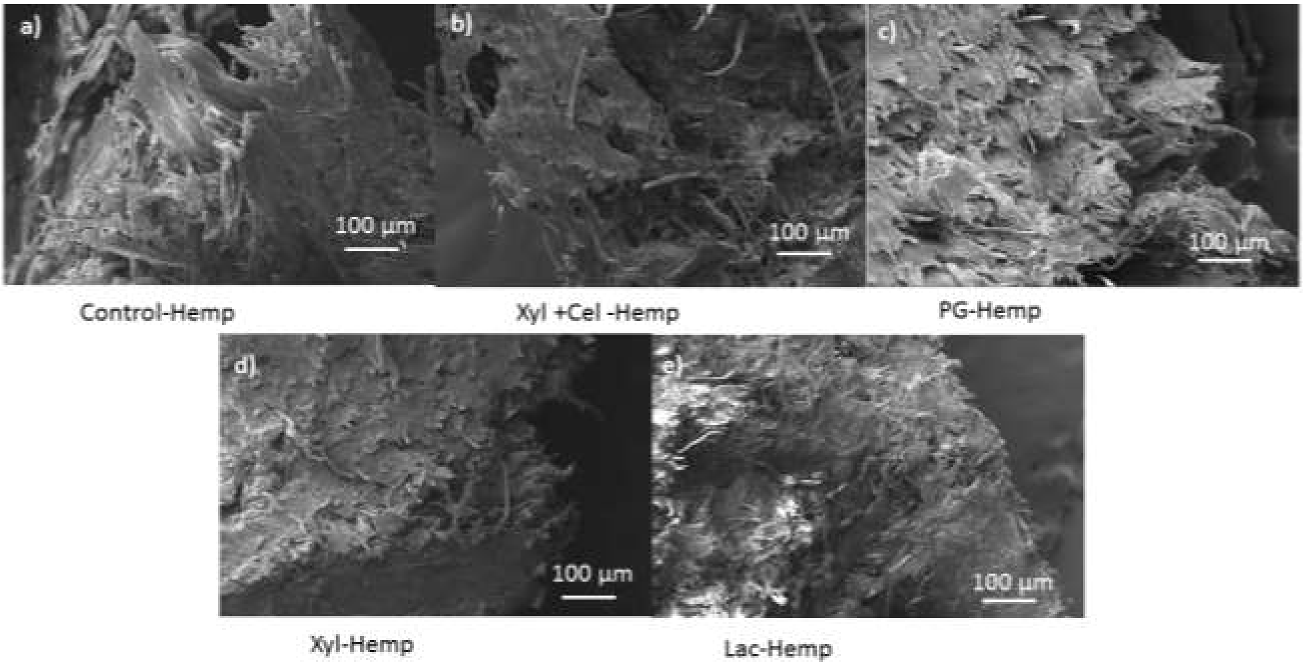


Figure 6.8 SEM micrographs for enzyme treated hemp reinforced composites treated (a) control, b) Xyl + Cel – xylanase + cellulase, c) PG – polygalacturonase, d) Lac – laccase, and e) Xyl – xylanase treated fibre reinforced composites)

6.4 CONCLUSION

Specific degradation of hemp and flax fibres using Xyl + Cel or PG produced fibres that were better suited for reinforcing NFPP composites, as evidenced by the better water resistance and thermal stability of the composite material. These improvements likely resulted from the removal of the pectic and hemicellulosic content by polygalacturonase and xylanase + cellulase, respectively. Furthermore, the removal of these components in most cases had no significant influence on the mechanical properties of the composites. This can be very advantageous because the enzymatic treatments resulted in little or no change in the mechanical properties.

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CHAPTER 7.

Supplementation of epoxy with tall oil rosin acids to produce plastics[6]

7.0 RATIONALE

Epoxy is one of the more versatile material for many applications including laminates, composites, and adhesives. Nevertheless, epoxy is derived from petro-chemical based material and is neither renewable nor biodegradable. In this chapter, we detail the replacement of a given fraction of epoxy with tall oil rosin acids (TORAs), a waste product from the pulping industry that is renewable and sustainable. The end goal is that supplementation of epoxy can be done, so as to reduce the cost without affecting the properties of the manufactured plastics.

7.1 INTRODUCTION

Recently, there has been an increasing demand for natural products applicable to the industrial space because of the concerns of waste disposal, the depletion of non-renewable resources, and environmental issues. Renewable resources have the potential to replace petro-chemical-based platforms through the design of bio-based systems that can compete with the former in terms of cost. The use of biomass or byproducts from the biomass processing for production of composites and plastics has been identified as one of the key areas [1-3].

A very important trend has been the development of protocols to produce plastics using renewable feedstocks in an attempt to reduce the dependence on petro-chemical based materials and to aid in the mitigation of possible harmful environmental effects. Researchers have used different proteins such as egg albumin [4], soy protein [5], and carbohydrate sources such as corn [6] to produce plastics with properties mimicking those of the conventional polymers. Despite being sourced from renewable resources, most of these systems are limited by large amounts of biological waste as a result of fermentation, poor mechanical, and water resistance properties [11]. The unique feature of most bio based polymer systems is the availability of large numbers of functional groups that can be exploited to

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[6] A version of this chapter has been prepared for peer-review.

produce plastics via crosslinking or polymerization. Based on a survey of the current literature and the possible technologies of the future, I postulate that production of polymers or plastics from renewable sources which are part of an existing technology (such as the mining or pulping sectors) would be integral. In that case, the production of polymers or plastics from waste materials from established industries would be an integral technology platform.

Mekonnen *et al.* (2013) [7] studied the possibility of thermosetting proteinaceous plastics from hydrolyzed specified risk material (SRM). SRM which was traditionally used in animal feed has been identified as one of the main source of bovine spongiform encephalopathy (BSE). As a result, rendering plants are faced with costly disposal of this abundant and low value material. These researchers produced plastics from thermosetting epoxy resin with the hydrolyzed SRM and these plastics were characterized with excellent mechanical, water resistance, and thermal properties. This study highlighted how one industry (Rendering) can produce a low value material that can be transformed into a high value material in another sector (Plastic manufacture), effectively creating a closed cycle biorefinery. As a result, in this research, we aim to study the use of a waste material from the pulping industry as a possible supplement/curing agent for epoxy plastics.

Tall oil has been widely used in industrial applications of late. It has been used as additive during the production of diesel, production of glue, and nylon [1]. Tall oil is a by-product of the paper and pulping industry. After the kraft pulping of coniferous wood, tall oil can be recovered from the black liquor. The chemical composition of tall oil depends on the species and age of pine, as well as the processing involved. Tall oil consists of primarily fatty acids, rosin acids, and unsaponifiable matter (e.g. sterols, waxes, and hydrocarbon) in an approximate ratio of 5:4:1 [1].

In the present work, tall oil was separated into different fractions using distillation. The heavier fraction, comprising mainly of tall oil rosin acids (TORAs) was used for the subsequent study. The use of this heavy fraction of polycyclic compounds, primarily abietic acid was used to supplement epoxy to produce plastics. The thermal and mechanical properties were unaffected up to 15 % replacement with the TORAs in the epoxy blends.

7.2 EXPERIMENTAL

7.2.1 Materials

Tall oil was obtained from a kraft pulping mill in Alberta. The tall oil was distilled to produce the different fractions. Tall oil rosin acid was the viscous and black liquor remaining after the process. Araldite® 506 epoxy resin and 2, 4, 6- Tris (dimethylaminomethyl) phenol (mol wt. 265.39) were obtained from Sigma-Aldrich (Minnesota, U.S.A).

7.2.2 Supplementation of epoxy resin with TORAs- Production of plastics

TORAs and epoxy resin were heated in a convection oven at 60 °C for 2 hours to reduce the viscosity. Mixtures of 5, 15, 25, and 35 % (w/w) TORAs, 5 % (w/w) hardener (2, 4, 6- Tris (dimethylaminomethyl) phenol) and the remainder in epoxy were mixed vigorously for 5 minutes before being placed in the molds. The weight ratios were selected based on possible number of functional groups capable of crosslinking the TORAs fractions. The different formulations are presented in Table 7.1.

Table 7.1 Relative percentage of different components used to produce the plastics

System	% Epoxy (w/w)	% TORAs (w/w)	% Hardener (w/w)
Control	95	0	5
5 %	90	5	5
15 %	80	15	5
25 %	70	25	5
35 %	60	35	5

High concentrations (25 and 35%) of TORAs when mixed with epoxy, impeded mixing, and tend to cure faster (3 hours) when compared to the lower concentrations which took at least 15 hours to cure. Curing took place at 165 °C for 5 hours followed by post curing at 180 °C for an hour. Plastics were cooled and placed in desiccator overnight. It should be noted, in the preliminary reactions, the absence of a hardener resulted in poorly cured samples.

7.2.3 Characterization of plastics

7.2.3.1 Infrared spectroscopy

Samples were finely comminuted, mixed with KBr, and compressed into a pellet for analysis. A Shimadzu 8400 S, taking 100 scans for each samples with a resolution of 4 cm^{-1} was used for all analyses. The spectra were obtained by smoothing with a factor of 10, to improve the sharpness of the peaks. All spectra were reproducible for each sample.

7.2.3.2 Differential scanning calorimetry

A thermal analyzer (Q_{100} TA Instruments, USA) was used to study the thermal transition temperatures of the plastics under a nitrogen atmosphere. For each test, approximately 5 mg of sample was weighed into an aluminum pan and heated at 10 °C per min from 0 to 175 °C. Triplicate analysis was done for each system.

7.2.3.3 Thermal gravimetric analysis

The thermal stability of the plastics was determined using a Q_{500} series, TA instruments (USA). Plastics samples (5-10 mg) were placed in platinum pans and heated at 10 °C per min from 20 to 600 °C under a nitrogen atmosphere.

7.2.3.4 Mechanical properties

Test specimens of dimensions 4 × 0.5'' were obtained using silicon molds. Specimens were conditioned in a room maintained at a relative humidity of 45 ± 5 % and temperature of 22 ± 2 °C overnight before testing. Mechanical properties namely; tensile strength, modulus, and elongation at break were determined using an Instron (Norwood, MA) equipped with a 5 kN load cell at a crosshead speed of 10 mm/min. ASTM D639-14 was followed for all the mechanical properties measured.

7.2.4 Statistical analysis

Statistical analyses were done as outlined by George *et al.* (2014) [3]. Treatments were done in triplicates and results reported as mean ± standard deviation. The statistical analyses of the data were conducted using the statistical software package SAS Version 9.4. The Kruskal Wallis Test was applied to the data populations involved, with a 95 % confidence level ($P < 0.05$).

7.3 RESULTS AND DISCUSSION

7.3.1 Reaction chemistry and possible crosslinking

Tall oil rosin acids comprise primarily abietic acid. A possible reaction scheme for the crosslinking of epoxy with abietic acid is shown in Figure 7.1.

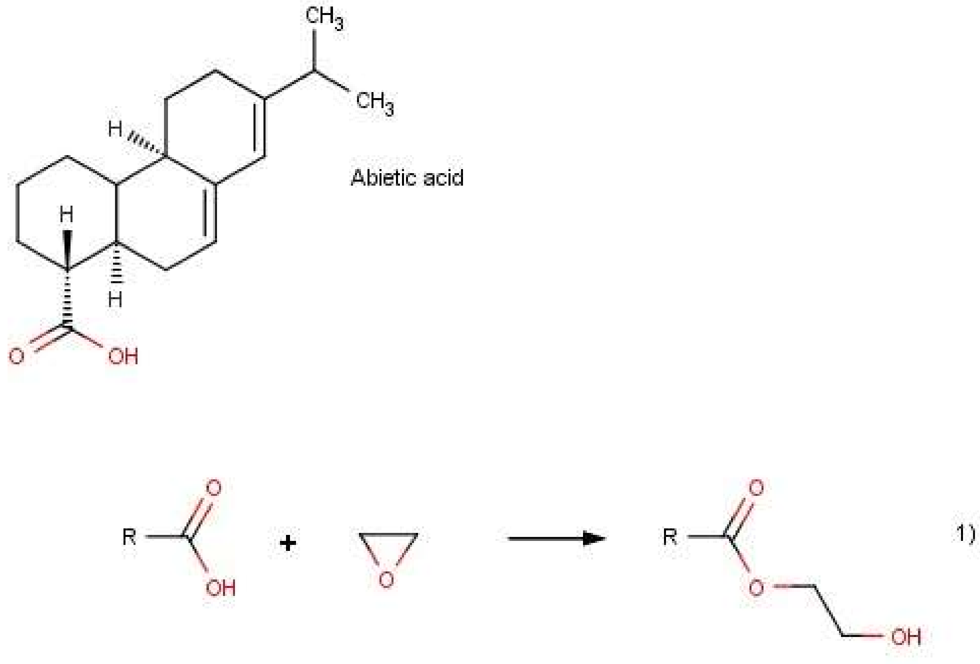

Figure 7.1 Plausible reaction between epoxy and the hydroxyl group of abietic acids found in tall oil rosin acids

It is possible that the carboxylic or hydroxyl groups in the rosin acids can react with epoxy as shown in Figure 7.1. The epoxide functional group can react with a variety groups, including carboxylic and alcohol groups. In this study, TORAs have hydroxyl and carboxylic groups that can be used to crosslink the base polymer, epoxy. The final product went through a number of transitions, from liquid to viscous rubbery and finally solid state. Because of the nature of the chemical reaction taking place between the hardener (2, 4, 6-Tris (dimethylaminomethyl) phenol), and the TORAs, the solid state is irreversible. It is very important to note, given that each moiety of tall oil rosin acid has one reactive site (the carboxylic group), the TORAs acts more as a filler than a curing agent in this research study.

7.3.2 Infrared spectroscopy

IR spectra for epoxy, the hardener, TORAs and the plastics are given in Figure 7.2. It can be observed that the epoxide peak at around 915 cm^{-1} reduces in intensity when compared to the plastic spectrum. Also, the peaks at approximately 1400 cm^{-1} in the plastics are as a result of aromatic stretches from the TORAs bonds, confirming the reaction of the epoxide groups with the rosin acids. The -OH groups present at 3215-3400 cm^{-1} in the TORAs significantly diminished in the plastic samples. This also confirms the reaction of the acid with the epoxy groups. Finally, the peak at approximately 2820 cm^{-1} corresponds to the C=H stretch of aldehydes. It is not clear as to how carbonyl compounds may be introduced or produced, but one plausible reason may be from the TORAs, since traces of lignin fragments (rich in aldehyde groups) can be found in the complex mix. The peaks reported here were in agreement with the ones published my Mekennon *et al.* (2013) [7] when they investigated the thermosetting properties of specified risk material. It should be noted, the spectra obtained for epoxy was confirmed using National Institute of Standards and Technology reference spectra.

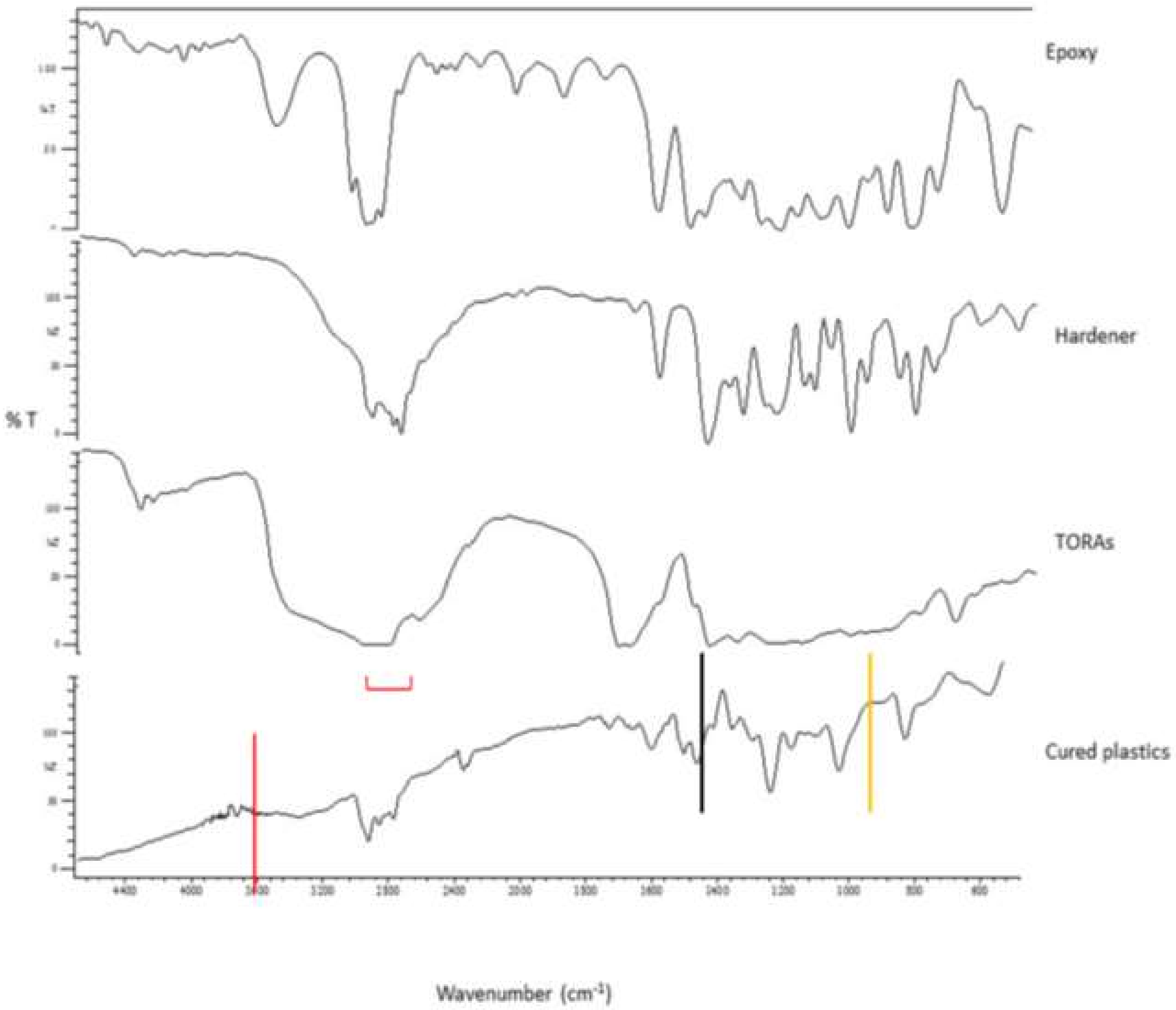

Figure 7.2 Infrared spectra for epoxy, hardener, TORAs, and plastics.

7.3.3 Thermal properties

The thermal properties of the plastics were investigated using Differential scanning calorimetry (DSC) and Thermogravimetric analysis (TGA). TGA was used to determine the thermal stability of the plastics at different temperatures. The onset decomposition temperature is reported in Table 7.2. When compared to the virgin epoxy blend, replacement up to 15 % (w/w) with TORAs can be used without altering the thermal stability of the produced plastics. On the other hand, as the concentration of TORAs increases in the plastics, there was a corresponding decrease in the temperature needed for degradation of 10 % of the samples. It was observed, the thermal stability of the plastic gradually decreased with increasing TORAs concentration in the formulation. Plausible reasons for this may be the variation in crosslinking of the different formulations, superior thermal behavior of virgin epoxy, and the heterogeneous nature of the TORAs.

Table 7.2 Thermal properties for the different plastic blends

System	Onset decomposition temperature (^oC)	Temperature for 10 % degradation (^oC) -T_{10}	T_g	Peak of curing
Control	316 ± 6.23^a	385 ± 7.21^a	95.1 ± 3.85^a	141 ± 1.09^a
5%	314 ± 7.57^a	385 ± 6.56^a	92.7 ± 2.30^a	138 ± 0.72^a
15%	316 ± 8.22^a	367 ± 4.93^b	90.4 ± 3.28^a	136 ± 2.87^b
25%	292 ± 3.61^b	369 ± 1.15^b	79.8 ± 3.22^b	122 ± 3.62^c
35%	275 ± 6.03^c	355 ± 4.36^c	67.6 ± 1.94^c	126 ± 2.71^c

Systems with different letters are significantly different from each other at $p<0.05$

Differential scanning calorimetry (DSC) can be used to determine the peak of curing and glass transition temperature of the plastic samples. Curing was observed for all TORAs/epoxy formulations between 125-145 °C. Curing involves the exothermic reaction when the epoxide group of the epoxy resin and the reactive functional group in the TORAs react. As observed in Table 7.2, there is a gradual decrease in the peak of curing, plausibly as a result of relative availability of functional groups in TORAs. Mekonnen *et al.* (2013) [7] and Zhao *et al.* (2012) [8] reported similar pattern of results. Zhao *et al.* (2012) [8] studied the effect of varying amounts of curing agents in epoxy blends and reported on the thermal properties. They reported similar curing, as the agent concentration increases, the curing temperature decreases.

Another very important parameter for characterization of plastics is the glass transition temperature (Tg). This is the temperature where polymers goes from a hard glass like state to a rubbery state, and can provide useful insight into the structure and property of polymeric materials. As reported in Table

7.2, there is a reduction in Tg with increasing TORAs concentration in the formulations. This difference can be explained by the variability in chemical reaction and because of the heterogeneous nature of TORAs. Also, TORAs are characterized with the polycyclic structure, which when introduced into the epoxy crystalline region may have caused kinks, resulting in voids. As a result, less energy or heat is needed to transform the material from the brittle to rubbery state, via bonding disruption.

7.3.4 Mechanical properties

An insight into the polymer integrity under stress conditions can be obtained by evaluating the mechanical properties of the plastics formulated. The tensile strength, tensile modulus, and elongation at break are presented in Table 7.3. According to Mekonnen *et al.* (2013) [7], mechanical properties of plastics depend on the crosslinking density and network structure of the resulting thermoset. Supplementation with 15 and 25 % of epoxy with TORAs did not adversely affect the tensile strength of the plastics. Good dispersion and crosslinking between the epoxy resin and TORAs plausibly contributed to this. On the other hand, replacement of epoxy with TORAs resulted in decrease in stiffness or tensile modulus of the plastics. One plausible reason for this may be the introduction of kinks in the polymer chains of the plastics as a result of the structure of the TORAs, resulting in flaws. Wang *et al.* (2005) [9] investigated the mechanical properties of epoxy nanocomposites with exfoliated clay and reported significant decrease in tensile strength with increasing clay content. They mentioned that flaws in the existing nanocomposites, as a result of clay particles getting trapped within bubbles during sample preparation may have resulted in the decrease in tensile properties observed. Hence, as the volume of clay filler increases, so do the flaws within the final structure. As similarly reported in this present study, there is a decrease in modulus with increasing TORAs mass fraction. One of the earlier studies looking at reducing cost and amount of epoxy used in plastics was reported by Manzione *et al.* in 1981 [10]. They utilized dissolved rubber to produce rubber-modified epoxies via careful control of compatibility and curing. The range of tensile modulus reported in that study (0.935 - 1.256 GPa) was lower than observed in the present chapter. As a result, it may be possible to substitute epoxy with TORAs up to 35 % and still produce plastics with comparable mechanical properties.

Finally, as the percentage of TORAs increased in the plastics, the elongation at break (%) increased. This is in agreement with the decrease in T_g observed earlier. As the concentration of TORAs increased, there is the possibly of a larger number of chain disruptions (polycyclic rings), hence allowing the macrostructure of the polymer to stretch further without failing. In summary, the supplementation of epoxy resin with TORAs has the potential to reduce cost and produce plastics with mechanical strengths that are comparable up to 15 % replacement or up to 35 % replacement, depending on the application.

Table 7.3 Mechanical properties for formulated plastics

System	Tensile strength (MPa)	Tensile Modulus (GPa)	Elongation at break (%)
Control	24.5± 1.90^{a}	2.46 ± 0.07^{a}	6.77 ± 0.54^{a}
5%	22.5 ± 1.07^{a}	1.99 ± 0.11^{b}	5.78 ± 0.78^{a}
15%	24.4 ± 0.93^{a}	1.91 ± 0.22^{b}	5.54 ± 0.98^{a}
25%	14.7 ± 1.92^{b}	1.66 ± 0.04^{c}	9.02 ± 0.71^{b}
35%	13.6 ± 0.24^{b}	1.91 ± 0.10^{b}	8.86 ± 0.62^{b}

$^{a,\ b,\ \&\ c}$ Systems with different letters are significantly different from each other at $p<0.05$

CONCLUSION

TORAs can be used as a suitable filler or supplementary agent for epoxy based plastics. Infrared spectroscopy confirmed partial chemical reaction of the epoxide groups with the carboxylic groups of the TORAs species. Also, supplementation up to 15 % of the epoxy resin can be done without affecting the thermal properties of the plastics. Also, up to 35 % replacement with TORAs can be used to produce plastics with mechanical properties better than many reported studies. In summary, this study outlined a successful path for utilizing an abundant and possibly hazardous material from the pulping industry to partially supplement epoxy base plastics.

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CHAPTER 8.

Life cycle assessment of biomass as a possible replacement for glass fibres as reinforcement in composite materials[7]

8.0 RATIONALE

A life cycle assessment is performed on chemical methods for enhancement of hemp fibres for composite applications. Specifically, chemical methods studied within this thesis study, involving the grafting of sulfonic acid derivatives onto the surface of hemp fibres, was compared to established and previously published methods. Also, the subsequent reinforcement of polypropylene, a petroleum based resin, and polylactic acid, a renewable polymer, using these treated fibres were assessed. The end goal was to determine whether the studied systems are environmentally better than the other methods.

8.1 INTRODUCTION

Natural fibre reinforced composites materials have been studied for different applications, including the housing and the automotive. Natural fibres such as hemp, kenaf, and sisal fibres have been used to reinforce polymers for composites applications because of advantages such as low density, renewability, chemical resistance, and comparable specific mechanical strength to carbon and glass fibres [1, 2]. Despite these features, natural fibres need to be enhanced to be compatible with petroleum-based polymers. As a result, researchers have studied a number of different methods for improving on the limitations of natural fibres. These methods, including mercerization [3, 4] and acetylation [5], have been used to produce enhanced fibres. But there has been little or no research on assessing the effectiveness of these methods in terms of environmental and economic impacts. In fact, the use of natural fibres or any form of biomass in composites materials does not automatically renders it sustainable. In other words, natural may not always be environmentally friendly. Hence, over the past decade, researchers have tried to couple their chemistry with evaluating the sustainability of these systems wherever possible.

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[7] A version of this chapter has been prepared for peer review.

A good evaluation of the fundamental loops and processes of the entire life cycle of a new material, such as the stages involved with raw material production, treatment of the material, final fabrication of the composite, and the paths for material end of life is becoming more necessary to decide the green tag for a specific material. Life cycle assessment (LCA) is a tool designed for assessing the environmental impacts of a given method, process, or product (PRé Consultants, 2014). The data provided can be used to improve on a given process or method. Common impact categories assessed in LCA include global warming, acidification, eutrophication, ozone depletion, production of photochemical matter, and many other indicators. These impacts are grouped into three main damage assessment criteria, namely, the effect on Human Health, Ecosystem, and Resources.

In term of phases, most LCA consist of four main stages. These parts are based on ISO 14040 - 14042, (1997- 2000). The main phases are goal and scope, life cycle inventory, life cycle impact assessment, and life cycle interpretation. The goal and scope outlines the main motivation for a given study, the unit of study (functional unit), and the factors considered (system boundaries). Life cycle inventory consists of grouping and listing pollutant emissions and consumption of resources per a given functional unit. With an idea of the inventory, the impact assessment can be done. Basically, this entails classification and evaluation of the different environmental impacts of the pollutants during a given cycle. Finally, the interpretation stage involves studying the results to determine better paths or possible improvements. When these four components are done cohesively, a good representation of a process or product can be modeled and studied (PRé Consultants, 2014) [6].

There is limited research reported on methods for treating natural fibres and their products. De Vegt and Haije (1997) [7] compared rotor blades produced from flax fibre reinforced epoxy, carbon reinforced epoxy, and glass fibre reinforced polyester. A better environmental score was given to the natural fibre system, because less energy and resources were required to cultivate the fibres. Wang *et al.* (2013) [8] did a comparative analysis on sheet molds made from natural fibres and glass fibres. They concluded that kenaf reinforced sheets performed better than their glass fibre counterparts based on the environmental factors studied. In fact, the global warming potentials of kenaf reinforced sheets and kenaf soy resin based sheets were 45 and 58 %, respectively, when compared to the glass reinforced sheets. The mode of natural fibre usage can significantly influence the effectiveness of the composites. As Dissanayake *et al.* (2009) [9] reported, when flax fibres are processed into a yarn, there is little or no improvement in environmental impacts when compared to glass fibre reinforced polymers. But, sliver (pre-spun fibres), when used to reinforce the polymer, had a significant influence on the impacts. These minute preferences of one technique over another have shown to significantly influence the overall impact reported by various studies. One of the first studies that looked at specific applications was reported by Wotzel *et al.* (1997) [10]. They fabricated an Audi A3 panel made from a hemp fibre epoxy composite. They considered the cultivation of the fibres, harvesting, processing of fibres, and manufacturing of the composite materials. The properties of the natural fibre composite was inferior to the control, but exhibited better environmental impacts. In these previous studies researchers have preferred to compare functional materials, but in the present study, we seek to actually compare the methods used to enhance the natural fibres.

In order to provide optimal environmental solutions, we intend on comparing the environmental impacts of two sulfonic acids (aniline-2-sulfonic – A2S and 3-aminotoulene-4-sulfonic acids – A3TS) to established and accepted chemical methods used for fibre enhancement (mercerization, acetylation, and propionylation). Each sulfonic acid was compared to the established methods, and the main environmental impacts were reported. Network trees and environmental impacts were determined and reported. The studied methods had less of an adverse impact on the environment when compared to the established methods.

8.2 METHODOLOGY

8.2.1 Goal and scope definition as per ISO 14040

The present work is a cradle-to-manufacture study comparing the main environmental impacts related to treatment of biomass for composite applications. A few chemical methods studied in our research group were compared to established mercerization and alkylation methods. Also, the subsequent use of these fibres to reinforce different polymers was modeled. The Life Cycle Assessment study was developed according to the ISO 14040 and 14044 methodology and done using SimaPro 8.0 (SimaPro 8.0, 2014).

The inventory data were obtained from the SimaPro database. Hemp information was obtained from Alberta farmers. It should be noted, no fertilizer or pesticide was used during the cultivation of the fibres. The specific aims developed for this study were:

- To determine if the studied chemical methods when compared to the established methods are advantageous from an human and environmental point of view
- To identify key environmental parameters and phases in the whole cycle which have significant influences
- To compare the environmental impacts of glass fibre production relative to production and treatment of hemp fibres
- To investigate the potential impacts of reinforcing polypropylene and polylactic acid with glass fibre, the studied fibres

8.2.1.1 Functional unit

In most LCA studies, a tangible product is used as a functional unit. For example, La Rosa *et al.* (2014) [11] compared an eco-designed sandwich made from traditional and bio based polymers. Similarly, Xu *et al.* (2008) [6] examined the environmental impacts for producing wood fibre reinforced polypropylene composites. In this study, the functional unit examined was 1 kg of reinforcing hemp fibres after chemical treatment (dried mass). Also, composites made from the treated fibres and polymers were assessed.

According to the Canadian Hemp Trade Alliance, hemp is classified as *Cannabis sativa L.* (Cannabaceae). There have been fluctuations in hemp growth in Canada over the years, but in 2013, it was reported that approximately 66,671 acres were licensed to grow commercial hemp. Each plant is comprised of roughly 30 % bast fibre, 60 % hurd, and 10 % chaff. The production of composite from hemp requires the high tensile bast fibres. Prior to reaching the processing facilities, hemp plants are retted to separate the hemp's outer bast and core fibres. It can be thought of as a controlled biodegradation and usually involves leaving the plants in the field. Once the fibres are fully retted, in this specific study, they were transported to Alberta Innovates Technology Futures facility in Vegreville, Alberta, for decortication (fibre separation). Each stage of the hemp production was modeled, from growth until chemical treatment, and then subsequently used to reinforce polymers.

8.2.1.2 System boundaries

The main system boundaries for the comparative analysis are shown in Scheme 1. A bit detail of each step is given below. Ecoinvent 3 database was used for most inputs, otherwise stated.

a) *Cultivation of hemp fibres.* The tillage, growth, and harvesting techniques native to Alberta Hemp Growers Association was modeled. Hemp can be classified as an ideal rotation crop because its long taproot structure assists with conservation of the topsoil. Also, the natural degradation of leaves and stems improves soil nutrients. The hemp fibres produced for this study were obtained from farms on the outskirt of Edmonton, Alberta. Routine retting measures were used and fibres were extracted via hackling (combing of fibre samples to remove short broken fibres) and scutching (removes broken stalks and remaining shives and also short fibres) at Alberta Innovates Technology Futures, Vegreville Facility, Alberta. As previously mentioned, hemp was grown exclusively in the absence of herbicides or pesticides and the fibres were extracted in a chemical free and waste free (leaves, stalks returned to fields) process. In cases where data was not available or unclear, a report done by Gonzales-Garcia *et al.* (2010) [12] where they examined the environmental impacts associated with the production of hemp and flax fibres for paper pulp was used. Data from Ecoinvent v3 database was used as final option (PRé Sustainability Consultants, 2012). Information relevant to hemp fibre is given in more detail in Appendix II.

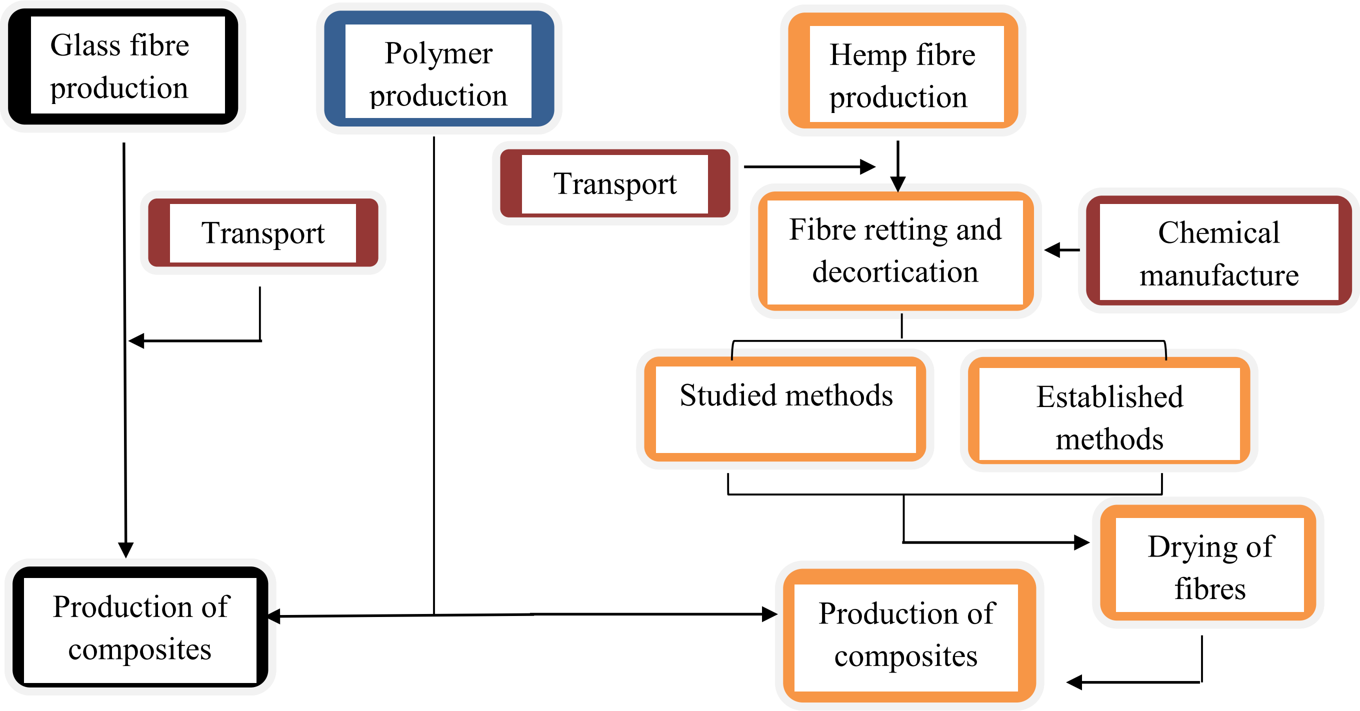
b) *Chemical manufacture.* Information needed on the chemicals needed for modeling the treatment of the fibres or pulp, namely, aniline-2-sulfonic acid (A2S), 4-aminotoulene-3-sulfonic acid (AT3S), acetic anhydride (AA), propionic anhydride (PA) and sodium hydroxide were obtained from the database. The information for most of the chemicals was found in the respective databases. In cases where the chemical was missing, data was imported and modeled similar to the manufacture of the given chemical.

c) *Treatment of fibres.* Hemp fibres were modeled to be treated in 100 g batches. Given the nature of the chemical treatments, there were variation in energy required for each process. Table 1 gives an approximation of the main chemicals and other requirements for hemp fibre treatments.

d) *Production of glass fibres and resins.* All materials were assumed to be commercial and can be sourced locally within Alberta, Canada, with minimal transportation impacts.

e) *Drying and removal of excess reagent.* As can be observed in Table 8.1, drying of the fibres and the energy for heating during treatment accounted for the total electricity. For A2S and A3TS systems, no energy was needed for fibre treatment, just for drying fibres.

f) *Production of composites*. Extrusion and injection molding were used to model the production of the composite materials. The fibre content (glass and hemp fibres) was maintained at 20 % (w/w) for all modeled systems.



Scheme 8.1 Outline of the different components of the study

8.2.1.3 Assumptions

The main assumptions made to fit this specific model are outlined below:

- Replanting of hemp was neglected

- CO_2 absorbed by the plants during their vegetative cycle were not estimated nor reported. Hence, as per convention (La Rosa *et al.* 2014) [11], it was assumed the CO_2 originating from the biomass is equivalent to the amount that was withdrawn from the atmosphere during growth, thus does not contribute to global warming.
- End of life of the treated fibres and eventual composite materials were not considered because the aim was not limited to a specific application.
- Fertilizer and pesticides are not necessary for the cultivation of hemp in Alberta
- No need for allocations because there were no co-products or byproducts considered in this study
- Glass fibre and resins are mostly sourced from Mississauga, Ontario, but in this study, we assumed that these raw materials can be obtained in Alberta.

8.2.2 Life-cycle inventory

Based on the general framework provided by ISO 14040-44 (2006), an inventory analysis was carried out to quantify the environmentally significant inputs and outputs of the systems under study, by means of a mass and energy balance. Different data sources were used. To ensure quality, the LCA worked within the ISO standards on data quality.

8.2.2.1 Geographical representation

The geographical area from which the data is relevant or obtained to satisfy the goal of the study is very important. Data for energy, materials, processes, and transportation are based on sources local to Alberta. Glass fibre and resin can be obtained from Mississauga, Ontario. Data from the processing facilities at Alberta Innovates Technology Futures were used for modeling the compounding of the treated fibres, or pulp, or glass fibres with the polymers. The parameters for each system are outlined in the Table 8.1.

Table 8.1 Chemical and other requirements for treatment of 1kg hemp fibres.

Process/chemical	Chemicals (per kg fibre)	Electricity [a] (kWh)	Water (kg)	Transportation [b] (tkm)
Chemical treatment of hemp fibres				
A2S	0.346 kg A2S	1.17	10	0.603
	0.05 kg $SOCl_2$			
	4.2 kg Pyridine			
A3TS	0.374 kg A3TS	1.17	10	0.603
	0.05 kg $SOCl_2$			
	4.2 kg Pyridine			
Established chemical methods				
Mercerization	5 kg NaOH	2.50	50	0.603
	0.05 kg HCl			
Acetylation (AA)[c]	10.8 kg acetic anhydride	2.85	-	0.603
	15.82 kg acetone			
Propionylation (PA)[c]	10.2 kg propionic anhydride	2.92	-	0.603
	15.82 kg acetone			
Resin and glass fibre parameters				
Glass fibre	1.0 kg			5
Polypropylene	4 kg	25		-
Polylactic acids	4 kg	25		-

[a] Calculated using Xu *et al.* 2008 [6]

[b] Distance for transport of hemp fibres and shipping of chemicals not considered.

[c] Data derived based on publication by Tserki *et al.* (2005) [5]

It can be observed, that the electricity requirement is the same for most of the resin and glass fibre stages because of the dependence on extrusion and injection molding.

8.2.2.3 Temporal representation

The data presented and modeled in this study represents current products and practices as updated per SimaPro 8.0. The manufacturing of composites were not modeled for one specific application because that can be a matter of just attaching the correct configuration of a die to the injection molding equipment. Instead, the focus is on the effect of these processes on the environment.

8.2.3 Life cycle impact assessment methodology

The main software, methods, and a descriptive outline are summarized below. These descriptions were obtained from the PRé Consultant Handbook obtained with the SimaPro software.

SimaPro. LCA software allows to model processes, products, and pathways using Monte Carlo analysis and integrated ecoinvent database. The software was developed by PRé Consultants based in the Netherlands.

Ecopoints. This method provides a characterization of toxicity, with a clear separation of scientific and societal weighting in the evaluation phase. The categories considered that are presented in this chapter are human toxicity, marine toxicity, acidification, and eutrophication. The method was developed in Switzerland via a few integral collaborations.

Eco-indicator. Eco-indicators focuses on the effect of emissions on the ecosystem. The three possible types of environmental damage are deterioration of ecosystems, human health, and damage to mineral and fossil resources.

EcoInvent. Collection of life cycle inventory (LCI) data with agricultural, transport, energy supply, materials, and processing. This method was pioneered by the Swiss Centre for Life Cycle Inventories.

Given the methods used, it may be appropriate to review a few of the key factors that will be outlined in this study.

Acidification. A consequence of acids (or any compound transformed into an acid) emitted to the atmosphere and then deposited into surface soils and water. Importantly, acidification reduces the fertility of soil by affecting the microbes, disintegration of organic matter, and loss of plant nutrients. The acidification potential is primarily based on the contributions of SO_2, NO_x, HCl, NH_3, and HF.

Ozone depletion and climate change. Ozone is formed and depleted naturally in the stratosphere. As more ozone is depleted, more ultraviolet rays in sunlight can reach earth's surface affecting health of humans and other organisms. Industrialization, deforestation, and pollution have increased atmospheric concentrations of greenhouse gases that cause climate change (Ewart *et al.* 2015) [13]. Also, these indirectly lead to food and water scarcity in many part of the globe. Key sources of GHG with respect with this study included:

i) Glass fibre - production energy (melting and spinning) and emissions from glass melting (volatile organic compounds, particulates, and gases).
ii) Hemp fibres – production energy used to power agricultural equipment and the release of gases from decomposition and oxidation reactions
iii) Polymers/resins – production energy (either from fermentation of biopolymers or conventional synthesis of resins) and emissions during the production and use phases.

Human toxicity. Persistent chemical reaching undesirable concentrations in the three segments (air, soil, and water) leads to damage in humans, animals, and eco systems. The toxicological factors are created using scientific estimates based on acceptable daily intake.

Eutrophication. Eutrophication can be defined as the potential for nutrients to cause over fertilization of water and soil resulting in increased growth of biomass. Essential nutrients for growth include nitrogen, carbon, and phosphate. The presence of these conditions spurs an explosion of algal growth of many water ways, preventing air and other much needed nutrients to percolate into stream (Lebret *et al.* 2009) [14].

Fossil change. Minerals used in glass fibre production are naturally occurring and abundant, but are non-renewable resources. This index is calculated for fossil fuels, metals, minerals divided by the quantity of resource used by the estimated total world reserves of that resource.

A basis understanding of some of the key parameters have been explained, the next phase would be to model the systems and determine the outputs.

8.3 RESULTS AND DISCUSSION

The impact of the different methods are considered in this section. Note, the following two phases of the life cycle assessment touch on LCIA and Interpretation. The corresponding impact categories, network trees, and comparative analysis are presented.

8.3.1 Life cycle inventory (LCI) and assessment (LCIA)

The environmentally significant inputs and outputs, by means of mass and energy balance of the different systems, were determined based on the ISO 14040-44 framework. A lifecycle impact assessment was carried out for the two sulfonic acid methods and three established chemical methods under study. In SimaPro 8.0 (2014), several assessment representations are provided. SimaPro 8.0 (2014) provides 17 impact categories, but in this chapter we decided to present the 7 relevant contributors based on previous reports (PRé Consultants, 2014) [6] and rankings are based on contribution to the overall impact. The individual impact categories and the burden on the environment and society are presented in Table 8.2. The standard method, acetic anhydride (AA) treatment of hemp fibres, contributed the most to climate change because of the chemicals needed for the carbonylation of methyl acetate during the production of AA. Methyl iodide is one of the few intermediates during the production, and subsequent substitution can lead to compounds capable of adversely affecting the climate balance. A2S treated fibres were characterized with higher human effects when compared to the established methods. The presence of the parent compound, aniline, contributes to this because of its toxicity. Alkylation (AA and PA) of hemp fibres had a greater contribution to freshwater eutrophication, based on the number of species per year affected. Finally, the studied chemical methods had less of an effect on depletion of fossil reserves, except when compared to caustic treatment. One plausible reason for this may have been the low concentrations of A2S and AT3S required compared to the other chemicals.

Table 8.2 Potential environmental impacts associated with treated 1 kg of hemp fibres using the different chemical methods studied and the established methods.

Impact category[a]	Units	Treatment				
		A2S	A3TS	Caustic	AA	PA
Climate change	DALY[b]	5.67E-6	5.61E-6	3.38E-6	1.06E-4	7.03E-7
Ozone depletion	DALY	1.82E-9	1.39E-9	1.88E-9	6.95E-9	3.12E-11
Human toxicity	DALY	1.24E-6	1.6E-9	7.68E-7	9.84E-6	6.21E-8
Terrestrial acidification	Species.yr	2.08E-10	2.42E-10	8.56E-11	2.04E-9	5.31E-11
Freshwater eutrophication	Species.yr	4.94E-11	6.34E-11	6E-11	7.19E-10	5.19E-10
Marine ecotoxicity	Species.yr	7.8E-12	9.71E-12	4.83E-12	7.67E-11	3.89E-13
Fossil depletion	$	0.319	0.203	0.098	6.8	6.14

[a] Characterization method: ReCipe endpoint (H)

[b] Disability adjusted life years

There are few common modes that SimaPro can represent these results. The following section highlights each form, and the main features (PRé Consultants, 2014) [6].

- Characterization

The contribution of a substance to a given impact is multiplied by a characterization factor that expresses the relative contribution of the item relative to impact category. A common example is the assessment of greenhouse gases. 1 kg of methane has the same effect of 21 kg CO_2 on the atmosphere, in other words there is a relationship or factor that allows direct comparison.

- Normalization

The comparison to a given reference or fixed value gives rise to the normalization values. In other words, the values are divided by a reference. The reference can be chosen freely, but in most instances the average yearly environmental load in a country or continent, divided by the number of inhabitants is used as the reference [6]. Normalization allows for easy comparison of different methods because there is always a benchmark or reference that is assigned as 100%.

- Weighting

In SimaPro 8.0 (2014), weighting of impact categories is allowed. In simple terms, the impact result are multiplied by a weighting factor, and then added together for a total score.

Just as important as the individual factors listed in Table 8.1, the collective effect on the environment, humans, and resources are relevant. Damage to human health, ecosystem quality, and resource were evaluated using ReCipe Endpoint (H) which uses a damage approach. Data results reported in Table 8.3 and Figure 8.2 shows that the studied methods performed better than the established chemical methods for modification of hemp fibres. The data in Table 8.2 represents the actual values as it affects the environment or the life of a person, but Figure 8.2 was presented to give a comparative view of the performance of each system relative to each other, by normalizing the system with the highest impact at 100 % and modeling the rest relative [15].

1) Damage to Human Health (HH). This category is expressed as number of life lost and the number of years disabled. These two criteria are combined as the Disability Adjusted Life Years (DALYs). The effects included within this category are climate change, ozone layer depletion, carcinogenic effects, respiratory effects, and ionizing radiation. Acetic anhydride and propionic anhydride affects human health significantly. There were some improvements for the studied methods in this category.

2) Damage to Ecosystem Quality. Expressed as the loss of species over a certain time. The main contributors are land use, ecotoxicity, eutrophication and acidification. There were no significant improvements in ecosystem quality with the studied methods. One plausible reason for this may be the possibility of waste production for each process.

3) Damage to Resources. Given the economic climate and the limitation on resources, this category is of utmost importance. Included within this category is the surplus energy needed in the future to extract lower quality mineral and fossil resources. In most cases, expressed as currency. When compared to the grafting of acetyl and propyl groups onto the surface of hemp fibres, the studied methods (A2S and A3TS) resulted in less damage to resources [15, 17].

4) Global warming potential. GWP was evaluated using the International Panel on Climate Change (IPCC) GWP 2013 100a. GWP is significantly lower when fibres were modeled with the studied methods relative to the established methods. This supports the claim, that the studied methods can be used instead of acetylation, because the studied methods have the potential to improve compatibility with resins.

Table 8.3 Life cycle assessment of the two studied methods (A2S and A3TS) versus established methods

Item	Units	Studied methods		Established methods		
		A2S	A3TS	Caustic	AA	PA
Human health	DALY	9.54E-6	1.02E-5	1.92E-5	1.46E-4	1.13E-4
Ecosystem Quality	Species.yr	9.96E-8	3.53E-8	6.66E-8	6.53E-7	5.12E-7
Resources	$	0.172	0.229	0.405	6.97	6.22
GWP[a]	kg CO_2 eq	4.09	1.09	8.5	77	60.9

[a] Global warming potential (Characterization method: IPCC GWP 100a)

The studied methods (A2S and AT3S) had less of an effect on human health. The use of water instead of large quantities of organic solvents coupled with low concentrations of reagent may have accounted for this pattern. Also, there is less of an impact on the ecosystem when compared to the alkylation methods, but A2S had greater impact when compared to caustic treatment of fibres. Importantly, the studied methods utilized fewer resources and released less greenhouse gases when compared to the established methods. Figure 8.2 compared the different methods, all normalized and a better idea of the trends can be observed. As can be observed, alkylation (PA and AA) have high burden on resources, human health, and the ecosystem.

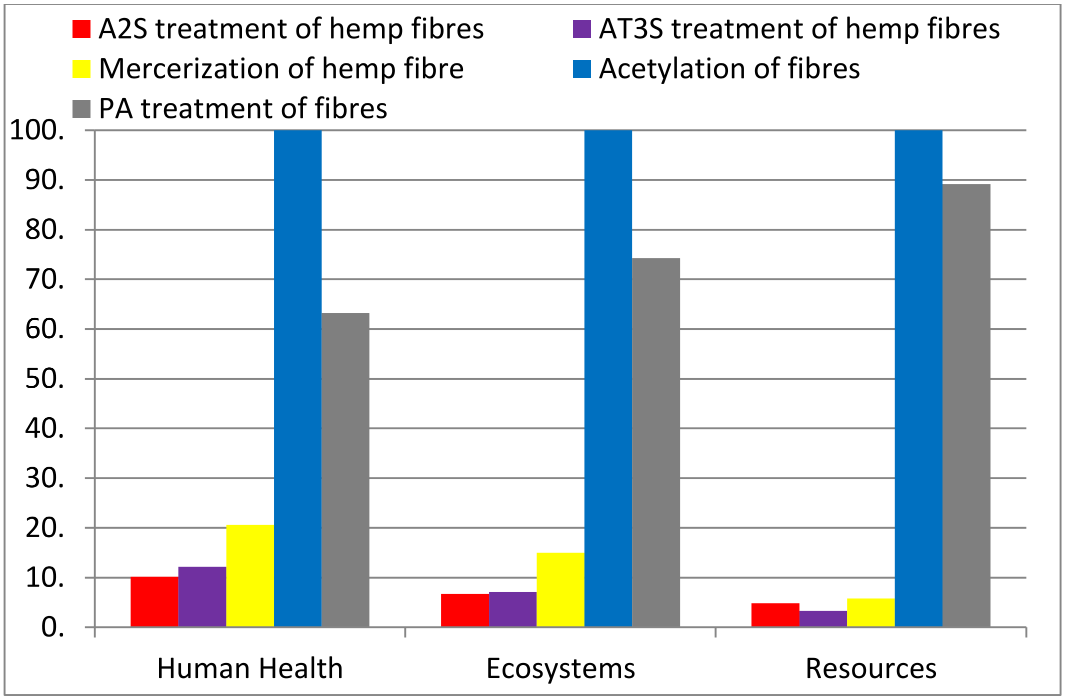


Figure 8.2 Comparison of the impacts of the studied methods relative to the established methods: Damage assessment

Data presented in Table 8.3 show significant reductions in Global Warming (GWP 100) for the studied methods (A2S), reflecting lower power consumption and subsequent CO_2 and other greenhouse gases emission. From Figure 8.3, it is obvious sulfonic acids (A2S and A3TS) treatment of hemp fibres has less of an impact on the environment, human health, and natural resources. Should be noted, the results presented in Table 8.2 are different from Figure 8.3 because the numbers in the figure has been normalized to a given value (allows for better comparison among systems), while the Table values are the actual calculated numbers.

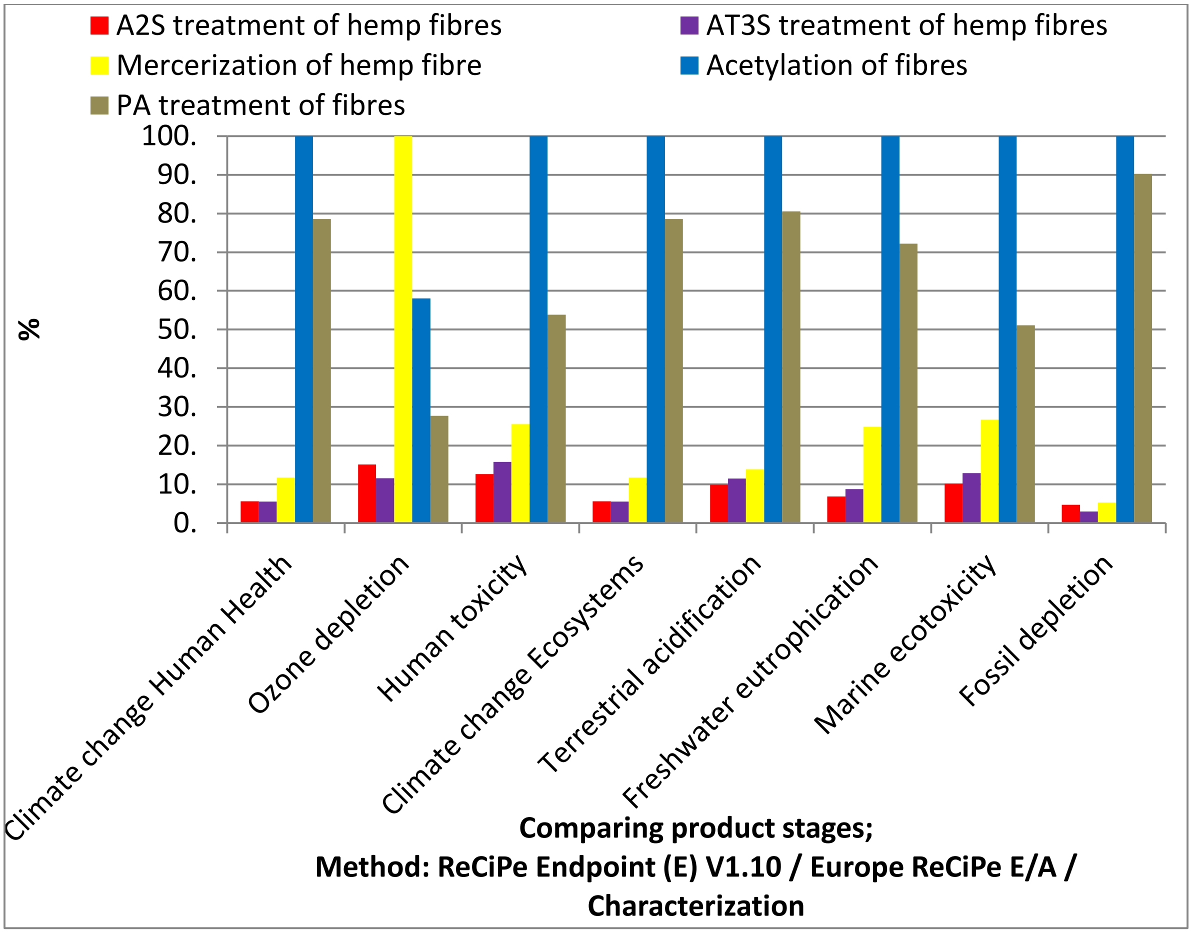
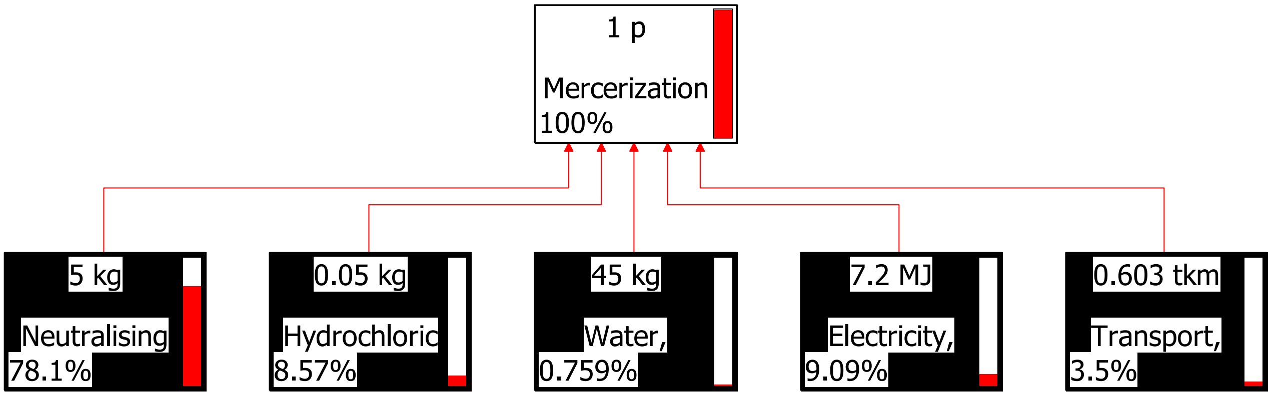


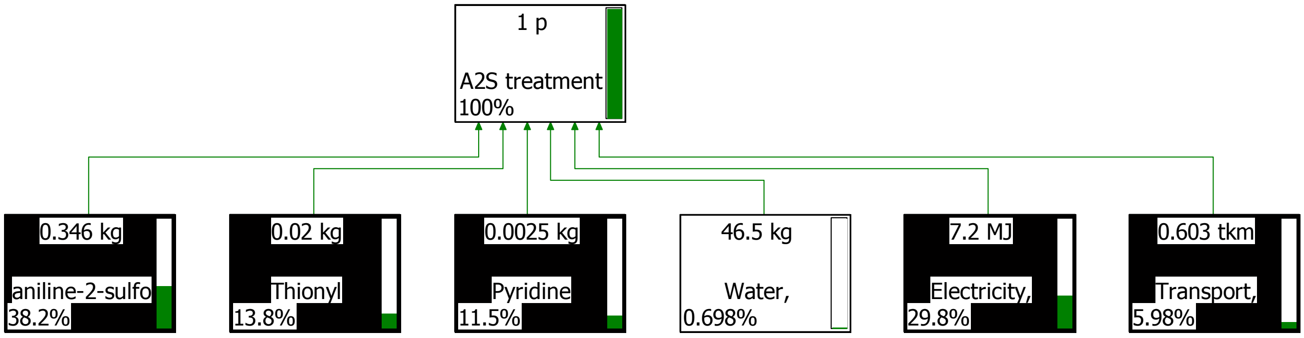
Figure 8.3 Comparison of impact categories for A2S or A3TS versus established methods

8.3.2 Network trees for the different methods

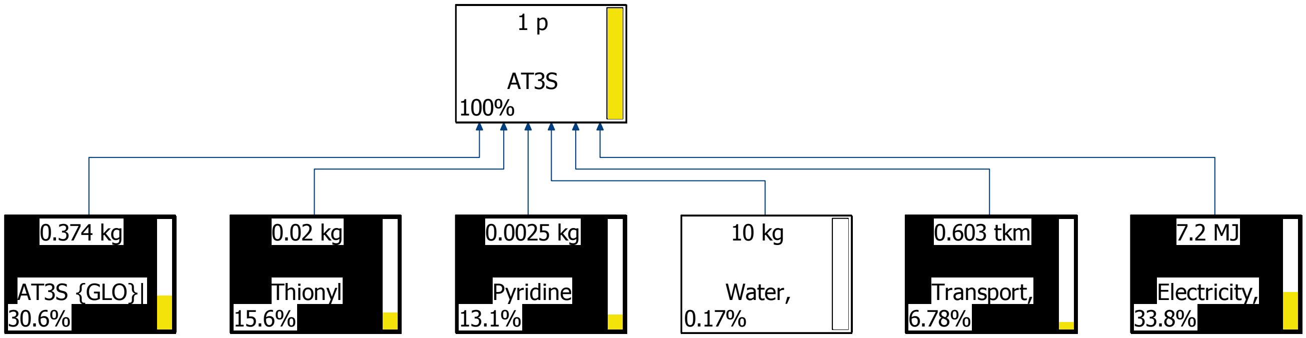
Flowcharts of each process, showing the impact of each component involved in the treatment of hemp fibres for composite materials are presented in Figure 8.4. The major impact in all cases is due to the chemicals needed for treatment. For mercerization of hemp fibres, if the caustic can be recycled after each cycle of treatment, the process may be more environmentally friendly and have fewer impacts on the factors discussed. Sulfonic acid treated hemp fibres exhibited significant contributions from pyridine and thionyl chloride during the reaction. Also, A2S and AT3S treated fibres required considerable energy for the reaction (29.8 and 35.1 %, respectively contributions to process). The energy requirement contributed largely for both of these processes because of the relative little dependence on the chemicals, as opposed to the established methods.


1 p
Mercerization
100%
5 kg
Neutralising
78.1%
0.05 kg
Hydrochloric
8.57%
45 kg
Water,
0.759%
7.2 MJ
Electricity,
9.09%
0.603 tkm
Transport,
3.5%

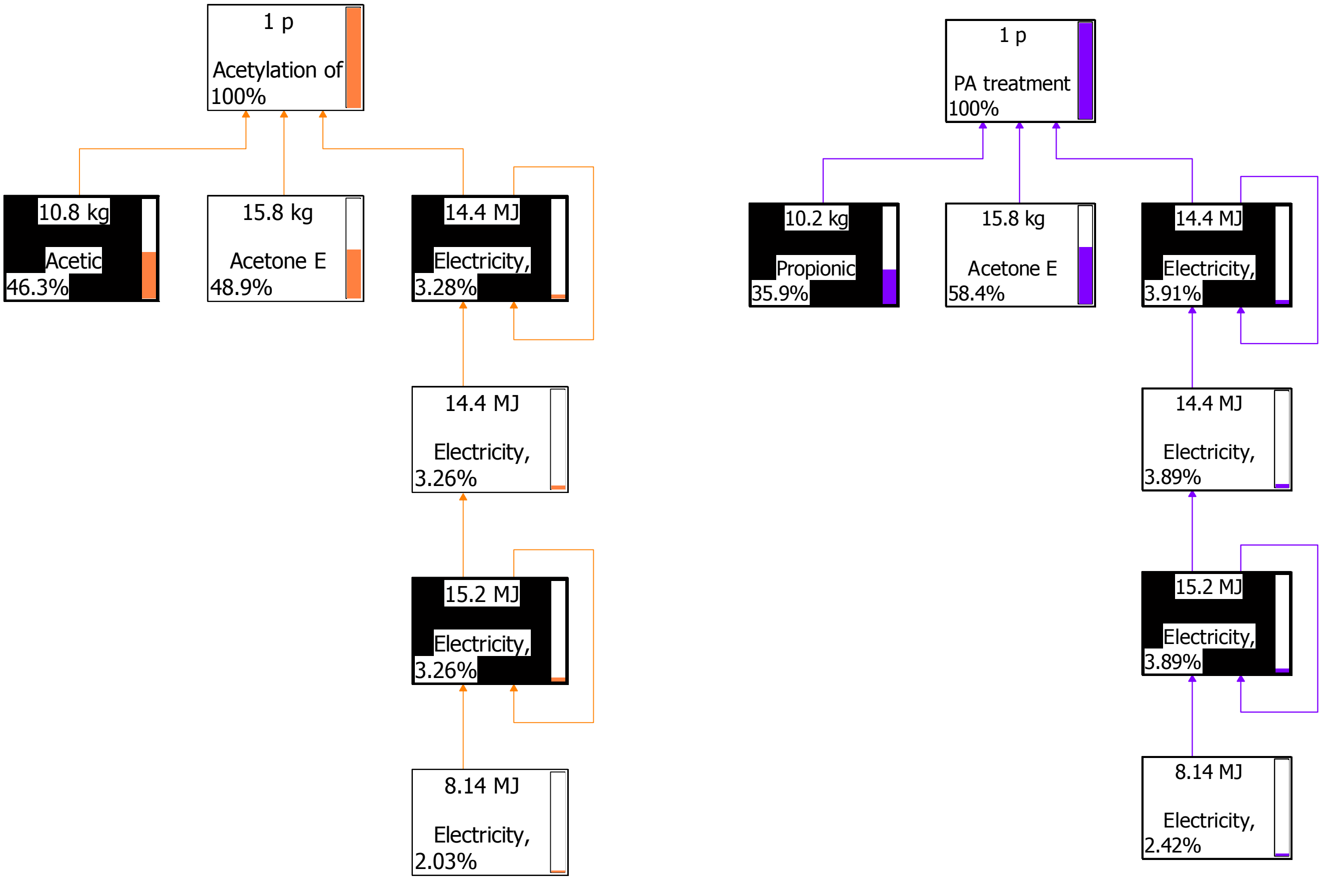
a)


1 p
A2S treatment
100%
0.346 kg
aniline-2-sulfo
38.2%
0.02 kg
Thionyl
13.8%
0.0025 kg
Pyridine
11.5%
46.5 kg
Water,
0.698%
7.2 MJ
Electricity,
29.8%
0.603 tkm
Transport,
5.98%

b)


1 p
AT3S
100%
0.374 kg
AT3S {GLO}|
30.6%
0.02 kg
Thionyl
15.6%
0.0025 kg
Pyridine
13.1%
10 kg
Water,
0.17%
0.603 tkm
Transport,
6.78%
7.2 MJ
Electricity,
33.8%

c)



d) e)

Figure 8.4 Network tree showing the main contributors during a) caustic, b) A2S, c) A3TS, d) AA, and e) PA treatment of hemp fibres

8.3.3 Comparison of glass fibre and chemically treated hemp fibres

The production phase of glass fibre is modeled against the treatment of hemp fibres for composite applications. Glass fibre is present as a component in the Eco invent database, while the data presented in the previous sections are used to model the treated hemp fibres. Hemp fibre cultivation, extraction, and treatment are considered. To facilitate comparison, the best-established method, mercerization, is also included. The main environmental impacts are summarized in Table 8.4.

Table 8.4 Impact properties of producing glass fibre and hemp fibres for composite applications

Impact category[a]	Units	Hemp fibre treatment			Glass fibre
		A2S	A3TS	Caustic	
Climate change	DALY[b]	5.67E-6	5.61E-6	3.38E-6	4.76E-5
Ozone depletion	DALY	1.82E-9	1.39E-9	1.88E-9	2.19E-9
Human toxicity	DALY	1.24E-6	1.6E-9	7.68E-7	1.39E-4
Terrestrial acidification	Species.yr	2.08E-10	2.42E-10	8.56E-11	2.64E-9
Freshwater eutrophication	Species.yr	4.94E-11	6.34E-11	6E-11	1.4E-10
Marine ecotoxicity	Species.yr	7.8E-12	9.71E-12	4.83E-12	2.23E-8
Fossil depletion	$	0.319	0.203	0.098	0.74

The production of glass fibre has higher impact within most of the sub categories. Notably, there is high human toxicity associated with glass fibre, probably as a result of fibre spinning and extraction. As expected, there is a higher demand for fossil fuel resources when producing glass fibres when compared to treating hemp fibres. The evidence is that replacing glass fibres with hemp fibres can potentially reduce the impact. The main damage for caustic treatment on Human Health was due to ozone depletion and climate change (from Table 8.4). Based on these numbers, it is safe to say that glass fibre production has a greater environmental, human, and ecosystem impact. This conclusion is supported by data presented in Table 8.5. The four main indicators for assessing any process or products are summarized.

Table 8.5 Key impact categories for different reinforcing agents

Item	Units	Hemp fibre treatment			Glass fibre
		A2S	A3TS	Caustic	
Human health	DALY	9.54E-6	1.02E-5	1.92E-5	4.72E-3
Ecosystem Quality	Species.yr	9.96E-8	3.53E-8	6.66E-8	1.4E-3
Resources	$	0.172	0.229	0.405	2.72E-3
GWP[a]	kg CO_2 eq	4.09	4.04	8.5	14.2

[a] Global warming potential (Characterization method: IPCC GWP 100a)

Interestingly, glass fibre production utilized fewer resources during production. Hemp fibres require a lot of land and water for growth, hence the higher numbers. Finally, the global warming potential for all the treated hemp fibre systems are significantly lower than the production of glass fibre. These numbers reflect the motivation for using natural fibres to reinforce polymers, assuming the properties of the produced materials are comparable.

8.3.4 Comparison of natural fibre based and glass fibre reinforced composites

The next step would be to compare the composites made from the different treated fibre and resins types. It is very important to note that the performance of the composite materials from glass fibre and hemp reinforced polymers were assumed to be similar. Polypropylene and polylactic acid were considered as a petroleum and a renewable polymer, respectively. Table 8.6 summarizes the key inputs for comparing among the different systems. It should be noted that all composites were modeled using extrusion followed by injection molding. The energy requirements were obtained from previous publications and approximate energy use from specialists at Alberta Innovates Technology Future, Alberta, and Saskatchewan Research Council, Saskatchewan.

Table 8.6 Key inputs for each composite system modelled.

System	Inputs			
	Mass of resin (kg)	Mass fibre (kg)	Electricity (kWh)	Transport (tkm)
A2S + PP	4	1.73	25	20
A2S + PLA	4	1.73	25	20
A3TS + PP	4	1.73	25	20
A3TS + PLA	4	1.73	25	20
Glass fibre + PP	4	1	25	30
Glass fibre + PLA	4	1	25	30

Conditions adopted and modified from Joshi *et al.* (2004) [17] and Corbière-Nicollier *et al.* (2001) [15].

It should be noted that the unit on the y axis for Figure 8.5 is expressed as *Eco-indicator point* (Pt). The absolute value of the points is not very relevant because the main purpose is to compare the relative differences between the processes. As a side note, the value of 1 Pt is representative for one thousandth of the yearly environmental load of one average European inhabitant. This value is calculated by dividing the total environmental load in Europe by the number of inhabitants and multiplying it by a 1000 (scale factor). The synthesis of polylactic acid via fermentation has been pioneered and lately commercialized. As a result, it is considered on two fronts, as an emerging competitor against petroleum based resins, and its renewability index. Polypropylene production is standardized and used directly from the database. The data used to model the previous sections (fibre treatments) were employed here in addition to the polymer factors. The main impacts of each system are shown in Figure 8.5, 8.6, and Table 8.7. The effect of using a renewable polymer (PLA) with natural fibres or glass fibres increased the impacts on every category. A few plausible reasons for this may be the production of large quantity of bio-waste along with high energy demand as a result of long fermentation times during the production of PLA. The performance of the A2S and A3TS treated hemp fibre reinforced polypropylene exhibited less impacts on all four sub categories when compared to glass fibre and the established fibre treated composites.

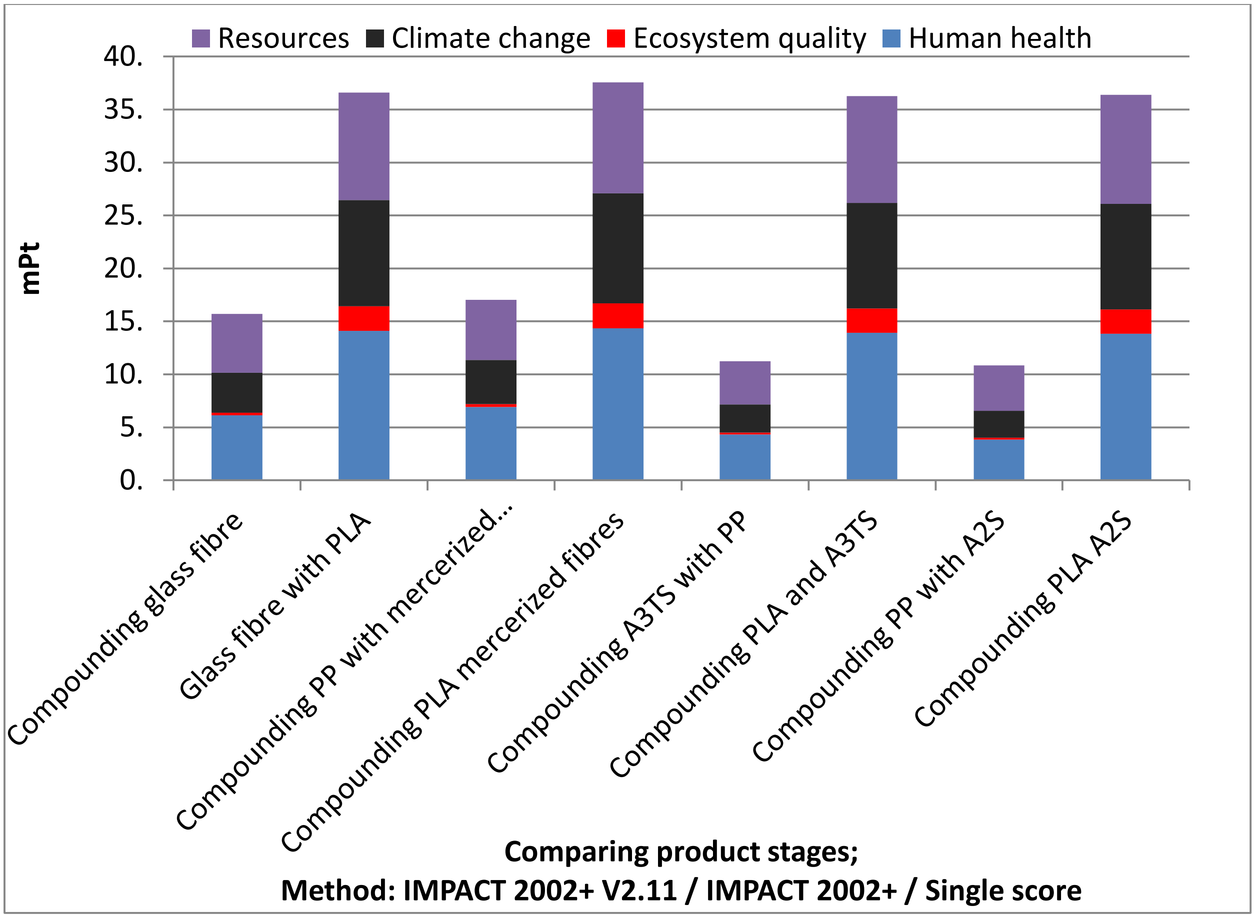


Figure 8.5 Single score comparison of the four main impact criteria for composites made from different fibre and resin types using Impact 2002 + V2.11 methodology.

Interestingly, the use of PLA as a polymer system required the use of significantly more resources per composite material plausibly because of the nutrients, chemicals, and solvents needed to produce this material. Additionally, the production of fermentation waste, would adversely affect the environment as characterized by the high impact.

Table 8.7 specifically addresses two of the other key environmental impacts of using these materials for applications. The global warming potential and water scarcity indices are presented, using hemp fibres improved both of the factors. It should be noted, this life cycle impact assessment does not consider end of life situations, and with respect to the hemp fibres, and degradation may return nutrients to the soil and also water vapor. On the other hand, incineration of glass fibre composites would increase the GWP potential of this particular system. Another key aspect, given that PLA is biodegradable and renewable, the use of this material may be feasible if end of life is considered.

Table 8.7 Key environmental indicators for production of composites using different fibre treatment and resins types.

System	GWP (kg CO_2 eq)[a]	Water scarcity index (m^3)[b]
Compounding glass fibre with PP	40.2	113
Glass fibre with PLA	105	107
Compounding PP + mercerized fibres	44.4	127
Compounding PLA+ mercerized fibres	108	117
Compounding PP + A2S fibres	27.5	60.4
Compounding PLA + A2S fibres	104	113
Compounding PP + A3TS fibres	28.5	64.9
Compounding PLA + A3TS fibres	104	112

[a] Global warming potential (Characterization method: IPCC GWP 100a)

[b] Water Scarcity V1.01.

The final parameter used to compare the different systems was the Ecological footprint as determined via Ecological footprint V1.01. A3TS or A2S reinforced PP produced the lowest footprint in most cases. All the systems contributed primarily via CO_2 emissions to the ecological footprints. Also, it can be observed, the PLA systems were characterized with a substantial portion of land occupation because of the feedstock for synthesis within the model was considered to be corn. Given the low yields and the efficiency associated with microbial synthesis of PLA from sugars (derived from predominantly corn), a fair bit of land is required for feedstock cultivation. Nuclear footprint comes solely from the electricity supply, primarily from grids within regions where the software was developed (Europe).

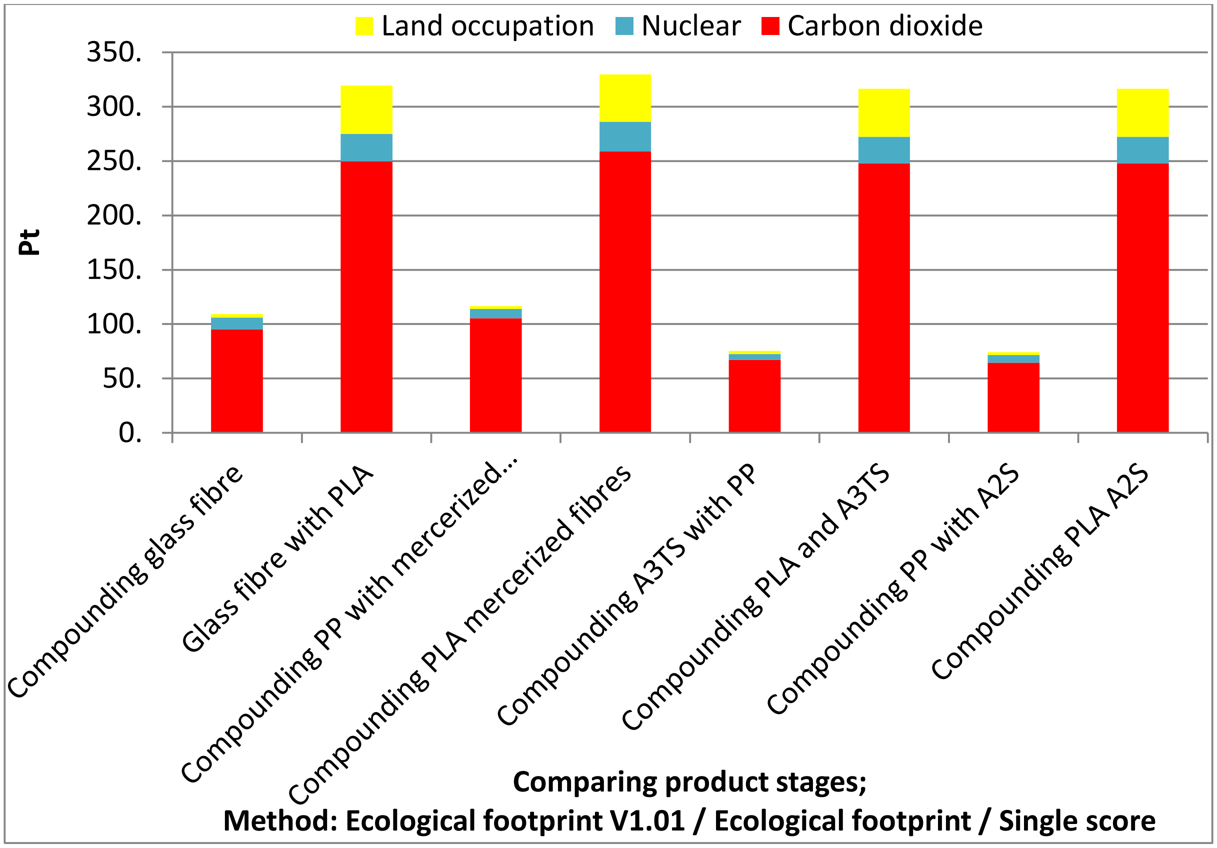


Figure 8.6 Single score representation of the ecological footprint for different fibre and resin types, determined via Ecological footprint V1.01

8.3.5 Interpretation

8.3.5.1 Sensitivity analysis

Sensitivity analysis can be used to determine uncertainty involved with each calculation step done in the life cycle assessment by repeating the calculations or comparison. In this case, the methods studied (A2S and A3TS) when compounded with polypropylene were compared with glass fibre reinforced polypropylene.

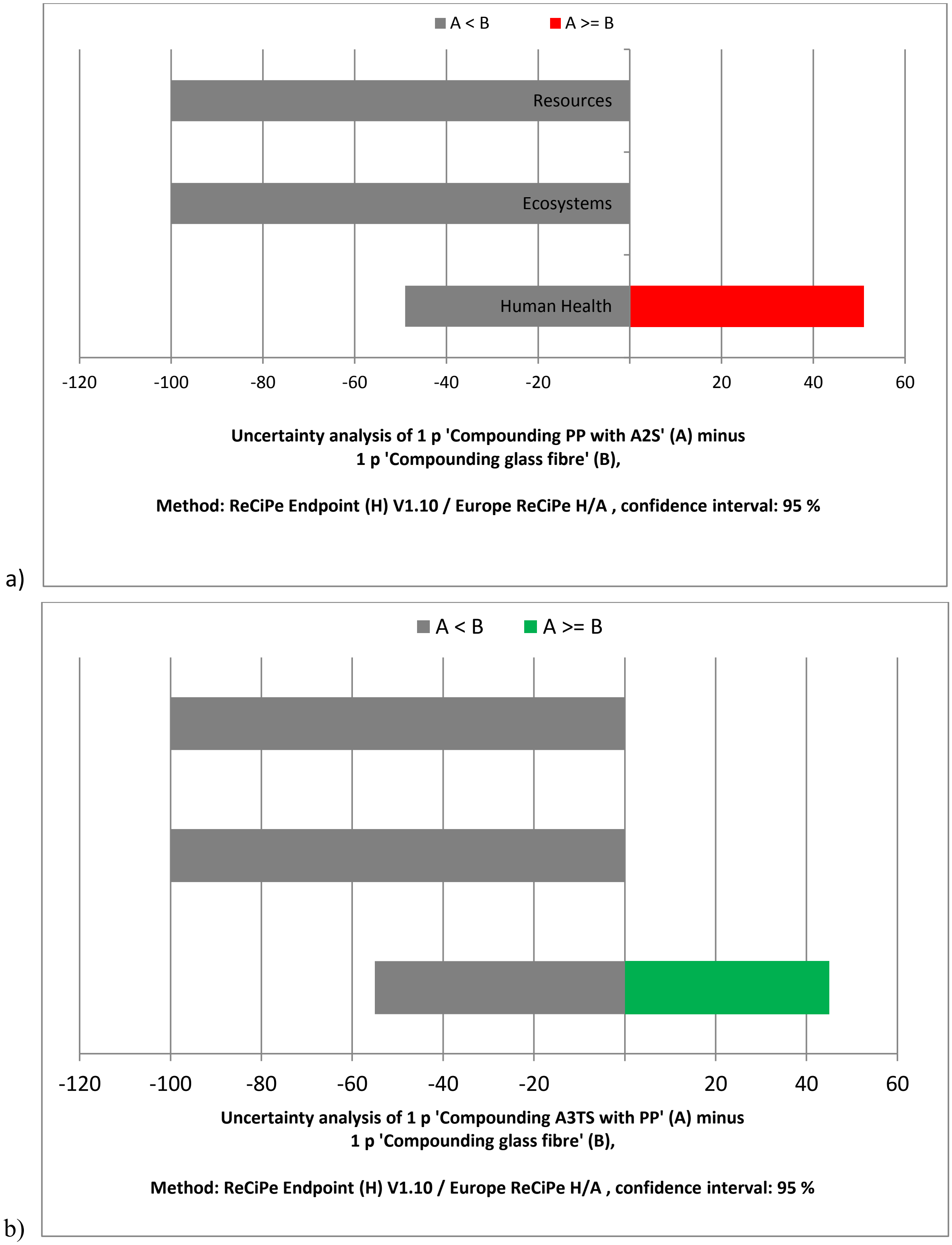


Figure 8.8 Sensitivity analysis estimation for a) A2S (red) and b) A3TS (green) treated fibres reinforced polypropylene against glass fibre reinforced polypropylene (grey).

Based on the data presented in Figure 7.8, of the 100 calculations performed, 100 % of those resulted in glass fibre (grey bar) reinforced polypropylene having a higher impact on the resources and ecosystems. On the other hand, approximately 51 % and 45 % of the calculations yielded that the A2S and A3TS, respectively, natural fibre composite had a higher human health impact than the glass fibre composite material.

7.4 CONCLUSION

The study presents data results regarding the environmental impact assessment assigned to treating 1 kg of hemp fibres or TMP with different chemical methods. Based on the results obtained from modeling using SimaPro 8.0 (2014), it seems the major contributions to the impact in all the methods studied comes predominantly from the chemical used and the electricity required during the treatments and for drying of the fibres. Primary data are useful for reducing the data uncertainties associated with LCA, but this study highlights a few methods that can be used to treat natural fibres for composite applications. The studied methods for hemp (A2S and AT3S) and pulp (iodomethane and hexanoyl chloride) had less impact on the environment, humans, and resources. If these fibres / pulp are used to reinforce polypropylene or polylactide acid, they have less of an environmental impact when compared to glass fibre reinforced composites. It should be noted, there is much more that can be optimized, such as the effect of treatment on mechanical properties of the fibres or the process efficiency, but in this study we only evaluated the environmental aspects of the different processes as reported or done. LCA is a useful tool for choosing clean production processes, avoiding hazardous, and toxic materials, and process redesigning to accommodate energy conservation.

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CHAPTER 9.

General Conclusions

Renewable and sustainable materials have been of great focus because of global ecological and financial concerns. Natural fibres such as hemp and flax fibres is a feasible substitute for glass fibre in composite materials. The main goal of this research thesis was to study the macrostructure of bast fibres and other lignocellulosic sources and how this may impact these materials as potential reinforcement for polymeric systems. Also, the influence of chemical and enzymatic treatments on the surface and bulk properties of biomass was a key motivation. This research demonstrated that enzymes can be used to remove components of natural fibres, rendering them better candidates for composite applications. Enzymatic treatment removed the hygroscopic pectic and hemicellulosic contents which accounted for 10-20 % of the fibre mass, depending on the type of fibre. There is potential to use this stream of primarily oligosaccharides and reducing sugars for fermentation and the enzymes can be recycled.

Chemical modification of the hemp fibres involved using derivatives of sulfonic acid for enhancement of fibres for composite applications. The chemical reaction proceeded at low temperature and in an aqueous reaction medium. Treated fibres were characterized with increased hydrophobicity and thermal stability. Infrared spectroscopy and Secondary ion mass spectroscopy confirmed the presence of chemical signatures and chemical fragments, respectively, of the different systems. The chemical treatment developed in this study may be applied for enhancing the surface and thermal properties of natural fibres specifically suited for composite applications.

The effect of the different treatments on the chemical composition, chemical functionality, and structural features of natural fibres are well known. One gap pertaining to natural fibre modification is that of the influence of these treatment types on the physical properties of natural fibres. Atomic force microscopy was used to estimate the surface roughness and adhesion forces of the different fibre types. Contrary to what is reported or believed, treatment of natural fibres, using either chemical or enzymatic methods, lead to a significant reduction in roughness at a nano scale. Also, depending on the treatment, interaction with a polar silicon nitride tip can be used to determine the adhesion forces on the fibre surfaces. Hemp fibres treated with the sulfonic acid derivatives were characterized with a reduction in adhesion with the tip, indicating an increase in surface hydrophobicity. This section of the thesis demonstrated that AFM can be a powerful tool to determine the forces and roughness of treated fibres.

The enzyme treated hemp and flax fibres were utilized as a reinforcing agent of composite materials. The treated fibres were used to reinforce polypropylene at a fixed mass ratio of 20 % (w/w) of fibre. The study showed that there was no significant change in mechanical properties except for laccase treated flax fibres. In fact, removal of hemicellulosic and pectic material produced a material better suited for reinforcing polypropylene. Flax fibres are characterized with a higher lignin content when compared to hemp fibres and it is plausible the removal of this random adhesive component might have resulted in fibrils with less strength. There was significant improvement in thermal properties for most of the studied systems. Again, removal of the thermally labile hemicellulose and pectic material concentrated the cellulosic and lignin components which are much more stable to heating. Lastly, when compared to the untreated fibre samples used to reinforce polypropylene, the studied systems exhibited improved water resistance. In summary, this study was successful in demonstrating that selective removal of chemical components can result in composites with properties similar to conventional platforms.

The importance of assessing methods, products, and pathways are becoming relevant with growing technologies and the need to replace conventional petroleum commodities. In this thesis, we compared the environmental effect of the studied sulfonic acid modification of hemp versus established published methods, and the production of glass fibre. The methods studied had less of an environmental and human impact. If used to compound PP and PLA, the studied methods again exhibited better properties. Also, enhancement of pulp samples using two other chemical methods (iodomethane and hexanoyl chloride) returned better environmental and human effects when compared to the production of glass fibre. In the end, we demonstrated that the studied methods pioneered in our laboratory were better candidates for reinforcing both petroleum (PP) and renewable (PLA) based polymer systems.

The final piece to the thesis puzzle focused on developing an epoxy based plastic with a renewable index. As a result, abundant, inexpensive, and renewable tall oil rosin acids (TORAs) were used to supplement/cure epoxy. In the presence of a hardener, replacement of up to 35 % (w/w) epoxy with the TORAs was studied. The biggest challenge was trying to get a homogenous reaction mixture with the three individual components. Nevertheless, replacement up to 15 % (w/w) of epoxy produced plastics with similar mechanical properties to the virgin epoxy resin. There was a corresponding decrease in stiffness (Young's modulus) and T_g as the replacement ratio increased. The heterogeneous nature of the TORAs and the polycyclic structures of some of the components could have resulted in random kinks in the cured plastics resulting in the two properties mentioned previously. In conclusion, it was demonstrated that epoxy based plastics can be produced with a renewable component taken from a waste stream of the pulping industry.

The reaction chemistries, enzymatic treatments, and modeling demonstrated in this thesis are not only limited to natural fibres, but can be applied to other lignocellulosic material such as other types of fibres, forest residues, and processed pulps. The chemical method outlined in this thesis were assessed using LCA modeling and is very promising in comparison to other methods. Finally, the research in this thesis demonstrates many avenues where value can be created in Alberta, from integrating the pulping industry to the potential of becoming a hub for natural fibre enhancement given the facilities and knowledge.

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Appendix I – Other work not part of thesis

Other peer reviewed articles published or submitted but not part of this thesis are listed:

1) **George, M**., Mussone, P., Bressler, D. (2015). Improving the accessibility of hemp fibres using caustic to swell the macrostructure for enzymatic enhancement. Industrial Crops and Products, 67, 74-80.
2) **George, M**., Mussone, P., Bressler, D. (2015). Surface and bulk transformation of thermo mechanical pulp using fatty acyl chlorides: influence of reaction parameters on surface, morphological and thermal properties. Journal of Wood Chemistry and Technology (Accepted).
3) **George, M**., Mussone, P., Bressler, D. (2015). Rapid hydrophobic and thermal enhancement of pulp samples: grafting of iodomethane on the pulp surface (Under peer review - Industrial Crops and Products).
4) **George, M**., Mussone, P., Bressler, D. (2015). Enhancement of natural fibres using tall oil rosin acids: surface and thermal characterization (To be submitted - Material Chemistry)

Industrial Crops and Products 67 (2015) 74–80

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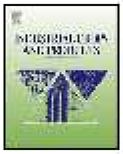

Improving the accessibility of hemp fibres using caustic to swell the macrostructure for enzymatic enhancement

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ABSTRACT

The main aim of this study was to investigate the effect of mercerization prior to enzymatic treatments on the surface, on the thermal and morphological properties of hemp fibres. Hemp fibres were treated with sodium hydroxide (5%, w/v) and five different enzyme systems. The enzymes studied were xylanase, xylanase + 10% cellulase, pectinmethylesterase, polygalacturonase and laccase. A previous report utilizing enzymes alone (George et al., 2014) was used as a baseline to determine the impact of caustic swelling of the hemp fibres coupled to enzymatic treatment. Thermal gravimetric analysis (TGA) was used to study the effect of each system on thermal properties of the hemp fibres. Scanning electron microscopy (SEM) was used to monitor the changes in the morphological features of the hemp fibres, while X-ray photoelectron spectroscopy (XPS) was used to study the influence of each treatment of the surface chemical composition. Force tensiometry was used to investigate the effect of the different systems on the surface polarity of the treated fibres. The additional treatment with NaOH + hemicellulases was found to result in enhanced thermal properties when compared to previous systems. SEM micrographs confirmed greater fibre bundle rupture and increase in surface roughness of the base + enzyme treated samples. This work demonstrates that mercerization prior to enzymes, depending on the mode of action and arrangement of chemical components of the fibre, can be an effective method for improving the thermal and surface properties of fibres for composite applications.

1. Introduction

As a result of the growing environmental sensitivity, new legislations and regulations, industries are forced to develop and implement ecologically friendly materials. The engineering of materials that are sustainable, characterized by low carbon footprint and renewable content has been the focus of the past few decades. Specifically, the use of natural fibres instead of glass fibre for composite applications is a key focus. Natural fibres have many advantages compared to synthetic fibres, including decreased weight and they are most often recyclable and fully biodegradable (Bledzki et al., 1996). Additionally, natural fibres are obtained from renewable sources and cause decreased wear of processing equipment and yet have comparable strength and stiffness to synthetic fibres (Wambua et al., 2003; Mohanty et al., 2000). Nevertheless, natural fibres have a few inherent disadvantages in composite applications that have been the focus of much optimization research. These limitations include moisture uptake, low thermal stability and quality variations due to fibre processing (Oksman et al., 2003).

Many strategies have been investigated to improve the properties of natural fibres for composites applications. For example, chemical methods such as mercerization (Mwaikambo and Ansell, 2002), acetylation using acetic anhydride and propionic anhydride (Bledzki et al., 1996) and silane treatment using a number of quaternary functionalized silane groups (Xie et al., 2010) have been reported. The effect of compatabilizers such as maleic anhydride on the swelling properties (Naik and Mishra, 2007) and the mechanical properties of natural fibres have studied and thoroughly investigated (Mishra and Naik, 2005). Despite the success of these methods, environmental concerns associated with chemical waste disposal and exposure of processing workers to harmful chemicals have limited many of these technologies to the laboratory scale.

The use of biological methods such as enzymatic processing and microbial fermentation offer an answer to many of the limitations. Evidence for this is seen in the increase of publications in the area of biological agents for modifying natural fibres in the past decade. Pommet et al. (2008) successfully optimized a process for depositing bacterial cellulose onto the surface of loose hemp fibres and mates. They showed that the deposition of nanosized bacterial

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Appendix II – Source and information about natural fibre samples

Source of natural fibre samples.

Hemp:

Location grown – Rod Lanier's farm east of Lethbridge

Variety – X59

Grown for seed, combined and the residue baled into big round bales and shipped to AITF Vegreville (500km) on a flatbed truck

Seeded mid-May harvested mid-October- approximately 140 day growth period

The decortication process involves 2 passes through a hammermill type decorticator and the cleaning duvex, followed by 2 passes through the fine opener and the step cleaner, then rebaled and shipped to AITF Millwoods another 120km

No herbicides were used in the growing of this hemp.

Flax:

Location grown on Rudy Warawa's farm at Mundare

Variety – Bethune

Grown for seed, combined and the residue baled into big round bales and shipped by truck to AITF Vegreville (10km)

Growth period approximatly 125 days.

The decortication process involves 2 passes through a hammermill type decorticator and the cleaning duvex, followed by 2 passes through the fine opener and the step cleaner, then rebaled and shipped to AITF Millwoods another 120km

No herbicides were used in the growing of this Flax.

Appendix III – Future directions

Possible future work that can build off the work done in this thesis include:

1) Investigate the feasibility of producing chemicals or fuels from the sugar stream produced during enzymatic enhancement of natural fibres. Given approximately 10 -25 % (wt.) of fibres mass is removed during treatment, there is the possibility to create a closed bio refinery concept around composites production and synthesis of high value materials.

2) Biodegradation studies can be done to supplement the data on the natural fibre composites and the plastics produced from blending epoxy with tall oil rosin acids. This will enable a better understanding of the renewable index and degradation mechanism for these bio-based products.

3) The "green" index of products, services, and processes is very important. Going hand and hand with this, is the need to determine the economics of each process. As a result, techno economic models can be developed to study the feasibility of the technologies studied in this thesis, including:

- Enzymatic treatment versus chemical treatment
- Novel chemical treatment (sulfonic acid study) versus established chemical methods
- Cost savings from replacing epoxy with a cheap and abundant (TORAs) resource

In summary, this thesis has answered many questions and filled a few gaps in the international literature, but there is room for further understanding and evaluating the methods studied.