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Extraction Methodologies and Physicochemical Characterizations of Nanocellulose Isolated from Kudzu for Potential Sustainable Packaging Applications

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ABSTRACT

Flexible packaging is an integral part of the food supply chain due to its unique thermal, mechanical, and barrier properties. The many advantages (e.g., lightweight, product protection, reduction in food waste, communication medium, etc.) come with certain drawbacks (low recyclability rate, pollution of ecosystems, etc.). Still, the heavy reliance of the food industry on these products, many of them being non-sustainable polyolefins, is unlikely to diminish in the foreseeable future. Thus, more sustainable alternatives that do not sacrifice performance (machinability, shelf-life, etc.) are needed before pollution becomes irreversible and public outcry insurmountable. Nanocellulose, especially in the form of cellulose nanocrystals (CNCs), can be incorporated into sustainable polymer matrices to enhance mechanical, thermal, and barrier properties. Cellulose is the most abundant polymer (polysaccharide) found in nature. More research to find novel biomasses for CNC extraction would be welcome to the industry. One potential biomass source for the extraction of nanocellulose is the invasive deciduous perennial: kudzu (*peuraria montana* var. *lobata*). This legume is predominantly an agricultural nuisance. However, an industrial benefit may exist that would increase raw material supply for the degradable packaging market. However, no formal research exists for this application of kudzu. The purpose of this research is to: (1) identify a CNC extraction methodology for kudzu, and (2) physiochemically characterize the product to evaluate its efficacy as a possible nanofiller in degradable packaging solutions.
DEDICATION

I dedicate this research to my mother, Betts Love, and my de facto grandmother, Tita Salloum. Without the love and support of these two incredible women in my life, I would be lost. I love y’all very much.
ACKNOWLEDGMENTS

I would like to acknowledge and thank my family for all their support. As aforementioned, I would not be able to have done this without my mom and Tita. I also would like to thank my dad for all his assistance and encouragement. My brother and two sisters are always an inspiring force in my life, and I am very blessed to have such good siblings. Last, but far from least, my girlfriend, Sallie – thank you for all your love, patience, and support during my time here. It is such a blessing to have you in my life.

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CHAPTER ONE

INTRODUCTION

Introduction to Sustainable Packaging Materials

Flexible plastic packaging is a lightweight protector of consumer-packaged goods (CPGs) that provides a good communication medium to the customer while reducing product waste. Its advantages also come with certain environmental issues. Yet, its heavy use in the CPG industry is unlikely to lessen in the near future unless society collectively decides to homestead. A conservative model predicts that the production of plastic will increase by 10.8 million metric tons each year in the next decade (Brandon & Criddle, 2019). This rate far outstrips the estimated plastic recycling rate of 0.8 million metric tons each year in a time of increasing public dissent (much of it due to misinformation) and decreasing landfill capacity. The environmental concerns when made worse when China, the biggest importer of recycled plastics, closed its doors to receiving American plastic waste (Brandon & Criddle, 2019).

Thus, the advantages of plastic are undeniable to the food industry and numerous other sectors. However, it is in the interest of plastic-producing companies to heed the sustainability concerns and explore alternatives before pollution becomes irreversible and public outcry insurmountable, likely leading to crippling government interventions. Legislation to prohibit or curtail the utilization of single-use plastic is increasing drastically, and non-governmental organizations, like the Ellen MacArthur Foundation, are pushing the most prominent plastic-producing corporations to search for alternatives.
Biopolymers have attracted increasing interest as an alternative to conventional petroleum-derived plastics in the food packaging industry. Biobased and compostable polymers are undergoing development to replace conventional polyolefins. However, the performance, processing, and costs of the biopolymers present significant hurdles to overcome. For instance, the costs of biopolymers range from 3-10 times the price of conventional polyolefins (Rodriguez-Perez et al., 2018). Biopolymers also struggle to meet the desired thermal stability, molecular weight (MW), melt strength, and modulus needed for processing, as well as the barrier and tensile properties required for consumer product protection (Lucas-Freile et al., 2018; Petersen et al., 1999; Mensitieri et al., 2011).

Biopolymers often exhibit inadequate shelf-life performance, poor mechanical properties, and low thermal stability (Brandon & Criddle, 2019). Their poor mechanical and thermal properties make food protection an issue, and they can be challenging to run on typical polymer processing, converting, and packaging equipment. The very properties that make a film compostable (such as structures that are subject to hydrolytic attack) often undermine its ability to be run efficiently on machinery such as blown, cast, extrusion, print, and package lines. Additionally, biopolymers historically have poor oxygen transmission rate (OTR) and water vapor transmission rate (WVTR). Poor performance in these metrics negatively affect the shelf-life of some products. However, proven processes, such as the addition of nucleating agents to increase crystallinity, can rectify some of these weaknesses. Therefore, more research must be conducted to generate sustainable nucleating agents that can be utilized as additives in sustainable packaging structures. Sustainable additives can thereby enhance the desired properties of the sustainable matrix.
while not taking away from the degradability of the package. By researching and providing sustainable additive materials on the market, it will be financially and operationally easier for companies to offer sustainable packaging options as potential replacements for petroleum-based plastics in the CPG marketplace.
Introduction to Cellulose as a Sustainable Packaging Material

Cellulose, the most abundant polymer found in nature, is a valuable biopolymer that can be incorporated into many sustainable polymer matrices (Habibi et al., 2010). It is located in the cell walls of many biomasses (plants, bacteria, tunicates, etc.), making it a sustainable and renewable option instead of conventional polymers produced mainly from limited resources (Chakrabarty & Teramoto, 2018; Yang et al., 2007). This biopolymer is available in various terrestrial and marine environments, including seaweed and kudzu (Doh et al., 2020). It exhibits a high modulus, between 100-220 GPa, with a relatively low density in its nanoform (nanocellulose) (Mondragon et al., 2014). The high modulus makes it a potential additive candidate into sustainable matrices for improving the mechanical properties of the composite. To fully leverage this mechanical strength, it must be in its highly crystalline (>80%) form and thus isolated from the amorphous faction of cellulose plus the other plant components. However, nanocellulose tends to agglomerate due to its strong intermolecular hydrogen bonding. Thus, the two main issues in obtaining useful nanocellulose composites: (1) amorphous factions, water, and biomass material must be separated from the native cellulose to isolate the crystalline cellulose, and (2) the crystalline cellulose must be dispersed throughout a matrix without significant agglomeration.

There is no issue in obtaining a feedstock for this research. As mentioned, cellulose is abundantly available in many different biomasses worldwide, including the infamous kudzu (Pueraria montana var. lobata) vine in South Carolina. Kudzu will serve as a welcomed biomass for the extraction of nanocellulose in this research with the hope that the sustainable packaging industry can find a use for this invasive plant.
Purpose and Objectives

Due to the growing interest in biopolymers, the purpose of this research is to determine the efficacy of extracting nanocellulose from kudzu, characterize said nanocellulose, and compare it with extracted cellulose nanocrystals (CNCs) from seaweed biomasses in prior research (Doh et al., 2020). The potential industrial consumption of a predominantly troublesome and invasive species clearly has its inherent advantages, especially with its target use being the growing sustainable packaging market. Numerous biomasses (cotton, hemp, flax, spruce, bamboo, seaweed, etc.) used acid hydrolysis to extract nanocellulose with varying degrees of concentration, time, temperature, and type of acid (Bondeson et al., 2006; Brito et al., 2012; Chen et al., 2016; Doh et al., 2020; Dong et al., 1998; Donaghy et al., 1990; Hamad & Hu, 2010; Luzi et al., 2014; Mondragon et al., 2014; Lu & Hsieh, 2010). However, throughout the literature review of this field, the most widely accepted chemical utilized for acid hydrolysis of CNCs was sulfuric acid (H₂SO₄) due to the stabilizing effect of the esterification of the hydroxyl groups into sulfate groups (Lu & Hsieh, 2010). This effect is advantageous when creating a homogenous dispersion in a matrix (Beck-Candanedo et al., 2006). Furthermore, different biomass parts (i.e., the vine versus the leaf) may yield different results. According to the literature review, the process to isolate nanocellulose from kudzu has never been documented, and consequently, its resulting characteristics have yet to be formally studied. The objectives of this research are:

1) To evaluate the effectiveness of previous isolation methods on two parts of kudzu biomass: the aerial and vine regions
2) To characterize the resulting isolated nanocellulose particles and discern if they can
provide any value to the sustainable packaging landscape
REFERENCES


BACKGROUND AND HISTORY OF CELLULOSE

Cellulose is the most abundant polymer on earth, with up to 100 billion tons of cellulose produced each year by all the world’s ecosystems (Habibi et al., 2010; Sundarraj & Ranganathan, 2018). A naturally occurring polysaccharide extracted from various biomasses, cellulose is considered a biopolymer (Karuppusamy et al., 2015). It is a fibrous material commonly found in trees, plants, and many other types of vegetation. Anything from single-celled organisms to crops, higher plants, bacteria, and algae all contain cellulose. Although, the amount of cellulose can vary drastically depending on the source (Habibi et al., 2010; Pandey et al., 2015, Sundarraj & Ranganathan, 2018). Cotton has the highest yield of cellulose out of the crops, as it is 90% pure cellulose, whereas trees and higher plants have a range of 40-50% pure cellulose (Sundarraj & Ranganathan, 2018). Cellulose is found abundantly in the plant cell wall embedded in a primarily hemicellulose and lignin matrix. The French chemist, Anselme Payen, was the first to extract, separate, and chemically define the renewable polymer in 1838 (Karappusamy et al., 2015). In 1937, the American botanist Wanda Farr built on Payen’s revolutionary discovery and pioneered research into cytoplasmic cellulose production during plant cell wall formation (Farr, 1937).

The first known cellulose production in its isolated form was in the 1850s (Habibi et al., 2010). However, indigenous peoples have been using cellulosic fibers in a mixed form for clothes, twine, baskets, and other items since the earliest days of mankind (Fatma,
The first commercial application of cellulose was in 1870, when the Hyatt Manufacturing Company produced a cellulose thermoplastic (Sundarraj & Ranganathan, 2018). Hyatt named it celluloid, and it was inspired by the work of a British scientist, Alexander Parkes, who first produced it by reacting nitrocellulose with camphor (Gordon, 2017; Sundarraj & Ranganathan, 2018). Commercially, it first served as a replacement for the expensive and cruelly sourced ivory utilized in billiard balls (Gordon, 2017). It was later used for movie films and clothing, but its high flammability (due to the nitrocellulose) limited many of its commercial opportunities (Gordon, 2017).

Cellulose first came into the world of packaging in the 1920s. Its revolutionary discovery as a packaging product was built on the pioneering research of the Swiss chemist Jacques Brandenberger, who coined the name for the cellulose-derived film: cellophane (Inskeep, 1952). He originally intended cellophane to be a water-wicking replacement for tablecloths. While dining at a restaurant and witnessing a spilled glass of wine, he observed the resulting commotion of the waiters to change the tablecloth and thought there must be a better solution (Gordon, 2017). After much experimentation, Brandenberger realized his tablecloth solution might have a better fit in packaging since it was too stiff to function as a tablecloth but flexible and transparent enough to protect products needing transportation (Gordon, 2017). He immediately started his own company, La Cellophane, in 1912. However, the mass production of cellophane certainly presented Brandenberger’s company with some challenges (Gordon, 2018). Nevertheless, by 1923, DuPont entered into an agreement with Brandenberger to help develop a more robust process to produce cellophane. In short, they treated bleached sulfite wood pulp with caustic soda and carbon
disulfide to form viscose. They then extruded the viscose into a sulfuric acid bath that coagulated and regenerated it into a continuous web (Inskeep, 1952). In the beginning years of the partnership between La Cellophane and DuPont, cellophane’s most significant setback for food packaging was its poor water vapor barrier (although it had water resistance) (Gordon, 2017). However, by the late 1920s, the Dupont chemist duo, Charch and Prindle, found the solution of applying a nitrocellulose lacquer to enhance the moisture vapor barrier (Gordon, 2017; Inskeep, 1952). This discovery made sales grow significantly, and total sales of cellophane reached $100 million in 1950 (Gordon, 2017).

Various Industries started to gain interest in micro and nanoforms of cellulose. In the 1950s, a researcher for American Viscose Corporation, named Orlando Battista, figured out a way to commercially isolate cellulose using hydrochloric acid treatment into its microcrystalline form (Battista & Smith, 1962). Today, microcrystalline cellulose (MCC) is widely used in the food, pharmaceutical, and medical device industry. Also, around 1950, Bergt Rånby discovered that further acid hydrolysis of cellulose could isolate it to its nanoform (Rånby, 1951). Marchessault et al. built on this work and helped discover the chiral nematic liquid phase that cellulose nanocrystals (CNCs) form in solution when treated with sulfuric acid under the right conditions (Habibi et al., 2010). The nematic phase is when liquid crystals (having the orientation of solid crystals while flowing like a liquid) configure in a parallel orientation within mesophases rather than in well-defined planes. Chiral, sometimes called cholesteric, nematic phases also form into helical structures which display unique optical properties. This discovery opened the door to the present-day impregnation of CNCs into various polymer matrices to form composites with unique
mechanical and optical properties. The stable colloidal CNC suspensions can enhance composite properties while maintaining compostability (Irvin et al., 2019; Stark, 2016). Hence, characterizing this sustainable nanomaterial in terms of its efficacy as a potential nanofiller in packaging composites will be the focus of the ensuing experimentation.
Applications in Packaging and Other Markets

By the 1950s, cellophane was a widespread packaging material for various foods and household products. However, its time as a market leader eventually ended. Cellophane was widely replaced by petroleum-derived films, such as biaxially oriented polypropylene (BOPP), in the 1990s (360 Market Reports, 2019). It is currently making a bit of a resurgence as it is marketed as an eco-friendlier choice relative to BOPP. Other cellulose derivatives are also prevalent in the current packaging industry. As already discussed, nanocellulose, which is cellulosic material with at least one of its dimensions in the nanoscale, has garnered a tremendous amount of research interest in the past twenty years (Stark, 2016). There are three main types of nanocellulose: cellulose nanocrystals (CNCs), cellulose nanofibrils (CNFs), and bacterial nanocellulose (BNC) (Jovic, 2021). All have the potential for major impacts in the packaging industry due to their low-density, high strength, optical properties, and barrier enhancing properties (Stark, 2016). The main differences between the categories are that CNCs are produced chemically, whereas CNFs are produced mechanically or chemically, and bacteria secrete BNC via fermentation (Reshmy et al., 2020). They can all exist as standalone materials (i.e., neat CNF film), however it is more common to use CNCs and CNFs as reinforcements in a polymer matrix. The main benefit of nanocellulose is that it is a renewable and degradable material. Hence, much of the research around nanocellulose focuses on its incorporation into biopolymer matrices (i.e., biopolymer packaging films). The most common biopolymer films used in nanocellulose composites are polyhydroxyalkanoate (PHA) films, polylactic acid (PLA) films, starch films, and thermoplastic starch (TPS) films (Oksman et al., 2016). Since these
sustainable films are an essential part of the potential application for nanocellulose, it will be helpful to review each one briefly.

Polylactic acid is a biopolymer generated mainly from cornstarch. It is the most commercially available biopolymer, and it also has the widest processing window relative to the other biopolymers. The PHA family of biopolymers are produced by providing certain carbon feedstocks with the correct Redfield ratio (such as sugar, fatty acids, crude glycerol, agro-alimentary, and urban waste streams) to genetically engineered microbes. With the addition of these feedstocks, the microbes can then intracellularly produce degradable polyesters (Rodriguez-Perez et al., 2018). PHAs have a narrow processing window, and the MW quickly degrades when subjected to the temperatures used to process polyolefins (Stark, 2016). Nanocellulose has also gained interest in conjunction with starch and thermoplastic starch (TPS) biopolymers because starch is also a hydrophilic material (like dissolves like principle). Starch biopolymers also lack mechanical and thermal stability. Starch and CNCs are soluble in one another which increases adhesion. Hence, CNCs can rectify some of the mechanical shortcomings of starch because there would exist good adhesion. However, the CNCs would need to be well dispersed in the matrix to achieve optimal mechanical performance, and nanocellulose dispersions are hard to obtain. Mechanical techniques to improve the nanocellulose dispersion in polymers are sonication and high-pressure homogenization (Doh et al., 2020; Khalil et al., 2015; Luzi et al., 2014). Furthermore, in the case of poor adhesion, grafting of nanocellulose is a chemical technique widely utilized and researched. Since cellulose has high functionalization given the presence of its hydroxyl groups (specifically at C2, C3, and C6 shown in Figure 2.1),
grafting various polymeric chains onto the hydroxyl groups of cellulose is an effective method that allows for better adhesion and dispersion in the targeted matrix (Chakrabarty & Teramoto, 2018; Karuppusamy et al., 2015; Khalil et al., 2015).

![Chemical structure of cellulose showing the hydroxyl groups and their corresponding carbon atoms. Adapted with permission from Habibi et al. Copyright 2018 American Chemical Society.](image)

**Figure 2.1.** Chemical structure of cellulose showing the hydroxyl groups and their corresponding carbon atoms. Adapted with permission from Habibi et al. Copyright 2018 American Chemical Society.

Outside the field of packaging, cellulose and nanocellulose have many other uses. Nanocellulose is commonly used in the food and pharmaceutical industry as a binding agent, and obviously, cellulose is integral to the paper, construction, and textile industries (Habibi, 2010). In the paper industry, the primary source of cellulose is softwood used in the kraft pulping method (Sundarraj & Ranganathan, 2018). In 2009, approximately 3.6 million tons of wood pulp were dissolved to form cellulose fibers (Sundarraj & Ranganathan, 2018). Most of the world’s manufactured cellulose is in the paper, pulping, and textiles industry and is usually found in the form of cellulose II (Habibi et al., 2015; Wang, 2008). The mercerization process utilized in these industries converts cellulose I into II using amine complexes and caustic soda (Habibi et al., 2015, O’Sullivan, 1997).

On the other hand, the cellulose most relevant to this research is mainly cellulose I and in the nanoscale form. Nanocellulose I production requires different processing and will be discussed later in detail. Furthermore, rayon, which undergoes a production process much like cellophane that incorporates dissolving cellulose in a caustic solution to produce
viscose, is an important fiber in the textile industry (Gordon, 2017). With its lower water absorption than native cellulose, cellulose acetate plays a predominant role in biomedical applications (Liu et al., 2021; Pandey et al., 2015). Gun cotton is nitrocellulose (sometimes called cellulose nitrate) and is used in propellants, rockets, and as a printing ink base (Liu et al., 2021). Lastly, cellulosic ethanol continues to be investigated in the energy industry as a potential renewable energy source (Sundarraj & Ranganathan, 2018). Thus, although cellulose has been utilized since the earliest days of mankind, it will remain relevant in the modern era, and new purposes will likely continue to be discovered.
Market Overview

Historically, two cellulose commodities, specifically cellophane and paper, have been widely used as packaging materials. Both are making a resurgence due to their perceived environmental friendliness relative to today’s conventional plastics. For example, cellophane was largely phased out of food packaging in the 1980s and 1990s and replaced with oil-derived films such as biaxially oriented polypropylene (BOPP) that exhibit better thermal, mechanical, barrier properties. Yet, after decades of negative growth, cellophane is now making a comeback in the present day, boasting sales of 6% growth each year due to its perceived sustainable nature (360 Market Reports, 2019). Futamura Chemical, based out of Japan, is the leading supplier of cellophane as of 2019 (360 Market Reports, 2019). Innovia Films (USA) developed NatureFlex™ in 2008, which is a certified compostable film (according to ASTM D6400) derived from cellulose (Khalil et al., 2016; Reshmy et al., 2020). Paper follows a comparable and even more pronounced trend as a reemerging packaging material due to the major brands trying to appeal to the perceptions (whether based in reality or not) of the environmentally concerned millennial demographic.

Although there has been much academic interest and growing commercial interest, nanocellulose is not considered a commodity by most at this stage. However, it can be incorporated into commodity packaging materials, like paper and plastic, to add value by increasing sustainability and alleviating mechanical weaknesses in composites (Reshmy et al., 2020). For instance, nanocellulose can be added to paper as a coating to increase the oil and grease barrier (OGR) (Reshmy et al., 2020). It can be used in biopolymer composites as a coating or a filler to increase barrier, mechanical, and thermal properties.
It has various applications in high-end markets (printed electronics and batteries), mid-range markets (construction and food science), and low-end markets (paper and packaging) (Reshmy et al., 2020). The total nanocellulose market is valued at $350 million in 2021 and is projected to reach between $682-932 million by 2026 with a compound annual growth rate (CAGR) of 19.8-21.0% (Expert Market Research, 2020; Jovic, 2021). This aggressive projection is driven mainly by the consumer-packaged goods (CPG) companies increasing the demand for sustainable packaging materials. Composite and packaging applications account for almost half (around $140 million) of the total nanocellulose market share (Expert Market Research, 2020). By nanocellulose type, microfibrillated cellulose (MFC) makes up the most significant share of the market, followed by CNFs, then CNCs, and lastly BNC (Jovic, 2021). The biggest producer of CNCs is CelluForce Inc. out of Canada (tradename CelluForce NCC®), and Nippon Paper Industries out of Japan leads CNF production (tradename Cellenpia™) (Expert Market Research, 2020; Jovic, 2021; Reshmy et al., 2020). Similarly, Canada and Japan are the leading countries of nanocellulose production, with the United States lagging but expected to make up ground in the next five years (Expert Market Research, 2020; Reshmy et al., 2020). The biggest competitors to nanocellulose in the packaging landscape are the conventional plastics (such as the polyolefins) which generally exhibit far superior mechanical, thermal, barrier and processing properties. There are emerging compostable plastics (such as PLA and PHA polymer) that may already exhibit adequate properties without the need for nanocellulose addition. If sustainable additives are needed in these plastics, there are also other options besides nanocellulose such as nanoclays.
**Materials Science: Structure**

Materials Science is the study of the relationships between the processing, structure, properties, and performance of a material (Callister & Rethwisch, 2010). As shown in Figure 2.2, all four elements of Materials Science affect each other, and no single element should be examined in a vacuum. Thus, before delving deep into the properties and the structure of nanocellulose, it is essential to note that the extraction and processing of nanocellulose dramatically influence the structure, properties, and thus performance of the final product.

![Figure 2.2. The Processing-Structure-Properties-Performance Triangle of Materials Science demonstrates the interrelationship between the four components (Callister & Rethwisch, 2010).](image)

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**Figure 2.2.** The Processing-Structure-Properties-Performance Triangle of Materials Science demonstrates the interrelationship between the four components (Callister & Rethwisch, 2010).
The morphology of cellulose depends on the source, cultivation, extraction methodology, mechanical treatment, chemical treatment, and scale of the cellulosic material (Khalil et al., 2015). Shown in the first image on the left in Figure 2.3, raw cellulose is found in the trunks or vines of vascular plants. This form is called a bast (or phloem) fiber, but cellulose fibers can also be classified as the leaf, seed, grass, and fruit fibers as well (Habibi et al., 2015). The source of cellulose fibers can yield various mechanical, thermal, and dimensional properties. One property of distinction is the aspect ratio, or the fiber length (L) divided by the diameter (D). The season of harvest affects the preceding properties as well (Khalil et al., 2015). For kudzu cellulose fibers in the southeastern United States, the fiber is strongest in early spring (Ulundag et al., 1996). Additionally, the method of cultivation and processing (such as retting, carding, and enzyme treatment) has downstream effects on the fiber structure and performance (Fatma, 2019; Khalil et al., 2015; Luzi et al., 2014).

There are numerous methods employed in the isolation of cellulose. High-pressure homogenization, steam explosion, ultrasonication, grinding, carding are a few mechanical isolation techniques. However, these mechanical methods do not isolate cellulose into nanocellulose. Cellulose can be isolated into various orders of magnitude with respect to its size. Some methods even allow cellulose to be isolated into fibers with diameters in the micrometer and nanometer dimensions (Peneder, 2017). There exist several chemical methods to obtain nanocellulose such as TEMPO-mediated, alkaline, and acidic treatments as well (Habibi et al., 2010). Furthermore, techniques like acid hydrolysis or enzymatic hydrolysis produce cellulose nanofibrils and nanocrystals when executed under precise
conditions (Khalil et al., 2015). The methodology of choice depends on the desired composition, structure, size, morphology, and ultimately the desired performance of the final product. Most isolation practices combine mechanical and chemical methods to generate anything from woody fibers, cellulose microfibril bundles, and nanofibrils to highly crystalline (>80%) nanocrystals, as shown further to the right in Figure 2.3 (Khalil et al., 2015).

Figure 2.3. Structure of cellulose at various degrees of abstraction. Taken from Peneder, 2017.

In its higher plant native form, cellulose accounts for 15-30% of the mass in the primary wall shown in Figure 2.4 (Sticklen, 2008). Moreover, it is found in even higher concentrations in the secondary plant walls, where it constitutes up to 40% of the wall mass depending on the source (Sticklen, 2008). These secondary walls, which measure up to 30 times thicker than the primary wall (S2 in Figure 2.4), are responsible for much of the mechanical properties of the plant (Habibi et al., 2010; Li et al., 2012). In this portion, the cellulose chains wind into helical formations of cellulose microfibril bundles. These wound microfibrils are embedded in a matrix of lignin (10-25%), hemicellulose (20-40%), and pectin (35%) (Sticklen, 2008).
Figure 2.4. Composition and structure of the plant cell wall. (a) Cellulose microfibrils embedded in the cementing matrix of the primary wall; (b) Cross-sectional view of the primary and secondary cell walls; (c) Another cross-sectional view of the cell wall structure (left), and perspective view (right) the cellulose microfibrils integrated into the structural support of all the cell wall components (Sticklen, 2008). Reprinted with permission from Springer Nature. Copyright 2008 Springer Nature.
Xyloglucans, or more broadly hemicellulose materials, are vital in binding the cellulose to the rest of the matrix (Habibi et al., 2015). Hemicellulose is composed of various branched saccharides, namely xylose, arabinose, glucose, galactose, and mannose (Mandal & Chakrabarty, 2011; Yang et al., 2006). Furthermore, the main function of pectin is to balance the pH of the extracellular matrix (Sticklen, 2008). The main components of pectin are homogalacturonan, rhamnogalacturonan I, rhamnogalacturonan II, arabinans, galactans, and arabinogalactans (Sticklen, 2008). Lastly, the complex assortment of aromatic compounds containing ester and phenyl bonds, which are more largely referred to as lignin, provide defense against unwanted organisms and pathogens entering the cytoplasm (Sticklen, 2008). Lignin is the second most prevalent biopolymer on earth and is even more compositionally diverse than hemicellulose (Karuppusamy et al., 2015). It also gives the plant compressive strength and stiffness. Due to its more hydrophobic structure than cellulose, lignin helps hydrate cellulose (by keeping water in) as it binds and encases factions of cellulose (Karuppusamy et al., 2015; Khalil et al., 2015). Consequently, this hemicellulose, pectin, and lignin matrix acts as a glue that holds the rigid cellulose bundles in place within the cell wall. Using an analogy from the construction industry, the cellulose is the rebar (the more crystalline material) in the plant cell that provides much of the strength of the structure. In contrast, the hemicellulose, pectin, and lignin matrix (the more amorphous material) is the cement, gluing and holding the cellulose fibril bundles together while also providing some compressive strength and structural integrity (Sticklen, 2008). In cotton, cellulose is found in its pure form without the lignin, hemicellulose, and
pectin “cement” thereby making it an ideal candidate for cellulose extraction (Khalil et al., 2015). However, cotton as a source of CNCs has already been extensively researched.

The crystallinity of native cellulose can be attributed to its unique structure. Like any polymer, it is a chain of monomers. Specifically, the monomer of cellulose is cellobiose, which is a dimer of the simple sugar glucose ring in the D enantiomer configuration (Habibi et al., 2010; Khalil et al., 2015). Pyranose is a term for saccharides that contain five carbons and one oxygen in a six-membered ring. Hence, cellobiose is the linkage of two pyranose glucose rings in the $^4C_1$ chair conformation. This polysaccharide dimer, shown as cellobiose seen in Figure 2.5(a), is formed when water is eliminated to create the 1,4-glycosidic bond between the C1 hydroxyl atom (nonreducing end) and the C4 hydrogen (hemiacetal reducing end) of the D-glucose molecule (Habibi et al. 2010; Khalil et al., 2015). The hydroxyl groups of the cellobiose rings are in the equatorial position, or the $\beta$-configuration. This configuration optimizes both the intramolecular and intermolecular hydrogen bonding network depicted in Figure 2.5(b). This network of cellulose chain, which can take on various conformations, permits cellulose its highly crystalline and linear form (Habibi et al. 2010; Khalil et al., 2015). Lastly, anhydrous means literally without water. Thus, combining all these traits, cellulose is chemically described as $\beta$-1,4-D-anhydroglucopyranose.
Figure 2.5. Chemical structure and network of cellulose chains. (a) The chemical structure of β-1,4-D-anhydroglucopyranose, or cellobiose, monomer; (b) Inter and intramolecular hydrogen bonding of the different conformations of cellulose. Cellulose I_α conformations are shown on top, and the cellulose I_β conformations are shown on the bottom. Adapted with permission from Habibi et al. Copyright 2018 American Chemical Society.

Although the chemical of structure of cellulose is consistent regardless of the source, the geometry of the hydrogen bonding network determines its polymorphic or allomorphic structure (Habibi et al., 2010). Mechanical and chemical treatments allow for interconversion of cellulose into its polymorphic and allomorphic types (O’Sullivan, 1997; Habibi et al., 2010). Cellulose can exist in six different polymorphs (I, II, IIIα, IIIβ, IVI, IVII). However, native cellulose only occurs as cellulose I with two allomorphic structures: cellulose I_α and I_β (Habibi et al., 2010). Figure 2.5(b) shows the primary hydrogen bonding
arrangements of $I_\alpha$ (top two structures) and $I_\beta$ (bottom two structures). Cellulose found in bacteria and algae is mainly composed of cellulose $I_\alpha$ (accounting for around 70% the total cellulose content). In contrast, higher plants contain about 70% of the $I_\beta$ allomorph (Atalla & Vanderhart, 1984). The differences between the two allomorphs derive from the biosynthesis of the microfibrils manufactured in the rosettes of the plasma membrane shown in Figure 2.4(b) (Atalla & Vanderhart, 1984; O’Sullivan, 1997). The rosettes house the plant cellulose synthase proteins that polymerize the cellulose chains (Habibi et al., 2010). Cellulose $I_\alpha$ in algae and bacteria has a less complex structure because it is biosynthesized simultaneously with the construction of the rest of the cell wall.

On the other hand, cellulose $I_\beta$ in higher plants, such as kudzu, exhibits a more intricate architecture as it is biosynthesized after the formation of the other cell wall components. Consequently, it must form around existing infrastructure making its structure more complicated (Atalla & Vanderhart, 1984). Small differences in the structure cause small differences in hydrogen bonding arrangements that lead to large differences in the properties. Both networks contain extensive intermolecular hydrogen bonding (primarily between the C6 hydroxyl and C3 oxygen atom) and intramolecular hydrogen bonding (primarily between the C3 hydroxyl and the pyranose oxygen atom) (Habibi et al., 2010). This hydrogen bonding arrangement of the equatorial hydroxyl groups characterizes the linear pattern and planar stacking of the chains (O’Sullivan, 1997). However, there are also crucial differences in the unit cell parameters. For instance, cellulose $I_\alpha$ is triclinic, whereas cellulose $I_\beta$ is monoclinic. Unit cell differences account for the slight variations in the hydrogen bonding networks (Habibi et al., 2010). These subtle variations induce significant
distinctions in the crystalline structure, making cellulose I\textsubscript{β} the more thermodynamically stable allomorph (Habibi et al., 2010). Theoretically, the increase in crystallinity would also make cellulose I\textsubscript{β} the more mechanically and thermally advantageous nanofiller in packaging applications.

However, the fibers are not purely crystalline. Like other polymers, cellulose exists in two morphologies: crystalline and amorphous (Khalil et al., 2015). Regions of long-range order (crystalline) cellulose chains with strong hydrogen bonds are alternated with regions of random chain entanglement (amorphous) that exhibit less hydrogen bonding and hence less crystallinity. The tightly packed crystalline parts are more thermally and mechanically stable and consequently less susceptible to chemical attack than the disordered amorphous areas. The difference is critical to this research, as one objective of this study is to isolate the mechanically and thermally enhanced crystalline regions by removing as much of the amorphous part as possible. Just as cellulose was biosynthesized from bundling together nanofibers comprised of cellobiose monomers (~1 nm) to form microfibrils (100-350 nm), macro networks of cellulose can be reduced back down to its high crystalline nanoscale morphology by using isolation methods (Habibi et al., 2009; Khalil et al., 2015; Peneder, 2017; Wang, 2008). The nanoscale morphology has gained much research interest over the last decade due to its highly crystalline and thus mechanically advantageous nature. Thus, concerning the structure of cellulose, this research will focus on solubilizing and discarding the amorphous parts of the lignocellulosic material to leave only the CNCs to utilize the desired mechanical, thermal, sustainable, and biocompatible properties for subsequent packaging applications.
Material Science: Properties

As depicted in Figure 2.2, the properties of cellulose are highly dependent on its previously described structure. Cellulose in its nanocrystal form has many advantages in the packaging industry. It presents a high tensile strength (7.5-7.7 GPa) and elastic modulus (110-220 GPa with an average of around 170 GPa) while maintaining a low density (1.61 g/cm) (Habibi et al., 2010; Stark, 2016). Cellulose thermally degrades between 275-400°C (Chirayil et al., 2016). Since CNCs are more crystalline than other lignocellulosic material (and other polymer for that matter), they have the potential to enhance the thermal properties of composites. With their high reactivity, CNCs act as nucleating agents that can increase the crystallinity of the surrounding polymer (Habibi et al., 2010). They also have fascinating optical properties when the crystallinity index is above 80% (Hamad & Hu, 2010). Implementations of CNCs into a matrix can increase optical clarity up to a critical concentration (Chakrabarty & Teramoto, 2018). Sulfuric acid hydrolyzed CNCs self-assemble into a chiral nematic ordered phase in film and solvent suspensions (Habibi et al., 2010). The chiral nematic order of CNCs can be leveraged to design specific optical characteristics. For instance, these properties are utilized in idiosyncratic watermarking and electrical engineering applications such as printed flexible electronics (Habibi et al., 2010; Stark et al., 2016). Similarly, CNCs are more conductive than natural cellulose (Shimazaki et al., 2007). However, like other cellulose-derivatives, the most significant setback of CNCs is their hydrophilicity which limits some of their industrial uses (Habibi et al., 2010; Stark, 2016). Work is being conducted to find reliable methods for modifying CNCs to become less hydrophilic when desired (Chakrabarty & Teramoto, 2018; Habibi et al., 2010;
Karuppusamy et al., 2015; Stark et al., 2016). It is important to note the wide range of CNC properties for future studies, but this research will primarily focus on the superb mechanical properties of CNCs.

Given that CNCs can exhibit moduli of between 100-220 GPa, it is crucial to understand how to fully leverage this mechanical strength in packaging applications. The most essential trait for unleashing the full mechanical potential of these additives is the aspect ratio (L/D). Larger aspect ratios are desired for increased mechanical performance (Clyne & Hull, 2019; Mondragon et al., 2014; Reshmy et al., 2020). This can be visualized by examining the diagram in Figure 2.6 and the plots in Figure 2.7 which illustrate the shear lag model of cylindrical, short fibers in composites.

The shear lag model is widely used to approximate the mechanical behavior of short-fiber composites under loads (Clyne & Hull, 2019; Cox, 1952). Two critical assumptions are made for this model to hold validity: (1) the adhesion between the fiber and matrix is good, and (2) there is no shear strain in the fiber itself (Clyne & Hull, 2019; Cox, 1952). The drawing in Figure 2.6 represents the transfer of tensile stress ($\sigma_m$) from the polymer matrix (white space) to a fiber embedded in the matrix (grey rectangle) which is aligned parallel to the axis of force. The fiber center is denoted at point 0 in the diagram due to the axis being the fractional distance (F.D.) from the fiber center. Consequently, the fiber ends are denoted at point -1 on the left end of the fiber and point 1 on the right end. According to the shear lag model, the mechanism of stress transfer from the matrix to the fiber is through shear stresses at well-adhered interfaces between the fiber and the matrix (Clyne & Hull, 2019; Cox, 1952). The model does not account for normal stress transfer from the matrix.
matrix to the ends of the fibers. Nevertheless, the shear stresses at the interface can be resolved by transferring the stress to the fiber itself, given that there is good adhesion between the matrix and fiber. Thus, the stress in the fiber builds up with distance from the fiber’s ends and towards the fiber center (Clyne & Hull, 2019; Cox, 1952). The tensile stress is zero at the ends of the fiber, and the stress is maximized at the center as seen in the plots of Figure 2.7. The increasing stress towards the center of the fiber causes more axial strain in the fiber center. This fiber strain can be observed in the diagram of Figure 2.6 as the space between the dotted reference lines in the fiber. Near the fiber ends, the matrix is significantly warping near the interface due to the difference in strain between the fiber and the matrix. Fracture of the composite is more likely where the strain of the fiber does not equal the strain of the matrix due to excessive shear stress remaining unresolved. Longer fibers, or fibers with larger aspect ratios, have sufficient length to allow for the stress to build up until the strain of the fiber is equal to that of the matrix (Clyne & Hull, 2019; Cox, 1952). In this region, there will be minimal shear stress at the interface and a low probability of fracture. However, where strains are unequal (for instance the warping of the matrix near the fiber ends), there will be an increase in shear stresses at the interface and a higher likelihood of fracture.
Figure 2.6. Diagram showing the shear lag model approximation of a short-fiber composite under a tensile load ($\sigma_m$).

The shear lag model plots (Figure 2.7) of cellulose nanocrystals (Young’s modulus = 100 GPa) with three different aspect ratios (aspect ratios: L/D = 5, L/D = 10, and L/D = 50) are impregnated with a common sustainable plastic, PLA (Young’s modulus = 4.107 GPa) (Pinto et al, 2015). When the composite undergoes a constant tensile stress ($\sigma_m$), the stress is transferred to the CNC via shear stresses built up at the interface (Clyne & Hull, 2019). As shown in Figure 2.7(a), the model with the larger aspect ratio (L/D = 50) quickly reaches a plateau in tensile stress over most of the CNC’s length. This effect causes the shear stress (Figure 2.7(b)) between the CNC and the PLA matrix to be essentially zero over much of the CNC (L/D = 50) length. In comparison, the smaller aspect ratio CNCs (L/D = 5 and 10) never reach the peak tensile stress plateau. The smaller aspect ratio CNCs have a more linear shear stress shape, although the curve for the CNC (L/D = 10) is marginally flatter than that of the CNC (L/D = 5). Consequently, the strains in the smaller aspect ratio CNCs never match that of the PLA polymer. The composite is more likely to fail at an interface containing non-zero shear stresses between the CNC and the matrix. Thus, since the higher aspect ratio CNC has low shear stress along much of the CNC’s
length, the adhesion between the CNC and matrix is not as compromised as the lower aspect ratio CNCs. This phenomenon allows composites with higher aspect ratio CNCs to handle more stress and strain before it fails. Besides the aspect ratio and modulus of the CNC itself, other factors that significantly affect composite strength are the modulus of the matrix, volume fraction of the CNC, Poisson’s ratio of the matrix, CNC/matrix adhesion, and orientation of the CNC (Clyne & Hull, 2019). This research will not be engineering a composite, so this study will focus on characterizing the aspect ratio of the extracted CNCs to predict the mechanical properties of sustainable composites that would utilize the CNCs produced in this research.
Figure 2.7. The effect of a fiber’s aspect ratio on the mechanical properties of a matrix. Example prediction of the axial stress in a fiber (a), and the shear stress at the interface between a fiber and a matrix (b) using Cox’s shear lag model. This example is of a PLA/10% CNC composite. The applied axial tensile strain is 0.001, and the Poisson’s ratio of PLA is 0.30 (Mirkhalaf & Fagerström, 2021).
Materials Science: Extraction, Processing, and Manufacturing

The third point of the Materials Science Triangle (Figure 2.2) that affects a material’s performance is the processing of said material (which includes the extraction and manufacturing of the material). The primary methods used to extract cellulose nanocrystals are through enzymatic and acid hydrolysis. This research will use acid hydrolysis, and the general process is illustrated in Figure 2.8. The acid hydrolysis of cellulose was pioneered by Bengt Rånby in 1951, who first described that cellulose exists as bundled microfibrils that are fundamentally composed of highly ordered micelles mixed in with amorphous regions (Hamad & Hu, 2010; Khalil et al., 2015; Rånby, 1951). The hydronium ion produced by the hydrolyzing sulfuric acid transversely attacks the glycosidic bonds of the accessible amorphous regions (Mozdyniewicz et al., 2015). Theoretically, the glycosidic attack leaves the tightly packed crystalline micelle domain intact and substitutes sulfate esters throughout the CNCs (Khalil et al., 2015). These negatively charged sulfate groups are critical in minimizing aggregation (via repulsion forces) and forming stable solutions (Doh et al., 2020; Habibi et al., 2010; Wang, 2008). Sulfation of CNCs is a key distinction from Battista & Smith’s revolutionary work in 1961 to produce MCC with hydrochloric acid, which does not impart a charge and thereby does not effectively prevent aggregation. Since sulfuric acid can hydrolyze the tightly packed crystalline areas, it is of utmost importance that the hydrolysis parameters (concentration, time, temperature) are precisely controlled. Tightly controlled kinetics allow for only the dissolution of loosely packed amorphous chains, thereby leveraging the excellent mechanical properties of crystalline nanocellulose (Dong et al., 1998; Hamad & Hu, 2010).
Figure 2.8. General overview of the widely accepted process used to extract CNCs.

Before hydrolysis, the lignocellulosic fibers are first pulverized into a powder and then pretreated to open the lignocellulosic matrix for increased exposure of the cellulosic material to acid hydrolysis. Retting and carding of fibers are two environmentally inert and ancient mechanisms that can be employed as pretreatments to maximize CNC yield (Luzi et al., 2014; Tanner et al., 1993; Uludag et al., 1996). The retting process, which simply immerses the plant into water, capitalizes on the degrading function of the microbes naturally present in the plant stem or vine (Tanner et al., 1993; Uludag et al., 1996). Pectin and plant proteins are consumed by inherent micro-organisms leading to the more accessible release of the lignocellulosic fibers from the surrounding plant tissue, such as the vine sheath (Tanner et al., 1993; Uludag et al., 1996). This process then employs a natural method to remove non-cellulosic components (e.g., pectin) before ever treating the fiber with any artificial chemicals (Luzi et al., 2014; Sundarraj & Ranganathan, 2018). Similarly, carding is a process heavily used in the textile industry that disentangles fibers
while also cleaning them of any undesired components (Luzi et al., 2014). Previous research found that carded hemp fibers demonstrated increased cell wall surface area over the control allowing for more efficient downstream accessibility of the targeted components (Luzi et al., 2014). Nevertheless, these natural and prehistoric procedures cannot reduce the fiber to its nanocellulose components. To produce CNCs, the use of man-made chemicals and energy-demanding technology is currently unavoidable.

A low concentration of hydrochloric acid can be used as a pretreatment that initiates bond cleavage of any loosely bound amorphous materials, such as lignin, in the lamella and cell wall via a process called depolymerization (Doh et al., 2020). The depolymerization step helps to enhance the surface area contact of the lignocellulosic material by increasing the free volume between the fibers. It starts the process of hydrolyzing the hemicellulose material as well (Chirayil et al., 2014). Furthermore, an alkali treatment with a combination of sodium hydroxide and potassium hydroxide is effective at breaking down the hydrogen bonds between the targeted cellulose and the amorphous lignin, hemicellulose, and pectin (Chirayil et al., 2014; Hashim et al., 2017; Sung et al., 2017). Alkaline reactants also cleave ester bonds between lignin and hemicellulose in addition to swelling the fiber to loosen the microfibrils further, increase surface area, and make the cellulose more accessible for chemical removal (Hashim et al., 2017).

The bleaching step is an acetylation process comprised of adding sodium hypochlorite to the lignocellulosic fiber in a low pH-controlled reaction. This forms lignin-chlorine complexes, which can then be dissolved to further remove lignin complexes
(Chirayil et al., 2014; Mandal & Chakrabarty, 2011). Finally, the bleached and swelled fibers can then be reacted with sulfuric acid to remove the amorphous domains of the cellulose microfibrils to produce the CNCs. Mechanical dispersion through sonication followed by immediate lyophilization is recommended to ensure that the CNC particles do not form hydrogen bonds and agglomerate in suspension (Doh et al., 2020; Khalil et al., 2015).

Other than the previously mentioned processes of kraft pulping and hydrolysis, additional methods for isolation are under development. Steam explosion is a more cost-effective and environmentally friendly way to remove hemicellulose and lignin rather than using hazardous chemical treatments. However, it is not as effective as acid hydrolysis at removing amorphous cellulose domains (Chirayil et al., 2014; Saelee et al., 2016). Hydrolyzation of hemicellulose and depolymerization of lignin occurs with steam explosion. However, if the goal is to get down to the nanocellulose scale, then steam explosion does not suffice and must be used in combination with acid or enzymatic hydrolysis (Chirayil et al., 2014; Sun et al., 2005). To reduce the heavy reliance on hazardous chemical treatments, studies have combined steam explosion, high-pressure homogenization, and a cellulase-free xylanase enzyme to isolate CNFs. However, CNFs are larger with diameters between 4-20 nm and aspect ratios greater than 100 (Chakrabarty & Teramoto, 2018; Saelee et al., 2016). Cellulose nanocrystals are generally defined as having aspect ratios below 100 (Chakrabarty & Teramoto, 2018). No known research has converted lignocellulosic material down to CNCs with just the steam explosion method.
Enzymatic hydrolysis of cellulose presents another eco-friendly alternative to acid hydrolysis due to its minimal downstream effects during disposal. Filson et al. took recycled pulp (a source rich in cellulose without much industrial use as it leads to poor paper quality) and reacted it with the endoglucanase enzyme to produce CNCs (Filson et al., 2009). This is a promising study in finding an alternative to acid hydrolysis. Yet, the enzymes suited for degrading amorphous cellulose are not very efficient in removing the lignin and hemicellulose attached to the cellulose fibers, so it still requires chemical pretreatment (such as alkali treatment) to work effectively (Li et al., 2012). Additionally, there are indications that enzymatic hydrolysis does not achieve the same thermal stability and repulsion effect of the produced CNCs compared to acid hydrolysis (Luzi et al., 2014).

Sulfuric acid hydrolysis is also not the only option when trying to achieve the repulsion effect to minimize aggregation. Researchers widely utilize 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO)-mediated oxidization to substitute the C6-hydroxyl groups with more negatively charged carboxyl groups for fibril separation (Chakrabarty & Teramoto, 2018; Filson et al., 2009). Phosphoric acid can be used as a substitute for sulfuric acid during acid hydrolysis while achieving the repulsion in solution, but it still shares the same environmental concerns as sulfuric acid (Habibi et al., 2010; Hamad & Hu, 2010). Investigation into the combination of the synergistic effects between steam explosion, high pressure homogenization, alkali/bleach treatments, TEMPO-mediated oxidation, enzyme mixtures, and acid hydrolysis of lignocellulosic materials will likely continue to minimize the environmental impacts of extracting CNCs from biomasses. Processing parameters can be tweaked to maximize performance or CNCs, but
environmentally responsible methods for processing must be further developed to shore up the sustainability of the CNC lifecycle.
Social Aspects

Petroleum-derived plastics replaced Cellulose-derived plastics in the late 20th century due to the superior performance of petroleum-based plastics (which entails structure, properties, and processing of the material as shown in Figure 2.2). However, cellulose-based plastics are now making a comeback, as sustainability is more at the forefront of the consumer’s mind and, resultantly, the industry’s marketing strategies. For many companies, a transition back to cellophane is an easier move towards sustainability than implementing some of the more cutting-edge sustainable polymers (such as PHAs). This is attributed to the fact that cellophane has already shown robust supply capacity, machinability, and market endurance. Furthermore, it has already been deemed generally recognized as safe (GRAS) by the United States Food and Drug Administration (FDA). Yet, the perception that cellophane is environmentally friendly is false. Although it comes from a renewable source, the viscose process that is required to produce cellophane often uses chemicals with high concentrations of sulfur and yields harmful by-products (Reshmy et al., 2020).

Although the use of cellophane may be past its prime, cellulose in its nanoform brings new hope to its place in the sustainable packaging industry. As previously described, nanocellulose can be incorporated into an array of sustainable polymers to rectify some of their weakness and allow them to perform at a more similar level to that of the traditional petroleum-based plastics (Chakrabarty & Teramoto, 2018). Its enhancing properties come with certain downsides, such as the problematic industrial processing of CNCs (Stark, 2016). Much like any manufactured good, the social aspects of this material are tightly
bound to the manufacturing procedures. And due to the large-scale manufacturing impediments, it remains too early to determine the widespread social impact of nanocellulose. Being a hydrophilic non-thermoplastic polymer that tends to agglomerate, it is intrinsically difficult to extrude CNCs when compounded with the typical polymers in the sustainable packaging industry. Issues have included the molten curtain frequently breaking, the CNCs thermally degrading, and the CNCs significantly agglomerating during processing (Oksman et al., 2016). So, although it has gained academic notoriety (where small-scale solvent casting is the preferred method), it has yet to be widely adopted in large-scale industrial processes (Oksman et al., 2016). However, recent advances in continuous melt processing such as co-rotating twin-screw extrusion, solid-state pulverization, grafting, and chemical pretreatment are making up for some industrial deficiencies (Oksman et al., 2016; Stark, 2016).

Due to relatively recent technological advances allowing for isolation of materials to their nanoscale, many questions of the safety of nanomaterials are left unanswered. These nanomaterials may potentially be hazardous to human health in that organs can absorb them at higher rates due to their minuscule size, increased surface area, and enhanced chemical functionality and reactivity (Callister & Rethwisch, 2010). Preliminary studies indicate that there is little toxicity concern in the case of nanocellulose, but more studies need to be performed (Deloid et al., 2019). For these reasons, nanocellulose has yet to be regarded as GRAS by the FDA (DeLoid et al., 2019).
Trends and New Developments

Nanocellulose has generated a lot of excitement in the world of academia over the past 15 years (Oksman et al., 2016). Over 6,000 papers published concerning nanocellulose, highlighting its wide breadth of potential uses (Oksman et al., 2016). Recent trends and new developments demonstrate the diversity of nanocellulose functions that extend far beyond packaging and composites (Habibi et al., 2010). Despite the obstacles regarding industrial processing and market durability, nanocellulose use has grown in the food and cosmetics industries because of its superior stabilizing properties and hygroscopicity (Reshmy et al., 2020). There are also many biomedical applications, such as tissue scaffolding and drug-delivery mechanisms (Karuppusamy et al., 2015). Furthermore, freeze-dried nanocellulose aerogels are used in wound dressings, tampons, and diapers (Reshmy et al., 2020). Water purification, flexible electronics, and microelectronics also present markets of growth for nanocellulose (Karuppusamy et al., 2015; Reshmy et al., 2020).

Japan and Canada are leading the industrial development of nanocellulose mostly in the composite engineering (aerospace, automobile, biomedical, construction, packaging) landscape, but companies in the United States, like American Process Inc., are growing with more American competitors entering the marketplace (Jovic, 2021; Reshmy et al., 2015). The effect of nanocellulose on the packaging industry could be substantial due to its dynamic range of functions. It can be used as fibers impregnated by a composite, as a coating on a vast array of packaging materials, as flexible electronics printed on a film, as a laminated layer in a multilayer structure, or as a standalone film (Habibi et al., 2015;
Moreover, the combination of chitosan and nanocellulose films leads to unique antimicrobial properties. Similarly, chitin nanofibers and CNCs have been incorporated together as spray-coatings on PLA and poly(vinyl alcohol) (PVA) films leading to enhanced mechanical, thermal, and barrier properties (Irvin et al., 2019; Satam et al., 2018). Chitin nanofibers are cationic, while CNCs are anionic (when hydrolyzed with sulfuric acid). Due to their opposite charges, the mixture of the two polymers lead to synergistic effects such as increased adhesion and interfacial densification (Irvin et al., 2019; Satam et al., 2018). Thus, if academia continues to prove viable and reliable sources of CNCs and the manufacturing process can be improved to make large-scale production both cost and resource-efficient, it is likely that CNCs will continue to develop in marketplaces.
Kudzu (*Pueraria montana var. lobata*)

As discussed, the performance of nanocellulose can be greatly dependent on the source. The biomass used in this research is from the Leguminosae family, Papilionoideae subfamily, Phaseoleae tribe, Glyciniae subtribe, genus *Pueraria*, species *montana* variety *lobata*. This is more commonly known as kudzu, “the vine that ate the South” (Keung, 2002). In the southeastern United States, it is infamously known as an invasive vine that overtakes the surrounding vegetation and strangles it due to its rapid growth (Gulizia & Downs, 2019). In its native environments (China, Japan, and other parts of Asia), the rapidly growing vine is kept at bay by numerous evolutionary counterforces. However, since kudzu has only been in the United States since the late 19th century, the vine is free to spread rapidly without much competition. In fact, under optimal conditions, the kudzu vine has been measured to grow a foot per day and up to a hundred feet per year (Keung, 2002; Shurtleff & Aoyagi, 1977). This robust legume flourishes in various climates and soil contents. It resides anywhere from high altitude environments and rocky terrains to tropical climates and fertile lowlands. However, it thrives in temperate rainforests with at least 40 inches per year of rain and a soil pH between 5.0-7.1 (Keung, 2002; Shurtleff & Aoyagi, 1977). This wide adaption to different ecosystems and soil qualities makes it an extremely versatile and hardy cover crop.

Kudzu requires a high degree of photosynthesis to survive. The plant has grown very adept at achieving this goal by climbing up anything it can find including trees, telephone poles, and various man-made structures. It demonstrates the expression “survival of the fittest,” as it receives ample sunlight by strangling, climbing, and drowning out all
other vegetation (Keung, 2002; Shurtleff & Aoyagi, 1977). It is a suffocating, semi-woody vine that is a strong climber whose sole purpose is to capture sunlight with its trifoliated leaves, as seen in the left picture of Figure 2.9 (Shurtleff & Aoyagi, 1977).

As seen in the right picture of Figure 2.9, the vine exhibits diameters between 1-2.5 cm (Keung et al, 2002). Three to five vines originate from a crown that sits in the topsoil connected underneath by bulbous and tubular-shaped roots that form a sizeable horizontal network 3-10 feet below the ground (Shurtleff & Aoyagi, 1997). These large starchy roots (2-18 cm in diameter) contain an abundant energy reservoir that makes it a drought-resistant and fast-spreading plant in the warm months. The roots also firmly entrench the legume into the surrounding soil (Keung, 2002; Shurtleff & Aoyagi, 1997). The deeply

Figure 2.9. Pictures of the kudzu leaves (left) and vines (right) harvested behind the C-1 parking lot in Clemson, SC, USA.
entrenched roots and drought-resistant nature of kudzu make it a good candidate for preventing soil erosion. The United States government started an extensive campaign in 1935 (Soil Conservation Service) to incentivize farmers to plant it for this purpose (Shurtleff & Aoyagi, 1977). Furthermore, like other legumes, kudzu has nitrogen-fixing bacteria in its roots which convert the free nitrogen found in the atmosphere into nitrates which replenish depleted soil (Keung, 2002; Shurtleff & Aoyagi, 1977). The protein and carbohydrate-rich content of kudzu, which has been utilized in Asian cultures for millennia (along with medicinal uses), makes it a viable fodder for livestock (Keung, 2002; Shurtleff & Aoyagi, 1977). Due to the recognized benefits and governmental sponsorship, kudzu cultivation became rampant in the 20th century and covered 3 million hectares of American soil by 1950 (Keung, 2002).

Nevertheless, the properties that make kudzu such a hardy plant also come with the trade-off of being extremely hard to exterminate and control (Keung, 2002; Shurtleff & Aoyagi, 1977). Given its exceptionally rapid propagation, it is now widely considered an agricultural nuisance. It is safe to say its removal and harvesting for alternative purposes would be welcomed by local populations in the Southeastern United States. A newly found use for this noxious weed would significantly improve its perception, help regulate its proliferation, and provide a renewable resource to whichever industry can find it a value-added purpose (Lou et al., 2002). Thus, in all its abundance around the city of Clemson, kudzu will serve as the biomass for the extraction of nanocrystalline cellulose in this research.
This research believes that the packaging industry can find a use for kudzu. More specifically, this research will demonstrate that cellulose derived from kudzu can add value to the degradable packaging market in the form of CNCs that act as nanofillers in sustainable polymer composites. Since the properties of CNCs are highly dependent on the source, many feedstocks have been researched to determine the most efficient source for the isolation of nanocellulose as shown in Table 2.1 (Chakrabarty & Teramoto, 2018). Logically, higher cellulose compositions would lead to higher CNC yields. Furthermore, a higher aspect ratio is important when incorporating the CNCs into a polymer matrix to enhance mechanical, thermal, and barrier properties (Doh et al., 2020). Not only must the yield be high enough to make the environmental impact of processing (energy and hazardous materials utilized) worth it, but it would also be advantageous to use a waste feedstock that has no other value. Doh et al. demonstrated that two other plentiful but problematic species, the *Sargassum natans* (also known as sargassum seaweed) and *Laminaria japonica* (also known as kombu seaweed), could be treated to produce CNCs that enhanced mechanical and thermal properties when disseminated into a polymer matrix (Doh et al., 2020; Doh et al., 2020). The sargassum seaweed contains approximately 20% cellulose, and the kombu seaweed contains 17% cellulose (Rabemanolontsoa & Saka, 2013; Shi et al., 2011). Hence, even relatively low cellulose comprising sources can produce useful CNCs. Cellulose makes up 33% of the kudzu vine composition (Anele et al., 2020; Lou et al., 2002; Tanner et al., 1979; Wilke & Rosenberg, 1977). The rest of the vine is composed of hemicellulose (11%), lignin (14%), solubles (41.4%), and ash (0.3%) (Lou et al., 2002; Tanner et al., 1993). However, the kudzu aerial parts (leaf and stem)
contain less cellulose (14-20%), less lignin (4-6%), and more hemicellulose (10-20%) on average when compared to the vine (Anele et al., 2020; Gulizia & Downs, 2019). It is important to note that the range of composition percentages is highly dependent on if the kudzu was harvested in the early or late season (Gulizia & Downs, 2019; Uludag et al., 1996).


<table>
<thead>
<tr>
<th>Source</th>
<th>Composition (%)</th>
<th>Aspect Ratio (L/D)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cellulose</td>
<td>Hemicellulose</td>
<td>Lignin</td>
</tr>
<tr>
<td>Cotton</td>
<td>95</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Flax (retted)</td>
<td>71</td>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td>Flax (unretted)</td>
<td>63</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Hemp</td>
<td>70</td>
<td>22</td>
<td>6</td>
</tr>
<tr>
<td>Kombu</td>
<td>17</td>
<td>31</td>
<td>0</td>
</tr>
<tr>
<td>Kudzu (aerial)</td>
<td>14-20</td>
<td>10-20</td>
<td>4-6</td>
</tr>
<tr>
<td>Kudzu (vine)</td>
<td>33</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>Recycled Pulp</td>
<td>98</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sargassum</td>
<td>20</td>
<td>43</td>
<td>8</td>
</tr>
<tr>
<td>Sisal</td>
<td>73</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Sugarcane Bagasse</td>
<td>40</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>30</td>
<td>50</td>
<td>15</td>
</tr>
<tr>
<td>Wood</td>
<td>40-47</td>
<td>25-35</td>
<td>16-31</td>
</tr>
</tbody>
</table>
Furthermore, the cellulose of kudzu has been reported to have a relatively high complex viscosity and a high degree of polymerization (DP) above 1720 (Harland, 1952; Li, 2003). With sufficient acid concentration, the DP will reach a level-off degree of polymerization (LODP) independent of the source's initial DP before hydrolysis (Hamad & Hu, 2010; Mozdyniewicz et al., 2015). However, to reach this LODP, the yield will be sacrificed because higher sulfuric acid concentrations are needed. Sulfated cellulose chains (amorphous and sometimes crystalline) are more likely to be water-soluble and thus discarded in the supernatant, diminishing yield (Hamad & Hu, 2010). Moreover, Hamad and Hu (2010) also show that crystallinity reaches a maximum of 90% around the LODP, and the nanocrystal reaches a minimum diameter of 8-10 nm, independent of the initial DP. However, LODP is likely to vary based on the source, leading to different dimensions, viscosities, optical properties, and surface reactivities of the nanocellulose (Vanderfleet & Cranston, 2021). Vanderfleet & Cranston (2021) also demonstrated that, when CNCs are isolated from two somewhat different biomass sources (such as softwood and hardwood) using the same or similar protocols, their functional properties are very similar. The thought is that severe hydrolysis conditions overtake any subtle chemical or structural differences in the biomasses. However, kudzu (a higher land plant) and seaweed (a marine plant) are two very different sources, so the careful study and delineation of properties between their respective extracted CNCs are of keen interest to this research.
REFERENCES


CHAPTER THREE
MATERIALS AND METHODS

Kudzu Collection and Nanocellulose Preparation

*Harvesting and retting of kudzu*

The following procedure was separated into two parts: Treatment 1 (T1), which was executed in August 2020, and Treatment 2 (T2) in August 2021. Kudzu (*Pueraria montana* var. *lobata*) was harvested in the late summer months of August 2020 for T1 and August 2021 for T2. Both the aerial part (leaf/stem) and the vine were collected from a heavily infested area in Clemson, South Carolina, USA for T1. As for T2, just the vine was collected from the same place. The aerial parts were washed with running tap water and then dried at 30°C for 7 days in a laboratory drying oven. The aerial parts were then pulverized for 5 minutes using a commercial blender to produce a fine powder and then set aside to undergo chemical treatment.

The kudzu vine underwent a retting process for both T1 and T2 like that of the Uludag et al. study (Uludag et al., 1996). This process utilized the enzymes naturally produced by the bacteria found in the kudzu vine to degrade the outer sheath and expose the fiber bundles (Uludag et al., 1996). Vines were approximately 1 cm in diameter. They were then cut to lengths of 15 cm and placed in a container of distilled water to soak. The soaking time was determined to be the time needed for the pH to decrease to a constant level of pH 5.5 (Uludag et al., 1996). This period was approximately 3-5 days. After retting, the outer sheaths were peeled off under running tap water, and the fiber bundles were then
isolated and dried in the laboratory oven for one day at 30ºC. Finally, the fibers were pulverized using a commercial blender and put back in the oven for 3 days at 30ºC.

Isolating nanocellulose from kudzu

After the leaf and vine powders were dried, the samples in T1 and T2 were subjected to a four-step treatment (depolymerization, bleaching, acid hydrolysis, and mechanical dispersion). The purpose of depolymerization was to break easily accessible bonds and increase surface area. Bleaching was utilized mainly to break benzyl ester bonds between lignin and hemicellulose through various alkali treatments (Sun et al., 2005; Tarasov et al., 2018). Acid hydrolysis was used to break down any remaining alkali-resistant bonds (such as benzyl ether and phenyl glycosidic linkages) and remove amorphous cellulose factions (Doh et al., 2020; Tarasov et al., 2018). Mechanical dispersion kept the nanocellulose particles from agglomerating to be better dispersed into a matrix (Doh et al., 2020; Khalil et al., 2015). Due to this study being the first known attempt to isolate nanocellulose from kudzu, the procedure was primarily adapted from previous methods (Doh et al., 2020; Feng et al., 2015; Huq et al., 2012; Liu et al., 2017; Sung et al., 2017). Treatment 1 (T1) was executed first. Then procedural changes were made, aiming to increase CNC crystallinity index (CI), and the new procedure was named Treatment 2 (T2). The major differences between the treatment groups T1 and T2 are denoted in Figure 3.1. All chemicals used were of analytical grade.
Figure 3.1. General overview of the experimental design that denotes the major differences between the T1 and T2 treatment procedures.

Treatment 1 (T1) Procedure

To begin the depolymerization step for T1, the pulverized powders from the aerial part and the vine were separately placed in hydrochloric acid (HCl) solutions (0.2 M) at 1:15 proportion (w/v) and magnetically stirred for 2 hours at 30ºC. The colloidal suspensions were then washed with distilled water by centrifugation cycles at 15,000 g for 10 minutes to remove the supernatant. Once neutral pH was obtained, the residues of both the aerial part and vine were separately placed in distilled water at a 1:60 proportion (w/v). Then, 4% sodium hydroxide (NaOH) was added to the solutions until the pH of the solutions were approximately pH 10. These solutions were immediately placed on a hot-plate magnetic stirrer to continuously mix and heat the colloidal suspensions for 3 hours at 75ºC. After completion, the suspensions were washed by several centrifugation cycles at
15,000 g for 10 minutes to remove excess NaOH and isolate the remaining residues. Finally, the residues were placed in the laboratory oven for 3 days at approximately 65°C.

The bleaching step was conducted by treating the polysaccharide residues with 10% potassium hydroxide (KOH), using a 1:20 solid to liquid ratio, for 3 hours at 30°C. After this treatment, the residues from the aerial parts were set aside, and further treatment was aborted due to the miniscule amount of yield following this step. However, the treatment of the vine residue was continued. After washing with distilled water, the vine residue was treated for 12 hours with 6.5% (w/v) sodium hypochlorite (NaClO) at room temperature. The solution was then washed with distilled water by several cycles of centrifuging the samples at 15,000 g for 10 minutes. Then the pH was adjusted to pH 5 by gradual addition of glacial acetic acid (CH₃COOH). Once the desired pH was reached, the solution was stirred for 2 hours at room temperature. This step further removed the remaining hemicellulose by hydrolysis of glycosidic linkages and some of the lasting lignin by catalyzing the β-O-4 ether bonds (Tarasov et al., 2018; Sun et al., 2005). A second round of bleaching was conducted using 30% hydrogen peroxide (H₂O₂) (g/5 mL solid to liquid) for 70 minutes at 80°C to further ensure the removal of lignin and any remaining hemicellulose (Chen et al., 2016). The solution was washed with distilled water and centrifuged (15,000 g for 10 minutes) for several cycles to remove the supernatant. The extracted bleached residue was then ready for acid hydrolysis.

During the acid hydrolysis step, the bleached residue was converted to nanocellulose by removing amorphous regions using 51% sulfuric acid (51% H₂SO₄). The residue was stirred in a beaker with 51% H₂SO₄ for 30 minutes at 45°C with a 1:15 solid
to liquid ratio. The solution was then washed with distilled water centrifuged at 15,000 g for several cycles to obtain a nanocellulose suspension with a pH close to that of distilled water. The pH of this suspension was then adjusted to approximately pH 7 with 4% NaOH. Finally, the colloidal suspension was mechanically dispersed using an ultrasonicator (Q500 Sonicator, Qsonica, USA) at 30% amplitude for 15 minutes. The suspension was then immediately lyophilized to prevent aggregation of the nanocellulose and yield a fine powder.

_Treatment 2 (T2) procedure_

The depolymerization step of T2 was the same as T1 except that only the raw vine powder was treated. No ariel parts of the kudzu were used in T2 due to their poor performance observed during T1. Hence, after the depolymerization of the raw vine powder, the bleaching was conducted by treating the residues with 10% KOH (1:20 solid to liquid) for 3 hours at 80°C (a higher temperature than T1). After treatment, the residue was separated from the supernatant by centrifuging several times for 10 minutes at 15,000 g. Then, 6.5% NaClO was added to the residues at a 1:15 (solid to liquid) ratio, and the pH was adjusted to pH 5 with acetic acid. Once the desired pH was reached, the solution was stirred for 2 hours at 75°C. The sample was subsequently centrifuged for several cycles of 10 minutes at 15,000 g. A second round of bleaching was conducted by treating the sample with 30% H₂O₂ at 80°C for 70 minutes. The residue was then washed to remove supernatant by several cycles of centrifugation (15,000 g for 10 minutes). The T2 bleached sample was dried overnight in the oven at 65°C. Once dry, a small sample was set aside for characterization, and the rest underwent the next step (acid hydrolysis).
For the T2 acid hydrolysis step, the extracted cellulose was treated with 51% H₂SO₄ and 64% H₂SO₄ (1:15 solid to liquid ratio), separately. Both reactions occurred at 45°C for 30 minutes. However, both solutions almost immediately turned dark brown when the sample was added to the acid solution, as seen in Figure 4.1. Nanocellulose therefore could not be extracted with the T2 treatment.
Characterization Methods

Morphology analysis

The morphology of the T1 nanocellulose sample was characterized using transmission electron microscopy (TEM). The nanocellulose samples were diluted with distilled water at 0.2-0.5% (w/v) for TEM sample preparation. The suspension was sonicated for 5 minutes, and then a drop was placed onto a SiO-based grid and allowed to dry overnight. The high-resolution transmission electron microscope (TEM, H-9500, Hitachi, USA) imaged the sample grids at an accelerating voltage of 300 kV. The dimensions (length and width) of the imaged nanocellulose samples were calculated using the ImageJ software (National Institute of Health, Bethesda, MD, USA). Ten different crystals in the image were measured for length (L) and diameter (D) to calculate the average aspect ratio (L/D) of the sample.

Fourier transform infrared spectrometry (FTIR) analysis

The T1 untreated vine, the T1 bleached vine, and the T1 acid hydrolyzed vine underwent separate FTIR analyses. A milligram of each sample was individually placed on the crystal beam of the FTIR spectrometer (Nicolet iS10, Thermo Fisher Scientific, USA). Using the attenuated total reflectance (ATR) technique, each sample underwent 128 scans in the infrared wavenumber range of 500-4000 cm\(^{-1}\) with a resolution of 4 cm\(^{-1}\).

X-ray diffraction analysis

Multiple different samples from the two treatment groups underwent X-ray diffraction (XRD) using the Rigaku Ultima IV X-ray diffractometer (Tokyo, Japan) to determine crystallinity of the samples. For the T1 group: the T1 untreated vine sample, the
T1 bleached vine sample, and the T1 acid hydrolyzed sample all underwent XRD analysis. And from the T2 group: the T2 untreated vine sample, the T2 depolymerized vine sample, and the T2 bleached vine sample underwent XRD analysis. Using a scanning ratio of 2 degrees/min and a range of 5-40 degrees, the samples were radiated with copper K-\(\alpha\) (wavelength = 1.5418 Å) using a voltage of 40 kV and a current of 40 mA. The Segal method was used to determine the crystallinity and was calculated as follows (Segal et al., 1959):

\[
\text{Crystallinity Index} \, (\%) = \frac{I_{200} - I_{am}}{I_{200}} \times 100
\]

where \(I_{200}\) is the peak intensity of the (002) plane (\(2\theta \approx 23^\circ\)), and \(I_{am}\) is the peak intensity of the amorphous fraction (\(2\theta \approx 19^\circ\)).

**Thermogravimetric analysis**

Different samples from the two treatment groups were analyzed with thermogravimetric analysis (TGA) (Hi-Res TGA Model 2950, TA, USA). From the T1 group, the T1 untreated vine sample, the T1 bleached vine sample, and the T1 acid hydrolyzed sample all underwent TGA analysis. From the T2 group, the T2 untreated vine sample, the T2 depolymerized vine sample, and the T2 bleached vine sample all underwent TGA analysis. The samples were tested by placing 2-10 mg on an aluminum pan in the device, where it was burned under a nitrogen gas atmosphere. The heating cycle was from 30°C to 600°C with a ramp rate of 10°C/min. Derivative thermogravimetric (DTG) data was also generated from the TGA analysis to better understand each treatment group's heat degradation profile. The DTG data were calculated using the following formula:
Derivative Weight (\( \% \) weight change \( \text{min} \)) = \(- \frac{m_i - m_{i-1}}{m_o} \times \frac{t_i - t_{i-1}}{100} \)

where \( m_i \) is the mass of the sample at the \( i \)th index, \( t_i \) is the time (in minutes) at the \( i \)th index, and \( m_o \) is the mass of the original sample before heating. The data were corrected for noise using an exponential smoothing software package.

**Statistical analysis**

Most results in this study are descriptive, so the statistical analysis was only used to compare the dimensions of the nanocellulose obtained in this study versus the dimension of nanocellulose from previous studies. For instance, the average aspect ratio, length, and diameter of the extracted nanocellulose was compared to that of the Doh et al. (2020) extracted seaweed nanocellulose samples. Analysis of means using a t-test (as well as a Wilcoxon signed-rank test where appropriate) was used to determine any statistically significant difference between the aspect ratios, lengths, and diameters of the respective nanocellulose extractions (\( p \leq 0.05 \)).
REFERENCES


CHAPTER FOUR
RESULTS AND DISCUSSION

Qualitative Analysis

As mentioned in Chapter Three, two samples did not survive the treatments of this study. The yield of the kudzu leaf after the depolymerization was too low to continue with further treatment. Although the leaf is a good source of fodder due to its high crude protein content, it has a lower amount of cellulose (14-20%) compared to the vine (33%), as shown in Table 2.1 (Anele et al., 2020; Gulizia & Downs, 2019; Tanner et al., 1993). Furthermore, the leaf's comparatively low acid detergent fiber resulted in significant degradation during acid treatment to be efficiently utilized in this study (Anele et al., 2020; Gulizia & Downs, 2019; Tanner et al., 1993). On the other hand, the vine successfully produced nanocellulose (top of Figure 3.1) with the T1 treatment. The T2 treatment yielded cellulose after bleaching, but the 51% and 64% H₂SO₄ degraded the cellulose chains indicated by the dark brown color (bottom of Figure 3.1) and did not yield nanocellulose. This dark brown color may be attributed to the breakdown of cellulose by the pathway of cellulose→furan→benzene, in which 5-hydroxymethylfurfural (5-HMF) plays an intermediary role (Nomura et al., 2021).
Figure 4.1. Qualitative results of the T1 acid hydrolysis versus the T2 acid hydrolysis.
Characterization Analysis

*Morphology analysis*

The TEM image in Figure 4.2 clearly reveals that the kudzu vine was isolated into its cellulose nanocrystal (CNC) form upon completion of the T1 procedure. The microscope used a resolution in the nanoscale range, and thus the rod-shaped particles indicated that the sample did in fact contain CNCs. Furthermore, CNCs are defined as having an aspect ratio between the range of 1-100, and the results in Table 2.1. exhibited an average aspect ratio of $6.43 \pm 1.78$ for the T1 kudzu vine sample (Doh et al., 2020). Thus, the particles shown in the sample were within the CNC aspect ratio range. When compared to the Doh et al. samples (who used a similar procedure to T1), the average aspect ratio of the kudzu vine CNCs (Table 2.1.) was equivalent to the sargassum seaweed (no statistical significance found) but smaller than that of the kombu seaweed (statistical significance found). The significantly higher length of the kudzu vine CNCs as compared to the seaweed CNCs could be attributed to kudzu’s higher DP (Li, 2003; Vanderfleet & Cranston, 2011). However, the lower average aspect ratio of kudzu CNCs ($6.43 \pm 1.78$) relative to the kombu seaweed CNCs ($11.13 \pm 1.84$) was a product of the larger diameter of the kudzu vine CNCs (statistical significance found). A reasonable explanation for the larger diameter could be the lower content of hemicellulose in the kudzu vine as compared to seaweed sources. This is in accordance with previous studies. Mozdyniewicz et al. (2016) reported a higher fibril width with decreasing hemicellulose content. Duchesne et al. (2001) explained that the lower hemicellulose content allowed the cellulose fibrils to aggregate and recrystallize upon treatment, thereby forming a larger structure.
Figure 4.2. TEM observation of the samples that underwent the T1 procedure. Rod-shaped nanocellulose particles were clearly distinguished in this nanoscale microscope image.
Table 4.1. The dimensions (length, diameter, and aspect ratio) of the imaged kudzu vine CNCs measured using ImageJ software and statistically analyzed versus the Doh et al. seaweed CNCs.

<table>
<thead>
<tr>
<th>Source</th>
<th>Length$^{1,2}$ (L) (nm)</th>
<th>Diameter$^{1,2}$ (D) (nm)</th>
<th>Aspect Ratio$^{1,2}$ (unitless)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kudzu Vine (T1 Procedure)</td>
<td>285.01 ± 150.33$^a$</td>
<td>42.78 ± 13.26$^a$</td>
<td>6.43 ± 1.78$^a$</td>
</tr>
<tr>
<td>Sargassum (Doh et al., 2020)</td>
<td>49.30 ± 11.73$^b$</td>
<td>8.55 ± 2.11$^b$</td>
<td>6.11 ± 1.85$^a$</td>
</tr>
<tr>
<td>Kombu (Doh et al., 2020)</td>
<td>228.14 ± 46.07$^c$</td>
<td>20.96 ± 5.11$^c$</td>
<td>11.13 ± 1.84$^b$</td>
</tr>
</tbody>
</table>

(1) Data are mean ± S.D.
(2) Values with different superscripted letters within a column denote that there was statistical significance found by t-test (p ≤ 0.05).

**FTIR analysis**

The FTIR data from the untreated vine, the T1 bleached vine, and the T1 acid hydrolyzed vine were analyzed to gain insight into the chemical structure of the samples. As a general trend, the T1 bleached vine and T1 acid hydrolyzed vine spectra differed from the raw vine sample in some key wavenumber bands, as shown in Figure 4.3. Although differences between the T1 bleached vine and T1 acid hydrolyzed spectra were present, they were not as large as the differences between the untreated vine and the treated samples. These distinct differences showed that the lignin, hemicellulose, and other non-cellulosic materials were removed with the performed treatment. The untreated vine sample showed three peaks specific to the presence of lignin and hemicellulose that were reduced in the T1 bleached vine spectra and were non-existent in the T1 acid hydrolyzed vine spectra. First, the peak at 1236 cm$^{-1}$ in the untreated vine sample, which is characteristic of the aryl-alkyl ether linkages in lignin, disappeared in the T1 bleached vine and T1 acid hydrolyzed
samples, demonstrating the elimination of lignin in these treated samples (Morán et al., 2008). Second, the untreated vine sample had a band in 1500-1600 cm⁻¹ range, which indicated the presence of lignin aromatic rings. This band was significantly reduced in the T1 bleached sample and not observed in the T1 acid hydrolyzed sample. The band at 1507 cm⁻¹ indicates the vibrating aromatic rings, and hence the absence of any band in this range demonstrated the removal of lignin with treatment (Luzi et al., 2014; Morán et al., 2008). Lastly, the untreated vine exhibited a pronounced peak at 1733 cm⁻¹, whereas the T1 bleached vine showed it to a much less extent, and the T1 acid hydrolyzed registered no peak at this wavenumber. This could be attributed to the atomic vibrations of several groups present in hemicellulose and lignin: (1) the acetic and uronic acid ester groups unique to hemicellulose, or (2) the carboxylic acid ester groups in ferulic and p-coumaric acids characteristic to both lignin and hemicellulose (Alemdar & Sain, 2008, Chandra et al., 2016; Chirayil et al., 2014; Sun et al., 2005). The removal of these peaks furthermore demonstrated the isolation of cellulose from the surrounding hemicellulose and lignin components in the cell wall matrix via the performed treatments.
Figure 4.3. FTIR spectra of (a) the untreated vine, T1 bleached vine, and T1 acid hydrolyzed vine samples in the 4000-500 cm$^{-1}$ region, and (b) expanded region between 1500-500 cm$^{-1}$. 

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The FTIR data also provides insights into the cellulose structure of the samples. In Figure 4.3(b), the peak around 896-897 cm\(^{-1}\) was associated with the deformation of the \(\beta\)-glycosidic C-H linkages of cellulose (Alvarez & Vázquez, 2006; Chen et al., 2016; Chirayil et al., 2014; Oh et al., 2005). As the treatment increased, this peak became more pronounced. A broad band with a relative strong intensity was indicative of a cellulose I structure, whereas a weak intensity was associated with relatively amorphous cellulose (Nelson & O’Connor, 1964). The T1 bleached vine and T1 acid hydrolyzed samples demonstrated a more crystalline structure based on this peak intensity compared to the untreated vine sample. Furthermore, peaks between 1105-1110 cm\(^{-1}\) are hallmark bands of the cellulose I structure (Chen et al., 2016). The peak at 1105 cm\(^{-1}\) shifted to 1109 cm\(^{-1}\) after the 51% acid hydrolysis, which signaled the cellulose I structure. On the other hand, the subdued shoulder for the untreated vine sample in this region further indicated the relative amorphousness of this sample compared to that of the treated samples. Similarly, a shift from 1424 cm\(^{-1}\) (untreated sample) to 1428 cm\(^{-1}\) (treated samples) was representative of C-H\(_2\) scissoring motion, where a strong 1428 cm\(^{-1}\) peak indicated cellulose I and a less intense peak around \(\sim 1420\) cm\(^{-1}\) suggested a decrease in crystallinity (Nelson & O’Connor, 1964). For the treated samples, the band at 1161 cm\(^{-1}\) was associated with the C-O-C stretching of the antisymmetric glycosidic linkages in cellulose I (Liang & Marchessault, 1959). This band was shifted down to 1155 cm\(^{-1}\) in the untreated vine sample, which indicated a more amorphous structure (Nelson & O’Connor, 1964). Compared to the weak 1155 cm\(^{-1}\) peak for the untreated sample, the increased intensity of the 1161 cm\(^{-1}\) peaks of the treated samples could be explained by the removal of lignin and hemicellulose.
(Chandra et al., 2016; Chen et al., 2016; Marchessault, 1962). As the lignin and hemicellulose were removed, the treated samples were allowed to fall into more compact and crystalline structures relative to the more heterogeneously structured, untreated sample. Additionally, the peaks in the 1047-1055 cm\(^{-1}\) range further demonstrate the isolation of cellulose in the treated samples. This range corresponded to the pyranose structure of cellulose, and its increasing intensity with treatment pointed to the increasing content of cellulose and decreasing content of non-cellulosic components (Doh et al., 2020; Sun et al., 2005; Yahya et al., 2015). To the right of this range is the 1032 cm\(^{-1}\) band, which showed the vibrations of the C-O stretching in cellulose and was most pronounced in the T1 acid hydrolyzed vine sample (Zhuang et al., 2020). This increased intensity further demonstrated the enhanced isolation of cellulose with the hydrolysis treatment. However, it is essential to note that spectra of cellulose, lignin, and hemicellulose overlap at specific regions, so caution should be taken in reporting conclusive results. The region around the 1032 cm\(^{-1}\) wavenumber is one area of overlap. It could indicate C-O stretching in cellulose as previously stated, but this band is also simultaneously characteristic of C-H in-plane deformations found in lignin (Zhuang et al., 2020). Consequently, the possibility of residual lignin in bleached and acid hydrolyzed vine samples could not be eliminated via FTIR analysis.

The bands associated with bound water and hydrogen bonding also helped distinguish the degree of crystallinity in the samples. It is advantageous for most packaging materials to be as hydrophobic as possible. The broad peaks in the 1603-1640 cm\(^{-1}\) provides insight into relative amount of water that was trapped in the structure via hydrogen bonding
(Chandra et al., 2016; Chen et al., 2016; Chirayil et al., 2014; Oh et al., 2005). As shown in Figure 4.3(a), the peak intensity for the range was higher for the raw sample. Hence, there were fewer carboxylate groups and bound water molecules in the T1 bleached vine and T1 acid hydrolyzed vine samples, making them more ideal candidates for packaging materials (Chirayil et al., 2014; Nacos et al., 2006).

Furthermore, key bands in the 1316-1370 cm\(^{-1}\), 2900 cm\(^{-1}\), and 3300-3400 cm\(^{-1}\) regions (Figure 4.3(b)) are linked to the degree of hydrogen bonding (Doh et al., 2020). The 1368-1370 cm\(^{-1}\) peaks indicated the symmetric stretching of the C-OH bonds, whereas the 1316-1317 cm\(^{-1}\) indicated the asymmetric C-H deformations, and the 1335 cm\(^{-1}\) peak demonstrated the O-H bending in crystalline cellulose (Chen et al., 2016; Higgins et al., 1961; Luzi et al., 2014). The decrease in intensity or absence of these bands in the untreated vine spectra pointed to a lower degree of hydrogen bonding. Thus, it can be concluded that hydrogen bonding was increased in the treated samples, and thus a more crystalline structure was formed in these samples (Nelson & O’Connor, 1964). To further expand on this point, the peaks at ~2900 cm\(^{-1}\) in Figure 4.3(a) became progressively more intense and acute from the untreated sample to the treated samples. These bands were associated with the C-H stretching vibrations present in the cellulose rings as the degree of hydrogen bonding increased both inter and intramolecularly (Oh et al., 2005). The broad peak centered around 3300 cm\(^{-1}\) has historically been sensitive to the extent and type of hydrogen bonding present (Alvarez & Vazquez, 2006; Chandra et al., 2016; Chirayil et al., 2014; Oh et al., 2005). Hence, the higher the wavenumber in this 3300-3400 cm\(^{-1}\) range corresponded to more intramolecular hydrogen bonding occurring between the O3-H and
the pyranose O5 (Oh et al., 2005). The lower the wavenumber, the more intermolecular hydrogen bonding occurs between O6-H and the O3 of the neighboring cellulose molecule.

The integrated areas under the peaks for the T1 bleached vine sample and the T1 acid hydrolyzed vine sample in this region were approximately equal, indicating similar degrees of crystallinity. This finding was corroborated by XRD analysis (Figure 4.5(a)). Moreover, the area under the curve for the untreated sample was significantly lower, and thus it was expected to have a more amorphous nature relative to the treated samples. Thus, the FTIR results demonstrated that the treatments did increase the crystallinity of the treated samples by removing the non-cellulosic parts of the raw vine (lignin, hemicellulose, etc.), however the treated sample appeared to have a similar composition (isolated cellulose) and crystallinity index (CI). More conclusive evidence for the CI of the samples was collected by analyzing the XRD results.

**XRD analysis**

The XRD results are shown in Figure 4.4, and the calculated crystallinity indexes (by the Segal method) are reported in Table 4.2. Previous studies reported that the cellulose I structure will demonstrate peaks when $2\theta$ is approximately 15-16 degrees, 22-23 degrees, and 34-35 degrees (Chirayil et al., 2014; Doh et al., 2020; Luzi et al., 2014; Mandal & Chakrabarty et al., 2011; Sung et al., 2017). Peaks at $2\theta=22-23^\circ$ are characteristic of the distance between the stacked sheets of adjacent cellulose chains that are held tightly together by hydrogen bonding (Luzi et al., 2014). The $2\theta=15-16^\circ$ and $2\theta=34-35^\circ$ peaks are indicative of the orientation of chains along the fiber length (Luzi et al., 2014). Observed pronounced peaks with treatment in both Figure 4.4(a) and Figure 4.4(b) demonstrated that
the treatment successfully isolated cellulose I. These findings agree with the previously reported FTIR results. It is interesting to note that the T2 procedure had more structural variation between treatment steps, as shown in the XRD patterns and the reported crystallinity indices. This could be due to the prolonged exposure to NaClO (significant oxidizing agent) followed by the separate treatment with acetic acid (significant hydrolyzing agent) combining to have a strong oxidization and subsequent hydrolytic effect. The acetic acid (when it is not combined with NaClO) may have already begun to hydrolyze the easily accessible glycosidic bonds (made easier by the preceding oxidation step) of hemicellulose and amorphous cellulose even before sulfuric acid hydrolysis was reacted (Mozdyniewicz et al., 2016). This effect would also account for the slight decrease in CI from the T1 bleached vine sample (90.8%) to the T1 acid hydrolyzed sample (90.0%). If the amorphous factions were already degraded by acetic acid, then the sulfuric acid would only decrease particle size and crystallinity by chain scission of crystalline parts.
Figure 4.4. XRD patterns of the samples that underwent the T1 procedure (a) and the T2 procedure (b).
The results from Table 4.2 further support the claim made in the FTIR analysis that non-cellulosic and amorphous components were increasingly degraded in each progressive treatment step. This was especially apparent in the bleaching step where it accounted for the 11.4% increase in crystallinity in T1 and the 18.9% increase in T2. This approximate 11-19% increase in CI with treatment was confirmed with previous studies (Mandal & Chakrabarty, 2017; Sung et al., 2017). Moreover, the 90.0% CI of the T1 acid hydrolyzed vine was higher than the sargassum seaweed CI (85.1%) and higher than the reported CI of pure cellulose samples (41.4-60%) (Doh et al., 2020; Sung et al., 2017). However, the CI for acid hydrolyzed kudzu vine was not as high as the impressive CI of the kombu seaweed CNCs (98.9%) isolated by Doh et al. (2020) using similar methods. Nevertheless, a crystallinity of 90% was as close as some sources can reach to perfectly crystalline cellulose (Hamad & Hu, 2010). It was above the 80% benchmark needed to self-assemble into a chiral nematic phase that offers enhanced optical and mechanical properties (Hamad & Hu, 2010). In fact, purely crystalline CNCs may be disadvantageous in packaging applications since some degree of amorphousness is beneficial in selecting for certain viscoelastic and rheological behaviors (Hamad & Hu, 2010). Lastly, it is essential to point out that the untreated vine crystallinity indexes had significant differences. The CI from T1 (harvested in August 2020) was 79.4%, whereas the CI from T2 (harvested in August 2021) was 54.7% even when the location, month of harvest, and vine diameter were controlled. This highlights the unpredictable nature of CNC biomass sources even when selecting within the same species and area of harvest.
Table 4.2. The calculated crystallinity indexes (using Segal method) of the kudzu vine under the two different treatments and at the different stages within those treatments.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Treatment 1 (T1)</th>
<th>Treatment 2 (T2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated Vine Crystallinity</td>
<td>79.4</td>
<td>54.7</td>
</tr>
<tr>
<td>(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depolymerized Vine Crystallinity</td>
<td>N/A</td>
<td>57.2</td>
</tr>
<tr>
<td>(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleached Vine Crystallinity</td>
<td>90.8</td>
<td>73.6</td>
</tr>
<tr>
<td>(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid Hydrolyzed Vine Crystallinity</td>
<td>90.0</td>
<td>N/A</td>
</tr>
<tr>
<td>(%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Thermal analysis

The thermal degradation profiles of samples after different treatments are shown in Figure 4.5. All samples demonstrated some weight loss in the range from 25-125°C which can largely be attributed to the evaporation of bound water (Doh et al., 2020; Mondragon et al., 2014). However, some samples indicated more weight loss than others. This can be explained by the presence of less lignin as treatment increases from untreated vine to acid hydrolyzed vine. The amorphous lignin envelopes the more crystalline cellulose in the plant cell wall, and one of its functions is to act as a barrier that helps cellulose retain its bound water (Khalil et al., 2015). Upon removal of the lignin with successive treatment, the cellulose retained less water, and the water attached to the hydrophilic cellulose could be quickly evaporated. Thus, the T2 bleached vine sample only lost ~0.5%, the T1 acid hydrolyzed vine sample lost ~1.5%, and the T1 bleached vine sample lost ~4% of its weight in this range. Compare these to the weight loss of the untreated vine (~5.5%) and the sample
after depolymerization (~7%) in this 25-125°C region, and it can be determined that more extensive treatment results in less bound water in the samples. The higher water evaporation in the T1 bleached vine sample relative to the T2 bleached sample and T1 acid hydrolyzed sample may be attributed to higher contents of the characteristically water-entrapping lignin. This is an important hypothesis that will be later discussed in detail. However, more treatment generally yields higher crystallinity, and the more crystalline samples were less likely to allow water to bind and become entrapped because of the more densely packed factions (Mondragon et al., 2014). The lower weight loss of the acid hydrolyzed sample as compared to the untreated sample could be explained by (1) the acid hydrolysis step was an intrinsically dehydrating mechanism, and (2) the presence of sulfate groups and acetyl groups in the remaining amorphous faction that replaced the hydroxyl groups of cellulose led to lower hydrogen bonding and overall higher hydrophobic character (Mandal & Chakrabarty, 2011). The higher weight loss observed with the depolymerized sample for the raw vine sample in this region could be explained by the depolymerization process breaking down the cell walls of the kudzu powder. Hence the exposed lignin, hemicellulose, pectin, and cellulose had more ambient contact area allowing for easier water sorption and subsequent evaporation.
Figure 4.5. TGA (a) and DTG (b) curves of the kudzu vine under various stages of treatment with the two treatment groups (T1 and T2).
Hemicellulose largely decomposes exothermically in the 220-315°C range, and the maximum degradation of pure hemicellulose has been reported to occur around 260-270°C (Yang et al, 2007). As seen in Figure 4.5, the untreated vine sample, the depolymerized vine sample, and the T1 bleached vine sample all exhibited significant increases in derivative weight in this range. Although other lignocellulosic materials were degrading in this range, much of this increase could be attributed to hemicellulose. The depolymerization step and T2 bleaching step remove a large portion of the hemicellulose, which could be observed in the higher weight loss and derivative weight in the region of 220-315°C as compared to the further treated samples (Doh et al., 2020). The bleaching process further removed hemicellulose, and whatever hemicellulose remnants remained were largely dissolved and discarded during acid hydrolysis (Doh et al., 2020). Hence, the weight of the T2 bleached and T1 acid hydrolyzed vine samples were much less affected around the max degradation temperature.

Pyrolysis of pectin exothermically occurred in a relatively wide range of 200-400°C (Mondragon et al., 2014). The bleaching process, specifically the treatment with KOH, is reported to be an efficacious way to remove non-cellulosic material while leaving much of the cellulose intact (Doh et al., 2020; Sung et al., 2017). As demonstrated in Figure 4.5, the TGA curves of the bleached and acid hydrolyzed samples stayed above the curves of the depolymerized and untreated samples. It was also observed that the T2 bleached vine curve was less affected in this range than the T1 bleached sample. This was expected as the T2 sample was reacted with KOH at a much higher temperature. Therefore, the increased reaction kinetics of T2 compared to T1 appeared to achieve more breakdown of pectin (and
hemicellulose for that matter). This could further be understood by examining the DTG shoulders around and above 200°C (Figure 4.5(b)) in the untreated raw vine, the depolymerized vine, and the T1 bleached vine samples. The shoulders detected were indicative of pectin and hemicellulose burning off at higher rates than the T2 bleached vine and T1 acid hydrolyzed vine samples since there were only trace amounts of hemicellulose and pectin in these samples (Mondragon et al., 2014).

Due to its varied composition and aromatic structure, lignin has the broadest range of thermal degradation from 150-900°C (Yang et al., 2007). It decomposes exothermically until approximately 550°C, where it can then thermally degrade all the way to 900°C endothermically (Yang et al., 2007). Since lignin decomposes at such a wide range, it is difficult to determine the exact point of maximum degradation. However, samples with higher lignin concentration will exhibit a broader thermal degradation profile due to the difficulty of decomposing the aromatic rings and its diverse branches (Mandal & Chakrabarty, 2011; Yang et al., 2007). This effect was observed in the broader DTG curves of the untreated vine, depolymerized vine, and T1 bleached vine samples relative to the narrow T2 bleached vine and T1 acid hydrolyzed samples (Figure 4.5b). This finding relates to the crucial premise that the T1 bleached sample may have contained more lignin than the T2 bleached and T1 acid hydrolyzed samples. In fact, the DTG curve of the T1 bleached sample was the broadest in the whole group except for the untreated vine, which could suggest the significant presence of lignin. Even more, its DTG curve exhibited a sharp increase in the 150-250°C range, where lignin would start to show significant increases in degradation (Yang et al., 2007). Mandal & Chakrabarty (2011) reported that
reacting lignocellulosic material concurrently with NaClO and acetic acid in a pH adjusted solution was crucial in creating lignin-chlorine complexes that could be dissolved out of the matrix. The T1 bleaching was not a concurrent reaction process, whereas T2 bleaching process was simultaneous. Thus, the T1 bleached vine sample was far more likely to contain lignin relative to the T2 bleached vine and T1 acid hydrolyzed vine (lignin was dissolved with sulfuric acid).

Furthermore, cellulose is a less branched and more homogenous material than lignin, pectin, and hemicellulose, thereby making it stronger, denser, and more thermally stable (Yang et al., 2007). Therefore, the onset of thermal degradation will happen at a higher temperature. Yet, once degradation initiates in the cellulose, it will be in a narrower range due to the lack of impurities. This suggests that the presence of impurities (such as lignin, pectin, and hemicellulose) in the lesser treated samples contributed to a lower onset of thermal decomposition temperature since the non-cellulosic components lack the inherent crystallinity of cellulose and provide more accessible active sites for thermal degradation (Doh et al., 2020; Lee et al., 2018; Mandal & Chakrabarty, 2011; Mondragon et al., 2014; Yang et al., 2007).

As alluded to in the previous paragraph, cellulose is reported to have the narrowest window of thermal degradation between 315-400°C (Yang et al., 2007). It also has a crucial distinction from the non-cellulosic components: it degrades endothermically at temperatures below 500°C (Yang et al., 2007). This can be attributed to the different mechanism during heating. Much of the cellulose mass will largely volatize (endothermically) into carbon monoxide (CO) and carbon dioxide (CO₂), whereas much of
the mass in non-cellulosic components will largely char (exothermically) at temperatures below 500°C (Yang et al., 2007). However, the amount of residue appeared to have been larger in the T1 acid hydrolyzed sample. This could be attributed to several (but not mutually exclusive) hypotheses. First, the more severely treated samples have higher crystallinity, thus making them more thermally stable (Doh et al., 2020; Mondragon et al., 2011). In addition, the more severely treated samples underwent more chemical reactions and were likely to have more molecules grafted onto the parent chain such as acetyl groups (T1 bleached vine), Na-cellulose formations (T2 bleached vine), and sulfate groups (T1 acid hydrolyzed vine) (Mondragon et al., 2014). The sulfated groups of the T1 acid hydrolyzed sample also gave the structure flame resistance, which could have also accounted for its low charring rate and larger residue after TGA analysis (Mandal & Chakrabarty, 2011). Similarly, this same phenomenon could explain why the T2 bleached sample exhibited a higher onset of thermal degradation temperature than the T1 acid hydrolyzed sample.

In general, the presence of sulfate groups have been reported to hinder thermal stability in many facets: (1) sulfate groups act as initiators for thermal decomposition, (2) the negative charges and relative bulkiness of sulfate groups create more interchain space and thereby expose more of the nanocellulose surface area to the heat source, (3) acid hydrolysis with sulfuric acid not only attacks and cleaves the glycosidic bonds of the amorphous nanocellulose, but it penetrates into crystalline parts causing chain scission and lower molecular weight (Lu & Hsieh, 2010; Mandal & Chakrabarty, 2011, Mondragon et al., 2014). It is well understood that treating with sulfuric acid lowers thermal stability (Doh
et al., 2020; Lu & Hsieh, 2010; Mandal & Chakrabarty, 2011; Mondragon et al., 2014). Another possible reason the T1 acid hydrolyzed sample revealed a lower onset of thermal degradation temperature is the increased thermal conductivity of nanocellulose (Shimazaki et al., 2007). This is achieved by the decrease in photon scattering of nanocellulose as compared to larger cellulose molecules which tend to have a less efficient photon pathway leading to poorer thermal conductivity (Shimazaki et al., 2007). The increase in thermal conductivity of the nanocellulose led to a more efficient and quicker thermal degradation relative to the T1 and T2 bleached sample (Lu & Hsieh, 2010). Generally, it was observed that with increased treatment, the onset temperature increased, and the DTG curve peaks moved to the right. Thus, it can be concluded that thermal stability was observed to increase with more treatment due to the removal of the amorphous non-cellulosic parts that allowed for the reorganization and tighter packing of crystalline cellulose factions.
REFERENCES


Shimazaki et al, 2007


CHAPTER FIVE

CONCLUSIONS

It can be concluded that the kudzu vine presents a novel source for nanocellulose extraction. TEM results confirmed that cellulose was present in its nanoform after the T1 procedure. The average aspect ratio was found to be $6.43 \pm 1.78$, which classifies as a cellulose nanocrystal (CNC). As compared to the aspect ratios in Cox’s shear lag model (Figure 2.7), CNCs with an average aspect ratio of $6.43 \pm 1.78$ would not be as mechanically reinforcing in a matrix as nanocrystals or fibers with higher aspect ratios. The average length of the CNCs were impressive ($285.01 \pm 150.33$ nm) due to the large DP of kudzu cellulose. Yet, the large average diameter ($42.78 \pm 13.26$ nm) decreased the average aspect ratio. It is thought that the relative lack of hemicellulose present in native kudzu compared to other sources creates a clustering effect of cellulose chains via intense hydrogen bonding between the hydroxyl groups of neighboring cellulose molecules. This causes an agglomeration of stacked chains and thus a larger average CNC diameter. However, the CNCs extracted from the sargassum seaweed in the Doh et al. study (2020) had an average aspect ratio smaller than $6.43 \pm 1.78$ (statistical significance found). Despite their small average aspect ratio, the sargassum seaweed CNCs still enhanced mechanical properties in a matrix. The comparable crystallinity and aspect ratio of the kudzu CNCs with respect to CNCs extracted from other biomasses (specifically the Doh et al. sargassum seaweed) points to its potential as a mechanically enhancing nanofiller in sustainable packaging polymers. It has the crystallinity needed to form self-assembled chiral nematic structures in a matrix and a sufficient aspect ratio to thereby enhance the mechanical and
optical properties of the targeted polymer matrix. Future studies should aim to increase the aspect ratio by reducing the diameters of the kudzu CNCs. Adding treatment steps such as carding, high-pressure homogenization, and steam explosion (to name a few) may be able to disrupt the hydrogen bonding of the cellulose chains in the CNCs (decreasing the diameter) while not causing chain scission (thereby keeping the superb length).

Confirmed by the results of FTIR and TGA, non-cellulosic materials (lignin, hemicellulose, pectin, etc.) were observed to be solubilized and discarded from the kudzu vine via the depolymerization (T1 and T2), bleaching (T1 and T2), and the acid hydrolysis step (only T1). The XRD results demonstrated a CI of 90.0% for the T1 acid hydrolyzed CNCs. The T1 bleached vine already exhibited a high CI (90.8%) before the acid hydrolysis step. However, upon further examination of its thermal properties, it appeared the acid hydrolysis is needed to remove remnants of lignin, increase the thermal stability, and protect against agglomeration via the repulsion effect of the acquired sulfate groups. On the other hand, the kudzu leaf is not an adequate biomass for CNC extraction, at least by means of this concentrated chemical treatment. A low concentration of HCl acid proved to be too intense for the kudzu leaf and quickly degraded the whatever present cellulosic component.

Furthermore, CNCs could not be extracted using the T2 procedure. After the T2 bleaching step, the 51% and 64% sulfuric acid degraded the substance into dark brown slurries (Figure 4.1). The critical difference between the treatment T1 and T2 may have been the concurrent treatment of NaClO with acetic acid in the T2 bleaching step. It appeared that the treatment of the T1 sample with NaClO followed by the addition of acetic
acid in the T1 bleaching step led to better results during acid hydrolysis. Thus, it is recommended T1 procedure is used when trying to isolate CNCs from kudzu vines and similar biomasses.

Given that the subsequent acetic acid treatment yielded such a high CI, it may be beneficial in future studies to determine if higher concentrations (> 51%) of sulfuric acid would yield even greater CI in the T1 process. Future research should also incorporate these kudzu T1 acid hydrolyzed CNCs into various sustainable matrices to study their impact as a nanofiller. Research is already underway to examine the mechanical, thermal, and barrier effects of implementing these specific kudzu CNCs into starch matrices. It may be warranted to increase adhesion by grafting and blending the CNCs with synergetic polymers to enhance composite performance.

Finally, there remain some broader concerns from this area of research that are seldom discussed. As witnessed, the process to extract these “sustainable materials” requires the use of very hazardous chemicals, which have the potential for detrimental environmental impacts if utilized in high volumes. If this process is to become more commercially prevalent, sustainable extraction methods need to be further developed, such as using enzymatic hydrolysis or steam explosion to produce CNCs. It would be a shame to widely adopt a sustainable end-product that appears eco-friendly to the consumer without having a sustainable industrial process.

To conclude, there remains some unpredictability when selecting a source for cellulose and CNC extraction. Just like the human species, biomasses have an incredible amount of diversity even within species. Climate, soil content, time of harvest, and the
method of extraction impact the efficacy of CNC isolation. Even when these parameters are controlled, variation may still exist in chemical makeup and physical structure. It would be prudent to have a diverse portfolio of potential sources for CNC extraction, and now the field has one more option with the addition of the kudzu vine as a source. However, the primary source should be native to the ecosystem, easily harvested, and replenished with minimal evolutionary impact on the neighboring species. If the story of kudzu has taught society anything, it would be do not hinder the natural evolution of living organisms until the system is fully understood and all the costs and benefits are carefully considered. Otherwise, it is possible that the next couple of centuries will be spent frantically searching for a way to correct that oversight.
REFERENCES