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Cellulose

Edited by Alejandro Rodríguez Pascual and María E. Eugenio Martín

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Published in London, United Kingdom







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http://dx.doi.org/10.5772/intechopen.75244
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First published in London, United Kingdom, 2019 by IntechOpen
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, 7th floor, 10 Lower Thames Street, London, EC3R 6AF, United Kingdom
Printed in Croatia

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Additional hard and PDF copies can be obtained from orders@intechopen.com

Cellulose
Edited by Alejandro Rodríguez Pascual and María E. Eugenio Martín
p. cm.
Print ISBN 978-1-83968-056-4
Online ISBN 978-1-83968-057-1
eBook (PDF) ISBN 978-1-83968-058-8

Meet the editors

Dr. Alejandro Rodríguez Pascual is a professor in the Chemical Engineering Area at the Universidad de Córdoba. His research-line focused on the integral use of the different fractions of the lignocellulosic materials; cellulose, hemicellulose and lignin. More than 100 indexed articles published, >80 International Congresses, co-authored 3 books, 2 book chapters and edited 1. Supervisor of 9 doctoral theses, currently supervising 2. He collaborates with several Spanish and international research groups. Member of the Ibero-American Network for Research on Pulp and Paper and coordinator of a research group made in Provalor and Nanocelia (both CYTED thematic network). Member of several Scientific Committees in international congresses. President of the Organizing Committee of the 1st and 2nd Int. Workshop on Biorefinery of Lignocellulosic Materials. He is an expert evaluator in: European Research Agency (REA) on FP7-SME 2012 and 2013, and H2020-BBI-PPP-2015.2.1. FET-OPEN 2019; ANEP (General Directorate of Scientific and Technical Research), Spain 2014, Torres Quevedo program; Ministry of Economy, Spain "Retos" 2015 and 2018; Fondazione Cariplo, Italy, 2015; Tec21, France, 2014; SGS enterprise, Spain 2016-2017; Executive Agency for Higher Education, Research, Development and Innovation Funding, Romania, 2016-2017, 2019. He is also a member of Editorial Boards of several journals and peer-reviewer of Q1 and Q2 journals.

María E. Eugenio Martín is a tenured scientist at INIA-CIFOR. Her research focused on the application of biotechnology to the pulp and paper industry using different raw materials, and in biorefineries of lignocellulosic materials to produce mainly bioethanol, nanofibrillated cellulose, biodegradable grasses, asphalts, graphene, etc. She has completed her training in these fields at EFPG (France), KTH (Sweden) and at the University of California (Irvine). She has been the main researcher of various projects from which two patents have been granted, and is currently the main researcher of projects P2018/EMT-4348 SUSTEC-CM and RTI2018-096080-B-C22. She is the author of more than 70 scientific articles and has participated in more than 50 national and international congresses.

Contents

Preface

This book presents important information about the structure of cellulose as well as its uses and applications.

In Chapter 1, "Insights from over 10 Years of Cellulosic Biofuel Modeling", Daniel Inman et al. present insights gained from more than ten years of system dynamic modeling of cellulose to the biofuel industry in the United States. They use a publicly available Biomass Scenario Model to explore the impact of logistics, economies of scale, and shared industrial learning on the developing cellulose-to-biofuels industry in the United States. One theme from this study as well as from the work performed over the last decade is the importance of the movement of the system toward maturation, both in terms of the supply system and the conversion processes. Mature processes imply lower investment risk, better yields, and better process economics.

In Chapter 2, "Alternative Raw Materials for Pulp and Paper Production in the Concept of a Lignocellulosic Biorefinery", María E. Eugenio Martín et al. study the use of non-wood raw materials to obtain cellulosic fibers. There exists a renewed interest in the use of non-woody raw materials due to them being an abundant source of low-cost fibers. In addition, they are sometimes the only exploitable source of fibers in certain geographical areas, mainly in developing countries. Moreover, the great variety of characteristics, fiber dimensions, and chemical composition of these alternative raw materials give them great potential to produce different types of papers. The pulp and paper industry is an excellent starting point for the development of lignocellulosic biorefineries, possessing the necessary technology and infrastructure as well as extensive experience in lignocellulosic biomass transformation.

In Chapter 3, "Influence of Size Classifications on the Structural and Solid-State Characterization of Cellulose Materials", Sunday Samuel Oluyamo and Mathew Adefusika Adekoya show the influence of size classification on the properties of cellulose materials. Their study focuses on the influence of size classifications on the structural and solid-state characterization of cellulose obtained from wood dust. The isolated cellulose exhibits good mechanical and solid-state properties with promising applications in device utilization.

In Chapter 4, "An Update on Overview of Cellulose, Its Structure and Applications", Praveen Kumar Gupta et al. review the chemistry of cellulose, its extraction, and the properties that help various industries to make the most of it. Cellulose is one of the most ubiquitous organic polymers on the planet. It is a significant structural component of the primary cell wall of green plants, various forms of algae, and oomycetes. It is a polysaccharide consisting of a linear chain of several hundred to many thousands of $\beta(1\rightarrow4)$ linked D-glucose units. There are various extraction procedures for cellulose developed by using different processes like oxidation, etherification, and esterification that convert the prepared celluloses into cellulose derivatives. Since it is a non-toxic, bio-degradable polymer with high tensile and compressive strength, cellulose has widespread use in various fields such as

nanotechnology, pharmaceuticals, the food industry, cosmetics, the textile and paper industry, and drug-delivery systems.

In Chapter 5, "Microbial Cellulases: An Overview and Applications", S. K. Jayasekara and R. R. Ratnayake present a review on cellulases, which are a complex group of enzymes secreted by a broad range of microorganisms including fungi, bacteria, and actinomycetes. They discuss the structure, function, possible applications, and novel biotechnological trends of cellulase enzymes. Furthermore, they examine the possibility of using low-cost, enzymatic pretreatment methods of lignocellulosic material in order to use it as an efficient raw material for biofuel production.

In the final sixth chapter, "Multi-Finishing of Polyester and Polyester Cotton Blend Fabrics Activated by Enzymatic Treatment and Loaded with Zinc Oxide Nanoparticles", Al-Balakocy N.G. et al. discuss the possibility of applying enzymatic treatments for fabric surface activation that can facilitate the loading of zinc oxide nanoparticles (ZnO NPs) onto polyester (PET) and polyester cotton blend (PET/C) fabrics prepared by sol-gel method.

We would like to thank the management at IntechOpen for their support while editing this book.

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Section 1

Reviews

Chapter 1

Insights from over 10 Years of Cellulosic Biofuel Modeling

Daniel Inman, Emily Newes, Brian Bush, Laura Vimmerstedt and Steve Peterson

Abstract

We present insights gained from over 10 years of system dynamic modeling of the cellulose to biofuel industry in the United States. We use a publicly-available Biomass Scenario Model to explore the impact of logistics system, economies of scale, and shared industrial learning on the developing cellulose-to-biofuels industry in the United States. One theme from this study as well as from the work performed over the last decade is the importance of the movement of the system toward maturation, both in terms of the supply system and the conversion processes. Mature processes imply lower investment risk, better yields, and better process economics.

Keywords: system dynamics, biofuels, biomass, modeling, renewable energy, cellulosic biofuel

1. Introduction

The Biomass Scenario Model (BSM), developed by the U.S. Department of Energy (DOE), is used to explore the emerging biofuels industry in the United States. Over the course of the last decade, the model has evolved along with the biofuels industry. This evolution includes numerous upgrades to the model and associated software, updates to the underlying data, and public release of the model (https://github.com/NREL/bsm-public).

The BSM has supported multiple analysis studies focused on various components of the feedstocks-to-biofuels supply chain; links to publications and reports associated with these studies can be found on NREL's OpenEI BSM wiki pages (https://openei.org/wiki/Biomass_Scenario_Model). Two important themes, which serve as focal points for this chapter, have emerged from our analyses: (a) the importance of feedstock logistics and (b) the impact of shared industrial learning. We present illustrative results from the publicly-available version[1] of the BSM that explore both themes.

1.1 Biofuels in the United States

Biofuels—specifically soy-based biodiesel and corn-starch-based ethanol (**Figure 1**)—have benefited from government support within the United States. Both the ethanol and biodiesel markets have grown following the Energy Tax Act [1], a law passed by the federal government in 1978 to promote fuel efficiency with

[1] https://github.com/NREL/bsm-public; git commit # e62598a.

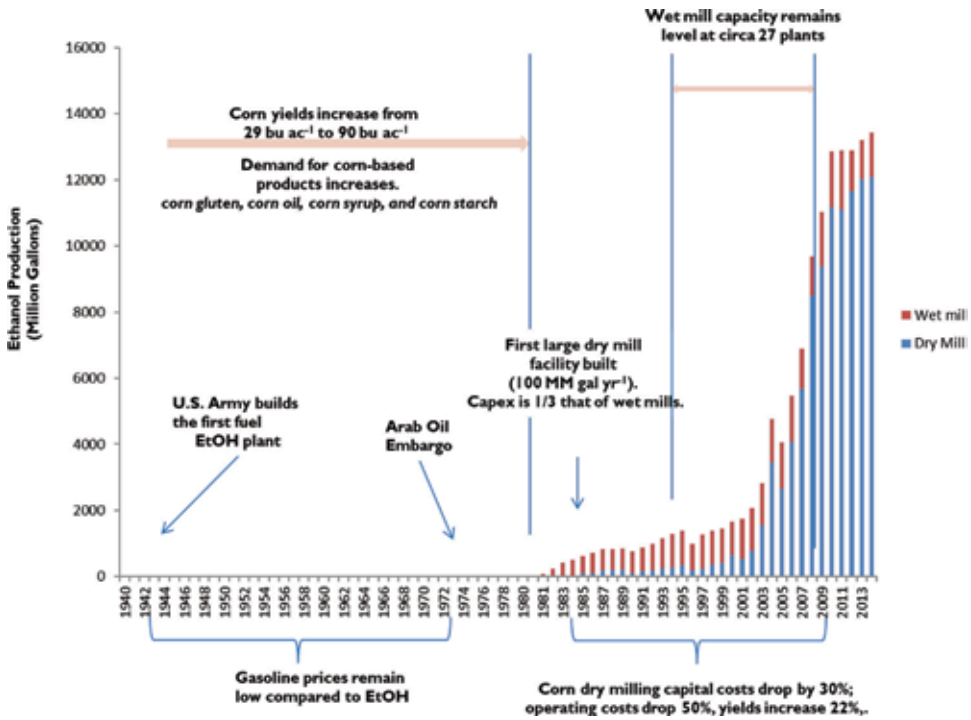


Figure 1.
Growth of the ethanol industry and a timeline of major biofuel legislation.

favorable tax incentives. Other government measures, such as guaranteed loans and research funds, helped de-risk the markets further [2].

Ethanol received another boost when methyl tertiary butyl ether (MTBE) was banned [3], which opened new markets for ethanol as an oxygenate in gasoline. Ethanol use in gasoline was reinforced a year later with the 2015 passing of the Energy Policy Act [4], which removed oxygenation requirements and mandated that refiners blend up to 10% ethanol by volume [5], adhering to the new Renewable Fuels Standard.

From 1978 to 2005, energy policies continued to favor the domestic ethanol industry through production tax credits and capitol grants, among other industry incentives. The passing of the Energy Independence and Security Act of 2007 slanted in favor of lignocellulosic ethanol—increasing biofuel volume requirements while incentivizing lignocellulosic feedstocks over corn starch through Renewable Identification Numbers (RINs) [5].

Ethanol continues to be the primary biofuel in the US. However, because of limits on blending, incompatible distribution and dispensing equipment, and limited market penetration of vehicles capable of using high ethanol blends (~ E-85), the overall biofuel market has been limited and less than anticipated volumetric goals reported in early legislation [6]. Additionally, much of the ethanol blended in the US is derived from corn-starch, which is classified as a "renewable fuel" by the EPA, meaning the fuel achieves a 20% reduction in CO_2 as compared to conventional gasoline. To develop a more environmentally sustainable biofuels industry in the US, corn -starch-based ethanol is limited to 15 billion gallons annually, whereas lignocellulosic biofuels are incentive through their eligibility for D5 and D3 RINs. Despite legislation that provides incentives for advanced and cellulosic biofuels, the market for such fuels has been slow to take off.

One factor that has limited the market for advanced and cellulosic biofuels is the development of integrated biorefineries. The technologies for converting lignocellulosic feedstocks into ethanol and hydrocarbons are underdeveloped. Technologies for

feedstock processing and handling have, at best, recently become commercial, and the markets for biomass feedstocks may not exist altogether.

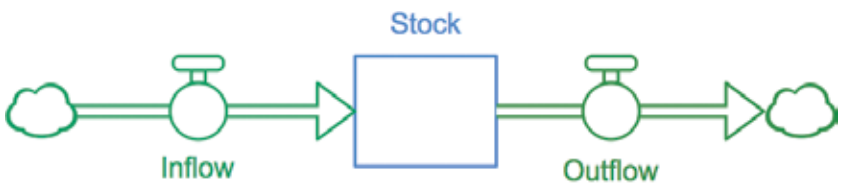
These biorefineries are gaining support from both public and private channels [7]. Among the former, both the DOE and the U.S. Department of Agriculture (USDA) have helped commercialize renewable, non-starch biofuels and development of feedstock supplies. Their R&D leadership in the sector has helped develop lignocellulosic feedstocks and has gone beyond biofuels to include growth in bioproducts and biopower [8]. The USDA is also empowering the sector through its Biorefinery Assistance Program, which guarantees loans for biorefineries [9], and through research into alternative feedstock species, and programs that incentivize producers [10].

1.2 The Biomass Scenario Model

Many of the physical processes, decision processes, feedbacks and constraints found in the biomass-to-biofuels supply chain are represented in the BSM [11]. The BSM is a system dynamics model developed under the auspices of the DOE as part of a multi-year project at the National Renewable Energy Laboratory. It is a tool designed to better understand biofuels policy as it impacts the development of the supply chain for biofuels in the United States and the economic agents influencing development through their decisions. The model is intended to generate and explore plausible scenarios for the evolution of a biofuel transportation fuel industry in the United States, representing multiple pathways leading to the production of fuel ethanol as well as advanced biofuels such as biomass-based hydrocarbons such as biomass-based gasoline, diesel, jet fuel, and butanol. The BSM, which is implemented using the STELLA [12] system dynamics simulation platform, integrates representations of resource availability, physical/technological/economic constraints, behavior, and policy to model dynamic interactions across the supply chain. It simulates the deployment of biofuels given technological development and the reaction of the investment community to those technologies in the context of land availability, the competing oil market, consumer demand for biofuels, and government policies over time. It has a strong emphasis on the behavior and decision making of various agents along the supply chain.

1.3 System dynamics modeling

System dynamics is used in a wide range of modeling applications to represent and simulate complex non-linear systems driven by multiple interacting physical and social components. As a modeling philosophy, system dynamics relies on three key concepts: stocks, flows, and system feedback [13]. **Figure 2** shows a basic stock-flow structure and corresponding mathematical representation. Below is a brief explanation of these concepts.



$$Stock\ (t) = \int_{t_0}^{t} [Inflow - Outflow]dt + Stock\ (t_0)$$

Figure 2.
A basic stock-flow structure and corresponding mathematical representation.

1.3.1 Stocks and flows

Accumulations, and the activities that cause accumulations to rise and fall over time, are fundamental to the generation of dynamics. System dynamics models are built up from stock and flow primitives. In the BSM, we use stocks to represent concepts such as prices, inventories, conversion facilities, and station owners who are contemplating investment in E85 tankage and dispensing equipment. Corresponding flows would include price changes; production, consumption, and shrinkage of inventories; investment or obsolescence of facilities; and deciding not to invest in tankage and equipment.

1.3.2 Feedback

Dynamic social systems can contain rich webs of feedback processes. Positive feedbacks tend to drive reinforcing growth in key quantities, while negative feedbacks support self-correcting behavior. In the BSM, we have sought to capture key feedbacks within and across each stage of the supply chain.

The BSM is built and designed using a top-down, modular approach representing the flow of feedstocks to flow down the supply chain to be converted into biofuels, with feedback mechanisms among and between the various modules. Our modeling approach respects the need for transparency, modularity, and extensibility. This enables standalone analysis of individual modules as well as testing of different module combinations. As shown in **Figure 3**, the model is framed as a set of interconnected sectors and modules. Each supply-chain element is modeled as a standalone module but is linked to the others to receive and provide feedback. The feedstock production module simulates the production of biomass as well as five major commodity crops (corn, wheat, soybeans, cotton, and other grains) through farmer decision logic, land allocation dynamics, new agricultural practices, markets, and prices. The feedstock logistics module models the harvesting, collection,

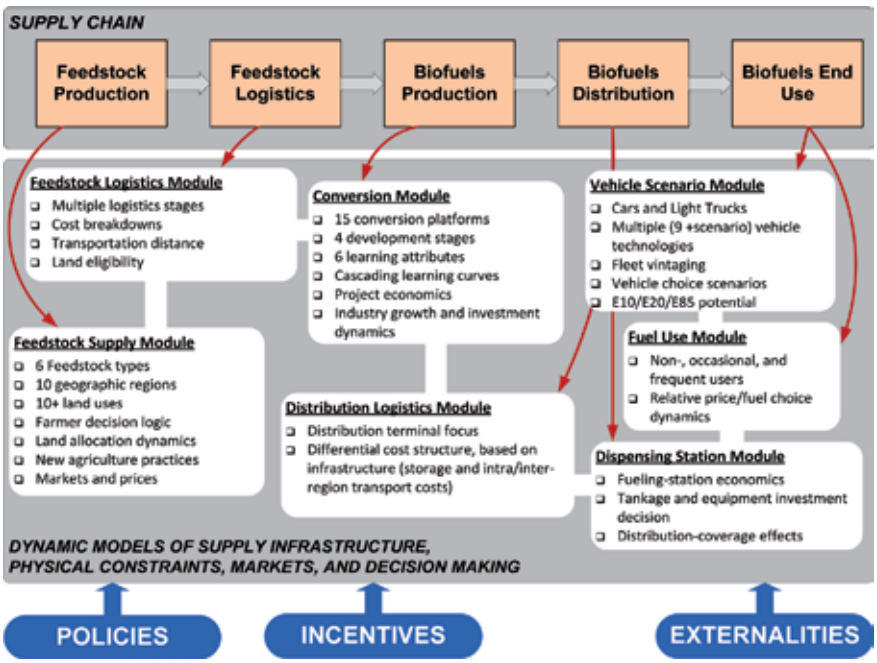


Figure 3.
The modules in the BSM represent elements of the biomass-to-biofuels supply chain.

storage, preprocessing, and transportation of biomass feedstocks from the field (or forest) to the biorefinery. The conversion module represents more than a dozen biofuel conversion technologies at pre-commercial and commercial scales. In the model, the biofuel produced in the conversion stage is then distributed to dispensing locations and end users. The model is solved numerically at a sub-monthly level and typically reports annual output for the 30–40-year timeframe. Modules receive and react to information in a response to, among other factors, industrial learning, project economics, installed infrastructure, consumer choices, and investment dynamics. The model is geographically stratified using the 10 USDA farm production regions [14] as a basis, which facilitates analysis of regional differences in key variables.

2. Modeling approach

We used the BSM to examine the impacts of (1) feedstock format and logistics, (2) biorefinery economies of scale, and (3) the impacts of shared industrial learning between fuel production technologies. In order to understand potential synergies between logistics, scale, and shared learning we modeled 10 combinations of feedstock logistics and economies of scale (**Table 1**). The feedstock formats and logistics considered include bale-based and advanced densified formats. At present, in the United States, the advanced densified logistics system is under development and we do not yet know the mechanism(s) for how these innovations may infuse into the broader market. Because of this, we model the transition from the current bale-based system to the advanced densified system based on the extent to which a commercial-scale industry has taken hold within a given region. In other words, the market demand has to be sufficiently large before large-scale investment in advanced logistics systems is warranted. Therefore, the transition to an advanced densified feedstock system is based on the number of commercial-scale biorefineries that are constructed within a given region during a model simulation. It should be noted that this study is not intended to assess the mechanism by which the biofuels industry transitions to more advanced feedstock logistics systems, but instead is focused on the system-level impact of the different feedstock logistics systems. The feedstock logistics systems modeled in this study are: Bale—feedstock is delivered to the biorefinery from within a 50-mile radius and is harvested using conventional

Combination	Format	Economies of scale	Shared learning
1	Bale	1	0
2	Densified A	1	0
3	Densified B	1	0
4	Densified A	≤2.5	0
5	Densified B	≤2.5	0
6	Bale	1	1
7	Densified A	1	1
8	Densified B	1	1
9	Densified A	≤2.5	1
10	Densified B	≤2.5	1

Table 1.
Feedstock format and economies of scale combinations explored in this study.

agricultural equipment and transported via truck in large round bales; Densified *A*—feedstock is harvested and collected using advanced equipment and is densified and delivered to a centralized depot from which the refinery receives feedstock, transition to an advanced system in which feedstock is harvested and collected using advanced equipment begins once the of one commercial-scale biorefinery (i.e. capable of processing 2,000 dry Mg per day) is constructed in the region; and Densified *B*—transition to an advanced system in which feedstock is harvested and collected using advanced equipment begins once five commercial-scale biorefineries (i.e. capable of processing 2,000 dry Mg per day) are constructed within a given region. We explored the impact of economies of scale by (1) holding the biorefinery scale constant at 2,000 dry Mg per day and (2) allowing the biorefineries to be constructed up to 2.5 times the base design case throughput of 2,000 dry Mg per day.

Shared learning (also known as spillover learning) is the process by which proximate industries have mutually beneficial conditions from accrued industrial learning (learning by doing). The process of industrial learning and shared learning has been documented in the literature [15, 16]. Examples of shared learning include knowledgeable employees working for different companies or different processes that use a technology purchased from a third party, movement of employees between firms, government-sponsored research being published in the open literature, informal sharing and/or trading of information through professional societies/conferences, and patents. In this study we explore two scenarios—(1) no shared learning between similar processes, (2) shared learning between similar processes (e.g., thermochemical processes learn from one another, biochemical processes learn from each other).

For this study, background model conditions include modeling incentives that are currently in-place and allowing them to end according to their legislative schedules. Specifically, we include the Low Carbon Fuel Standard of California, RIN credits, and the Biomass Crop Assistance Program [6, 17, 18]. For each of these we use historical data and allow them each to expire according to their respective schedules. The results and implications presented in this study should be viewed in the context of this minimal incentive environment.

3. Insights

3.1 Feedstock logistics and economies of scale

Insights reported herein should be considered in the context of the US Energy Information Administration's Reference oil price scenario. Overall, the impact of economies of scale is modest (**Figure 4**). However, the impact of feedstock format and logistics system is salient. The impact of feedstock format and logistics, without spillover learning, are shown in **Figures 5** and **6**. Moving from the status quo bale-based feedstock system to a densified advanced logistics system (Densified A and B) can facilitate higher volumes of feedstock production in response to higher demand for biofuels. Densified feedstock formats can be transported over longer distances, at lower costs, than bale-based systems, thus opening up larger areas of collection, enabling higher-throughput refineries, helping to insulate the system against risks associated with feedstock procurement (e.g., regional supply shocks such as those caused by drought, flooding, pests, etc.). Comparing simulations from the Densified A to those from Densified B, there is a clear advantage to moving to a densified feedstock supply system earlier in the simulation (Densified A transitions after construction of one commercial-scale facility whereas Densified B transitions after five commercial-scale facilities are constructed). Comparing feedstock and biofuel production levels, the system under the Densified A scenario begins growth earlier and reaches a sustained

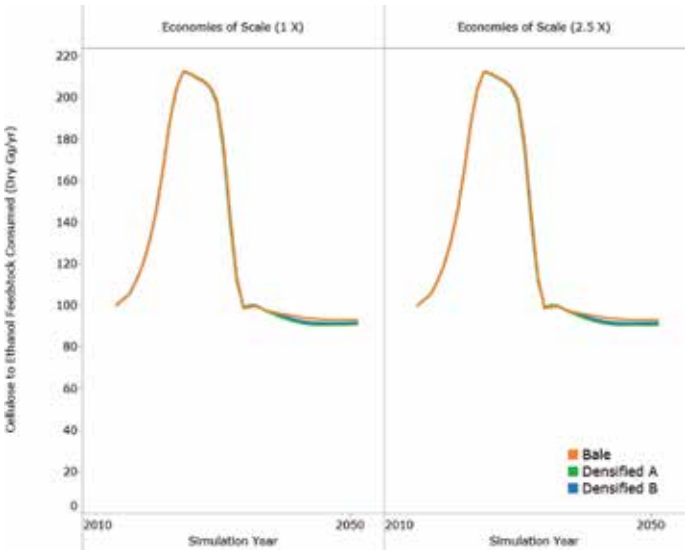


Figure 4.
Simulated cellulosic feedstock production for a 35-year period, in the United States, with and without economies of scale. Feedstock production volumes for three feedstock format and logistics systems. Bale—feedstock is delivered to the biorefinery from within a 50-mile radius and is harvested using conventional agricultural equipment and transported via truck in large round bales; Densified A—feedstock is harvested and collected using advanced equipment and is densified and delivered to a centralized depot from which the refinery receives feedstock, transition to an advanced system in which feedstock is harvested and collected using advanced equipment begins once the of one commercial-scale biorefinery (i.e., capable of processing 2000 dry mg per day) is constructed in the model; and Densified B—transition to an advanced system in which feedstock is harvested and collected using advanced equipment begins once the construction of five commercial-scale biorefineries (i.e., capable of processing 2000 dry mg per day) is constructed in the model.

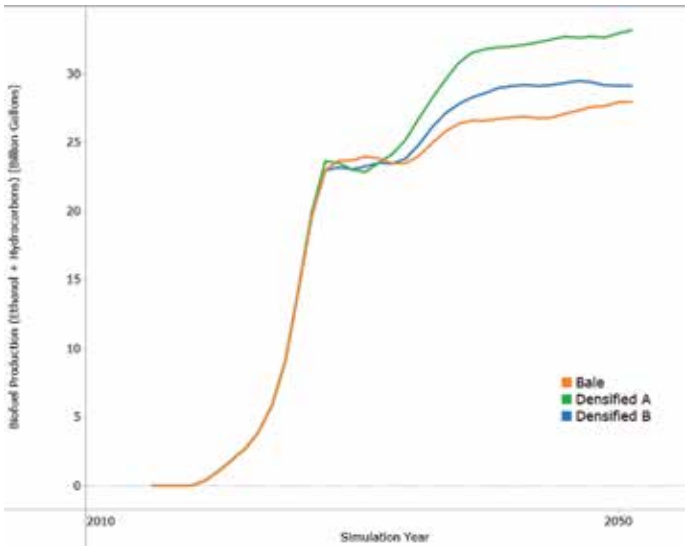


Figure 5.
Simulated cellulosic biofuel (ethanol and hydrocarbons) production for a 35-year period, in the United States. Fuel production volumes are shown for three feedstock format and logistics systems. Bale—feedstock is delivered to the biorefinery from within a 50-mile radius and is harvested using conventional agricultural equipment and transported via truck in large round bales; Densified A—feedstock is harvested and collected using advanced equipment and is densified and delivered to a refinery, transition to an advanced system in which feedstock is harvested and collected using advanced equipment begins once the of one commercial-scale biorefinery (i.e., capable of processing 2000 dry mg per day) is constructed in the model; and Densified B—transition to an advanced system in which feedstock is harvested and collected using advanced equipment begins once the construction of five commercial-scale biorefineries (i.e., capable of processing 2000 dry mg per day) is constructed in the model.

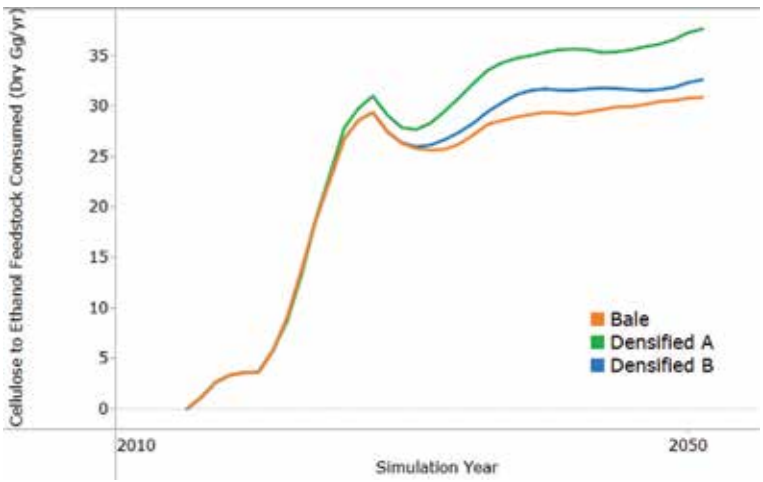


Figure 6.
Simulated cellulosic feedstock production for a 35-year period, in the United States. Feedstock production volumes for three feedstock format and logistics systems. Bale—feedstock is delivered to the biorefinery from within a 50-mile radius and is harvested using conventional agricultural equipment and transported via truck in large round bales; Densified A—feedstock is harvested and collected using advanced equipment and is densified and delivered to a refinery, transition to an advanced system in which feedstock is harvested and collected using advanced equipment begins once the of one commercial-scale biorefinery (i.e., capable of processing 2000 dry mg per day) is constructed in the model; and Densified B—transition to an advanced system in which feedstock is harvested and collected using advanced equipment begins once the construction of five commercial-scale biorefineries (i.e., capable of processing 2000 dry mg per day) is constructed in the model.

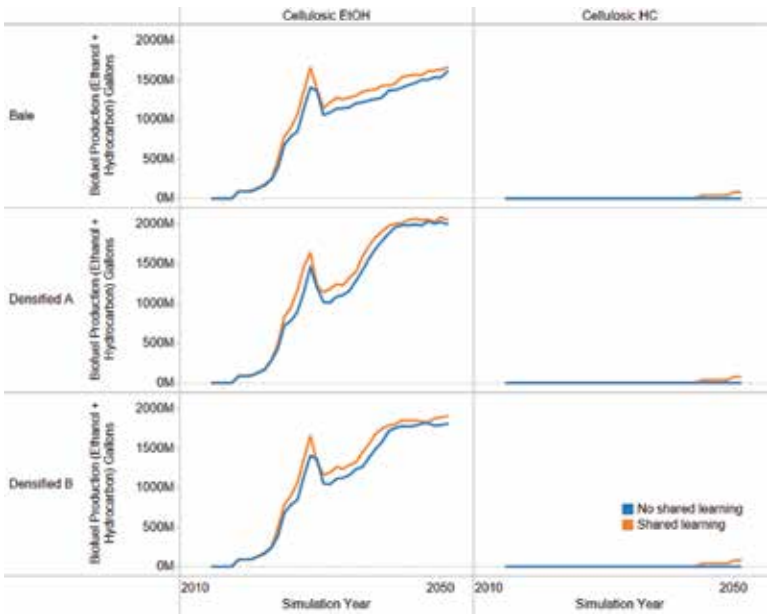


Figure 7.
Simulated cellulosic ethanol and hydrocarbon production for a 35-year period in the United States, showing impact of spillover (or shared) learning across technology pathways and for three feedstock format and logistics systems. Bale—feedstock is delivered to the biorefinery from within a 50-mile radius and is harvested using conventional agricultural equipment and transported via truck in large round bales; Densified A—feedstock is harvested and collected using advanced equipment and is densified and delivered to a centralized depot from which the refinery receives feedstock, transition to an advanced system in which feedstock is harvested and collected using advanced equipment begins once the of one commercial-scale biorefinery (i.e., capable of processing 2000 dry mg per day) is constructed in the model; and Densified B—transition to an advanced system in which feedstock is harvested and collected using advanced equipment begins once the construction of five commercial-scale biorefineries (i.e., capable of processing 2000 dry mg per day) is constructed in the model.

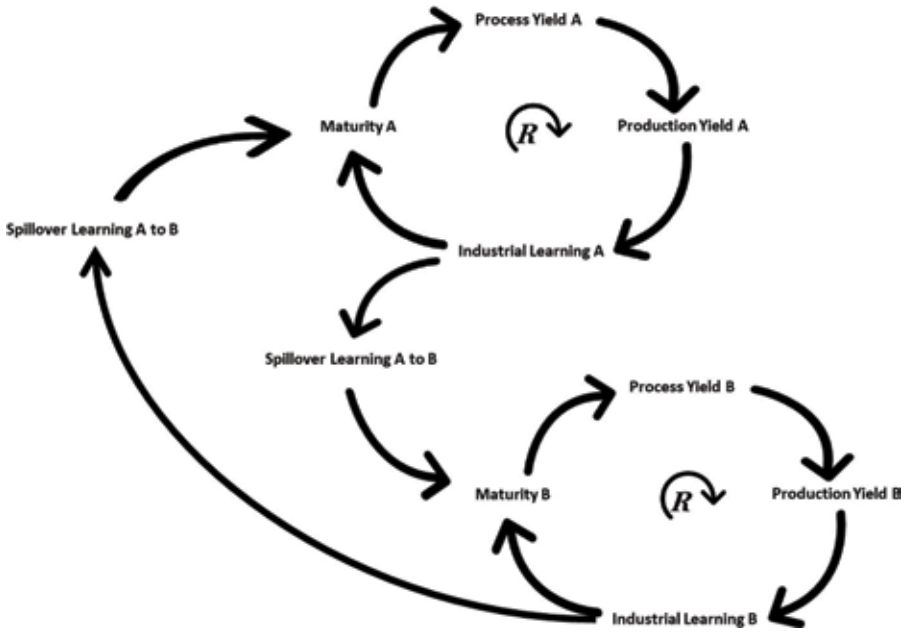


Figure 8.
Causal loop diagram illustrating the reinforcing (positive) feedback loop among learning, maturity, investment attractiveness, and production, for two generic fuel production pathways (A and B). Note: (+) sign at arrowheads means that input and output tend to vary in the same direction; (−) sign at arrowhead means that output varies in output varies in opposite direction from input.

higher level of output. Industrial learning is a high-leverage nonlinear system parameter in which small changes early on result in large differences later in the simulation.

3.2 Shared learning

Simulated lignocellulosic biofuel production with and without shared learning, in the United States, is shown in **Figure 7**. Shared learning has been shown to exert [15, 16]. Shared learning has a marked impact on both cellulosic ethanol and hydrocarbon production. In the latter case, without shared learning, cellulosic hydrocarbons do not experience any appreciable production. Industrial learning is a key system lever and acts on the system through a positive feedback loop, whereby higher learning rates result in stronger relationship between production and growth in maturity, which increases the investment attractiveness. A technology that attracts more initial investment will then have more fuel production, with associated learning advances. This increase in maturity, and the associated improvements in cost and performance, raises the attractiveness of future investment (**Figure 8**).

4. Summary

A key theme from this study as well as from the work performed over the last decade is the importance of the movement of the system toward maturation, both in terms of the supply system and the conversion processes. On the feedstock supply side, advanced supply systems have advantages relative to bale in terms of transport, handling, storage, and losses. From the conversion process perspective, mature processes imply lower investment risk, better yields, and better process economics.

Our simulations suggest that it is beneficial for the feedstock supply system to transition away from short-distance (i.e., <50 miles) transport of bales and/or any other low-density formats, to a densified system modeled after the modern commodity grain system, using larger collection radii and centralized depots. Our simulations also suggest that the temporal component is substantial—earlier transition to a high density, commodity logistics system leads to the largest gains in cellulosic feedstock production and utilization—in our model, densified A scenario accelerates maturation of the feedstock supply 21. By the end of our simulation, the Densified A scenario results in ~15% greater feedstock production.

Industrial learning (learning by doing) is a key system lever in developing industries such as the biofuel/bioproducts industry. Because the industrial learning process follows a positive feedback loop, small perturbations have large system impacts. Shared learning amplifies the industrial learning process. Advances across similar industries are shared among the industries, resulting in a substantial positive impact on the industry. A potential extension would be to look at what percent of learning needs to be shared across similar technologies for a substantial increase in overall biofuel production.

Author details

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Chapter 2

Alternative Raw Materials for Pulp and Paper Production in the Concept of a Lignocellulosic Biorefinery

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Abstract

The main source of cellulosic fibre used for pulp and paper production comes from wood, while non-wood fibres are used to a lesser extent. However, a renewed interest exists in the use of non-woody raw materials due to their abundance as source of low-cost fibres and because they are sometimes the only exploitable source of fibres in certain geographical areas, mainly in developing countries. Moreover, the great variety of characteristics, fibre dimensions and chemical composition of these alternative raw materials give them a great potential to produce different types of papers. On the other hand, the pulp and paper industry is an excellent starting point for the development of lignocellulosic biorefineries, possessing the necessary technology and infrastructure as well as extensive experience in lignocellulosic biomass transformation. Since its beginnings, the pulp and paper industry has been practicing certain aspects of the biorefinery concept, generating the energy necessary for the production of cellulosic pulp from the combustion of lignocellulosic waste and black liquors, recovering the chemical reagents used and generating high value-added products (e.g. tall oil) together with cellulosic pulp. However, the evolution of the pulp and paper industry to a lignocellulosic biorefinery requires technological innovations to make bioenergy and new bioproducts available alongside traditional products.

Keywords: alternative raw material, agricultural residues, annual plant (vegetables), pulp, paper, biorefinery

1. Introduction

Several successful industrial factories based on alternative raw materials for pulp and paper production already exist nowadays [1, 2]. Lignocellulose is the major structural component of plants and is by far the most abundant type of earthly biomass [1, 3]. It mainly consists of cellulose (40–60%), hemicellulose (10–40%) and lignin (15–30%), with minor amounts of extractives, proteins and inorganic compounds [1, 3]. Lignocellulose components can be found in both woody (e.g.

spruce, pine, eucalypt, poplar, etc.) and non-woody biomass, the latter including vegetables (e.g. bamboo, tagasaste, kenaf, abaca, etc.) and agriculture residues from harvesting and pruning operations (e.g. barley straw, wheat straw, orange tree pruning, olive tree pruning, etc.) and from agro-food industry [e.g. bagasse, empty fruit bunches from oil palm (EFB), etc.]. Cellulose is a linear and highly ordered polymer of cellobiose (D-glucopyranosyl β-1,4-D-glucopyranose), whereas hemicellulose represents a family of branched carbohydrate polymers containing both pentoses (e.g. xylose, arabinose) and hexoses (e.g. galactose, mannose, glucose) and showing often uronic acids (e.g. glucuronic acid) and acetyl moieties as sidechain groups [1, 3]. By contrast, lignin is a three-dimensional network buildup of dimethoxylated (syringyl, S), monomethoxylated (guaiacyl, G) and non-methoxylated (p-hydroxyphenyl, H) phenylpropanoid units, derived from the corresponding p-hydroxycinnamyl alcohols, which give rise to a variety of subunits including different ether and carbon–carbon bonds [4].

The main non-food use of lignocellulosic biomass is the production of cellulosic pulp from which a wide range of products can be obtained, highlighting the production of paper. At the beginning of the 1990s, there was the conviction that the arrival of new information technologies would reduce the consumption of paper; however the data of world consumption of paper and cardboard revoke this idea as it went from 240 million tonnes in 1990 to 413 million tonnes in 2016, of which 77.3 million tonnes are consumed in Europe [5]. In the past, the raw materials used in the manufacture of paper were herbaceous biomass such as flax, cotton, bamboo and cereal straw. It was not until the middle of the nineteenth century when woody materials began to be used, mainly due to the increased demand for paper because of the emergence and increased use of printing. Today, most of the cellulosic fibers used come from wood species, mainly hardwoods and softwoods [1, 2, 6]. Nevertheless, in recent years there has been an increase in consumer awareness of the need to preserve the environment, which is why they demand a more ecological production of paper, both in the use of raw materials and in manufacturing processes. With the same purpose, government bodies devote economic and human resources to research into alternative raw materials to conventional ones. For these reasons, a large number of studies on the use of non-woody materials, including agriculture residues and vegetables as alternative source for cellulosic pulp production, have emerged in recent years [1, 7–14].

Some of the advantages of using non-woody raw materials can be mentioned: (i) in developing countries with scarce forest resources, non-woody biomass provides an effective alternative to importing wood, paper, or cellulosic pulp. In these countries, there may be a large area devoted to food crops, which would provide considerable amounts of agricultural residues and agro-food industries [1, 15]; (ii) non-woody biomass increases the added value of agri-food crops by taking advantage of their residues (traditionally used for burning or agricultural amendments) to obtain a product in great demand such as paper [1, 15]; (iii) production of special papers, whose most suitable raw materials are certain vegetable alternatives to conventional woods [1, 16]; and (iv) since the morphological characteristics of the fibers and the chemical composition of the non-woody species are very varied, a wide range of papers can be obtained by properly selecting and/or mixing these raw materials [1, 14].

2. Availability of raw materials

The availability of raw materials is very important when approaching the industrial facility for the production of cellulosic pulp. Availability is related to

the production and location of the various lignocellulosic materials that can be used for the intended purpose. In the case of agricultural residues from harvesting and pruning operations, it can be said that they are very abundant in Spain. Specifically, it is estimated that the production of the most important agricultural residues, due to their abundance, such as cereal straw, sunflower stalks, vine shoots, cotton stems, olive, orange and peach tree pruning and vegetable and other similar crop wastes, represents about 50 million tonnes per year, with Andalusia contributing with more than 20% [1, 17].

Due to its abundance, it seems that the most recommended agricultural residue for the manufacture of paper pulp is cereal straw since it represents almost 20% of the agricultural residues considered in 2007, and the technology used in its collection is fully developed [1, 17–19]. Regarding the waste from the agri-food industry used for the production of cellulosic pulp, the bagasse from the extraction of sugar cane and waste from the palm oil industry (EFB) should be highlighted [20].

With regard to alternative vegetables for cellulosic pulp production, they can be classified in three groups: (i) plants of wild nature such as bamboo, different types of cane, esparto grass, etc. [21]; (ii) plants from plantations with industrial uses, such as sorghum, abaca, sisal, jute, hemp, kenaf, flax, etc. [7, 22]; and (iii) other plants, mainly herbaceous species, grasses and legumes, which produce high biomass yields when grown in intensive plantations (tagasaste, *Leucaena* spp., etc.) [23–25].

3. Storage of lignocellulosic materials

The prolonged storage of lignocellulosic raw materials is always necessary in the pulp and paper industry. In the case of raw materials that are harvested only at a specific period of the year, the storage is even more important. Therefore, these raw materials must be collected in order to meet the annual needs of a factory, so that it operates all year round, with the consequent better use of installed capacity. On the other hand, many alternative lignocellulosic materials are more easily deteriorated due to their non-woody special properties, such as straws, herbaceous vegetables, etc., mainly if they contain high percentages of humidity. In fact, of all the factors that influence the storage of this type of sources, the most relevant is the residual humidity. Given that these materials do not require too rigorous conservation, as they are not intended for food, the rule of allowing slightly higher humidity than the "Caurie safety" humidity obtained by adjusting the experimental data on equilibrium humidity and relative humidity of the environment of the adsorption isotherms to the Caurie equation can generally be adapted. Applying this standard and observing the experimental adsorption isotherms, it appears that wheat straw, vine shoots and cotton stems can be well conserved in environments with relative humidity below 60–70%, while other agricultural residues such as olive tree pruning or sunflower stems require lower values [26]. On the other hand, it has been verified that the recommended maximum relative humidity values, according to the standard followed in this work, coincide with those obtained experimentally when storing the different agricultural residues considered in environments with different relative humidity for 10 or 12 months. As the chemical composition of these agricultural residues is considered as well as their fibrous structure does not differ so much from other agricultural residues such as wastes from agro-food industries, forestry residues and vegetable materials in general, the above conclusions can be extended to all these alternative lignocellulosic materials.

4. Characterization of lignocellulosic materials

Theoretically speaking any plant containing a reasonable amount of fibres can be used as a raw material for pulp and paper production. In practice, this is not the case. Besides the abundance of the plant, a steady supply and many other requirements are necessary. The fibre content of the plant is important. The plant contains in addition to fibres many non-fibrous cells, e.g. parenchyma cells. Fibres themselves vary much in different plants regarding their length, width, fine structures or microstructures, as well as their chemical composition. In one and the same plant, there are different types of fibres. The same fibre type is not equal in dimension but contains a spectrum of different dimensions. For this reason, one speaks of "average fibre length". The length of the fibre is one of the most important parameters affecting paper strength [1].

Chemical characterization, which gives rise to the percentages of the main chemical constituents of lignocellulosic materials (generally cellulose, hemicellulose, lignin, as well as extractives and ash), is of great interest since it can indicate their possible applications for obtaining cellulosic pulps, in terms of the most suitable process to follow and the type of pulp that can be obtained. In this characterization, the contents of holocellulose, lignin, α-cellulose, hemicellulose, and extractives in water, 1% soda and ethanol-benzene and ash are determined as the most important. For this chemical characterization, TAPPI test methods, including TAPPI T 204 om-88, TAPPI T 211 om-93, TAPPI T 222 om-88 [27], and NREL analytical methods (National Renewable Energy Laboratory NREL/TP-510-42168) are usually employed [28].

When comparing the results obtained by different authors, a good concordance is generally observed for each specific material. Sometimes discrepancies appear that can be attributed to the different procedures used as well as to the different origins and varieties of the raw materials considered. For example, the chemical characterization results obtained for rice straw were analysed and compared with (i) some agriculture residues from harvesting and pruning operations and from agro-food industry (e.g. olive tree pruning, wheat straw, sunflower stems, sorghum stems, bagasse, vine shoots, and cotton stems); (ii) some vegetables (e.g. *Leucaena colinsi*, *Leucaena leucocephala, Chamaecytisus proliferus*, *Retama monosperma*, *Phragmites* spp., *Arundo donax, Prosopis juliflora*, and *Paulownia fortunei*); and (iii) softwoods (pine) and hardwoods (eucalyptus) [23, 29]. From this comparison it could be deduced that:

- The value of the hot water soluble content of rice straw (7.3%) is lower than that of the rest of agricultural residues, except for bagasse and cotton stems; it is higher than the values found for the vegetables considered, except for *P. fortunei*, and higher than the values for pine and eucalyptus.

- The value of soda extractives at 1% of rice straw (57.7%) is higher than the values corresponding to the rest of agricultural residues and vegetable considered, as well as those of pine and eucalyptus.

- The content of ethanol-benzene extractives in rice straw (0.56%) is lower than that of the materials considered: agricultural and agro-food residues, vegetables, pine and eucalyptus.

- The ash content of rice straw (9.2%) is higher than the values presented by the rest of agricultural residues and much higher than the values of pine and eucalyptus.

- The holocellulose content of rice straw (60.7%) is similar to the value found for olive tree pruning and lower than the values found for the rest of the agricultural residues considered, as well as those of the alternative vegetables considered and those of pine and eucalyptus.

- The content of α-cellulose of rice straw (41.2%) is lower than the values presented by the cotton stems, *L. colinsi*, *L. leucocephala*, *C. proliferus*, *R. monosperma*, pine and eucalyptus; higher than the values corresponding to olive tree pruning, wheat straw, *Phragmites*, *P. fortunei*, *Prosopis juliflora*; and similar to the values of the other species considered.

- The lignin content of rice straw (21.9%) is similar to the values corresponding to the cotton stems, *L. leucocephala* and *R. monosperma*; lower than the values found for *Phragmites* spp., *A. donax*, *P. fortunei* and pine; and higher than those of the other species considered.

In the same way, following the same example of rice straw, the experimental data on its physical characterization, which determines the size of its fibers, are compared with those of other lignocellulosic materials such as wheat straw, sunflower stalks, vine shoots, cotton tree stalks, olive tree pruning, sorghum stalks and pine and eucalyptus woods. After a biometric analysis with the rice straw studied, it is concluded that the length of its fibers (1.29 mm) is similar to that corresponding to the stems of sorghum, superior to those of the other agricultural residues considered and to that of eucalyptus but inferior to that of pine.

In summary, it can be stated that the alternative non-woody materials under consideration have acceptable chemical and physical characteristics for the production of pulp and paper [30].

5. Cellulosic pulp production

The manufacture of cellulosic pulp consists of the separation of cellulose fibers, which are cemented by the middle wall, composed mainly of lignin using physical or chemical methods [1, 2, 6]. In order to obtain cellulosic pulps from alternative non-woody materials, different chemical classical processes have been used (using chemical reagents such as soda, sodium sulphate and sodium sulfite) and organosolv (using organic solvents). In general, non-woody raw materials have a less density and more porous structure and, also in most of the cases, less lignin content, which means less energy and chemical requirements for fibre separation during pulp production. In addition, they have shorter growth cycles, reaching maturity faster than wood species, and in many cases the pulp yields obtained are higher [30].

5.1 Classical pulping processes

5.1.1 Soda pulping

Soda pulping is the oldest pulping processes known and consists of subjecting raw materials, cut and conditioned, to a cooking process with a given concentration of sodium hydroxide, at a specific temperature and cooking time, depending on the quality of the pulp to be obtained (chemical or semi-chemical) and the characteristics of the raw materials used [1, 2, 6]. A recovery of reagents and purification of black liquors is finally carried out. Each of these sections of the process can group

together different operations. Thus, for example, in the preparation of the raw material, a debarking is carried out in the case of woody plants or pith is removed in the case of some vegetables (e.g. sunflower stalks), a cutting or reduction in size to produce chips or flakes, a cleaning to remove impurities, and so on. In the pulping section, the operations of impregnation of the raw material, cooking or delignification to separate lignin, washing of the solid fraction resulting from cooking and draining of the same to eliminate the fluid used in the washing can be integrated. In the same way, the sections of reagents recovery and purification of residual black liquors are made up of different operations.

Soda pulps have been obtained from different alternative raw materials, specially agriculture residues such as wheat straw [31], sunflower stalks [32, 33], vine shoots [34], olive tree pruning [35], sorghum stalks [36, 37], tagasaste [24], EFB [20, 38], *H. funifera* [39] and rice straw [29, 38, 40], obtaining different yields depending on the conditions of soda concentration, temperature and cooking time used. Soda pulping has also been carried out using additives such as anthraquinone and parabenzoquinone, which accelerate the delignification process and stabilize carbohydrates, improving the yield of the process with respect to the conventional "soda" process when operated under the same working conditions. Assays have been carried out using wheat straw, olive tree pruning, rice straw and EFB. For rice straw and EFB, pulps have also been obtained using KOH in aqueous solutions [20, 40].

Miao et al. [22] also analysed the composition of the hemp root bast (HRT) to further subject it to a process of soda pulping and bleach it with an elemental chlorine free (ECF) bleaching sequence. These authors conclude that HRT is a suitable raw material to make paper obtaining a pulp with high viscosity and brightness (893 mL/g and 85.52% ISO, respectively). González et al. and Marrakchi et al. [41, 42] also applied soda pulping to orange tree wood and *Stipa tenacissima* stems, respectively. The first ones studied the influence of operational variables in both pulping and pulp beating (temperature, 155–185°C; time, 40–90 min; soda concentration, 10–16%; and number of PFI beating revolutions, 0 to 3000) on the yield and on the pulp refining degree as well as the physical properties of resulting paper sheets. These authors found an optimum compromise as regards operating conditions (170°C, 40 min, 13% soda concentration and 2700 number of PFI beating revolutions), obtaining a pulp with tensile index, burst index and tear index of around 59.11 Nm/g, 4.10 kN/g and 2.79 mNm^2/g, respectively; these values deviate from their maximum values in 5.8, 2.2, and 1.4%, respectively. The pulp yield under these operating conditions is 43.9%; the refining degree is of 39.5°SR with the advantage of an increased drainability in paper production. These conditions involve a lower temperature, time, soda concentration and refining than those required to maximize the studied paper properties; so it is possible to save energy, chemicals and capital for industrial facilities. On the other hand, Marrakchi et al. [42] analysed the composition and fibre characteristics of the *S. tenacissima* steams and of its corresponding soda unbleached and bleached pulps. They conclude that the properties of *S. tenacissima* fibers are intermediate between those of non-wood and wood plants and are most often close to those of eucalyptus fibers. After studying a refining process and characterizing paper sheets obtained, these authors demonstrate the high potentiality of this non-wood species for papermaking applications.

5.1.2 Kraft pulping

The pulp obtained by this procedure is usually called Kraft (strong) if used for raw papers or "sulphate" if they are going to receive a further bleaching, although

both denominations are used indistinctly. The name "sulphate" is due to the fact that it is the sodium sulphate, and not the sodium sulphide, the reagent that is replaced, although the real agent that acts during the reaction is the sulphide that is generated in the recovery treatment of residual black liquors [1, 2, 6]. The process can be divided into two parts: the first is the obtaining of the pulp, and the second is the recovery of the chemical reagents used from black liquors.

According to different authors [1, 2, 6], Kraft pulping process consists of the following stages:

i. The chips are taken to the reactor where they are cooked with white liquor (dissolution of sodium hydroxide and sodium sulphide), controlling the "liquid/solid" ratio.

ii. Pulping takes place during the established time, under appropriate pressure conditions.

iii. The black or residual liquor and the pulp are separated by filtration. The pulp is washed, and the black liquor is sent to the reagent recovery phase.

iv. Once washed, the pulp goes to the bleaching stage or to the raw paper manufacturing plant.

In the reagent recovery phase, organic compounds dissolved in black liquor are used to produce energy, thus reducing the rate of polluting effluents. The stages of recovery are as follows: (i) concentration of the black liquor in the evaporators; (ii) spraying of the concentrated black liquor in the oven, where the carbon reduces the sodium sulphate to sodium sulphide; (iii) the melted solids are discharged and dissolved in water, resulting in the green liquors; and (iv) the green liquor is sent to the causticizing stage, where the sodium carbonate reacts with the calcium oxide to form sodium hydroxide [1, 2, 6].

Some studies have been carried out to obtain Kraft pulps using alternative materials to traditional wood, including olive tree wood [43], *Cynara cardunculuns* L. [44], vine shoots [34], wheat straw [45] and kenaf [46]. Nevertheless, due to the more accessible structure of these materials compared to conventional wood materials, a soda process is usually applied to them, as this process is less pollutant. Thus, as an example, a factorial design of central composition experiments to find equations that relate the characteristics of the pulp and paper sheets with the operation variables have been realized using olive tree pruning [47, 48]. From these studies, it can be concluded that, in order to obtain pulp with suitable characteristics to be bleached to obtain paper and with good mechanical properties in the paper sheets, it is necessary to operate with an active alkali concentration of 25%, at 175°C during 90 min. The paper sheets obtained from olive tree pruning pulps were produced in different degrees of refining and were characterized attending their stretch index, burst index, and tear index. All paper sheets reach between 33 and 39 kN m/kg in the stretch index, between 1.5 and 2 kN/g in the burst index and 0.7–2.5 N m^2/g in tear index and not using a high refining degree (<45°SR) [47, 48].

5.1.3 Sulfite pulping

Sulfite pulps are obtained by cooking the lignocellulosic material with a solution of bisulfite and sulfur dioxide [1, 2, 6]. The cooking liquor is obtained by burning sulfur to obtain sulfur dioxide which is absorbed in a base of calcium, magnesium, sodium or ammonium. The most important variables of the "sulfite" process

includes impregnation of the chips with the cooking reagents, dimensions and quality of the chips, temperature, time, pressure, pH of the white liquor, concentrations of sulfur dioxide combined (total and free), "liquid/solid" ratio and raw material used. Several "sulfite" processes have been proposed, including acid sulphite, bisulphite, alkaline sulphite, multistage sulphite, high-yield sulphite, etc., to obtain dissolving pulp [1, 2, 6]. In addition to these variables, it has been proposed to use molybdate or anthraquinone as catalysts, achieving a stabilization of the polysaccharides and an acceleration in delignification.

The sulfite process has been studied for several alternative raw materials but not as much as the soda and Kraft processes. Then, different studies of sulfite process with olive tree [35, 49], sunflower stalk [50], bagasse [51] and wheat straw [52] have been reported.

5.1.4 Organosolv pulping

These processes are characterized by the fact that the separation of lignin from lignocellulosic materials is achieved by solubilization with organic solvents, which are subsequently recovered for a new pulping cycle, resulting in a concentrate rich in lignin, from which different by-products can be obtained [53]. Among organic solvents used, alcohols (ethanol, methanol, butanol, etc.) and organic acids (acetic and formic acids) are commonly employed for non-woody materials [1, 2, 18, 24, 34, 54–66]. Nevertheless, acetone and other solvents such as phenol, formaldehyde, ethanolamine, ethylene glycol and ethanol-water have also been used for these alternative raw materials [1, 2, 19, 23, 34, 38, 60, 67–71], demonstrating that these materials can be used for the manufacture of pulp and paper through different processes with acceptable characteristics.

5.1.4.1 Pulping using alcohols

These are the most widely used processes due to the selectivity that these solvents contribute to the separation of the lignin and their easy recovery by distillation. In the case of the ethanol process, the influence of the operating variables (ethanol concentration, temperature, time and liquid/solid ratio) on the characteristics of the pulp and paper sheets obtained from different alternative raw materials, including olive tree [62], wheat straw [1, 2, 18], tagasaste [24, 57], sunflower stalk and *P. fortunei* [54, 55] and vine shoots [34], has been studied. As an example, in the case of wheat straw, when pulping is carried out at 200°C, with an ethanol concentration of 75% for 60 min, acceptable good values are obtained for yield (37.6%), holocellulose (88.8%), α-cellulose (46.9%) and lignin (7.2%) [1, 2, 18]. Methanol and butanol have also been used on wheat straw [37, 61].

5.1.4.2 Pulping using organics acids

Along with the processes that use alcohols, the processes that use organic acids are the following most used. The most common are those that use acetic acid and formic acid, and different studies have been reported with EFB [58], rice straw [63], jute [66], rapeseed straw [56], cardoon stalk [64], and wheat straw [65].

The pulping of wheat straw with acetic acid and formic acid has been carried out, studying the influence of operation variables on the properties of the resulting pulps. Comparing the results obtained when operating for times ranging between 0.5 and 2 h, at temperatures of 75–125°C and 150–200°C, and with concentrations of 50–100% and 50–80% of the formic and acetic acids, respectively, it is concluded

that to obtain pulp with acceptable holocellulose (88.2%), α-cellulose (40.2%) and lignin (6.4%) contents are more effective than formic acid, operating at 50% concentration, 100°C and 2 h. This fact is mainly due to it requiring less acid and lower working temperature, with the consequent savings in chemical reagents and energy for heating [65].

5.1.4.3 Acetone process

Several studies have been studied with acetone solvent mainly on wheat straw [1, 2, 19, 60, 67]. From these studies it is concluded that it must be operated at 200°C, for 95–100 min and with 55–60% of acetone to obtain high holocellulose and α-cellulose values and low lignin and extractives, although the yield of the pulp is low [60]. To obtain good values of breaking length (3456 m), elongation (1.42%), burst index (1.36 KN/g) and tear index (3.86 mNm^2/g) of the paper sheets formed, a temperature of 200°C has to be used. On the other hand, if the brightness has to be high, it has to be operated at 140°C for 1 h with a concentration of 60% acetone [65].

6. Refining of cellulosic pulps

The refining of pulp is an operation that modifies, through the action of mechanical work and in the presence of an aqueous medium, the morphology of the fibres and their physicochemical structure, decisively changing the properties of the paper sheets obtained from the refined pulp [1, 2, 6]. Using a Sprout-Bauer refiner, the influence of refining pulp from different agricultural residues (wheat straw, sunflower stems, vine shoots, olive tree pruning, cotton stems and sorghum stems) on the corresponding pulp and paper sheets was studied [1, 2, 19, 32, 69]. In view of the results, it can be concluded that olive tree pruning pulp must be severely refined to obtain good quality paper, although the maximum values of the ring crush test (RCT) and the tear index are reached for refining grades of 45 and 55°SR, respectively. In the case of EFB soda-anthraquinone pulp, a study has been carried out in a PFI refiner, studying the influence of the cooking variables (soda concentration, temperature and time) and the number of turns in the PFI on the properties of the resulting paper sheets [20]. From this study it is deduced that under some operation conditions, 15% of soda, 170°C, 70 min and 2,400 turns in the PFI, the properties of paper sheets obtained deviate less than 12% from their optimum values (59.6 Nm/g for the traction index, 4.48% for elongation, 4.17 kN/g for the burst index and 7.20 mNm^2/g for the tear index), for a degree of refining of 47.5°SR, acceptable for the formation of paper sheets. Under these conditions, reagents, energy and immobilized capital are saved with respect to the maximum values of the operating variables used [20].

7. Bleaching of cellulosic pulps

The bleaching of cellulosic pulps is carried out for the elimination and/or modification of some constituents that add color to the raw pulp, generally using chemical reagents in one or more stages and trying to degrade the cellulose fibers as little as possible [1, 2, 6]. The main light-absorbing substances in the pulps are lignin and resins, so in order to bleach a pulp, these substances must be chemically transformed into a solid state in order to reduce their light absorption characteristics or

be oxidized, reduced or hydrolysed, to make them soluble in aqueous solutions and thus be able to be removed from the pulps.

The need to reduce pollution from bleached pulp mills has led to the study of new bleaching sequences [1, 2, 6], with research focusing in three main directions: (i) bleaching processes with reagents without elemental chlorine (ECF), which consist of the total substitution of chlorinated stages by compounds such as chlorine dioxide (without elemental chlorine), regardless of whether other bleaching agents totally free of chlorine, such as oxygen, hydrogen peroxide, etc., are also used; (ii) bleaching processes with totally chlorine free reagents (TCF), using reagents such as oxygen, hydrogen peroxide and ozone, mainly [72]; and (iii) biological bleaching processes involving microorganisms or enzymes produced by them.

ECF and TCF bleaching processes including enzymatic stages have been studied for different alternative raw materials. It is worth highlighting the TCF processes which have been studied using different chemical reagents individually (hydrogen peroxide, oxygen, ozone, sodium perborate and peracetic acid) or with OZP bleaching sequences (where Z is an ozone stage) [1, 2, 6].

Hydrogen peroxide has been used for the bleaching of Kraft olive tree pruning pulp with a Kappa index of 21, operating at a temperature of 70°C and a consistency of 10%, and following a factorial design of experiments in which the peroxide concentration varies from 1 to 5% and the time from 30 to 210 min, finding that it is recommended to use a low-medium concentration of peroxide (1–3%) and a long time (210 min) [73]. Comparing the results with those of bleached pulps with other reagents, it is concluded that the viscosity of the pulps is higher in the case of peroxide bleached pulps than those bleached with oxygen, ozone or chlorine dioxide. To improve the Kappa index and brightness values of peroxide bleached pulp, it is desirable to combine hydrogen peroxide with oxygen or to use the combination oxygen and ozone [74].

For the bleaching of abaca soda pulp with peracetic acid [75], the influence of the operating conditions on the Kappa index, viscosity and brightness of the pulp and on the breaking length and burst index of the paper sheets was studied. Following a factorial design of experiments, it is concluded that operating at 55°C, with 4.5% peracetic acid for 150 min, a brightness of 79.9% is obtained (only 6.5% lower than the maximum possible) and the maximum possible values for the breaking length (6547 m), burst index (5.0 kN/g) and viscosity (1519 mL/g).

Peracetic acid has also been considered in the bleaching of olive tree pruning, finding that it has to be operated at 55°C for 90 min, a consistency of 10% and an acid concentration of 2.5%, providing good values for brightness and Kappa index and improving the viscosity of the bleached pulp with respect to crude pulp [76].

In the bleaching of abaca soda pulp with sodium perborate [77], the influence of the concentration of reagent (1–5%), temperature (60–80°C) and time (1–2 h) on the characteristics of the bleached pulp and the resulting paper sheets has been studied. It is concluded that in order to obtain pulp with the highest possible values of viscosity (1601 mL/g) and breaking length (5943 m), it is necessary to operate at 60°C, 1% perborate and 60 min, achieving a brightness of 62.7%, only 11.9% below the maximum possible.

For abaca soda pulp, the bleaching processes using hydrogen peroxide, peracetic acid, sodium perborate and the OZP sequence were compared from the point of view of pulp yield and brightness, breaking length and burst and tear indexes of the paper sheets. Overall, the best results are achieved for peracetic bleached pulp (4.5%, at 55°C for 0.5 h), providing little loss of yield (<1%) and some values for breaking length (6.555 m), burst index (4.97 kN/g) and tear index (15.77 mNm^2/g), which only decrease, with respect to those of the raw starting pulps, by 7.0, 8.8 and 20.9%, respectively, while brightness (77.4%) increases by 56.7%; with the

additional advantage that by operating at a lower temperature and for less time than in the other bleaching processes considered, energy savings are produced for heating and immobilized capital for industrial installations. The pulp bleached with the OZP sequence has more brightness but loses more yield. Moreover, the characteristics of the paper sheets are worse, and the process requires higher costs of reagent, energy and immobilization [78].

The OZP sequence has been applied to EFB soda-anthraquinone and diethanolamine pulps [79]. For similar Kappa index values for the two pulps (14.2 and 17.3), the paper sheets of the raw soda-anthraquinone pulp exhibit higher values for tensile (25.8 Nm/g), elongation (2.35%), burst index (1.69 kN/g) and tear index (0.50 mNm^2/g) and brightness (60.6%) than the diethanolamine pulp, but the latter has a higher viscosity (659 mL/g). When OZP bleaching sequence is used, the diethanolamine pulp exhibits higher viscosity (783 mL/g), and the properties of the paper sheets are similar to or better than those of the soda-anthraquinone pulp: 22.2 as opposed to 20.4 Nm/g for the tensile index, 1.30 vs. 1.42 kN/g for the burst index, 0.71 vs. 0.70 mNm^2/g for the tear index and 71.3 vs. 77.5% for brightness [79].

7.1 Biobleaching

It is worth highlighting in this section that apart from xylanases, the use of laccases has been used for the bleaching of alternative raw materials [80–84]. As it is known, these enzymes need a mediator to make the bleaching more effective since thanks to them they are able to oxidize not only the phenolic part but also the non-phenolic of the lignin.

The work of Camarero et al. [80], who apply three different fungal laccases (from *Pycnoporus cinnabarinus*, *Trametes versicolor* and *Pleurotus eryngii*) and two mediators, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 1-hydroxybenzotriazole (HBT) to bleach flax pulp using a TCF sequence (enzymatic stage (L) plus hydrogen peroxide bleaching (P)), is noteworthy. These authors obtain delignification values of up to 90% after hydrogen peroxide bleaching when initial pulp is subjected to the enzymatic pretreatment (L). These results are improved when they apply a P stage under pressurized oxygen, obtaining a pulp with 82% ISO of brightness, and kappa index close to 1. Fillat et al. [81] also bleached flax pulp using natural mediators: syringaldehyde (SA), acetosyringone (AS) and p-coumaric acid (PCA) in combination with the laccase of *P. cinnabarinus* as a pretreatment prior to hydrogen peroxide bleaching stage. All mediators decrease the kappa index and increase the brightness of the bleached pulps after peroxide bleaching especially when SA was used. On the other hand, soda-anthraquinone pulp from orange tree pruning is also bleached by Fillat et al. [82]. In this case three different laccase-mediator systems (LMS) were used as pretreatment to an alkaline extraction plus a hydrogen peroxide bleaching: laccase from *Trametes villosa* (Tv), either in combination with 1-hydroxybenzotriazole (HBT) or with acetosyringone (AS) as natural mediator, and laccase from *Myceliophthora thermophila* (Mt) in combination with AS. The three laccase-mediator systems improve the bleaching sequence, with L-Tv + AS being the LMS that provides the highest delignification and improvement of optical properties. Finally, Martín-Sampedro et al. [83] also bleached soda pulp from olive tree pruning using not only a typical LMS but also adding xylanase jointly or prior to LMS to study the effect of this enzyme on the characteristics of the bleached pulps. The best results are found when both enzymes are applied in the same stage. In these conditions the lowest hydrogen peroxide consumption (63%), kappa index of 11.6 and brightness of 46% ISO are reached. Same authors [84] also bleached pulp from oil palm empty fruit bunches using laccase and xylanase. An enzymatic process with xylanase (X) and/or laccase (L) was incorporated before the alkaline extraction step

(E) and the hydrogen peroxide bleaching (P). Comparing with controls, the LEP sequence results in an improvement of optical properties (colorimetric properties and brightness) and a reduction of the kappa index. When both enzymes (xylanase and laccase) are used jointly, no improvement is detected; however, when the xylanase stage is applied before the laccase stage, the beneficial effects of laccase are boosted. Thus, the XLEP bleached pulp shows a brightness of 60.5% ISO, a kappa index of 5.4 although the hydrogen peroxide consumption increase (77.0 vs. 64.5% and 73.8% for EP and LEP, respectively).

8. Integration of the pulp and paper industry using alternative raw materials into the biorefinery concept

The concept of lignocellulosic biorefinery aims at the integral use of the main components of lignocellulosic raw materials to obtain energy, chemicals and products [85]. The pulp and paper industry is an excellent initial point for the establishment of this concept as it has the best infrastructure for biomass fractionation and conversion and a great deal of practical industrial experience. Then, the classical pulp and paper industry, including Kraft, sulfite and soda technologies, has been applying this concept for a long time as it not only produces paper as the main product (cellulosic fraction) but also recovers the reagents and produces energy from the residual black liquors (lignin-rich fraction) as well as the generation of bioproducts such as tall oils, which are sold to obtain high added value products (e.g. adhesives, detergents, etc.), and lignin for the production of chemicals or materials. In the future, the extraction of hemicelluloses prior to pulping will be included in order to make maximum use of lignocellulosic materials. A general scheme, which will be developed below including also gasification of lignin, is shown in **Figure 1**.

Using the same scheme-work of the pulp and paper industry with classical pulping methods, different organosolv pulping processes have been developed to produce cellulosic pulp and other products from different alternative raw materials such as agriculture residues [53], among them, those employing ethanol such as the Alcell© process for the production of cellulosic pulp, giving value to other biomass fractions, such as high-quality lignin in the residual black liquor with several potential industrial applications, and the Lignol© process, which also extracts lignin, as well as sugars for the production of ethanol, oligomers, furfural and acetic acid. However, one of the disadvantages of these processes lies in the incorporation of both extracts and a part of the hemicelluloses to the residual black liquors. For this reason, the possibility of carrying out a hydrolysis pretreatment of the polysaccharides with the original raw materials prior to organosolv pulping methods, using water at a high temperature (hydrothermal treatment), has been explored [53]. Then, a hydrolysis of the acetyl groups to acetic acid is produced, which acts as a catalyst solubilizing all or part of the hemicelluloses (autohydrolysis) and then resulting in a pretreatment aqueous fraction with oligomers (mainly gluco-oligosacharides and xylo-oligosacharides), sugars (glucose, xylose, arabinose), acetic, furfural or 5-hydroxymethyl-2-furfural (HMF) and some lignin. Oligomers are used as food additives or substrate for sugars, after hydrolysis and fermentation (xylose and arabinose could be fermented to ethanol or xylitol); and furfural and lignin derivatives have applications in the chemical industry [86, 87]. The disadvantage of this fractionation is the low selectivity towards cellulose, giving rise to a solid fraction structurally affected, which can limit its later use; but an adequate hydrothermal pretreatment achieves a solid fraction that can be used to obtain pulp and paper by classical or organosolv procedures, whose resistance can be improved using a relevant refining.

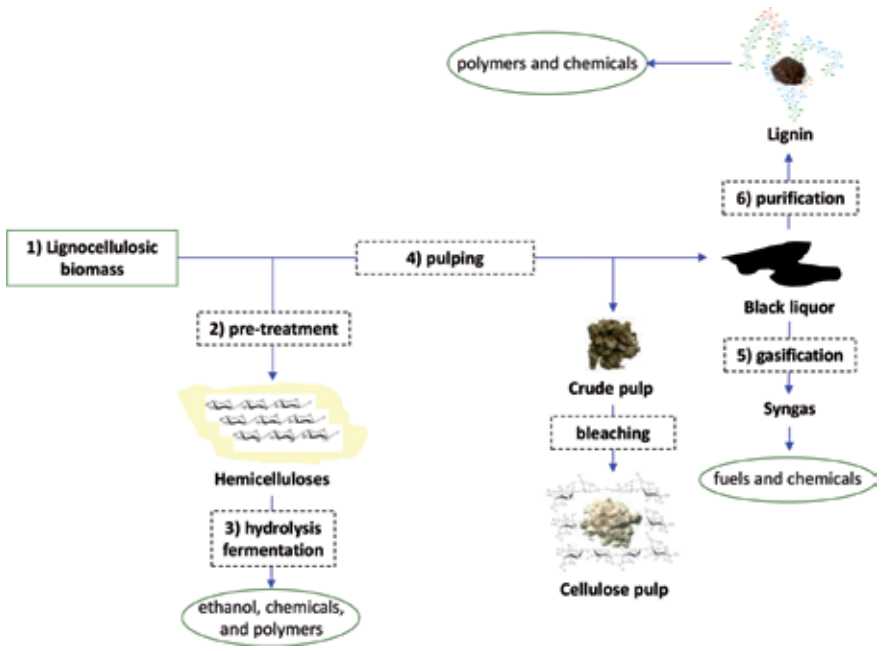

Figure 1.
Scheme of integration of pulp and paper industry into the biorefinery concept in the future.

In the pulping processes of the solid fraction coming from autohydrolysis or hydrothermal pretreatment, some residual black liquors are obtained, with lignin being the majority component. These liquors, after the separation of water and/or organic solvents used in cooking (which are recycled in the cooking process), are transformed into a concentrate rich in lignin. From these concentrates lignin can be obtained for different uses, and/or it can be subjected to gasification processes with the aim of obtaining high-quality products such as hydrogen, methanol, synthesis gas or dimethyl ether (DME) for motor applications [86–89].

8.1 Hemicellulose isolation by hydrothermal treatments

As commented above, one of the possibilities to convert the classical chemical pulp and paper industry into a biorefinery is to extract a portion of hemicelluloses from lignocellulosic materials prior to pulping, obtaining a liquid fraction enriched in hemicellulosic carbohydrates that can be converted into ethanol and/or chemical products. One of the options for the separation of hemicellulose from lignocellulosic materials is its depolymerization by autohydrolysis, also known as hydrothermal process, which does not require the addition of acids as it is auto-generated in the process [53, 85]. In addition to the process of autohydrolysis itself, the process of steam explosion is very significant (once autohydrolysis has taken place, the mixture undergoes a sudden decompression to produce the vaporization of the water contained in the fibers and the consequent disaggregation of the lignocellulosic matrix), as well as its variants, such as the Rash, Masonite, Iotech, Siropulper and Stake processes [53, 85].

These hydrothermal treatments can be carried out in a very wide range of operating conditions, with the temperature, time, solid concentration and particle size of lignocellulosic materials being the most influential variables [85]. In the case of autohydrolysis, the range of temperatures to treat lignocellulosic materials in an aqueous medium is in the range between 150 and 250°C. Under these conditions, the self-ionization of water generates protons that act as a catalyst for the hydrolysis

of the hemicellulose, reacting among others the acetyl groups (present in the form of esters in the hemicellulosic heteropolymers), which are released in the form of acetic acid. Its contribution to the generation of protons is 1700 to 1,000,000 times greater than that of water, so the contribution of aqueous protons to the hydrothermal process can be neglected once acetic acid has been generated. At the same time, there is total or partial solubilization of hemicelluloses and their conversion with good yields of oligosaccharides and monosaccharides, which can be used for different purposes [53, 85].

Other minor reactions associated with this type of process are the formation of products such as furfural from pentoses and HMF from hexoses; the generation of carbon dioxide by decomposition of carboxyl groups present in uronic acids; the condensation of some unstable molecules that intervene as reaction intermediates; the decomposition under severe conditions of products such as furfural, sensitive to acid concentration; the decomposition of HMF to formic and levulinic acids; and condensation reactions with lignin [90].

Different studies with traditional woody materials such as eucalypt have shown a pre-extraction of hemicellulose prior to pulping process by hydrothermal processes [91–93]. In the same way, these hydrothermal processes have also been applied to alternative raw materials such as paulownia [55], sunflower stems [54], rice straw [71], tagasaste [25] and *H. funifera* [94].

The influence of the temperature (160–200°C) of the autohydrolysis process applied to paulownia on the composition of the resulting solid and liquid fractions has been studied [55]. It is found that the maximum concentrations of glucose, xylose, arabinose, acetic acid, furfural, HMF and oligomers of the resulting liquid fraction correspond to when operating at maximum temperature.

A similar study carried out with sunflower stems concludes that at 190°C the highest values are obtained for the glucose, xylose and arabinose contents of the liquid fraction of the hydrothermal treatment, with a yield of 24.5%, while the yield of the solid fraction, which can be pulping, is 72.5% [54].

In the case of rice straw, the influence of temperature (150–190°C), time (0 to 20 min after reaching the working temperature) and liquid/solid ratio (6:10) on the hydrothermal treatment, on the lignin content, on the yield of the resulting solid fraction and on the composition of the corresponding liquid phase (glucose, xylose, arabinose and acetic acid) was studied [71]. It follows that in order to obtain high values of glucose (1.92 g/L), xylose (3.97 g/L), arabinose (0.99 g/L) and acetic acid (1.96 g/L) concentrations, it is necessary to operate at high temperature (190°C) and low-medium conditions for time (15 min) and hydromodule (9), which allows capital savings by not operating with the maximum time and using the maximum hydromodule value. The yield obtained for the solid fraction is 88.1%, and the lignin content is 24.43%.

Finally, tagasaste wood was submitted to hydrothermal treatment at 175–185°C [25]. Then, a liquor containing a substantially increased amount of oligomers (between 16.6 and 47.7% as percentages with respect to the content of the raw material in each polymer fraction) is obtained. In the case of *H. funifera*, a sulphuric acid-catalysed hydrothermal treatment (170°C, 0, 20 min after reaching operating temperature, 8 liquid/solid ratio, and 0.3% sulphuric acid), gives a liquid fraction containing 4.62% of glucose, 10.56% of xylose, 1.28% of arabinose, and a solid fraction with a solid yield of 57.0%.

8.2 Pulping of the solid fraction from hydrothermal treatment

Hydrothermal treatments under relatively mild operating conditions (temperature and time) do not cause significant alterations in the cellulose. In this way, solid fractions susceptible to delignification or pulping are obtained [53].

The solid fraction of the hydrothermal treatment of paulownia carried out at 190°C was subjected to pulping process with ethanol following a factorial design of experiments [55]. The conclusion of this work is that operating at 180°C for 30 min and an ethanol concentration of 20%, obtained pulp has acceptable values of Kappa index and viscosity, and their corresponding paper sheets have a brightness of 27.4% ISO, a tensile index of 28.87 Nm/g, a burst index of 1.22 $kPam^2/g$ and a tear index of 1.23 kNm^2/g.

In the case of sunflower stems [54], the solid fraction of a treatment carried out at 180°C is cooked with ethanol (70%, 170°C for 2 h and a hydromodule of 8) giving rise to a pulp with properties (36.3% of pulp yield, 69.1% cellulose, 12.6% hemicellulose, 18.2% lignin, 551 mL/g viscosity, 3.8 km breaking length, 1.23% elongation, 1.15 kN/g burst index and 2.04 mNm^2/g tear index) similar to that obtained by the soda process.

The influence of operating conditions (temperature from 160 to 180°C, time from 30 to 90 min and concentration of diethanolamine from 60 to 80%) on the pulping process of the solid fraction obtained from a hydrothermal treatment of rice straw (carried out at 190°C) on the characteristics of the pulp (yield, Kappa index, viscosity and degree of refining) and of the paper sheets obtained from them (length of rupture, elongation, burst index, tear index and brightness) was also studied [71]. It is deduced that it is convenient to operate at 162.5°C, 60 min and 70% of diethanolamine, since paper sheets present characteristics that deviate little from the optimal ones (less than 8% in the worst case), saving chemical reagents, energy for heating and immobilized capital for the installation, when operating with values of time and the concentration of diethanolamine medium and medium-low temperature, with respect to the maximums considered; likewise the values found for the yield and Kappa index deviate less than 14% with respect to the optimal values.

Autohydrolysed tagasaste wood was also submitted to ethanol and soda pulping procedures [25]. The autohydrolysis prior to ethanol pulping increases yields (53–60%); reduces Kappa index (28.8–34.6), but also viscosity (755–857 mL/g); and decreases paper strength (2.97–5.22 kNm/kg). However, applying a refining process to tagasaste pulp is found to improve its strength-related properties more markedly than in soda pulp from the same material (tensile index of 44 kNm/kg). In the case of *H. funifera*, the samples pretreated with sulphuric acid-catalysed autohydrolysis was subsequently submitted to soda, soda-anthraquinone, ethanolamine, ethylene glycol, diethanolamine and diethyleneglycol [94]. In this case, the best pulp of *H. funifera* pulp is obtained by cooking with 10% NaOH and 1% anthraquinone at 155°C for 30 min, exhibiting good values of yield (48.3%), viscosity (737 mL/g), Kappa index (15.2), tensile index (83.6 Nm/g), stretch (3.8%), burst index (7.34 kN/g) and tear index (3.20 mNm^2/g). Moreover, the soda-anthraquinone pulps of raw material have better properties than the pulps from solid fraction of hydrothermal treatments.

8.3 Use of residual liquors components obtained during pulping

The valorization of lignin-rich black liquors generated from pulping processes is another transition path from the traditional pulp and paper industry to future biorefineries. Generally, residual lignins from black liquors are used to obtain energy for processing plants, mainly by combustion. However, the aromatic structure of lignin makes it a potential source for the production of new bio-based high-value products and chemicals, increasing the sustainability and competitiveness of this pulp and paper industry [86]. Other different fractions of lignin and compounds such as various polysaccharides present in these black liquors, which may not have

specific applications or their transformation into high value-added products may not be profitable, can also be valorized by gasification process [89].

Pulp and paper industry is estimated that moves around 70 million tonnes of lignin annually [95], of which only just over 1 million tonnes are currently marketed, corresponding to lignosulfonates, and which have an established market for use in various uses such as plasticizers and dispersion agents, whereas Kraft lignins are used in the recovery tanks of products from the paper plants themselves and only market around 100.000 tonnes per year. Finally, only a few hundred tonnes of lignins from the soda process come onto the market each year, although this quantity is expected to rise rapidly to around 10,000 tonnes due to the fact that an increasing number of small paper mills, which use agricultural waste and non-wood species to produce cellulose, are introducing lignin recovery processes as the only way to meet environmental effluent treatment specifications.

8.3.1 Lignin applications

Depending on the biomass feedstock, pulping technology and conditions and isolation procedures, lignin has distinct features that may render them useful for different applications. Purity, molar mass and chemical functionalities are some of the characteristics to take into account [96]. So, a detailed knowledge of lignin structure, composition and purity is required in order to determine its behaviour in different potential applications. In this sense, characterization of residual lignins from Kraft and soda-anthraquinone pulping of agriculture residues such as olive tree pruning [97] and wheat or barley straw [98], as well as vegetables like *L. leucocephala*, *C. proliferus*, and *H. funifera* [99, 100], has been carried out.

Among the different characteristics of lignin, its high heterogeneity is one of the most important, which not only affects its structure but also its high distribution of molecular weights (range from 1.000 to 300.000 Da for the same sample) [101]. Therefore, fractionation is one of the ways of obtaining reactive lignins. The preparation of lignin with a defined molecular weight distribution can be carried out by means of different processes: ultrafiltration, selective extraction with solvents and differential precipitation.

The technique of ultrafiltration and nanofiltration is one of the methods being investigated today, with the dual intention of on the one hand reducing the organic load contained in the digestion solution, for its subsequent reincorporation into the pulping process without the loss of inorganic reagents, and on the other obtaining valuable organic resources for use in the development of high-value-added materials. By means of ceramic membranes capable of filtering the residual liquor until the separation of substances smaller than 1 kDa, low molar lignin fractions (1000 g/mol maximum) are obtained. After suitable purification processes, these lignins have a high phenolic hydroxyl content (and/or acid groups), high reactivity and low processing and handling temperatures. In this way, Toledano et al. [102] propose ultrafiltration as a fractionation process to separate different molecular weight lignin fractions from olive tree pruning organosolv black liquor.

Solvent extraction of lignin can be carried out primarily in two ways. In one case, lignin is extracted by a single solvent or a sequential use of multiple solvents. In the other case, a solvent is used to dissolve lignin and then precipitated using chemical (mainly with acids) treatments. Then, Domínguez-Robles et al. [103] used different proportions of acetone (40 and 60%) in water for lignin fractionation of two different sources (organosolv and soda wheat straw lignins), obtaining different fractions with different molar masses and functional groups. Finally, fractionation of the lignins by differential precipitation consists of extracting

different lignin samples as the pH of the solution is gradually lowered. It is the most commonly used method because the simple addition of a strong acid is sufficient, compared to the high costs of the other two methods. However, it has a disadvantage derived from the formation of colloids during precipitation, which can greatly complicate the filtration process. In this sense, Domínguez-Robles et al. [104] have proposed an acid precipitation of wheat straw lignin from soda black liquor using three different inorganic acids (phosphoric, sulphuric and chloride acids) at three different concentration levels, achieving pH values from 11 to 2.

Different lignin applications have been suggested depending on its properties. Then, poorly degraded lignin is employed as dispersants, surfactants and thermoplastic blends or copolymers [105–107] or as an aromatic compound platform to obtain fine chemicals such as polyols, benzene, xylene, toluene, vanillin, ferulic acid, etc. [87]. In contrast, extensively depolymerized lignin, therefore, with a high phenolic content, is suitable for coating, adhesives and composites [108–111]. In this sense, some examples of lignin valorization from alternative raw materials have been reported. Then, Borrero-López et al. [112] showed the possibility to produce olegels from soda lignin obtained from solid state fermented wheat straw; Tejado et al. [113] assayed soda-anthraquinone flax lignin and ethanol-water wild tamarind lignin to phenol-formaldehyde (PF) resin production; Domínguez-Robles et al. [103] investigated the use of soda wheat straw lignin as natural adhesive for the production of high-density fibre board; and Domínguez-Robles et al. [98] analysed Kraft, soda and organosolv wheat straw lignins as a binder material for electrodes in rechargeable lithium batteries.

8.3.2 Gasification of residual liquors components

Any proportion of the agricultural raw material non-suitable for pulp and paper production, in addition to lignin and other compounds such as various polysaccharides obtained in lignin separation processes, may be converted—via pyrolysis—into several types of fuels and petrochemical substitutes [1, 88].

As commented above, different fractions of lignin and other compounds such as various polysaccharides can be obtained in lignin separation processes. Some of these fractions may not have specific applications, or their transformation into high-value-added products may not be profitable, so they may be suitable for a gasification process [89]. This consists of the partial oxidation of the lignocellulosic residues to obtain carbon monoxide, hydrogen, methane, nitrogen and carbonic anhydride mainly, in proportions that depend on the raw material considered and the conditions of the process. Three types of processes can be distinguished: (i) exothermic, using oxygen or air to obtain carbon monoxide or a mixture of carbon monoxide and nitrogen (lean gas); (ii) endothermic, which use water vapor to obtain carbon monoxide and hydrogen (synthesis gas); and (iii) balanced or mixed, using oxygen and water vapor or air and water vapor to obtain carbon monoxide and hydrogen or a mixture of carbon monoxide, hydrogen and nitrogen.

Gasification gases can be used as fuels or to obtain chemicals. Among the latter, those obtained from carbon monoxide (methyl formate, formamide, formic acid, carbonyls, acrylic acid, etc.) and those obtained from carbon monoxide and hydrogen (ammonia, nitric acid, hydrazine, urea, hydrocyanic acid, aldehydes, explosives, etc.) can be distinguished. For example, pyrolysis of soda *H. funifera* lignin gives a gas mixture containing 1.13% H_2, 31.79% CO and 1.86% CH_4 by weight, whereas gasification of the same sample provides a mixture containing 0.18% H_2, 24.50% CO and 17.75% CH_4, also by weight [39].

9. Conclusions

The availability and concentration of wood in areas of easy access, the elevated fibre content, the cost of transport, the ease of storage as well as the stability of the raw material and its performance during the pulping process have supported the use of the wood in the pulp and paper industry. However, due to the numerous advantages of certain alternative raw materials (low-cost fibers, fast growth, low lignin content and fiber morphology, among others), they have proved to be a viable option as a starting raw material for the production of a wide range of different papers. On the other hand, taking into account the concept of lignocellulosic biorefinery, the pulp and paper industry is a good starting point since from its beginnings it not only produced pulp for paper but also energy. However, this industry needs different innovations to adapt even more to this concept. These innovations include the valorization of the extractives and hemicellulosic fractions through extraction prior to the pulping process, the valorization of black liquors through gasification or purification, the valorization of lignocellulosic waste through gasification or other processes such as saccharification and fermentation and also the introduction of new alternative raw materials to wood, as summarized in this work.

Acknowledgements

The authors are grateful to Spain's DGICyT, MICINN, for funding this research by Projects CTQ2016-78729-R and RTI2018-096080-B-C22 and the National Program FPU (Grant Number 454 FPU14/02278). The authors would also like to thank the Community of Madrid (Spain) for funding research through the project P2018/EMT-4348 (SUSTEC-CM).

Conflict of interest

The authors declare no conflict of interest.

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Section 2

Structure

Chapter 3

Influence of Size Classifications on the Structural and Solid-State Characterization of Cellulose Materials

Oluyamo Sunday Samuel and Adekoya Mathew Adefusika

Abstract

Influence of size classification on the properties of cellulose materials has been a subject of neglect over the years. Researchers had the opinion that there exist no significant difference between the characteristics of bulk particulate materials and sizes of their constituents. However, it has been affirmed that increase in crystallinity index, increases the strength properties of cellulose materials. Therefore, there is need to establish the influence of size classification as it affects the properties of cellulose materials. This study focused on the influence of size classifications on the structural and solid State characterization of cellulose obtained from wood dust. The structure of the cellulose composed principally of crystalline cellulose (I and II) and amorphous cellulose. The crystallinity and the inter-planar spacing revealed different structural properties for the two size classifications. The elemental composition consists of Carbon (C), Oxygen (O), Sodium (Na) and Chlorine (Cl) with Carbon having the highest percentage. The surface morphology of the isolated cellulose appears fiber -like for the size classifications examined. The isolated cellulose exhibits good mechanical and solid state properties with promising applications in device utilization. Within the limit of the research, size classification is noted to influence the characteristics of the cellulose materials.

Keywords: cellulose, size classification, crystallinity, structural characterization, solid state properties

1. Introduction

Wood is one of the hard fibrous structural tissue and abundant natural materials on earth. It is an organic material with a composition of cellulose, hemicellulose, and lignin which has been used for many years as a basic need in construction materials and other purposes [1–3]. The effect of particle sizes on the thermal and mechanical properties of wood had gained popularity in recent years due to its importance in improving the insulation properties of materials [4].

The environmental degeneration caused by solid waste from different activities had been a challenge to the waste management throughout the world. Nigeria, with a population over 180 million as at 2018, has the largest producer of residue and solid waste in Africa.

One important waste from wood is the wood dust. This by-product usually constitutes menace to man and his environment as the material is usually disposed of sometimes indiscriminately in different locations which most often constitute environmental pollution [3]. Studied had shown that if well harness, wood dust may attract economic values to the country rather than the usual pollution.

Cellulose is a formation of the composite, a versatile and widely natural-based material in nature that consists of glucose molecules which has various uses to man and used by man for thousands of years as building material, or energy source. It is a polymer that contains crystallites and displayed para-crystalline morphology [5]. The linear molecules are linked laterally by hydrogen bonds to form linear bundles which give rise to the crystalline structure. It has become one of the material's serving mankind for centuries and major subject in the history of polymer science in developing nation's economic and determination of polymeric crystal structures. Today, it is an important material which is widely used in industries (paper, pharm, food, etc.) and it has also served as an economic output in many countries of the world.

It has a general formula $(C_6H_{10}O_5)_n$, found in plants as microfibril and isolated from wide range of species from higher plants such as wood to green algae [6, 7]. It is a polymer that composed of amorphous and crystalline regions which varies depending on the plant species. Cellulose can be isolated from plants and non-plant sources. Isolation can be from a variety of sources such as (cotton, hemp, jute, sugarcane bagasse, rice straw, durra stalk, groundnut shell, etc.). The composition of chemical and cell dimensions depend on plants, origin and isolation method.

Cellulose can exist in its derivative forms namely rayon, cellulose acetate, cellulose nitrate, and ethyl cellulose. It is the main component of about half to one-third of plant tissues and categorized into three namely, α-cellulose, β-cellulose and γ-cellulose [8]. Solubility and precipitation nature are the major category upon which cellulose is based. In plants, it composed of a linear homopolymer of 1,4-β-glucopyranose units associated with hydrogen bonding and as a semicrystalline structure that is found and circulated from highly developed trees to primitive organisms [9, 10]. The chemical repeating unit is the β-1,4-linked glucose and structural repeat is β-cellobiose [11]. The repeating unit in cellulose is the anhydrocellulobiose and half a degree of polymerization (DP) gives rise to the number of repeating per molecule. It is higher in native cellulose than other group of cellulose which is usually due to the purification procedures. Van der Waals forces and hydrogen bonds tightly bound the glucose to each other to form crystalline structures called Elementary fibril. This consists of around 40 glucan chains, 40 Å widths, 30 Å tick and 100 Å long [12].

There are two main regions found in cellulose fibers. These are crystalline and amorphous. Crystalline are regions with a high order of microfibrils while less order of microfibrils is called amorphous. Amorphous material are materials that lack definite shape or formless. These regions vary proportions among the plants species. For this reason, the properties of cellulose materials depend largely on the material. The versatility of cellulose makes it important in it usage. The method of isolation or treatment, sources of cellulose give rise to different polymorphs with only a few exceptions. This may be due to molecular orientation and hydrogen bonding [3]. The polymorphic forms can be grouped into four: I, II, III, and IV which can be determined by XRD pattern. The first model of the crystalline structure of native cellulose of a monoclinic unit cell was developed by Mayer and Mish [13]. All native cellulose crystalline consists of CI with only a few exceptions and exhibits the same crystalline structure. It composed of two distinct allomorphs Iα(triclinic) and Iβ(monoclinic) depending on the biological origins. Iαstructure is metastable and dominated polymorph for most algae and bacteria, whereas Iβis dominant for

higher plant cell wall cellulose and in tunicates. Iα can be converted to Iβin alkaline solution by hydrothermal treatments at a temperature of 260°C. Native cellulose is organized in fibrils, which are represented by the association of cellulose molecules. The native cellulose of higher plants possesses a high degree of polymerization (DP) of up to 10.000 β- anhydroglucose residues [14]. This indicates that the molecular weight is above 1.5 million (g/mol). The increase in crystalline regions increases the rigidity and decreases the elasticity of the polymeric substance. The accessibility of cellulose molecules affects the ratio of the crystalline region and the amorphous region in the cellulose structure [15–18].

Modification of cellulose is identified by addition of crystalline allomorphs, II, III, and IV. Mercerization (Alkali treatment) and regeneration (solubilization and subsequent recrystallization) are the two main methods of preparing celluloses II.

However, when the time and the amount of chemical introduced in the treatment of native cellulose are not restricted to a predetermined pattern, it results to the production of cellulose (I and II).

Treatments with liquid ammonia with celluloses I and II produces celluloses III_I and III_{II}. In addition, heating of III_I and III_{III} produces celluloses IV_I and IV_{II} [19, 20].

Structure and morphology of cellulose give a clearer picture of understanding the behavior of cellulose during chemical modification. It also gives understanding on the morphological changing of materials after hydrolysis. There are three structural levels that describe the complex structure of cellulose. These are molecular level (molecular mass, potential intramolecular and chemical constitution), supramolecular levels (crystal structure and intermolecular hydrogen-bonding system) and morphological levels (organization of crystals into microfibrils, the existence of different cell wall layers in the fibers, and other cellulose morphologies). These levels determine both chemical and mechanical properties of cellulose.

One of the parameters used to study the total cellulose present in cellulosic materials is the crystallinity Index [21–25]. In addition, the presence of crystallinity in cellulose contributes greatly to its physical, chemical and mechanical properties [22, 26, 27]. The crystallinity index of cellulosic material has an influence on the stiffness, rigidity and the strength of the material. The increase in the crystallinity index (CI) is associated with high potential mechanical property and increase reinforcing capability of a cellulose material. Several techniques have been used to measure the crystallinity index. These techniques include; XRD, solid-state ^{13}C NMR, infrared (IR) spectroscopy and Raman spectroscopy. The crystallinity index has been used for years in interpreting cellulose changes after treatment (physicochemical and biological). It has been reported that crystallinity index varies significantly depending on the measurement method. Among these methods, XRD is the mostly employed. Three different methods are commonly employed in calculating the CI from the raw spectrographic data on the XRD [23, 28–32]. The first method was established by [33], proposed for cellulose I. In this method, consideration was based on the ratio of the peak height between the intensity of the crystalline and the total intensity after subtraction of background signal at 18° (2θ) degrees. The idea behind the Segal equation is that there are no crystalline peaks near 18° for cellulose I, therefore any observed intensity would be due to amorphism region. [33] found a maximum at 18° but other authors have found maxima at values even higher, such as 20–22° [34]. Thus, [30] showed that a perfectly crystalline cellulose would still only give a Segal CrI value of 92% when the crystal is approximately the size of a good cotton crystal (FWHM = 1.7°). Furthermore, for a 100% amorphism, a pattern would have to be completely flat; something that never happens. Because there is no fundamentally sound method that is well proven for crystallinity determination, Segal method results remain fairly simple to obtain and give helpful information. Segal with other methods (peak de-convolution method, and amorphous subtraction) all has fundamental flaws.

Available researches into isolation of cellulose focused mostly on thermal and mechanical properties. Hitherto, there is no available information about the influence of size classifications on the structural and solid state characterization of cellulose materials. Although, some school of thought have the notion that size classification has no significant influence on the properties of cellulose materials.

This effect is however well established for wood bulk and particle materials [2, 35, 36]. It is important to note that cellulose particles are presented in differing sizes in material processes and applications.

Guarea thompsonii is a species of plant from the family of *Meliaceae*. It is a hard wood that is naturally durable, resistant to impregnation, medium shrinkage and has a desired compressive advantage for concrete as a structural material.

Therefore in the present research, cellulose particles isolated from *Guarea thompsonii* are classified into two categories while the structural and solid state characterizations are determined.

2. Method

2.1 Material

Wood specie (*Guarea thompsonii*) was selected from a sawmill in the area of research and authenticated at the Department of Forestry and Wood Technology, Federal University of Technology, Akure (FUTA), Nigeria. The sample was processed into wood dust and sieved into two size classifications (424–599 μm and 600–849 μm) at the Department of Materials and Metallurgical Engineering (FUTA). Analytical grades chemical used were Sodium chlorite ($NaClO_2$) (Sigma-Aldrich, Steinheim, Germany), sodium hydroxide (NaOH) (British Drug House, Darmstadt, Germany), and acetic acid (Sigma-Aldrich, Steinheim, Germany).

2.2 Pre-treatment of material

The obtained sample after processed to wood dust were sieved using a Wiley mechanical sieve shaker (Pascal Engineering, Sussex, England) and the wood dust with two size classifications (425–599 μm and 600–849 μm) were obtained.

2.3 Pulping procedure

The two classifications of the wood dust were pulped in a water bath at 90°C under atmospheric pressure with the ratio of wood to liquor of 1:20, using 20% NaOH for 90 minutes. The pulped was obtained by filtration after digestion and washed thoroughly with water until it was free of residual alkali. The pulp yield was oven-dried at 105°C to a constant weight and stored for further processing.

2.4 Bleaching procedure

1000 mL of hot distilled water, 12 g of $NaClO_2$, and 3 mL of acetic acid were added to approximately 20 g of oven-dried pulp sample in a 2-L Erlenmeyer flask. The flask was covered and the mixture heated in a water bath at 70°C for 30 minutes with intermittent stirring. After the first 30 minutes in the water bath, another 12 g of $NaClO_2$ and 3 mL of acetic acid was added with intermittent stirring and sustained for another 30 minutes before switching the bath off. The sample was allowed to settle down for 24 hours in the water bath. After digestion, the bleach was obtained by filtration and washed thoroughly with water until it was free of residual alkali and chlorine. The obtained sample was dried at 105°C to a constant weight.

3. Theoretical consideration

3.1 X-ray diffraction method

One of the reliable techniques to determine the crystal structure of any material is the X-ray diffraction (XRD). The crystalline phase of the material can be obtained by examine the diffraction patterns.

The XRD patterns of the two size classifications were obtained using a Philips PW 3710 X'pert Pro diffractometer (Philips Analyical, Almelo, Netherlands) with a Cu-Kα monochromator of wavelength, λ = 1.540598 Åm, in the range of 10–50° (2θ) generated at 15 kV. All experiments were repeated twice and duplicate X-ray analyses were performed.

The Interplanar spacing (d-spacing) was calculated from the Bragg equation using [37, 38].

$$n\lambda = 2d\sin\theta \tag{1}$$

where n is the order of reflection, λ is the wavelength of the incident X-rays (m), d is the interplanar spacing of the crystal, and θ is the Bragg's angle (°).

The crystallinity index (CrI, %) was obtained from the XRD diffraction pattern. The patterns where engaged to determine the crystallinity parameters of cellulose derived from different size classifications of the wood dust samples. The crystallinity index was calculated according to [33], as followed Eq. (2),

$$CrI = \frac{I_{020} - I_{am}}{I_{020}} \times 100 \tag{2}$$

where I_{020} is the maximum intensity of the lattice diffraction and I_{am} is the low intensity peak at the amorphous region of the baseline at 2θ, approximately 18°.

The average crystallite sizes (L) in the isolated cellulose samples were calculated from the XRD line broadening using the Scherrer's equation

$$L = \frac{K \times \lambda}{H \times Cos\theta} \tag{3}$$

where K is a constant whose value is given as 0.91, θ is the Bragg's angle (°), and $H$is the intensity of the full width at half maximum (FWHM)corresponding to a high intensity peak of the diffraction plane.

The Surface chain W occupying a layer that is approximately 0.57 nm thick is given as

$$W = \frac{(L - 2h)^2}{L^2} \tag{4}$$

The determination of the monoclinic and triclinic structure for the two size classifications was calculated from the method developed by [37]. The isolated cellulose was categorized into Iα or Iβ predominant form by employing discriminant analysis. The function which discriminates between them (the monoclinic and triclinic structure) is given as:

$$Z = 1693d_1 - 902d_2 - 549 \tag{5}$$

where $d_1(nm)$ is the d-spacing of the Iβ $(1\bar{1}0)$ peak and $d_2(nm)$ is the d-spacing of the Iβ (110) peak. Z > 0 indicates that cellulose is rich in the Iα form and Z < 0 indicates that Iβ is the predominant form.

3.2 FTIR spectroscopy measurement

Fourier transform infrared (FTIR) spectroscopy is a mature analytical technique employed to examine the microscopic area of a materials. FTIR spectra of powder samples of cellulose were obtained using a Thermo Nicolet 5700 FTIR spectrometer (Nicolet, Madison, WI, USA). The Spectra were acquired over the range 500–4000 cm^{-1} at a resolution of 2 cm^{-1} for samples in pellet form prepared by mixing 1.0 mg of powder samples with 200.0 mg KBr spectroscopic grade. The spectra obtained were used for rapid information about the chemical structure of the cellulose samples.

3.3 Scanning Electron MicroscopeMeasurement

The morphological characterization of composite materials can be investigated by the Scanning Electron Microscopy (SEM). It is a popular and powerful technique for imaging the surface of a material (surface topology, morphology and chemical composition) [39]. The image resolution depends on the property of the electron and the electron probe interaction with the specimen. SEM analysis was performed on the isolated cellulose obtained of the two size classifications using FEI NOVA 200 NanoSEM equipment (FEI Company, Hillsboro Oregon, USA) with an accelerating voltage of up to 30 kV and a resolution of up to 1 nm to observe the morphology of the cellulose obtained.

4. Results

4.1 X-ray diffraction (XRD) of cellulose

X-ray diffraction (XRD) is a method generally used to determine the crystallinity of materials, interplanar distances etc. The free hydroxyl groups present in the cellulose macromolecules are likely to be involved in a number of intramolecular and intermolecular hydrogen bonds, which may give rise to various ordered crystalline arrangements [40, 41].

In order to evaluate and determine the intensities of the diffraction bands, establish the crystalline and amorphous areas and determine the crystallite sizes of the cellulose, the X-ray diffractogram (**Figure 1**) was adopted. The crystallographic planes from the diffractogram are labeled according to the cellulose structure.

The diffractograms showed that 12.414–14.755°C (2θ), 16.910–17.127°C (2θ), 18.01°C (2θ), 22.107–22.591°C (2θ) and 34.908–35.075°C (2θ) reflections were assigned to reflection assigned to the $(1\bar{1}0)$ crystallographic plane, (110) crystallographic plane, amorphous phase, (020) crystallographic plane and (004) crystallographic [37].

The X-ray diffraction pattern generated to evaluate the crystallinity of the cellulose samples in **Figure 1** showed a peak at 2θ = 22.591 and 22.308°C for *Guarea thompsonii of* 425–599 μm and 600–849 μm of size classifications. These distinct peaks obtained for the XRD of the samples is an indication that the cellulose are crystalline in nature.

The diffraction peak of cellulose shown around 2θ = 12.414–14.755°C was assigned to the crystalline plane of $(1\bar{1}0)$ for cellulose type I and II. Moreover, it is interesting that cellulose showed the doublet in the intensity of the main peaks (2θ = 20.2–20.4°C) which were also assigned to the crystalline planes of (110) for cellulose type I and II. This shows that mixture of cellulose I and II has been the most stable structure of technical relevance and can be produced by two processes:

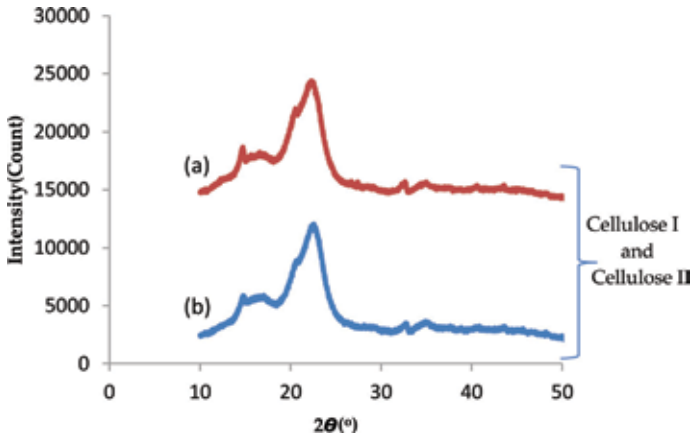

Figure 1.
XRD of isolated cellulose obtained for different size classifications for Guarea thompsonii *(a) 425–599 μm (b) 600–849 μm.*

regeneration (solubilization and recrystallization) and mercerization (aqueous sodium hydroxide treatments) [27].

From this result, it can be concluded that alkali treatment in pretreatment process led to the change of cellulose allomorph from type I; the native cellulose found in nature, to type I and II; the regenerated cellulose which is the most stable crystalline form. Moreover, the acid hydrolysis which removed the amorphous region out from the cellulose led to re-crystallization was the main cause to obtain the obvious peaks of doublet intensity at 2θ = 20.2–20.4°C.

4.1.1 Investigation of the crystallites structure of cellulose by XRD

The average dimensions of the elementary crystallites perpendicular to the $(1\bar{1}0)$, (110) and (020) crystallographic planes of the mixture of cellulose I and cellulose II with amorphous can be calculated using the Scherrer's equation, by measuring the full widths at half maximum (FWHM) of the different diffraction peaks, assuming that the finite size of crystallites dominate the broadening of the X-ray reflections. These values have to be considered as a lower bound since instrumental broadening and possible imperfections of the crystal lattice are neglected by the method.

The results obtained from the XRD profiles of samples in **Figure 1** are presented in **Tables 1** and **2**. In this, only the length perpendicular to the (020) plane could be calculated since the $(1\bar{1}0)$ and (110) reflections in the XRD profile overlapped each other. An average crystallite length of about 1.742 nm and 1.748 nm were obtained *Guarea thompsonii* of 425 μm −599 μm and 600 μm −800 μm size classifications.

The inter-planar spacing (d-spacing) values of the cellulose spectra for most prominent peak with crystal plane of preferred orientation along the (020) were 3.933 Å m and 3.982 Å m for *Guarea thompsonii of* 425–599 μm and 600–849 μm

Classification (μm)	**$(1\bar{1}0)$**		**(110)**		**(004)**		**(020)**	
	2θ	**d (Å)**	**2θ**	**d (Å)**	**2θ**	**d (Å)**	**2θ**	**d (Å)**
425–599	14.754	5.999	17.044	5.198	34.908	2.568	22.591	3.933
600–849	14.721	6.013	16.726	5.293	34.992	2.562	22.308	3.982

Table 1.
Band position (2θ) and d-spacing of crystalline cellulose of Guarea thompsonii.

Classification (μm)	$L(020)$ (nm)	Cr.I	W	Z-Values
425–599	1.750	56.89	0.12	−2.229
600–849	1.749	54.09	0.12	−8.428

Table 2.
Parameters obtained from the XRD analysis of the cellulose samples of Guarea thompsonii.

respectively. Other values of inter planar spacing for the remaining crystallographic planes were depicted in **Table 1**.

The crystalline interior chains W for the isolated samples were calculated by the fraction of cellulose chains contained in the interior of the crystallites. It was estimated as 0.12 for the two samples isolated in **Table 2**. This indicated that the proportion of crystallite interior chains, W, is similar for both samples examined.

Table 2 showed the calculated Z- values for the two size classifications (*Guarea thompsonii).* The values of the estimated isolated cellulose obtained wereless than zero ($Z < 0$). This shows that the cellulose samples belong to Iβ (monoclinic) dominant.

The degree of cellulose crystallinity is one of the most important crystalline structure parameters. The crystallinity index (CrI) calculated according to [33] showed that the crystallinity of cellulose obtained for 425–599 μm size classification was higher than that obtained for 600 μm to 849 μm size classification. The CrI value are 56.89 and 54.09% for *Guarea thompsonii* cellulose obtained from different size classifications of 425–599 μm and 600–849 μm, respectively. In contrast, 60.4% for *Eucalpyptus grandis* and 62.6% for *Pinus taeda* were also recorded in a study of structural characteristics and thermal properties of native cellulose [41]. This high percentage in crystallinity index might be associated with the reduction in the corresponding amorphous state of the material due to the probable dissociation of the bonds as a result of pulping. This can also due to significant increase in surface area-to-volume ratio of the molecules, and with the crystalline size of the samples reducing to nanoscale. The difference in the values obtained may be due to the chemical treatments for purification and crystalline or amorphous standard [42]. Moreover, high crystallinity index is associated to increase stiffness, rigidity and strength of the isolated cellulose obtained. As a result, sample with 425–599 μm size classifications has high potential mechanical property and reinforcing capability than sample with 600–849 μm size classifications [43].

4.2 Fourier infra-red spectroscopy (FTIR) of cellulose

Figure 2 shows the functional groups and the band positions for the isolated cellulose prepared by Fourier Infra-red Spectroscopy (FTIR).

Two main absorbance regions were pronounced by the samples considered. The absorbance regions were in the range of approximately 1110–870 cm^{-1} and 3630–2960 cm^{-1} wave number. The intensities regions were high in 425–599 μm size classifications than 600–849 μm due to the surface to volume ratio of the atom exposed to the FTIR machine.

The strong dominant broad peaks in the region are obtained from 3630 to 2960 cm^{-1} which is commonly observed for hydrogen. The absorption around 3320 cm^{-1} corresponds to the vibration of H- bonded OH groups while a peak near 2890 cm^{-1} is assigned to C-H stretching vibration. These inter- and intra- molecular hydrogen bonds in the cellulose has a strong influence on the physical and mechanical properties of the cellulose. The band near 1160 cm^{-1} is representative of the anti-symmetric bridge stretching of C—O—C groups. The band near 1300 cm^{-1}

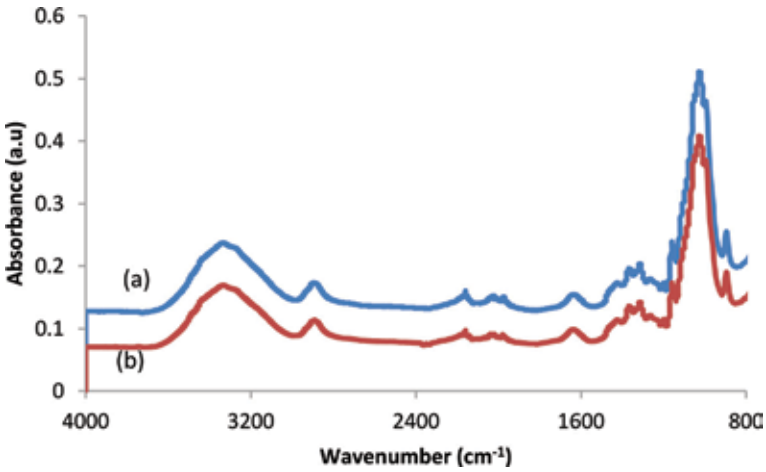

Figure 2.
FTIR spectra of isolated cellulose of different size classifications for Guarea thompsonii *(a) 425–599 μm (b) 600–849 μm.*

could be ascribed to CH_2 wagging vibrations in the cellulose while the absorption at 1200 cm^{-1} belongs to the C—O—H in-plane bending at C-6 [44, 45]. The band peak at 1610 cm^{-1} can be assigned as OH bending due to absorbed water because the OH bending mode is strongly perturbed by bound water [46]. Strong peaks at 1020 and 1090 $cm^{-1}$are indicative of C—O stretching at C-3, C—C stretching and C—O stretching at C–6 [45]. Finally, the absorbance peak observed at 899 cm^{-1} was assigned to the symmetric C—O—C stretching of β (1⟶4)-glycosidic linkage.

4.3 Scanning Electron Microscopy (SEM)

The Scanning Electron Microscopy (SEM) was an effective method for investigating the morphological characteristics of the composites. **Figures 3** and **4** show the SEM image of *Guarea thompsonii* of isolated cellulose obtained.

As shown in **Figures 3** and **4**, SEM images of the isolated cellulose of two samples depicted strings of fibers. This was in agreement with other authors' findings, although the length of the fiber may differ [47–52].

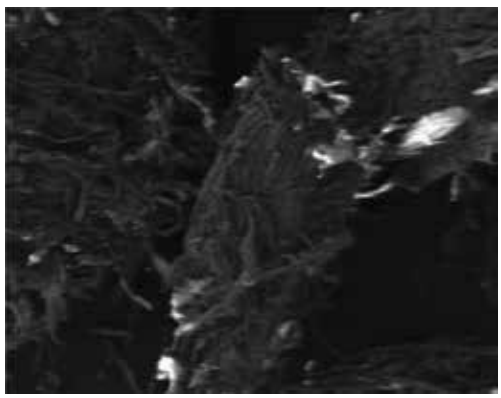

Figure 3.
SEM image of isolated cellulose for Guarea thompsonii *of 425–599 μm size classifications.*

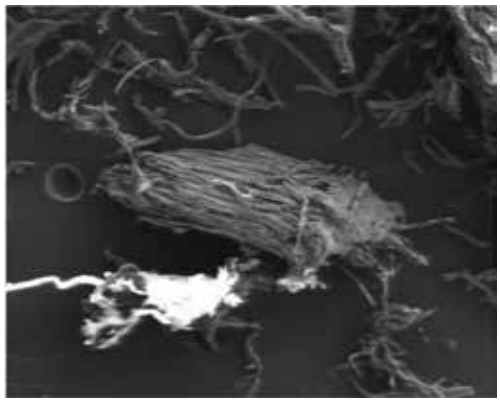

Figure 4.
SEM image of isolated cellulose for Guarea thompsonii *of 600–899 µm size classifications.*

4.4 Energy dispersive X-ray diffraction (EDX)

Energy dispersive X-ray diffraction (EDX) attached with SEM was used for elemental analysis of isolated cellulose. The EDX spectra (as shown in **Figures 5** and **6**) peaks correspond to the energy levels for which the carbon (C), oxygen (O),

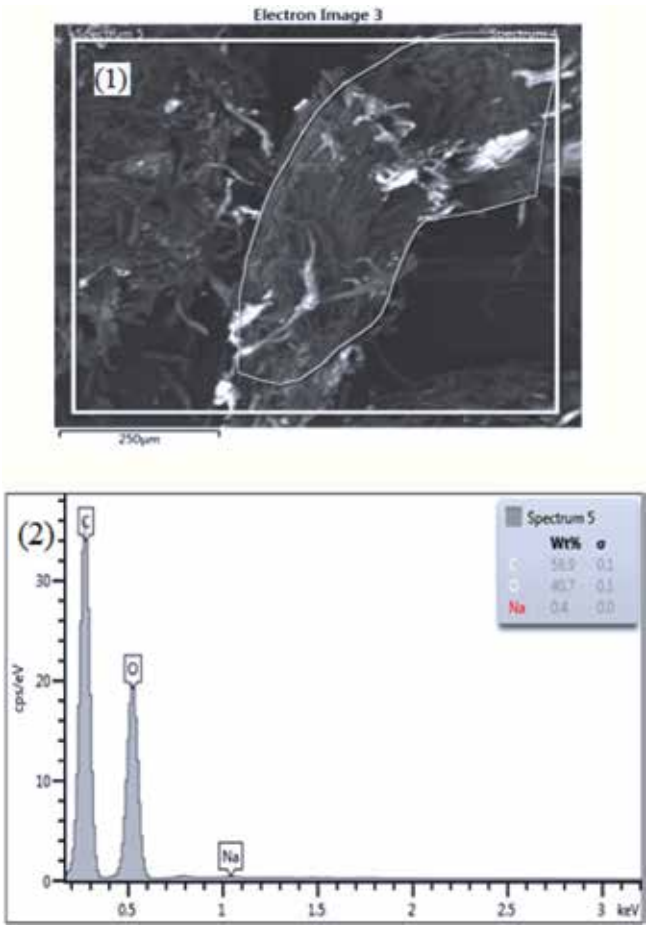

Figure 5.
(1) SEM image of isolated cellulose for Guarea thompsonii *of 425–599 µm size classifications; and (2) EDX spectrum of isolated cellulose for* Guarea thompsoniiof *425–599 µm size classifications.*

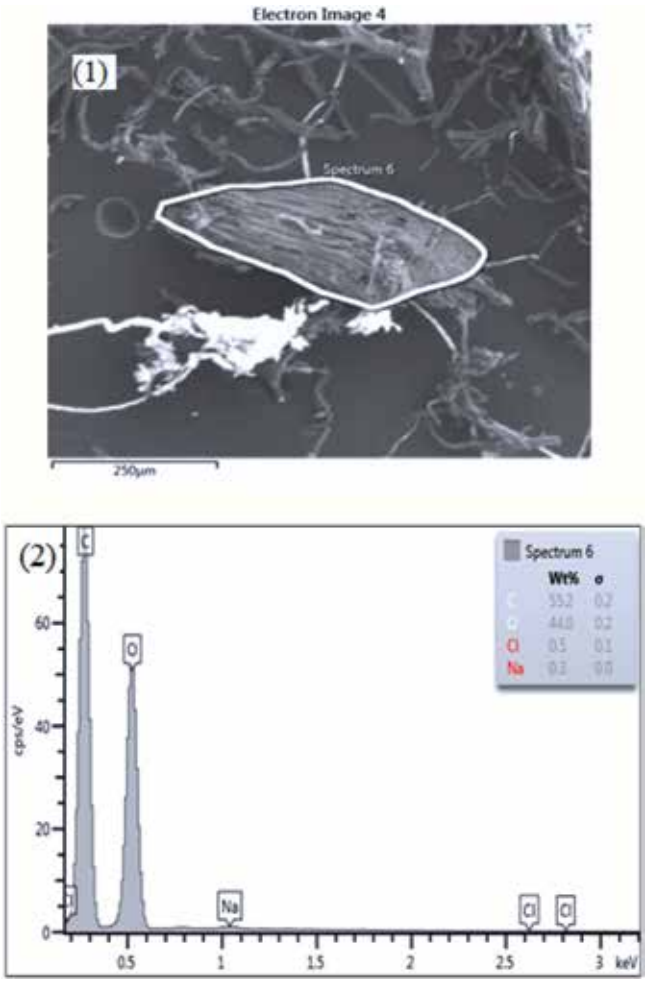

Figure 6.
(1) SEM image of isolated cellulose for Guarea thompsonii *of 600–899 μm size classifications; and (2) EDX spectrum of isolated cellulose for* Guarea thompsoniiof *600–899 μmsize classifications.*

chlorine (Cl), and Sodium (Na) were identified with carbon having the highest percentage among the elements observed in the spectra. The elemental compositions for the samples with size classifications 425–599 μm were 58.9 wt % carbon, 40.7 wt % oxygen and 0.4 wt % sodium and 55.2 wt % carbon, 44.0 wt % oxygen, 0.5 wt % chlorine and 0.3 wt % sodium for 600–899 μm. The impurities present could have been due to the $NaClO_2$ that was used in the bleaching process.

5. Conclusion

Wood dusts from two size classifications were isolated from XRD, FTIR, SEM and EDX. The isolated cellulose obtained is the mixture of cellulose I and II and amorphous with fiber-like shape. The two celluloses examined has a preferred orientations along the (020) plane for the most prominent peaks with a crystallinity index of 56.89% for size range 425–599 μm and 54.09% for size range 600–849 μm. The crystallinity index is high in 425–599 μm size classifications compare to 600–849 μm. This indicates that the strength properties is higher in 425–599 μm size classification and have reinforcement ability than 600–849 μm. It is important to

note that size classifications played a major role on the crystallinity index on the sample examined which is a basic factor that determine how high the mechanical properties is in a material.

Acknowledgements

We express our gratitude to the Material and Engineering Research Institute (MERI), Sheffield Hallam University (UK) for their support and excellent work done during the analyses of the powder material; The Federal University of Technology, Akure, Nigeria and Edo University, Iyamho, Nigeria are also appreciated for their support during the preparation of the samples.

Conflict of interest

No conflict of interest.

Author details

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Chapter 4

An Update on Overview of Cellulose, Its Structure and Applications

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Abstract

Cellulose $(C_6H_{10}O_5)_n$ is one of the most ubiquitous organic polymers on the planet. It is a significant structural component of the primary cell wall of green plants, various forms of algae and oomycetes. It is a polysaccharide consisting of a linear chain of several hundred to many thousands of $\beta(1 \rightarrow 4)$ linked D-glucose units. There are various extraction procedures for cellulose developed by using different processes like oxidation, etherification and esterification which convert the prepared celluloses in to cellulose derivatives. Since it is a non-toxic, bio-degradable polymer with high tensile and compressive strength, it has widespread use in various fields such as nanotechnology, pharmaceutical industry, food industry, cosmetics, textile and paper industry, drug-delivery systems in treating cancer and other diseases. Micro-crystalline cellulose in particular is among the most frequently used cellulose derivatives in the food, cosmetics, pharma industry, etc. and is an important excipient due to its binding and tableting properties, characterized by its plasticity and cohesiveness when wet. Bacterial cellulose's high dispensability, tasteless and odourless nature provides it with lot of industrial applications. Currently, about half of the waste produced in India contains about 50% cellulose which can be used productively. This chapter deals with the chemistry of cellulose, its extraction and its properties which help various industries to make the most of it.

Keywords: properties, chemistry, types, microcrystalline cellulose, cellulose nanocrystals, cellulose nanofibres, extraction, characterization, NMR, SEM, FTIR, BJH, biomedical applications, pharmaceutical applications, renewable energy applications, waste management, drug delivery, wound healing, scaffolding, implants, biofuels, consumables

1. Introduction

Cellulose is the most abundant biopolymer available in nature, since it is one of the major components of the cell walls of most of the plants [1]. It is a homopolymer of anhydroglucose, with the glucose residues linked in a ß-1,4 fashion [2]. Cell walls of plant cells attribute their mechanical strength to cellulose. Cellulose owes its

structural properties to the fact that it can retain a semi-crystalline state of aggregation even in an aqueous environment, which is unusual for a polysaccharide [3, 4].

As far as cellulose based products are concerned, paperboard and paper are the most commonly used ones [5]. Smaller amounts of cellulose when processed under appropriate conditions, can be converted to a wide variety of derivatives, these can be used in manufacture of few commercial products like cellophane and rayon [6].

Since cellulose is a homopolymer of a glucose derivative, it is a great source of fermentable sugar. It is cultivated in the form of energy crops for the production of ethanol, ethers, acetic acid, etc. Besides energy requirements the industrial demands of cellulose are fulfilled by wood pulp and cotton crops [7].

Cellulose also fulfils the dietary requirements of some animals, particularly ruminants and termite, they can digest cellulose with help of symbiotic microorganisms present in their gut, while some organisms secrete a group of enzymes called cellulases to aid the degradation of cellulose molecules [8]. Human beings are unable to digest cellulose due to lack of cellulases, thus cellulose acts as a hydrophilic bulking agent for faeces and potentially aids in defecation [9].

2. Overview

Among the various raw materials which nature has placed at our disposal for industrial purposes, cellulose has from time immemorial occupied a prominent position. Its abundance is attributed to the constant photosynthetic cycles taking place in higher plants, which can synthesize around 1011 ± 1012 tons of cellulose in a rather pure form. Since time immemorial it has served mankind either as a construction material or as a versatile starting material for chemical reactions for the production of artificial cellulose based threads and biofilms as well as for production of a variety of stable cellulose derivatives which are used for various industrial and domestic applications [10]. Cellulose was used for various biochemical conversions even before its polymeric nature was recognized and well understood. In the process of recognizing and understanding its polymeric structure, it led to the discovery of nitrocellulose, synthesis of organo-soluble cellulose acetate and the preparation of Schweizer's reagent (first cellulose solvent). Another area of great interest was nanocellulose, the nanostructure of cellulose has proven to be advantageous because of its applications in a variety of fields [11, 12].

Due to such great economical significance of tree cellulose, the current scientific focus is more towards cellulose biosynthesis as it is still not well understood [13]. Most of the recent findings concerning the molecular mechanism of cellulose biosynthesis in higher plants resulted from research in model herbaceous plants and fibre crops and have been reviewed recently. All these aspects trigger a researchers' curiosity and makes them want to dig deeper and unveil other properties and applications of cellulose.

3. Chemistry of cellulose

The Cellulose is made up of a D-glucose unit at one end and a C4-OH group, the non-reducing end, while the terminating group is C1-OH, the reducing end with aldehyde structure. Some technical celluloses contain extra carbonyl and carboxy groups, like the bleached wood pulp. The molecular structure is responsible for its significant properties: Chirality, hydrophilicity, degradability and chemical variability due to high reactivity from the donor group—OH. The superior hydrogen bonds add crystalline fibre structures to cellulose. **Figure 1** presents the four

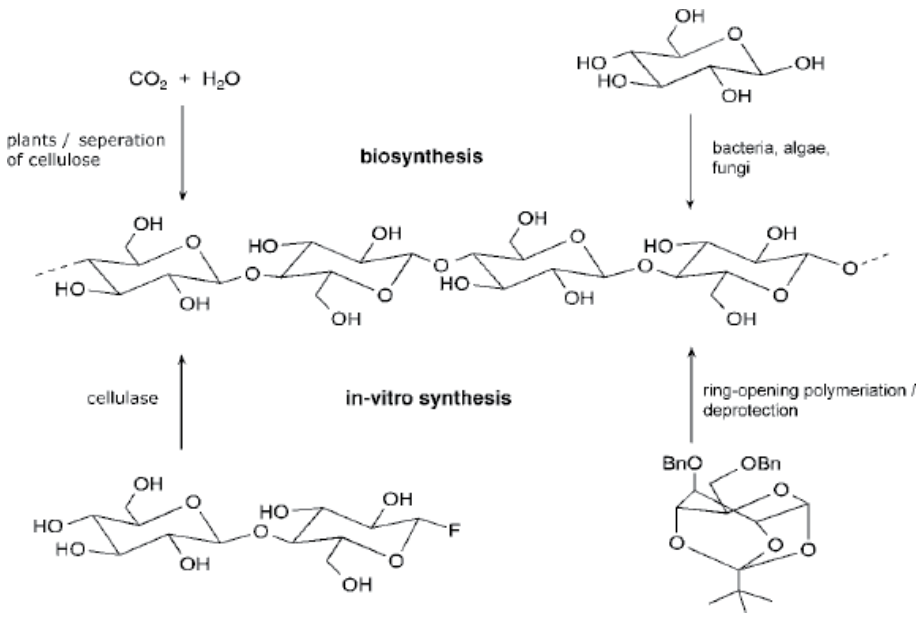

Figure 1.
Major pathways of formation of cellulose [14].

different pathways which determine the major processing routes. The most famous and highly used pathway is the manufacture of cellulose from plants. It is established that cellulose is found in its purest form from the seed hairs of cotton. The wood cellulose, on the other hand forms a composite with lignin and other polysaccharides, which is further separated by large scale chemical pulping and purification processes. Cellulose can be derived from algae, some specific bacteria, and fungi, apart from most plants. The supramolecular structures are used for research on cellulose structures, reactivity, and crystallinity with further note on development of biomaterials and new substances. Cyanobacteria are known to biosynthesize cellulose for nearly 3.5 billion years [15]. The first synthesis of cellulose in vitro is reported as the cellucellulase—cellulose formation by catalyzed cellobiosyl fluoride and the chemosynthesis was processed in a ring opening polymerization of the D-glucose moieties [16]. A lot of research is ongoing in the field and study of cellulose over the past decade. The structure and properties of cellulose are quintessential to perform modifications and processing of cellulose on the whole.

4. Properties of cellulose

The structure of cellulose has been constantly a subject requiring intensive research as it is formed by the hydrogen bonds between the network of hydroxy groups [17]. The progress was for more than a 100 years of intensive development on structure analysis methods like electron microscopy, X-ray diffraction and high

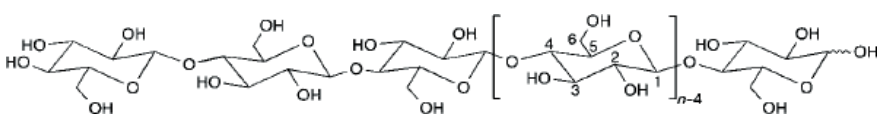

Figure 2.
Molecular structure of cellulose (n = DP, degree of polymerization) [14].

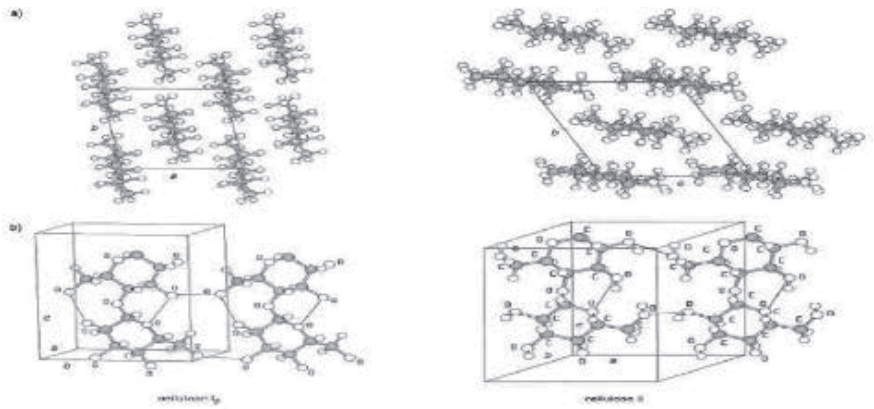

Figure 3.
The crystal structures of cellulose I and II: (a) projection of the unit cell along the a-b plane and (b) projection of UC parallel to (100) lattice plane, cellulose I and (010) lattice plane, cellulose II [14].

resolution solid state NMR spectroscopy. The complete detailed analysis is required for the procedures of synthetic reactions and cellulose based manmade products with extensive applications. The structure of cellulose as depicted in **Figure 2** consists of hydroxyl groups of β-1,4-glucan cellulose at C2, C3 and C6. The CH_2OH group is positioned relative to the C4 and C5 bonds along with a shear relativity with O5–C5 bonds. The solid state is equally likely to be represented in the crystalline (high order) and amorphous (low order). The crystal structure in particular is determined by the X-ray diffraction using a monoclinic unit cell which is made up of two cellulose chains in a parallel orientation and two fold screw axis [18]. The investigations with respect to the electron microbeam diffraction, combined X-ray and neutron diffraction have clearly indicated that the cellulose crystalline structures have a triclinic and monoclinic unit cell. The schematic representations of the I_β crystal structure, in **Figure 3**, indicate the two intramolecular chain-stiffening hydrogen bonds. The recent researches on the I_β crystal structure have different H-bonds and different conformations of neighbouring chains. The thermodynamically stable cellulose II can occur in other forms of crystal structures and is the most stable form of cellulose. The cellulose I can be treated with aqueous sodium hydroxide to form cellulose II.

5. Types of cellulose

5.1 Bacterial cellulose

Although cellulose is mainly produced by plants, many bacteria, especially those belonging to the genus *Gluconacetobacter are involved in the production of a* very peculiar form of cellulose with mechanical and structural properties that can be exploited in numerous applications. Bacterial cellulose are usually produced by *Gluconacetobacter hansenii* UCP1619 using the Hestrin-Schramm (HS) medium. But there are few limitations associated with bacterial cellulose like the production cost is high, use of expensive culture media, poor yields, downstream processing, and operating costs. Bacterial cellulose can also be produced by bacteria from genera *Sarcina*, and *Agrobacterium* [19]. *Bacterial cellulose produced by aerobic bacteria has unique physiochemical properties compared to plant cellulose* [20].

5.2 Cellulose acetate

Cellulose acetate is an important ester of cellulose. Cellulose acetate can be used for great varies of applications like for films, membranes or fibres, depending on the way it has been processed. A special field for using cellulose acetate is the synthesis of porous, spherical particles, so called cellulose beads [21].

5.3 Ethylcellulose

Ethylcellulose (EC) is a derivative of cellulose in which some of the hydroxyl groups on the repeating anhydroglucose units are modified into ethyl ether groups, largely called as non-ionic ethyl ether of cellulose. Ethylcellulose (EC) based microencapsulated drug delivery systems are being studied to achieve extended drug release and to protect the core substance from degradation [22].

5.4 Hydroxypropyl cellulose (HPC)

Hydroxypropyl cellulose (HPC) is one of the derivatives of cellulose which is soluble in both water and organic solvent. It can be used as a lubricant. It can also be used for the treatment of keratoconjunctivitis sicca, corneal erosions neuroparalytic keratitis etc. It is also used as a lubricant for patients having artificial eye [23–25].

6. Extraction and characterization of cellulose

Cellulose is one of the most abundant biomass materials in nature possessing some promising properties. In our work, let us look into the various procedures involved in extracting cellulose from different sources. The sources available are many which can be broadly classified into Agro-waste, Domestic-waste (and other means such as wood, plant and paper). The processes involved include usual chemical procedures like alkaline extraction, bleaching, acid hydrolysis and chlorination. The final products can be characterized by using techniques like thermogravimetric analysis (TGA), infrared spectroscopy (FTIR), X-ray diffraction (XRD), differential scanning calorimetry (DSC) and scanning electronic microscopy (SEM). Purified cellulose can be obtained successfully using the above mentioned procedures. In our study, we will be concentrating on the extraction of cellulose from agricultural residues and plants [26–28] (**Figure 4**).

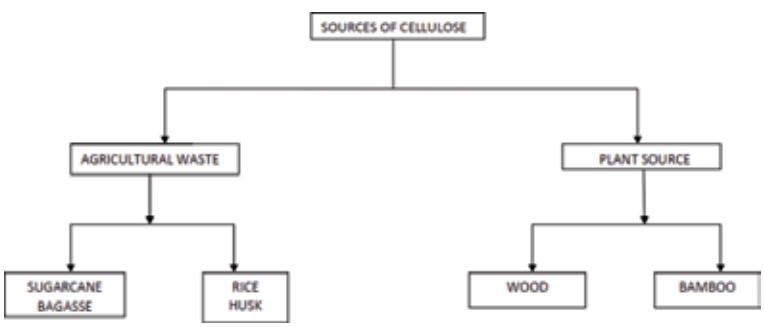

Figure 4.
Different sources of cellulose extraction.

6.1 Extraction of cellulose from agricultural residues

6.1.1 Extraction of cellulose from sugarcane bagasse

Sugarcane bagasse (SCB) is a key agricultural residue which has grown to be the point of interest in recent times. The extraction of cellulose from SCB was investigated using steam explosion and xylanase based environmentally friendly pretreatment for reducing chemical bleaching and to determine the characteristics of cellulose from sugarcane bagasse. SCB is a low value agriculture residue and about 40–50% of bagasse is the glucose polymer cellulose. There are several approaches to pretreat lignocellulosic materials to extract cellulose such as steam explosion, solvent extraction and alkaline treatment.

6.1.1.1 Methodology

Cellulose is extracted from SCB by using steam explosion and xylanase pretreatment and bleaching process. The dried SCB treated with steam explosion at a pressure 13 bar (195°C) for 15 minutes to obtain steam exploded SCB fibres. Then, the steam exploded SCB is treated with 20 μg of xylanase using fibre to liquor ratio of 1:10 for 1 hour at 50°C under constant agitation. Then, dried steam exploded SCB is treated with xylanase (fibre to liquor ratio of 1:10) and then bleached with 0.7% sodium chlorite ($NaClO_2$) adjusted to a pH of 4 by the addition of weak acetic acid at 70°C for 1 hour. Sodium chlorite and acetic acid at the same loading were added to the reaction every 1 hour till the cellulose turns white. The cellulose fibres thus obtained would then filtrated, washed with distilled water until the pH of water neutral and dried at 55°C for 24 hours. The steam explosion process includes saturating the dry material with steam at elevated pressure and temperature followed by sudden release of pressure, resulting in substantial resistance of degraded lignin fragments, facilitates the release of lignin, which is then readily available for further bleaching and thereby increases the efficiency of the bleaching process Thus, the use of enzymes to treat pulp before applying chemical bleaching help to reduce chemical required in bleaching stage, breakdown of lignocellulosic structure, hydrolysis of the hemicelluloses fraction, depolymerization of the lignin components and defibrillization. In terms of bleaching treatments, chlorine-based chemicals were typically used for bleaching process resulting in chemical hazard released into environmental. Xylanases are glycosyl hydrolase that can catalyse the hydrolysis of β-1, 4-glycosidic bonds in xylan by means of double displacement mechanical reaction. This treatment improves accessibility of bleaching chemical to the pulp by decreasing diffusion [29] (**Table 1**).

6.1.2 Extraction of cellulose from rice husk

Rice is the most cultivated cereal crop in the world. Rice husk is a major agrowaste that is generated in huge quantities. It accounts for 20% of the 600 million tons of paddy produced worldwide. People often dispose the rice husk waste using open burning which is an environmental threat causing damage to the land and the surrounding area in which it is disposed. This rice husk can be put into best use by exploitation of its cellulose content. Rice husk comprises of 33% cellulose, 26% hemicellulose, and 7% lignin. Thus, the use of rice husk as the primary source for producing cellulose fibres and nanocrystals is efficient. The strategy is to extract cellulose fibres from the rice husk using alkali and bleaching treatments.

Fibres	α-Cellulose (in %)	Hemicellulose (in %)	Lignin (in %)
Untreated SCB	44.5	21.8	22.5
Steam-exploded SCB	65.7	9.9	18.3
Steam exploded with xylanase treated	66.3	8.8	18.6
Bleached fibre	89.3	4.3	1.5

Table 1.
Extraction and characterization of cellulose from sugarcane bagasse by using environmental friendly method [29].

6.1.2.1 Methodology

Sodium hydroxide (99% purity) is used for alkaline treatment. Sodium chlorite, acetic acid and NaOH are used as bleaching agents while sulphuric acid is used for hydrolysis.

6.1.2.2 Preparation of cellulose nanocrystals from rice husk

1. **Treatment with alkali**: Cellulose is purified by treating with Alkali to remove lignin and hemicellulose on or after rice husk grits. The pulverised rice husk is preserved through an alkali solution (4% by weight of NaOH). The combination is poured into a plump bottom flask and action is performed at reflux temperature intended for 2 hours. The solid is formerly filtered and carry away numerous times by means of distilled water. This treatment is frequent thrice.

2. **Bleaching process:** Subsequent the alkali action, bleaching progression is completed by totalling a buffer solution of acetic acid, aqueous chlorite (1.7% by weightiness) and purified water at reflux (by means of a silicon emollient soak at 100–130°C) for 4 hours. The assortment is then permitted to cool and the additional distilled liquid is filtered. The bleaching procedure is performed four periods.

3. **Acid hydrolysis:** The acid hydrolysis action is performed on the fibres subsequently alkali action and decolourising at a temperature of 50°C by means of 10.0 mol L^{-1} of preheated sulphuric acid intended for 40 minutes underneath non-stop stirring. The fibre content varieties from 4 to 6% by heaviness. The hydrolysed substantial is eroded by centrifugation at 10000 rpm at 10°C for 10 minutes. This centrifugation stage is repeated many times before the suspension is dialyzed in contradiction of purified water for several days until persistent pH in the range of 5–6 is attained. The subsequent suspension is formerly sonicated for 30 minutes previously it is frozen for additional use [30].

6.2 Extraction of cellulose from plants

6.2.1 Extraction of cellulose from bamboo

This extraction method involves use of Bamboo fibres as a raw material for cellulose extraction. The chemicals used to produce cellulose nanofibres are toluene, ethanol, hydrogen peroxide, acetic acid glacial, titanium (IV) oxide, and sodium hydroxide. All the chemicals used here are of analytical grade.

6.1.2.3 Methodology

1. **Bamboo fibre preparation:** Green bamboo Culm of 1 m length is prepared. It is then ground using a planner machine to produce small chips and powder form excluding the internodes. This chips and powder mixture is put into an oven at 70°C for 72 hours to dry. The oven dried sample is ground and then sieved using 600 µm size sieve. The 600 µm mesh size fibres are used for the synthesis of cellulose fibre. This sample is then labelled as green bamboo fibre (GBF).

2. **Preparation of cellulose from bamboo fibre and de-waxing of bamboo fibre**: The 400 mL toluene and 200 mL of ethyl alcohol are filled into a round flask to produce toluene-ethanol of ratio 2:1. The round flask is placed on a heating element. A Soxhlet extractor is placed on top of the boiling flask and fixed firmly using a retort stand. About 10 grams of GBF is scooped into a membrane tube and placed into the extraction thimble. A Liebig condenser is placed on top of the extractor and then fixed firmly. The temperature of the heating element is observed using a digital thermometer and it is maintained at 250°C. The extraction process is continued till the colour mixture disappears. The process takes 2 hours with approximately 10–12 cycles of extraction. The extraction thimble is taken out using tweezers. The product is transferred into a beaker and stirred using a glass rod while adding toluene-ethanol mixture. The final product is filtered using a filter paper placed on a funnel. It is then distributed evenly using glass rod on a filter paper. It is then placed in an oven set to temperature 70°C for drying overnight and is kept for delignification processes. The dried sample is identified as dewaxed bamboo fibre (DBF).

3. **Delignification of bamboo fibre:** The delignification solution is prepared using 82.3 g of 35% by weight of hydrogen peroxide (H_2O_2) and 106.2 g of 99.8% by weight of acetic acid (CH_3COOH) in the present of titanium (IV) oxide as catalyst. Thirty grams of dry DBF sample is weighed and immersed into delignification solution contained in a round bottom flask. The flask is placed on the heating element and heated to 130°C. After 2 hours, the heater is switched off and cooled to room temperature. The treated product is then filtered using Buchner flask and rinsed with de-ionized (DI) water until the pH level reaches 7 and dried at 70°C for 1 day. The dried sample is placed in a bottle and kept in a cool and dark place for alkaline treatment. The sample so obtained is delignified bamboo fibre (DLBF).

4. **Mercerization:** DLBF is finally immersed in an alkaline solution in order to dissolve the pectin and hemicelluloses. 6% by weight of sodium hydroxide is used to treat the DLBF in a flask at room temperature. The mixture is stirred using auto shaker at 150 rpm, heated to 80°C for 2 hours, and stopped after 8 hours of proper stirring. The mixture is then rinsed continuously with de-ionized water until the product reaches pH 7. The treated product is finally filtered using Buchner flask, rinsed with de-ionized water until the pH level reaches 7, and freeze-dried at −85°C for 2 days [31].

6.1.2.4 Characterization of cellulose extracted from bamboo

After extraction of cellulose, it is generally characterized by using the following techniques:

1. **Fourier transform infrared spectroscopy (FTIR)**: The infrared spectra obtained using a FTIR Spectrometer model IRAFFINITY-1 CE. The spectra were to be taken at a resolution of 4 cm^{-1}, with a total of 60 scans for each sample. The transmittance range of the scans was 600–4000 cm^{-1}. The composition changes in GBF were investigated by FTIR spectroscopy (**Figure 5**).

2. **Scanning electron microscopy (SEM):** The bamboo fibre samples was vacuum-dried for 24 hours at 70°C, pressed onto a carbon tape adhered to a sample holder surface, and sputtered with titanium. Imaging of each sample was done using Hitachi M-3030 scanning electron microscope. All images were taken at an accelerating voltage of 5 kV with a magnification of 1500 time [33].

3. **Barrett-Joyner-Halenda (BJH) analysis:** In order to do BJH analysis the bamboo fibre sample is dried for 24 hours at 70°C and inserted into a capillary tube. The outgas had an approximately 7 hour duration with final outgas temperature of 350°C. After outgas process, the sample was analysed using Nova Quantachrome 4200e automated gas sorption instrument for 1.5 hours across a wide range of relative pressures at constant temperature (77 K) using liquid nitrogen (**Figure 6**).

4. **Thermogravimetric analysis (TGA):** Dynamic thermogravimetric measurements were performed using a Shimadzu DTG 60H instrument. The temperature programs for dynamic tests was run from ambient temperature 25–700°C. All measurements was made under a nitrogen flow (20 mL/min), while keeping a constant heating rate of 10°C min^{-1} and using an aluminium crucible with a pinhole (**Figure 7**).

6.1.2.5 Characterization of cellulose extracted from rice husk fibres

The chemical conformation of rice husk at individual phase of action is originate to be giving to the approaches conveyed by the Technical Association of Pulp and Paper Industry (TAPPI). The cellulose and hemicellulose contents are retrieved conferring to TAPPI standard T203 OS-74 though the lignin content is restrained according to TAPPI normal T222 OS-83. The silica ash content is calculated by

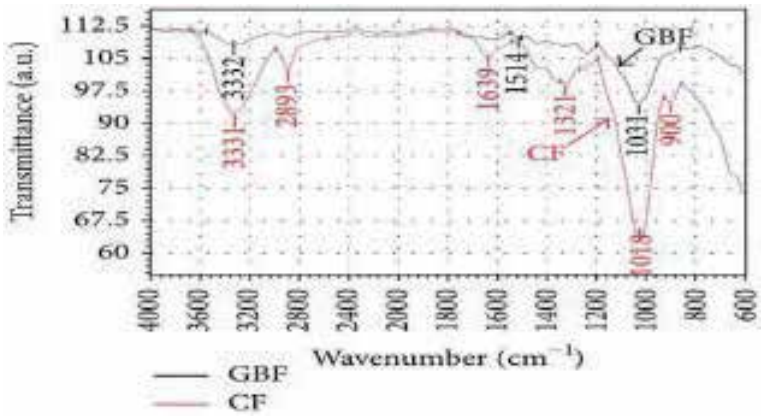

Figure 5.
Comparative differences of FTIR peaks into green bamboo fibre (GBF) and cellulose fibre (CF). The peak intensity at 1514 cm^{-1} from the spectrum of the GBF is credited to the C═C stretching vibration in the aromatic ring of lignin. Yet, the cellulose fibre did not show the C═C stretching at that region. This is an indication that the lignin was well removed by chemical process [30, 32].

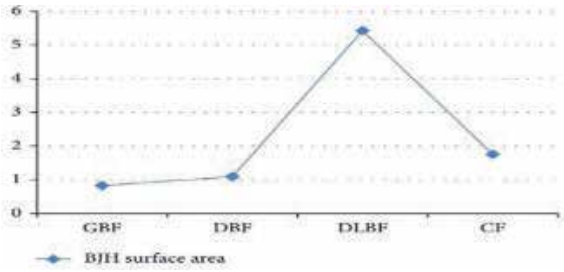

Figure 6.
BJH surface area for four samples. It was noted that there was an increase in the BJH surface area of GBF and a decrease after DLBF. The decreased surface area of cellulose fibre might be a result of the mechanical grinding process that creates smaller cellulose fibre thus reduced the surface area. This result also indicates that mechanical grinding should be avoided to obtain higher BJH surface area. The final cellulose fibre showed that BJH surface area is two times greater than the initial green bamboo fibre [30, 32].

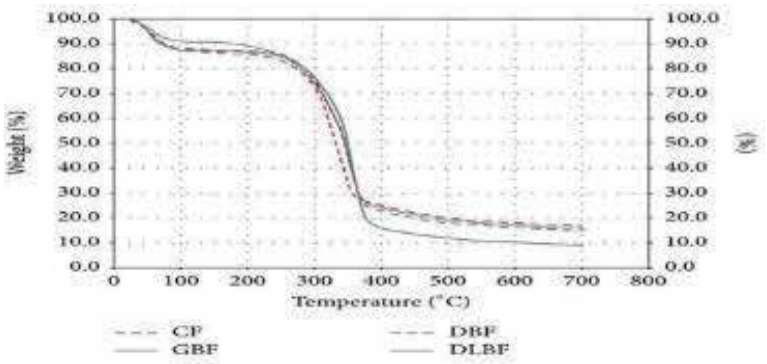

Figure 7.
The thermogravimetric analysis of (GBF, DBF, DLBF and CF) green bamboo fibre, dewaxed bamboo fibre, delignified bamboo fibre and cellulose fibre, respectively. The weight loss rate was obtained from the derivative thermogravimetric (DTG) data. The intersection of tangents drawn from thermogravimetric curve, one before inflection caused by the degradation and another from the cellulose degradation step indicates the onset degradation temperature [30].

means of the thermogravimetric examination (TGA) data. The arrangement of silica ash was found at temperatures 900°C as the residual ash at this point can be credited to the silica ash [30, 33, 34].

6.1.2.6 Scanning electron microscopy (SEM)

It is utilized to detect the superficial morphology of the rice husk fibres. The effect of the numerous chemical treatments is evaluated using an assessment of the raw, alkali treated, and bleached fibres. Rice husk fibres is reserved on the aluminium stub and raised in the oven at 60°C. The samples are then covered with gold by means of a vacuum sputter coater (model SC 500). The width of the gold layer is approximately 0.01–0.1 μm. The fast-tracking voltage is 15 kV. Transmission electron microscopy (TEM) is used to regulate the proportions of the cellulose nanocrystals attained from the rice husk fibres. A drop of a diluted suspension (1% by weight) is placed on the superficial of a clean copper grid and covered through a thin carbon film. As for contrast in TEM, the cellulose nanocrystals are undesirably stained in a 2% by weight solution of uranyl acetate for 10 seconds then carried away by means of 50% by weight of sieved alcohol. Later sample is dehydrated at ambient temperature before TEM examination is approved out through an accelerating voltage of 80 kV (**Figure 8**).

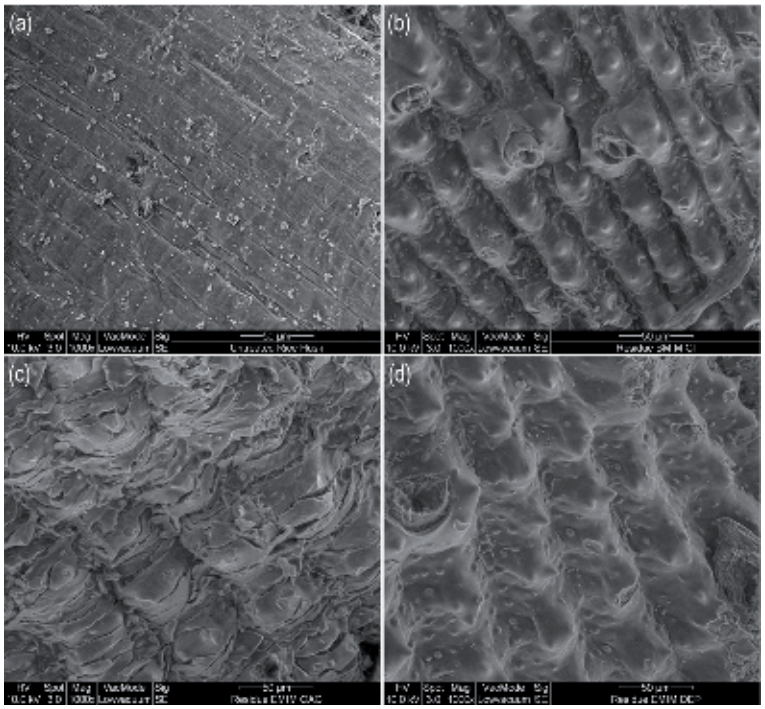

Figure 8.
SEM images of (a) untreated rice husk and rice husk residues of (b) [BMIM]Cl, 1-butyl-3-methylimidazolium chloride; (c) [EMIM] OAc, 1-ethyl-3-methylimidazolium acetate; and (d) [EMIM] DEP, 1-ethyl-3-methylimidazolium diethyl phosphate pretreatments [34].

6.1.2.7 Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared spectra are noted with the assistance of a Perkin-Elmer FTIR spectrophotometer. Un-treated, alkali-treated, blanched, and acid-hydrolysed rice husk fibres models are examined. Models are excellently crushed and mixed with potassium bromide. The combination is then flattened to pellet form. FTIR spectral investigation is achieved inside the wave number range of 400–4000 cm^{-1} (**Figure 9**).

6.1.2.8 X-ray diffraction (XRD)

X-ray diffraction is applied to identify the crystallinity of rice husk grits after numerous extraction methods. Each sample/material in the arrangement of milled powder is set aside on the sample vessel and levelled to attain complete and unvarying X-ray exposure. The trials are examined with the assistance of an X-ray diffractometer at room temperature (RT) by means of a monochromatic CuKα energy source (λ = 0.1539 nm) in the step-scan approach with a 2θ angle extending from 10 to 50°C with a stage of 0.04 and scanning period of 5 minutes. To characterize the crystallinity of the several samples, the crystallinity index CrI, is created based on the mirrored intensity data (**Figure 10**).

6.1.2.9 Thermogravimetric analysis (TGA)

The thermal stability of the different samples is determined by TGA measurements performed using a Mettler Toledo thermogravimetric analyser. The quantity

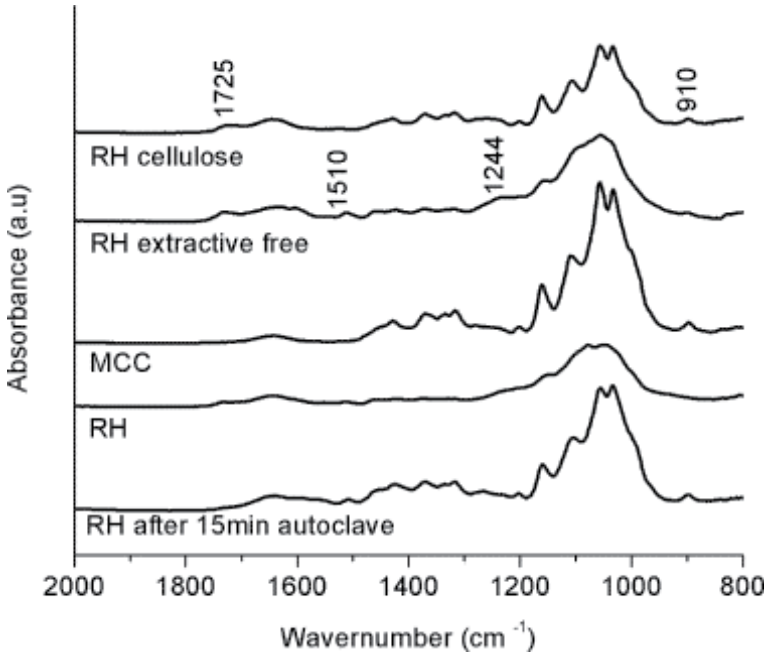

Figure 9.
FTIR spectra for RH (rice husk), RH extractive-free, alkaline treated RH (RH after 15 minutes autoclave), RH cellulose (after 30 minutes bleaching) and commercial microcrystalline cellulose (MCC) in the range from 2000 to 800 cm^{-1} [34].

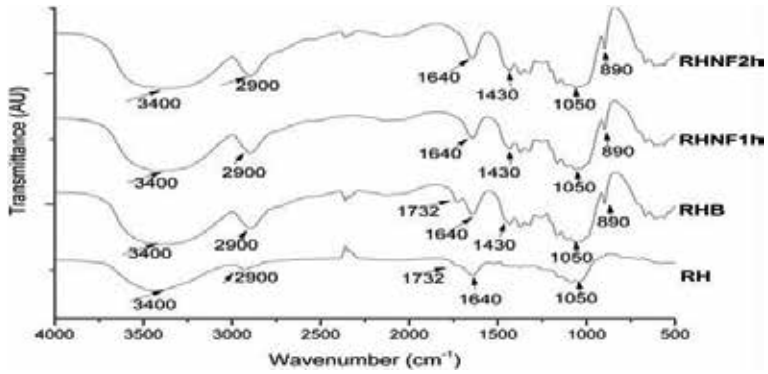

Figure 10.
X-ray diffractograms of rice husk (RH), bleached rice husk (RHB), rise husk nanofibrin after 1 and 2 hour of bleaching (RHNF1h and RHNF2h, respectively) [34].

of sample involved for individual measurement remained approximately 1 mg. Each measurement is achieved under a nitrogen based generated atmosphere through a gas flow of 10 mL min^{-1} by means of heating the substantial from room temperature to 900°C at a heating rate of 10°C min^{-1} [34].

7. Applications of cellulose

Cellulose is the most richly found organic polymer, as it is a major structural component of the primary cell wall of green plants, several algae and oomycetes. Cellulose is found in large quantities in frequently used materials like cotton (90%),

Wood (50%) and dried Hemp (57%). It has numerous applications in various fields, but it is most frequently used in the manufacturing of paper and cardboard or in derivative products like cellophane and rayon. It is also a major component of textiles made from cotton or linen. Further, its use is seen in the pharmaceutical industry as inactive fillers in drugs, in the form of powdered cellulose and microcrystalline cellulose. However, one of the most important uses of cellulose is in the production of biofuel and in the food Industry. This will be elaborated further in the following sections [33].

7.1 Use of cellulose in renewable energy

7.1.1 Biofuel

A drastic increase in the population of the world coupled with an exponential increase in technological advancements and need of the people, fossil fuels are being rapidly depleted. At such an hour, the term sustainable development comes into play. In order to develop sustainably, it is important to switch to a fuel that is more clean, green and more cost-effective. One such alternative, is cellulose derived biofuel. There are numerous advantages of using biofuel, the first and foremost being cost-effective. Recent studies have shown that due to increase in demand, the cost of biofuel is decreasing as ethanol costs lesser than petrol and diesel. Further, there is a significant reduction in the carbon emission. The raw material used for biofuel is simply a substrate that has cellulose in it. Since cellulose is so widely abundant, the cost is significantly lower.

The ethanol obtained from cellulose is used as an alternative substrate in the production of biofuel. It is considered a superior source due to its high energy efficiency and low cost as compared to other sources. This is a very good source for renewable energy as it is found most abundantly in stalks, leaves and stem of green plants. Other sources for ethanol include, feedstocks, including wheat straw, rice straw, sawdust, forest thinning and grasses perennial grasses and switchgrass. Cellulose can be broken down into fermentable sugars by using the fungus *Trichoderma reesei* or by using acid to convert them first into sugars and then into gas. The gut of termites also can be utilized for this purpose. Further, a group of bacteria collectively referred to as methanogens have the ability to digest cellulose and produce carbon di oxide and methane, which is further processed. One group of such bacteria called methanobacteria grow anaerobically on cellulosic matter and degrade it to produce methane. They are also found in the rumen of cattle and the dung of cattle. As is seen from this, it is quite easy to obtain a substrate for biogas production especially by using waste cellulosic material. Therefore, it is important that to utilize even the apparent waste material to ensure a reduction in wastage and optimum usage of its potential [34, 35].

7.2 Use of cellulose in consumables

Cellulose has numerous applications in the field of pharmaceuticals and food technology. Modifying the structure of cellulose with other chemical groups results in the production of structures that have better bio-compatibility, flexibility, stability, emulsifying effects. Further, cellulose being indigestible by human beings, tend to have zero calorific value and can thus have been added in food to serve several purposes. Compounds like HPMC, sodium carboxymethyl cellulose, hydroxyethyl cellulose and others are commonly utilized in the pharmaceutical industry and food technology industry. Some of these uses are enumerated below:

7.2.1 Hydroxypropyl methylcellulose

HPMC is widely utilized in the pharmaceutical industry not only because it is safe and nontoxic but also because it does not get engrossed orally and does not upsurge the energy of foods. It is utilized as a film-forming agent, thickener, blocker, sustained-release agent, blending agent and suspending agent in many dosage forms, thus forming the numerous pharmaceutical preparation consistently discrete, tough short of being wrecked due to sustained release effects or steady emulsion without stratification. It is regularly used as a matrix, adhesives, frame ingredients, the film creating material or in the creation of sustained or controlled release microcapsules and pellets [36].

7.2.2 Sodium carboxymethyl cellulose

It used as an emulsion stabilizer in injections, adhesion and film-forming materials which have proved to be effective in controlling wound infections and can reduce postoperative oedema and wound stimulation phenomena. Animal experiments have shown that sodium carboxymethyl cellulose is a safe and reliable carrier of anticancer drugs [37].

7.2.3 Hydroxyethyl cellulose

In ice cream, frozen milk drinks, it is added as a stabilizer to extend the storage life and improve the overflow property. It is also used as the stabilizer of beer foam.

7.2.4 Food

Due to its unique physical and chemical properties and its behaviour in water, it is today being increasingly used a food additive to improve the bulk and fibre content of foods without having a major impact on the flavour of the food. Since it is indigestible by humans, it has no caloric value and is thus used in excessive amounts in diet foods to create a sensation of fullness both physical and physiology without having consumed too many calories. It is also widely used an emulsifier and a thickening agent in whipped cream, sauces and ice cream [38].

7.3 Biomedical and pharmaceutical applications of cellulose

Cellulose, with its properties, as discussed in previous sections of this manuscript, is extensively used in the field of biomedicine and pharmaceuticals. The cost of several pharmaceutical products is extremely high due to production factors such as high cost, difficulty in procuring the material, complicated processing steps etc. These problems can be remedied by the use of cellulose, which is found abundantly in nature. The most productive use of cellulose would be the utilization of plant based waste materials which are produced in bulk by many industries such as the sugar production industry as well as in minor quantities by households. The applications highlighted below could be brought to mainstream commercial use with the appropriate optimization techniques and novel modifications to the various steps of the production and processing of cellulosic material.

7.3.1 Cellulose in coating of solid dosage forms and compressibility enhancers

Solid dosage forms including pills, tablets, granules, pellets, microcapsules and spherules can be coated, usually with the aim to protect the drug from adverse environmental factors such as humidity, oxygen, enzymatic or acidic degradation.

Coating may also be used to facilitate drug delivery systems with altered release mechanisms such as delayed release, extended release, step-by-step release, pulsatile release and sustained release. Derivatives of cellulose such as esters and ethers are also extensively used as coating materials. In the process of solid dosage form manufacture by direct compression, a problem that frequently occurs is low compactability of the drug, this is more seen more frequently when the amount of drug in the formulation exceeds 30%. Many attempts are being made to reduce the price of the final product by experimenting with various starting materials and test conditions [39].

7.3.2 Cellulose in drug delivery

From the advent of novel drug delivery systems, cellulose based models seemed like strong candidates due to their projected benefits. Since then various advances have been made with the aim to bring its use to common practice. There are still many hurdles to cross before this becomes a reality. Cellulose based drug delivery is an important step in green and sustainable pharmacy which focuses on toxicity reduction, biodegradability and less hazardous synthesis with respect to drugs and drug delivery systems. A very brief overview of the primary ways in which it is used is provided here. Cellulose nanocrystals (CNCs) have the potential to acquire a negative charge during hydrolysis. This coupled with their large surface area allow them to bind ionizable drugs such as tetracycline and doxorubicin permitting optimum dosing control. Sites for surface modification for multiple chemicals are provided by the multitude of surface hydroxyl groups. This is used in case of non-ionized or hydrophobic drugs which do not generally bind to cellulose. The open pore structure and high surface area of CNC based aerogels provide increased drug loading capacity and drug bioavailability. Extremely porous aerogel scaffolds were reported to attain sustained drug release [40].

Cellulose derivatives have also been researched in terms of drug delivery. For instance, cellulose acetate has been successfully used in several HIV drugs, five flavonoids, one pain reliever and two antibiotics among others. Hydroxypropyl methylcellulose has been used in oral drug delivery formulations [41].

7.3.3 Cellulose in scaffolding

Scaffolds are materials that have been engineered to cause desirable cellular interactions to contribute to the formation of new functional tissues for medical purposes by providing the microenvironment required by cells to proliferate, migrate and differentiate. It contributes the geometrical basis and building blocks to provide cell attachment. *Gluconacetobacter xylinus* sourced nanocellulose is an emerging biomaterial for this purpose. Bacterial nanocellulose has a very high affinity for water and therefore displays properties similar to those of hydrogels which provides an ideal environment to host cells. Studies have confirmed that human smooth muscle cells, bone forming osteoblasts and fibroblasts and human embryonic kidney cells can grow in the presence of bacterial cellulose scaffolds. The main challenge in the production of these scaffolds seems to be biodegradability as the cellulose, the enzyme required to breakdown cellulose is not present in humans. This property was reported to be enhanced by periodate oxidation [42].

7.3.4 Cellulose in biomedical implants

BNC is specifically nondegradable under physiological conditions and has been shown to be biocompatible. These properties further impart durable mechanical properties and long-term chemical stability which make it an exciting candidate for application in this field:

- Cardiovascular implants: Bacterial cellulose has an important application in artificial blood vessels. Compared to the material generally used for vascular grafts, these materials show less thrombosis and occlusion. Heparin hybridized bacterial nanocellulose scaffolds with anticoagulant properties have potential use in vascular tissue engineering. Potential use of BC in the production of heart valve replacements has been explored [43, 44].

- Bone and connective tissue repair: Nanocellulose are promising materials for the culture of various cells including osteoblasts and chondroblasts indicating that they have potential for bone tissue regeneration and healing. A membrane of BC and hydroxyapatite was developed as biomaterial for potential bone regeneration, which delivered prone growth of osteoblast cells, high level of alkaline phosphatase activity and greater bone nodule formation. It was also found that HAp crystals are partially substituted with carbonate resembling natural bones [45–47].

8. Conclusion

From the chapter we can conclude that cellulose is a highly versatile polymer which is easy to manufacture and extract. Its application in multiple fields has been discussed above. With increasing population, demand and technological innovations, renewable energy is gradually becoming imperative aspect of resource conservation and overall environmental health. Although various other polymers can be utilized for consumables, biomedical and pharmaceutical applications, the marked advantage of cellulose is that it is a biodegradable and environmentally friendly material. The intensive research on the chemistry of the compound, has resulted in the production of a wide variety of biodegradable products with a plethora of applications. An improved information of the many structural levels in which cellulose partakes will allow us to understand better practise of this exceptional and metastable molecular assembly produced by plant metabolic pathways. We have placed emphasis on the diverse applications of cellulose to promote more innovations that aim to bridge the gap between the amounts of cellulosic waste and its optimum utilization. Large amounts of cellulose based wastes are produced in every community across the world, which remain a largely untapped resource. Research that involves the conversion of this perceived waste into a widely used commodity would have the dual benefit of organic waste management and sustainable innovations.

Acknowledgements

The authors listed in this chapter wish to express their appreciation to the RSST trust Bangalore for their continuation support and encouragement. As a corresponding author, I also express my sincere thanks to all other authors whose valuable contribution and important comments make this manuscript in this form.

Conflict of interest

The authors listed in this chapter have no conflict of interest known best from our side. There was also no problem related to funding. All authors have contributed equally with their valuable comments which made the manuscript to this form.

Funding information

There was no funding provided for the above research and preparation of the manuscript.

List of abbreviations

DP	degree of polymerization
NMR	nuclear magnetic resonance
HS	Hestrin-Schramm
CMC	carboxymethyl cellulose
PKC	palm kernel cake
EC	ethylcellulose
HPC	hydroxypropyl cellulose
MCC	microcrystalline cellulose
TGA	thermogravimetric analysis
FTIR	Fourier transform infrared spectroscopy
XRD	X-ray diffraction
DSC	differential scanning calorimetry
SEM	scanning electron microscopy
SCB	sugarcane bagasse
GBF	green bamboo fibre
DBF	dewaxed bamboo fibre
DI	deionized
DLBF	delignified bamboo fibre
AmimCl	1-allyl-3-methylimidazolium chloride
IL	ionic liquid
DMSO	dimethyl sulfoxide
BJH	Barrett-Joyner-Halenda analysis
DTG	derivative thermogravimetric
CNC	cellulose nanocrystal
HIV	human immunodeficiency virus
ER	extended release
BNC	bacterial nanocellulose
BC	bacterial cellulose
HAp	hydroxyapatite
GTR	guided tissue regeneration

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Section 3

Applications

Chapter 5

Microbial Cellulases: An Overview and Applications

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Abstract

Cellulases are a complex group of enzymes which are secreted by a broad range of microorganisms including fungi, bacteria, and actinomycetes. In the natural environment, synergistic interactions among cellulolytic microorganisms play an important role in the hydrolysis of lignocellulosic polymer materials. In fact, it is the combined action of three major enzymes which determines the efficiency of this process. They are exoglucanases, endoglucanases, and β-glucosidase. Microorganisms produce these enzymes in a diverse nature which determines their efficiency in cellulose hydrolysis. During the cellulose degradation reaction, the enzyme targets the β-1,4-linkages in its polymeric structure. This is an essential ecological process as it recycles cellulose in the biosphere. The application of this same scenario for industrial purposes is identified as an emerging area of research. Biofuel production, textile polishing and finishing, paper and pulp industry, and lifestyle agriculture are among the key areas where cellulase enzyme shows a broader potential. The objective of this chapter is to discuss the structure, function, possible applications, as well as novel biotechnological trends of cellulase enzymes. Furthermore, possible low-cost, enzymatic pretreatment methods of lignocellulosic material in order to use it as an efficient raw material for biofuel production will be discussed.

Keywords: applications of cellulases, cellulase activity, cellulose, cellulolytic microorganisms, industrial applications of cellulase, pretreatment of cellulose

1. Introduction

Biomolecules derived from natural resources are playing a major role in manufacturing products needed for daily use. Enzymes are one of those molecules that are globally recognized for their multifarious applications in industries. For instance, their utility in brewing, dairy products, detergents, food and feed, pharmaceutical production, and paper and pulp industry is huge. One of those most widely used enzymes is cellulase. According to recent global cellulase market analysis reports, the demand for this enzyme is exponentially increasing.

Cellulose, the substrate of cellulase, is the most abundant polysaccharide present on earth. It is the main substance in plant materials. Anselme Payne was the very first person to discover and isolate this amazing compound from green plants [1]. It happened more than two centuries ago. From the past, cellulosic materials have played a crucial role in daily human life. They used it to fertilize their soil for crop cultivation. It was also fodder for their cattle. It was firewood for cooking, and they were igniting cellulosic material to generate heat whenever they needed to produce energy.

Currently, the role played by cellulose is not that simple. Especially, as it is recognized as a cost-effective raw material, the useful applications of cellulose in the industrial sector have become much more complex. This has laid a huge platform for scientists to do cellulose-based research in multidisciplinary approaches. One such area is hydrolysis of cellulose. In nature, this is usually accomplished by cellulases. Cellulase is catalyzing hydrolysis of cellulose.

However, cellulase is not a single enzyme. It is a group of enzymes which is mainly composed of endoglucanase and exoglucanases including cellobiohydrolases and β-glucosidase. Fungi, bacteria, and actinomycetes are recorded to be efficient cellulase enzyme producers in the natural environment. These microorganisms must secrete cellulases that are either free or cell surface bound. Their enzyme production efficiency and the enzyme complex composition are always diverse from each other. Although both aerobic and anaerobic microorganisms produce these enzymes, aerobic cellulolytic fungi, viz., *Trichoderma viride* and *T. reesei*, are excessively studied. The enzyme breaks β-1,4-linkages in cellulose polymer to release sugar subunits such as glucose. This notion is applied in industries either cellulose is utilized as a raw material or cellulose degradation is a must.

According to recent enzyme market reports, the key areas of the industry where cellulase enzyme is increasingly being applied are healthcare, textile, pulp and paper, detergent, food, and beverages. Its wide application in coffee processing, wine making, and fruit juice production is related to food and beverage segment. In other industrial applications, it is broadly used to produce laundry detergents and cleaning and washing agents. Cellulase is also being highly recognized as an effective alternative to available antibiotics for treatment of biofilms produced by *Pseudomonas*. Therefore, the potential of cellulases to fight against antibiotic-resistant bacteria is an amazing trend which will overcome problems in the healthcare sector [2].

Application of microorganisms or microbial enzymes for pretreatment of lignocellulosic material is currently earning a huge attention of the industry. This is a result of growing interest about depletion of fossil fuel resources in the world which have inspired the production of bioethanol from lignocellulosic biomass through enzymatic hydrolysis [3]. Lignocellulosic biomass is one of the best options as a low-cost, readily available, eco-friendly raw material. However, it is not found alone. Cellulose is forming lignocellulose in combination with hemicellulose and lignin which finally becomes a compact network structure [4]. Moreover, it has a crystalline structure which is hard to break down. Therefore, cellulose is insoluble in water and causes limitations in hydrolysis. That is why it is essential to pretreat lignocellulosic material in industries like bioethanol production. During pretreatment, it will loosen up the crystalline structure and facilitate the degradability to release fermentable sugar forms. There are several methods available for pretreatment of lignocellulose, viz., physical, chemical, and biological methods. Biological pretreatment using cellulolytic microorganisms and their enzymes is found to be the best way of addressing this problem.

By all means, cellulase is an enzyme which can cause a huge economic impact. However, there are some considerable bottlenecks of utilizing this enzyme in the industry. For example, the higher cost of cellulase and less catalytic efficiency are especially understood. Another important point is less understanding of the relationship between hydrolysis mechanisms and molecular structure of the enzyme. This knowledge is important to carry out further improvements in the enzyme to enhance its catalytic activity. Therefore, this chapter is discussing about the structure and function of cellulase in order to understand its mechanisms of action. The details on current applications of the enzyme have also been summarized here. Furthermore, the efforts have been taken to bring together information on novel

biotechnological trends of cellulase. Moreover, it is discussing about possible low-cost, enzymatic pretreatment methods that have been practiced for lignocellulosic materials in order to use it as an efficient raw material to produce bioethanol.

2. Cellulose

Before moving on to cellulase, it is essential to understand cellulose which is the substrate of cellulase enzyme. This section will provide a short description about cellulose.

2.1 Cellulose is a polysaccharide

Cellulose is a linear polysaccharide. In this polymer, D-glucose subunits are attached together by formation of β-1,4-glycosidic linkages between individual glucose molecules. The molecular formula of cellulose is $(C_6H_{12}O_6)_n$. The "n" indicates the degree of polymerization (DP). It symbolizes the number of glucose subunits connected with each other. This number is varying from hundreds to thousands. Two glucose repeating units together are called cellobiose. In other words, this polymer is made by β-(1 → 4)-D-glucopyranose units in $^4C_1$ conformation. It consists of long chains of anhydro-D-glucopyranose units (AGU) with each cellulose molecule having three hydroxyl groups per AGU with the exception of the terminal ends. Cellulose has both crystalline and amorphous regions in its structure in various proportions [5]. Those regions are intertwined to form the structure of cellulose. There are four major crystalline forms, for instance, Iα, Iβ, II, and III. This crystalline structure is a result of intramolecular and intermolecular hydrogen bonding between glucose monomers in cellulose. These hydrogen bonds construct a huge network that directly contributes to the compact crystal structure of cellulose polymer. On the other hand, this strong intramolecular and intermolecular hydrogen bond formation leads to poor solubility of cellulose.

2.2 Organization of cellulose

In plant cell walls, cellulose exists as different levels of structures, i.e., single cellulose chains, elementary fibrils (consisting of tens of single cellulose chains), and microfibrils (bundles of elementary fibrils). It is proposed that the macrofibril is composed by the attachment of several newly synthesized elementary fibrils. With the cellular growth, the macrofibrils divide to form individual microfibrils. Microfibril is consisting of a single elementary fibril. Although elementary fibrils and macrofibrils are composed of mere cellulose, microfibril has noncellulosic polymers like hemicelluloses along with cellulose. It is noted that others consider a microfibril as consisting of a number of elementary fibrils. Microfibril is an elementary fibril associated with noncellulosic polymers. Each microfibril might contain up to 40 cellulose chains and is about ~10 to 20 nm in diameter. Many such cellulose chains aggregate into bundles called micelles and micelles into microfibrils. Micelles are interconnected with few cellulose fibers. The plant cell wall structure is stabilized by the macrofibrils. The cross-links between hemicellulose and pectin matrices also support this stabilization process. Lignin is a complex polymer which usually fills the spaces between cellulose and pectin matrices. It forms covalent bonds with hemicellulose. This provides more mechanical strength to the plant cell wall. This structure which is present in plants is collectively called lignocellulose. Other components known as extractives including fats, phenolic, resins, and minerals are also present in lignocellulosic biomass.

3. Cellulase

3.1 Global demands for enzymes

Enzymes are known to be very useful in many industrial processes. Their broad applicability has created a significant market demand in the recent years. According to market reports on world enzyme demand (2017), they have recognized several key factors which lead to huge consumer demand for enzymes. Some of them are completely bound with economical advances. For example, increased per capita income in developing countries causes huge growth in consumer-related industrial applications [6]. A recent industry study done by Freedonia in January 2018 on "Global Industrial Enzymes" reveals that global demand for industrial enzymes is projected to grow 4.0% per year to $5.0 billion in 2021. This report also emphasizes the gains in personal incomes in developing countries as the key factor which is supporting growth in demand for enzymes. The development of scientific research on enzymes is mainly based on disciplines such as biotechnology,molecular biology and genetics. Continued advances in these areas of research, particularly related to DNA manipulation and sequencing, result in extensive increases in enzyme demand worldwide. Cellulase is one such enzyme which earns consecutively increasing demand. Therefore, collection of knowledge about this enzyme is essential for further development of fundamental and applied research on cellulase and for consequent application in human life.

3.2 Molecular structure and function of cellulase

3.2.1 Molecular structure of cellulase

It is produced by fungi, bacteria, actinomycetes, protozoans, plants, and animals. According to Carbohydrate-Active Enzymes database, there is information of the glycoside hydrolase families. Glycoside hydrolases, including cellulase, have been classified into 115 families based on amino acid sequence similarities and crystal structures. A large number of cellulase genes have now been cloned and characterized. They are found in 13 different families. Furthermore, there are 3D structures of more than 50 cellulases. All of cellulases cleave β-1,4-glucosidic bonds. However, they display a variety of topologies ranging from all β-sheet proteins to β/α-barrels to all α-helical protein.

In the structure of cellulase, there are catalytic modules and non-catalytic modules. The catalytic modules of cellulases have been classified into numerous families based on their amino acid sequences and crystal structures. The non-catalytic carbohydrate-binding modules (CBMs) and/or other functionally known or unknown modules may be located at the N- or C-terminus of a catalytic module. Usually, fungal and bacterial cellulase mainly has two or more structural and functional domains. Both aerobic and anaerobic microorganisms are producing this enzyme. Therefore, there are two types of cellulase systems: noncomplex and complex. A noncomplex cellulase system is produced by aerobic cellulolytic microorganisms, and it is a mixture of extracellular cooperative enzymes. In a noncomplex cellulase system, the common arrangement is joining of a catalytic domain with a cellulose-binding domain (CBD). A complex cellulase system is produced by anaerobic microorganisms and it is called "cellulosome." Cellulosome is assembled by joining a catalytic domain with a dockerin domain. The enzyme is a multiprotein complex anchored on the surface of the bacterium by non-catalytic proteins that serves to function like the individual noncomplex cellulases but is in one unit.

In addition to these two major domains in the cellulase structure, there are some other domains that are present in many cellulases, for instance, S-layer homologous (SLH) domain, fibronectin-type 111 domains, and NodB-like domain, and there are also other regions of unknown function. These domains are often connected by Pro and hydroxy amino acid (threonine and serine) enriched linker sequences. Among all these domains, catalytic and cellulose-binding domains are the most important because they are the domains which are considered participating in hydrolytic mechanisms of the enzyme.

3.2.2 Catalytic function of cellulase enzyme

Cellulase catalyzes the decomposition of cellulose polysaccharide by simply breaking down β-1,4-glycosidic bonds. Three major types of enzymes are generally involved in hydrolyzing cellulose microfibrils in the plant cell wall: endoglucanase, exoglucanase, and β-glucosidase. Complete cellulose hydrolysis is mediated by the combination of these three main types of enzymes. Endoglucanase usually attacks amorphous areas of cellulose. The random attack of this enzyme on internal bonds of loosely bound, amorphous areas of cellulose creates new chain ends. These new chain ends are then easily attacked by other types of enzymes. The highest activity of this enzyme usually occurs against soluble cellulose forms or acid-treated amorphous cellulose. The function of exoglucanase is to produce glucose or cellobiose units by attacking the reducing or nonreducing end of cellulose chains. Endoglucanase is different from exoglucanase because it is usually very active against crystalline cellulose substrates such as Avicel or cellooligosaccharides. Finally, β-glucosidase can hydrolyze cellobiose to glucose from the nonreducing ends, and it is inactive against amorphous or crystalline cellulose. Although an exact mechanism is not yet finalized, fragmentation of cellulose aggregations into short fibers has been observed and reported during the beginning of cellulose hydrolysis prior to releasing any detectable amount of reducing sugars. This is known as amorphogenesis.

There are two catalytic mechanisms of cellulases. They are simply introduced as retaining mechanisms and inverting mechanisms. Cellulases cleave glucosidic bonds by using acid-based catalysis. The hydrolysis is performed by two catalytic residues of the enzyme: a general acid (proton donor) and a nucleophile/base. The catalytic mechanism which occurs depends on the spatial position of the catalytic residues. The retention and inversion of the anomeric configuration of cellulose are the two mechanisms which hydrolyze cellulose. The "retaining" cellulases retain the same configuration of anomeric C bearing the target glucosidic bond even after a double-displacement hydrolysis with two key glycosylation or deglycosylation steps. "Inverting" cellulases inverts the configuration of the anomeric C configuration after a single nucleophilic displacement hydrolysis [7].

4. Applications of microbial cellulases

For many decades, cellulases have played a crucial role as biocatalysts. They have shown their potential application in a large number of industries. Textile, paper and pulp, laundry and detergent, agriculture, medicine, and food and feed industries are some of the major industries which employ microbial cellulases. According to Coherent Market Insights, the textile industry is the dominant market for cellulases in 2017. According to most of the enzyme market research reports published in 2018, food and beverages, textile industry, animal feed, and biofuels have been reported to be the major areas of applications.

According to another Global Cellulase (CAS 9012-54-8) Market Research Report published in 2018, Asia-Pacific is the largest consumer of cellulase, with a revenue market share nearly 32.84% by 2016. Furthermore, the reported data showed 29.71% of the cellulase market demand in animal feed, 26.37% in food and beverages, and 13.77% in the textile industry in 2016. This same report forecasts that the applications of cellulases will reach 2300 million USD by the end of 2025, growing at a compound annual growth rate (CAGR) of 5.5% during the 2018–2025 period. These data suggest that the application of cellulases in industries is drastically rising annually. Novozymes and DuPont from Denmark are key cellulase enzyme producers supplying these enzymes to the global market for industrial applications. From this point forward, in this chapter, our major effort was to discuss about the current applications of cellulases in major fields that have been listed above. The novel biotechnological trends emerging in those fields while understanding the key areas of research where further studies required also surfaced to an extent.

4.1 Textile industry

The textile industry is one of the largest industries in the world. The customer demand for fashion is increasing as they want uniqueness in styles, colors, and the clothes they wear. There was a significant growth in this industry during the last few decades as a result of this increasing customer demand. This enzyme has now become the third largest group of enzymes used in these applications [8]. This creates a very competitive market platform for manufacturers that are always looking for environmentally friendly approaches of giving their products a unique look. Cellulase is used for many purposes in the industrial sector.

Especially for textile wet processing, biostoning of denim fabric, biopolishing of textile fibers, softening of garments, and removal of excess dye from the fabrics are some of the major applications of this enzyme in the industry. Fungal cellulases from *Trichoderma reesei* are the mostly applied enzyme in the textile industry. Apart from that, actinomycetes from the genera *Streptomyces* and *Thermobifida* and other genera of bacteria, such as *Pseudomonas* and *Sphingomonas*, are some of the sources of enzymes to be used for decolorization and degradation of textile dyes [9].

Biostoning and biopolishing are well known for the best applications of cellulases in the current textile industry.

4.1.1 Biostone washing

The conventional washing process of denim usually has three steps. The denim fabric is first treated with amylase enzyme to remove the starch coating of the fabric. This process is called desizing. During this process, starch is broken down into maltose which is a water-soluble disaccharide composed of two glucose molecules. Then, the fabric is given treatment by providing abrasion to the material in pumice stones added to the washing machine. This wash was completely achieved by adding chemicals like sodium hypochlorite or potassium permanganate. This traditional process has several disadvantages. The addition of pumice stones must be done in larger quantities. This was affecting the machine's productivity in an adverse way causing tear effects. After the wash is completed, the manual removal of stones is needed. This is causing further reduction of the process efficiency. The excessive back-staining was another disadvantage of the traditional process. Backstaining is the reaction by which the removed dye molecules are deposited on the denim fabric again.

Application of microbial cellulases was found to be an efficient alternative for pumice stone washing. It was first staged in the 1980s. The use of stones is currently replaced by cellulases in a successful way. During this process, cellulases act on the

denim fabric which is made of tough cotton. The indigo dye which is used to color the fabric is trapped inside the cellulose fiber in this cotton material. Usually, the indigo dye is mostly attached to the surface of the yarn and to the most exterior short cotton fibers. When the fabric is treated with the enzyme, it hydrolyzes and breaks small fibers coming out of the fabric which loosens the dye. For this purpose, the β-1,4-linkages of cellulose chains will be broken down, and simple water-soluble sugars will be formed. This will remove the fibers which traps indigo dye. Then, the dye is easily removed from the fabric giving a faded look.

Trichoderma reesei acidic endoglucanase II has been found to be a very efficient candidate for biostoning [10]. The neutral cellulase enzyme extracted from *Humicola insolens* is also reported to be commonly applied in this process [11]. The use of cellulases has several advantages over stone washing with pumice stones including high productivity; less work-intensive, safer environment; short treatment times; and less wear and tear of machines. Currently, denim with a worn-out look has a huge demand in the textile market.

The major disadvantage associated with the application of microbial cellulases is again backstaining. The redisposition of dye on the fabric covers up the shaded look given by the treatment. In order to overcome this problem, several biotechnological approaches have been already experimented by researchers. Immobilization of cellulases on pumice stones is one such cost-effective way of doing this. It has also been observed that acidic endoglucanase causes a better abrasion and less backstaining compared to neutral endoglucanase. For example, the cellulase given by *Trichoderma reesei* is more efficient in preventing backstaining as compared to neutral endoglucanase of *H. insolens*.

The latest trend of biostone washing is to utilize an enzyme mixture composed of amylase, cellulase, and laccase [12]. The sizing is the process by which denim material surface is covered by a compound like starch to provide rigidity and stiffness to raw denim and provide strength and friction resistance during handling. During washing, this surface layer of starch must be removed first to facilitate interaction between cellulases and cotton fibers. The amylase hydrolyzes starch from the fabric and causes desizing. Cellulase hydrolyzes small cellulose fibers, and laccase usually causes bleaching of the fabric. Laccases (EC 1.10.3.2) with intrinsic electron-donating tendency can decompose indigo in the solution as well as on the fabric creating bleaching effect on denim garments. The indigo dye is converted into isatin and anthranilic acid like chemical forms. This prevents backstaining of dye on the fabric surface. Eventually, this will give a complete faded look to the denim fabric. The purpose of using a mixture is to improve the efficiency of biostone washing process by allowing those three enzymes to work together in a sequential manner.

In a recent study, it is reported that an alkali-stable cellulase in combination with xylanase from *Thermomonospora* sp. has a reduced tendency of backstaining [13]. However, the effluents generated during biostone washing must be pretreated to remove dye material and the intermediate chemical compounds present after the reaction. Otherwise, these dyes might pollute natural waterways and soil. Most of these dye residues are toxic and carcinogenic that would cause adverse health effects in humans and animals. Although biodegradability of enzymes is a positive advantage here, chemical by-products formed during dye removal must be neutralized.

4.1.2 Biopolishing and biofinishing

These two processes are simply similar to each other. Cellulosic fibrous materials like cotton and linen are always loosing appearance because of fuzz formation on the fabric surface. Fuzz occurred due to short fibers protruding out from the surface

of the fabric. Fuzz is sometimes loosely attached to the fabric forming a ball-like appearance which gives an unattractive look to the fabric. This is called pilling. The biopolishing process basically aims on removing microfibrils of cotton. It enhances fabric look, hand feel, and color by giving a smooth and a glossy appearance. This is also leading to improvement of color brightness, hydrophilicity, and moisture absorbance by the fabric [13]. The acidic cellulases produced by *T. reesei* and *Aspergillus niger* are found to be enormously effective in this process. Biopolishing is eco-friendly because the enzymes used in this process are readily biodegradable and nontoxic.

The repeated washing of a cotton garment makes it fluffy and dull. This is due to partially removed microfibrils on the fabric surface. Biofinishing by cellulases can remove these fibrils and give back the smooth surface and original color to the fabric. This will also give a soft hand feel to the material, and also this is a good way of removing stains and dirt spots that are trapped within the cotton fiber network [14, 15].

4.1.3 Bioscouring

This is the process that removes noncellulosic material from the surface of the cotton. This is usually done with cellulase alone or in combination with other enzymes such as pectinase. Pectinase digests the pectin substance present among cellulose fibers. This helps to remove the intact connection between the cuticle and the main body of the cellulose fiber. This helps to degrade the primary cellulosic wall of the fiber. The ultimate result is the destruction of the cuticle [16]. This reaction increases the softness of the fabric.

4.1.4 Biocarbonization and wool scouring

This is a kind of a biological mode of cleaning the fabric from the cellulosic or vegetative impurities with the help of enzymes. When a pure cotton or cotton blend fabric is prepared, some traces of unwanted cellulosic material still may remain in the fabric. They may result in imperfect finishing and lower quality of the fabric. The earliest methods of carbonization involved application of sulfuric acid. It was not only expensive but also corrosive, unsafe, and hazardous. Being a nonhazardous, non-corrosive, and eco-friendly method, enzymatic carbonization was a promising alternative. This method was perfect for removal of cellulosic impurities from the material because it was least affecting the color and the hand feel of the fabric. The removal of vegetative impurities from the surface of raw wool using cellulases is called wool scouring [17]. Cellulases can be used alone or in combination with other enzymes such as pectinases to increase the efficiency of this process. These methods are doing less damage to the fabric when compared to the treatment with sulfuric acid.

4.1.5 Defibrillation of lyocell

Lyocell is the generic name for a biodegradable fabric that is made out of treated wood pulp. This material is used in everything from clothing to cars. This is obtained from wood pulp using a solvent-spinning method. The solvent system which is usually applied is an organic compound called N-methylmorpholine N-oxide. Some main characteristics of lyocell fibers are that they are soft, absorbent, and very strong when wet or dry and resistant to wrinkles. One chief defect of this material is fibrillation. This is the formation of small tangled fibrils on the surface of the fabric. Cellulases can be efficiently applied to remove these fibrils and

give the fabric an increased softness and an improved appearance. This is also good for preventing fuzz and pill formation.

Although the applied enzymes are nontoxic and biodegradable in the above processes, the final effluent produced will show increased biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), and total suspended solids (TSS) to a certain extent because the effluent may contain digested sugar and cellulosic forms. Direct release of this effluent to natural water bodies may cause water pollution. Alkalinity and pH fluctuations of the effluent will also result in polluted water which is not good for human and animal consumption. This may cause health issues like skin irritations in humans. On the other hand, the enzymatic treatments need an incubation period to facilitate the reaction between enzyme and fabric. As this is a fermentation reaction, this may release certain odors to the environment which may cause air pollution to a certain extent. Moreover, textile dyes are removed during textile processing. Most of the dyes are toxic and some are highly carcinogenic. Mixing this type of dyes with water is definitely causing adverse health effects in humans. Therefore, direct release of effluent without applying any pretreatments to neutralize these toxic compounds will breach the stability in ecosystems to which they are released. Therefore, establishment of pretreatment facilities and water quality testing procedures are essential for these enzymatic textile processing plants.

Lyocell production has a different impact on the environment compared to the other textile polishing and finishing processes. The solvent which is used to manufacture this textile is N-methylmorpholine N-oxide. This is usually causing acute toxicity (oral, dermal, inhalation), skin irritation, serious eye damage and irritation, skin sensitization, and specific target organ toxicity. These are also hazardous to aquatic environments. These possible environmental impacts must be always addressed although application of enzymes in textile processing is eco-friendly.

4.2 Paper and pulp industry

This is one of the largest industrial sectors in the world. According to the World Wildlife Fund (WWF), the pulp and paper industry, which includes products such as office and catalog paper, glossy paper, tissue, and paper-based packaging, uses over 40% of all industrial wood traded globally [18]. On the other hand, the latest paper industry statistics reveal China, the United States, and Japan as the three countries where the largest paper production occurs in the world. Half of the total paper manufacture of the world is done by these three countries. However, Germany and the United States are the world's leading paper importers and exporters [19]. Moreover, the United States is reported to be the largest consumer of papers.

Papers and pulp are renewable resources. Therefore, recycling and reusing are two popular concepts related to this industry. Application of microbial cellulases is usually utilized for this purpose. The application of cellulases in this industry is broader. Starting from the 1980s up to now the possible applications are branching toward many areas. For instance, deinking, pulping, bioremediation of industry wastes, bleaching, and fiber enhancement can be taken.

4.2.1 Pulping

The drawbacks in mechanical pulping processes of woody raw materials such as refining and grinding resulted in pulps with higher amounts of fines, bulk, and stiffness. On the other hand, the process was high energy consuming which was not a profitable option for an industry. Meanwhile, biopulping using enzymes such as

cellulases is an energy-saving way, and also it is eco-friendly [20]. The substantial energy saving is reported around 20%–40%. During the refining process, it generates small particles of the pulps. These particles reduce the drainage rate during the paper-making process. These particles can be readily degraded by cellulases in order to increase the drainage ability of the pulp. Mixtures of cellulases (endoglucanases I and II) and hemicellulases have also been used for bio-modification of coarse pulp material to improve fiber properties. It is strengthening the hand sheets. On the other hand, biological pulping has the potential to improve the quality of pulp and properties of the paper while reducing energy costs and environmental impact [21].

4.2.2 Deinking

In traditional deinking, large quantities of chemicals are used which make the method expensive and environmentally damaging and increase the release of contaminants [22]. The main advantage of bio-deinking is the ability of avoiding the alkali use during the process. This prevents yellowing of the paper. Cellulases alone, or used in combination with xylanase, are beneficial for deinking of different types of paper wastes. In most of the applications, partial hydrolysis of carbohydrate molecules releases ink from the fiber surface. This is done by a mixture of cellulases alone or in combination of cellulases and hemicellulases. The advantages associated with enzymatic deinking are clean look of paper, enhanced brightness, as well as environmental pollution reduction..

4.2.3 Bio-modification and bio-characterization of fibers

Successful application of cellulase and hemicellulase mixtures has been reported to modify properties of fibers. Usually in the paper industry, the making of paper is made easier by improving the beatability, runnability, and drainage of paper pulp during the process. Modifications of fiber properties are also achieved through treatment of paper by cellulase enzyme [20]. Not only that but also the enzymatic hydrolysis helps in characterization of fiber using various techniques such as scanning electron microscopy (SEM) and HPLC [23].

4.3 Laundry and detergent industry

The application of enzymes in manufacturing enzymatic washing agents or biological detergents dates back to the 1960s. Using enzymes in detergent formulae is a common practice today. In fact, according to market reports, by 2014, the detergent industry was the largest single market for enzymes at about 25–30% of total sales [22]. Another market research report published in 2017 on laundry detergent market stated that its global market size valued at 133.3 billion USD in 2016. The latest trend in the industry is to use alkaline enzymes in large amounts. For instance, protease, cellulase, α-amylase, lipase, and mannanase are broadly applied in heavy-duty laundry and automatic dishwashing detergents..

The capability of enzymes to remove stains is the major focus of using them in manufacturing detergents. Cellulases are available in the market in different brands. For instance, Celluzyme® and Carezyme® are two main brands applied in detergent blends. These detergent blends are mainly applied in washing fabrics made of cotton and cotton blends. These detergents are making fiber modifications in the fabric in order to improve color brightness, softness, and particulate soil removal.

Cellulases extracted from fungi like *Trichoderma* sp. (*T. longibrachiatum*, *T. reesei*, *T. viride*, and *T. harzianum*), *Aspergillus niger*, *Humicola* (*H. insolens* and *H. griseathermoidea*), and *Bacillus* sp. have been excessively studied so far for

application in detergents. Alkaline cellulases are the most suitable additives to conventional detergents. It is because of their ability to remove soil and dirt particles from the interfibrillar spaces of the fabric. The cellulases remove the rough projections of cellulose fibers or cellulose aggregates attached to the fabric. This gives an increased gloss and smoothness to the fabric [23].

The most recent innovation is to use combinations of enzymes in detergents. Cellulases are used in combination with other enzymes like proteases and lipases. The combination of enzymes is used to increase efficiency on stain cleaning and fabric care. For instance, SaniZyme® is a four-enzyme liquid detergent containing lipase, cellulase, amylase, and protease. This is a bacteriostatic enzymatic detergent for the removal of blood, protein, mucous, fats, lipids, and carbohydrates from all types of endoscopic equipment and surgical instruments. Another example is Getinge Clean MIS Detergent® which is also a formulation which includes protease, lipase, amylase, and cellulase enzymes, surfactants, sequestering agents, and corrosion inhibitors (typical pH in use dilution 8) which is specifically designed to clean complex, minimal invasive instrumentation.

4.4 Agriculture

The application of cellulases in agriculture is usually reported in enhancement of crop growth and a control agent of plant diseases. For this purpose, combinations of cellulases, hemicellulases, and pectinases are broadly applied. Certain fungal cellulases are with the ability to degrade cell wall of plant pathogens. There are lots of details about application of bacteria such as plant growth-promoting rhizobacteria (PGPR) to improve plant performance. It is reported that these bacteria play a major role in reducing application of chemical fertilizers increasing plant development and also controlling potential plant pathogens and protecting plants from diseases. Moreover, many fungi including *Trichoderma* sp., *Geocladium* sp., *Chaetomium* sp., and *Penicillium* sp. enhance seed germination, support rapid plant growth, accelerate flowering, improve the root system and increase the crop yield. However, exact mechanisms behind these reactions are not yet clearly understood. But all these organisms have the ability to produce cellulase and related enzymes which may have a direct participation in these reactions. Some reports are about possible synergisms between bacterial cellulase production and bacterial antibiotic production against plant pathogenic fungi.

According to available information, it is evident that cellulolytic microorganisms are participating in many processes, viz., rhizosphere soil decomposition, increasing the availability of nutrient for the plant, controlling plant pathogens, facilitating root colonization, and penetration of cereal crops improving yields and nutritional contents. However, as there are no solid evidence to prove the mechanisms behind these, this area needs further research. The studies should be performed in order to characterize and improve applications of microbial cellulases in this field.

During traditional agriculture practices, especially in countries like Sri Lanka, farmers used to add straw and *Gliricidia* leaves like cellulosic materials into their fields. They observed that the incorporation of these types of plant material not only improved the quality of the soil but also increased the yield due to added nutrients. Therefore, it is obvious that in this type of processes cellulolytic microorganisms must have a direct contribution.

4.5 Medical applications

Medical pharmacology is currently a very active field of research that novel discoveries are coming into action. One such area is cellulases for development of

medicine. By the way, humans are not cellulase producers, but the recent research on health and medicine reveals the benefits of consuming blends of enzymes including cellulase. As a result of global demand for enzyme blends, cellulase produced by the natural fermentation process of *Trichoderma reesei* and *Bacillus licheniformis* has been included in commercially available enzyme blends. This type of enzyme blends target collective digestion of cellulose-rich fibrous substances such as fruits and vegetables, cereals, legumes, bran, nuts and seeds, soy, dairy, healthy greens, sprouts, and herbs along with fats (lipids), sugars, proteins, carbohydrates, and gluten. One such example is VeganZyme®. Apart from that digestive aids (e.g., Digestin, P-A-L Plus Enzymes, Polyenzyme Plus, etc.) to treat people suffering from metabolic disorders are evolving as a promising strategy in medicine.

In some records, the direct and indirect applications of cellulase in medicine have been mentioned apart from using it as consumable enzyme blends.

4.5.1 Indirect applications of cellulases for medical purposes

Cellulase of fungal origin in combination with chitinases and lysozymes has a reported use in chitosan degradation. To obtain chitosan, a partial degradation of chitin must take place. As cellulose, chitin is a structural polysaccharide present in animals such as marine animals like shrimp and insect exoskeleton as well as participates in the formation of some parts of fungal cell walls. Chitin is a poly-ß-1,4-N-acetyl-D-glucosamine, conforming crystalline microfibrils. This polysaccharide provides structural integrity, stability, and protection to animals. Chitosan is the most important semicrystalline derivative form of chitin. This is obtained by partial deacetylation of chitin (around 50%, soluble in aqueous solution) under alkaline conditions or enzyme hydrolysis. Chitosan and its derivatives have many medical applications, viz., surgical sutures, bone rebuilding, production of artificial skin, anticoagulant, antibacterial agent, hemostatic dressings, anticancer and antidiabetic agents (in combination with metals), hypocholesterolemic effectors, elaboration of cosmetics, production of biopharmaceutics, and encapsulation of diverse materials [24, 25].

Apart from these applications, a lot of reports have been published on several studies which discuss how cellulases hydrolyze chitosan and their potential biomedical effects. For instance, antitumor activity of cellulase-treated chitosan [26] and antimicrobial activity of low-molecular-weight chitosan obtained by *Trichoderma* commercial enzymes could be discussed. However, the lack of solid evidence is a common issue on this area.

4.5.2 Direct applications of cellulases for medical purposes

A bezoar is a mass found trapped in the gastrointestinal system. Phytobezoars, as its name suggests, are composed of indigestible plant material (e.g., cellulose). In other words, it is a gastric concretion formed by vegetable fibers, seeds and skins of fruits, and sometimes starch granules and fat globules trapped inside the gastrointestinal tract. This is a common problem frequently reported in patients with impaired digestion and decreased gastric motility. Although sometimes surgeries are needed to remove these stagnated substances, certain minor conditions can be treated by cellulases. The common application reported is fungal cellulases. As there are no much reports about application of bacterial cellulases, research can be conducted to find out the application of potential cellulolytic bacterial cellulases to treat this condition. However, what is most important is that any of these individual enzymes or enzyme cocktails should not adversely affect the healthy body cells.

Another possible direct application of cellulase in medicine is degradation of cell walls of pathogenic organisms. *Acanthamoeba* is a Protista which causes a very rare as well as serious corneal infection which leads to blindness. This is simply called keratitis. This amoebic keratitis is an acute sight-threatening corneal infection associated with contact lens misuse [27]. This organism has two stages in its life cycle: the cyst and trophozoite. Both these structures are available when the eye is infected by this organism. The cyst wall resembles the plant cell wall. It is plausible that amoebae secrete cellulose because its cyst wall is primarily composed of cellulose. There is evidence which has been found to prove this matter about the presence of cellulose in [28]. Therefore, it is possible to apply cellulases in controlling the pathogen which causes this disease. Cellulases can be used to break off the cyst wall and control the pathogen. However, it needs a lot of research before using cellulase as a treatment for the eye.

Pathogenic microorganisms usually form biofilms. A biofilm is an assemblage of microbial cells that is irreversibly associated (not removed by gentle rinsing) with a surface and is enclosed in an extracellular polymeric substance (EPS) matrix. Usually, pathogenic microorganisms form this type of assemblages. They may be found on a wide range of surfaces including living tissues and indwelling medical devices. Artificial hip prosthesis, central venous catheter, prosthetic heart valve, intrauterine device, and urinary catheter are some examples for indwelling medical devices that are commonly associated with biofilms. Most of the microorganisms produce extracellular polymeric substances composed of backbone structures that contain 1,3- or 1,4-β-linked hexose residues and tend to be more rigid, less deformable, and in certain cases poorly soluble. This is the exact linkages present in cellulose polymer. Therefore, cellulases can be further studied for their efficient application in removing this type of biofilms from medical devices.

4.6 Food and feed industry

4.6.1 Food processing industry

Food is essential for all living organisms to obtain nutritional support for their growth and well-being. The huge demand for food has laid a path to a very complex, interconnected global business that supplies most of the food consumed by the world's population.

Food biotechnology nowadays considers cellulases as an invaluable resource due to their increased applicability in a broad range of processes. Fruit and vegetable juice clarification, reducing the viscosity of nectars, concentrating purees, alteration of fruit sensory properties, carotenoid extraction, olive oil extraction, and the quality improvement of bakery products are among the various processes in food biotechnology that cellulase is exploited worldwide.

The cloudiness which is usually present in fruit and vegetable juices is a result of floating polysaccharide materials such as cellulose, hemicellulose, lignin, pectin, starch, metals, proteins, and tannins. The presence of these materials in the juice makes it low quality and draws less consumer demand. "Rapidase pomaliq" is a commercially available enzyme preparation composed of cellulase, hemicellulases, and pectinases obtained from *Trichoderma reesei* and *Aspergillus niger*. The application of this product in fruit juice clarification was beneficial to a considerable level. It is also reported that cellulase produced by bacteria such as *Bacillus* and *Paenibacillus* in combination with other enzymes such as pectinases and hemicellulases carries out the fruit and vegetable juice clarification. Apart from that, treatment of nectars and purees also found to be efficiently carried out by this enzymatic

process. Rheological parameters such as viscosity of these products are brought down to a commercially acceptable level.

Modification of sensory parameters of food is another important area where application of cellulases is highly recommended. The aroma properties, flavor, and texture of fruits are some sensory properties which play a crucial role in food biotechnology. The infusions of pectinases and cellulase enzymes have been found to be effective in altering the sensory properties of fruits and vegetables [29].

These enzymes are also applied in degradation of grape fruit peels to release sugars. These sugars will be used in many industries including food production. Another important application of cellulase is extraction of phenolic compounds from grape pomace.

During extraction of olive oil, malaxing (mixing) is an indispensable step. This period allows the tiny oil droplets to attach with bigger ones and increase the oil yield which is coming from the olive paste. The use of cellulases alone or in combination with other hydrolytic enzymes like pectinases in this step has been found to have an enhancing effect on the extraction as well as the quality of olive oil. The enzymatic treatment of olive oil at the extraction stage causes significant enhancements in phenolic content and antioxidant activity of olive oil, thereby ultimately improving its quality.

The enzyme cocktails of cellulases with other hydrolytic enzymes such as amylases, proteases, and xylanase result in increased loaf volume, improvement in bread quality, and production of softer crumb. Enzyme cocktail containing cellulases, hemicellulases, amylases, lipases, and phospholipases results in dough conditioning with improvement of flavor, prolonged shelf life, and increase in volume after baking [30].

Another important application of cellulases is in pigment extraction from plants and plant products. Natural pigments such as carotenoids are nowadays earning a huge consumer demand as food colorants because of their natural origin, less toxicity, and availability of a wide range of colors. Brightly colored fruit peels such as orange, sweet potatoes, tomatoes, and carrot cell walls are rich in carotenoids. These are applicable as natural food colorants. The treatment of fruit peels with enzyme cocktails including cellulase leads to carotenoid extraction.

4.6.2 Animal feed industry

In animal feed production, cellulases are applied to enhance digestibility of cereal-based food and to increase nutritive values for a higher quality of forages. There are reports about efficiently using *Trichoderma* cellulase as feed additives for significant improvements in feed conversion ratio as well as digestibility of the cereal-based food [31]. Forage feed of ruminants is quite complex in composition containing cellulose, hemicellulose, pectin, and lignin. There is a suggestion that it is possible to use cellulase preparations to enhance digestibility of forage [32, 33]. Usually, cellulase from bacteria such as *Bacillus subtilis* is used in this feed production and nutritional enhancement processes. Enzyme preparations containing cellulases and hemicellulases are utilized for numerous activities like milk yield, body weight gain, and feed. Another aspect of treating animal feed with these enzyme mixtures is to remove anti-nutritional factors present in grains and other cellulosic materials.

Furthermore, cellulases play an important part in increasing the rate and extent of fiber digestion. This can be used as a positive effect on natural gastrointestinal processes of the ruminants. The ultimate result will be the increased availability of absorbable nutrients by digestibility enhancement of fodder.

The partial hydrolysis of lignocellulose materials also leads to better emulsification of food in the animal digestive tract resulting in ultimate improvement of nutrients availability.

5. Pretreatment methods of lignocellulosic biomass for bioethanol production

Energy is the life blood of the modern world. Fossil fuel, among all energy sources, holds the highest consumer demand. Since the industrial revolution, there is a drastic increase in fuel consumption. As fossil fuels are not renewable resources, the depletion of its natural deposits is inevitable. Therefore, we are in an era of limited and expensive energy. With the recent rise in fossil fuel prices, along with growing concern about its adverse effects on environment such as global warming caused by carbon dioxide emissions and subsequent climate change problems, biofuels have been gaining popularity. In our study area, we are focusing on bioethanol production using cellulolytic microorganisms as well as fermentative yeast using cellulose as the substrate.

Bioethanol is a renewable form of energy. Especially, second-generation bioethanol production is an emerging trend because of the abundance of low-cost raw materials. The largest potential feedstock for this purpose is lignocellulosic biomass, which includes materials such as agricultural residues (corn stover, crop straws, and bagasse), herbaceous crops (alfalfa, switch grass), short-rotation woody crops, forestry residues, waste paper, and other wastes (municipal and industrial). Lignocellulose is the most abundant renewable biomass. The yield of lignocellulose can reach approximately 200 billon metric tons worldwide per year [34]. Bioethanol production from these feedstocks has certain advantages. It is an attractive method of disposing those lignocellulosic materials which is the nonedible part of plants. Less production of pollutants makes it environmentally friendly. Most importantly, bioethanol production using lignocellulosic biomass does not create any food insecurity because it does not utilize any food crops before harvesting. Finally, it is abundant all around the year as a raw material.

However, the major drawback of this production process is associated with the structure of lignocellulosic biomass. It mainly consists of lignin, cellulose, and hemicellulose which collectively form a very stable structure. In order to release fermentable sugars from this substrate, it needs to undergo a pretreatment process to break open the stable structure. One of the main focuses of this chapter is to discuss about the possible biological pretreatment of lignocellulosic biomass with special references to the current studies conducted by scientists. It also includes a description about our own attempts in this particular area of research.

5.1 What is biological pretreatment?

Biomass contains about 40–50% of cellulose, a glucose polymer; 25–35% of hemicellulose, a sugar heteropolymer; 15–20% of lignin, a non-fermentable phenyl-propane unit; and lesser amounts of minerals, oils, soluble sugars, and other components. Biological pretreatment of lignocellulosic biomass uses the ligninolytic potential of certain microorganisms (fungi and bacteria and actinomycetes) to reduce the recalcitrant nature which is mainly caused by lignin component of the feedstock and enhance its digestibility by hydrolytic enzymes [35]. The breakdown of lignin barrier changes the structure of lignocellulose and enhances the access to the cellulose and hemicellulose carbohydrate components present.

5.2 Advantages and drawbacks of biological pretreatment

Biological pretreatment seems to be a promising approach due to its low capital cost, low energy, and little dependence of chemicals, mild environmental conditions, eco-friendly nature, and the absence of inhibitor generation during the process which affects in bioethanol production. Moreover, this process does not release any toxic materials or any toxic effluents to the environment.

However, there are few limitations in this strategy. The main drawback against the industrial scale application is the prolonged incubation time consumed to achieve the efficient delignification [36]. This is because of low hydrolysis rate of the microorganisms. Another possible drawback that comes into mind is the possible consumption of carbohydrates as well as fermentable sugar formed by the same microorganisms used to pretreat the material. This is possible to take place because most of the lignolytic microorganisms are producing cellulolytic enzyme batteries as well. Then, the substrate left for fermentative organisms will be minimal which could consequently lead to lower bioethanol yields. Therefore, it is essential to have poor cellulolytic microorganisms for delignification process.

To minimize this type of drawbacks in a biological way, it is possible to use cocultures or biofilms of efficient ligninolytic microorganisms. Introduction of fermentative yeast isolates into the same microbial coculture would also be a perfect approach. However, developing the most efficient microbial consortium is not that simple. Excessive laboratory-scale studies are required to understand the optimum physiological as well as biochemical parameter setup.

5.3 Microorganisms employed in biological pretreatment

Fungi are found to be more efficient in degrading lignocellulosic biomass. For instance, white-rot fungi, brown-rot fungi, and soft-rot fungi can be taken. The first two are basidiomycetes and soft-rot fungi are classified in ascomycete group.

Among these efficient ligninolytic microorganisms that have been studied so far, white-rot basidiomycete fungi are found to be more versatile in the process. Most research has been concentrated on species such as *Phanerochaete chrysosporium* (*Sporotrichum pulverulentum*) which is considered as the model organisms for lignin degradation as it completely mineralizes lignin to CO_2 and water. *Ceriporiopsis subvermispora*, *Phlebia subseralis*, *Pleurotus ostreatus*, and *Lentinus edodes* are also some other white-rot fungi that have been studied. This is thanks to the ligninolytic enzyme systems produced by these fungi. Lignin peroxidase, manganese peroxidase, and laccase are the major enzymes involved in this process. Lignin peroxidase and manganese peroxidase enzymes catalyze H_2O_2-dependent oxidation of lignin, while laccase which is a copper-containing enzyme catalyzes demethylation of lignin components. These are a set of high-redox potential oxidoreductases.

Recently, some bacterial laccases have also been characterized from *Azospirillum lipoferum*, *Bacillus subtilis*, etc. Unlike fungi, the bacteria are considered as low potential for lignin degradation. However, the three groups of bacteria, namely, actinomycetes, α-proteobacteria, and γ-proteobacteria, are known to have ligninolytic systems. Some actinomycetes were studied for their role in lignin biodegradation [37]. These degraded lignin into low-molecular-weight fragments. Some studies have shown potential of *Penicillium camemberti* for lignin degradation.

The biological pretreatment can be performed by growing the microorganism directly on the feedstock or using the enzyme extracts. Solid-state fermentation is the method of choice for biological delignification. Thus, from the reports available, it is evident that white-rot fungi and actinomycetes can be used to remove lignin

from lignocellulosic substrates. However, further studies are required to shorten the incubation time and to optimize the delignification process.

5.4 Enhancement of biological pretreatment efficiency

The importance of enhancing enzymatic hydrolysis has been increased because of the urgent need for efficient biological pretreatment processes. For this purpose it is essential to search for high enzyme-producing organisms from the natural environment. Selecting the most effective strain and its culture conditions can make the process more efficient. Another important aspect is to find unique microbial communities for biological pretreatment. These communities can be called consortia. The efficient biodegradation of lignocellulosic biomass could be achieved by the synergistic action of various bacteria and fungi in a microbial consortium. There are a number of advantages in using a microbial consortium for biological pretreatment. The increase of adaptability, improved productivity, improved efficiency of enzymatic saccharification, control of pH during sugar utilization, and increase in substrate utilization are some of them. With the development of biotechnology and molecular biology, the production of hyperlignolytic mutants by genetic modification of wild-type species is one approach that could be studied further. Furthermore, complete understanding of the theoretical basis behind the mechanisms of actions of these hydrolytic enzyme systems is very useful in the process of enhancing hydrolytic efficiency.

Various process parameters affecting biological pretreatment like incubation temperature, incubation time, inoculums concentration, moisture, aeration, and conditions of pH have to be optimized. This must be done with well-planned laboratory-scale experiments. It is essential to pay attention to the microorganism used as well as the type of lignocellulosic material utilized because these parameters obviously change based on these two factors. Accessory enzymes are those enzymes which act on less abundant linkages found in plant cell walls. These include arabinases, lyases, pectinases, galactanases, and several types of esterases. Some studies have reported that addition of these accessory enzymes will improve hydrolysis efficiency.

Recently, several studies have been conducted in Sri Lanka on efficient lignocellulose-degrading microorganisms isolated from the natural environment. The effect of coculturing these fungal isolates for degradation of lignocellulosic material has also been reported. Coculturing of *Trichoderma* spp. with other cellulolytic fungi has found to improve the activity of lignocellulose-degrading enzymes compared to its monocultures [36]. In a different study, 18 basidiomycete isolates from the natural environment of Sri Lanka has been evaluated for their lignocellulose-degrading enzyme production. An *Earliella scabrosa* species with higher laccase activity (79,600 U/l) when cultured in 50 g/l rice bran has been reported [37]. Thus, it can potentially be used for industrial production of laccase using rice bran as a cheap carbon source for high laccase production.

6. Conclusions

The current progress in applications of cellulases is truly remarkable and attracting worldwide attention. It has already conquered the global market in an unbeatable way. Microbes are an attractive topic of interest for the production of cellulases due to their immense potential for cellulase production. However, it is apparent that more efficient species are still out there in the environment unnoticed by researchers. Further exploration and understanding of hidden mechanisms

behind the activity of these enzymes are much more important. Microbial cellulases are preferred for their potential applications in a broad range of industries. Their ventures are expanding day by day. More and more researches are required to produce scientific knowledge to meet the growing demands for microbial cellulase. The advances in the emerging fields such as biotechnology, microbiology, and molecular biology will open up novel strategies to magnify the still-unlocked potentials of these enzymes. Eventually, it will be able to fine-tune the areas which still are dragging on the way to their utmost success.

Acknowledgements

Special thanks go to Research Assistant, Mr. K. Mohanan and Technical Officer Mrs. Kumuduni Karunarathna at Bioenergy and Soil Ecosystems Research Project, National Institute of Fundamental Studies, Kandy Sri Lanka and the National Research Council (Grant No: 12-021) for financial support.

Conflict of interest

No conflicts of interest.

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Chapter 6

Multi-Finishing of Polyester and Polyester Cotton Blend Fabrics Activated by Enzymatic Treatment and Loaded with Zinc Oxide Nanoparticles

Naser Gad Al-Balakocy, Khaled El-Badry and Talaat Mahmoud Hassan

Abstract

The present work discusses the possibility of applying enzymatic treatments for fabric surface activation that can facilitate the loading of zinc oxide nanoparticles (ZnO NPs) onto polyester (PET) and polyester cotton blend (PET/C) fabrics prepared by sol-gel method. Activated polyester fabrics loaded by ZnO NPs were investigated by the use of scanning electron microscopy (SEM), electron dispersion emission X-ray (EDX) and Fourier transformed infrared spectroscopy (FT-IR). The functionality of activated polyester fabrics loaded by ZnO NPs was evaluated by analyzing its antimicrobial activity and UV protection efficiency. Antimicrobial activity of activated polyester fabrics and loaded by ZnO NPs was tested against Gram-positive (*Bacillus mycoides*), Gram-negative (*Escherichia coli*), and nonfilamentous fungus (*Candida albicans*). The level of UV protection was verified by the UV protection factor (UPF) of polyester fabrics. Activated post-treated polyester fabrics exhibited outstanding antimicrobial and UV protection efficiency. The achieved antimicrobial function and UV protection on the polyester fabrics are durable with repeated laundering processes even after five washing cycles.

Keywords: polyester fabric, alkali hydrolysis, cellulases, enzymatic hydrolysis, ZnO NPs, sol-gel, EDX, SEM, FT-IR, antimicrobial, UPF

1. Introduction

Loading of the nanoparticles onto the textile materials gained much scientific interest [1, 2]. Taking into consideration that functional groups such as carboxyl and hydroxyl have the possibility for binding nanometal oxides, both physical and chemical modifications suggested to date depend primarily on the incorporation of these groups to the textile material surfaces. Several surface modification methods for synthetic fibers have been described, for example, the use of chemical finishers based on carboxyl containing polymers [3]. Alkaline hydrolysis treatments are unspecific and result in strength and weight losses [4, 5]. Ionized gas treatment of PET materials using plasma

has also been investigated to introduce hydrophilic groups at the surface of the polymer [6]. However, the application of this method is limited because it is complicated to use, and it can be difficult to control the extent of the material modification [7].

Alternatively, surface activation of PET fabrics can be achieved by biological treatment with enzymes that introduce polar groups to the polymer surface. A number of hydrolytic enzymes, such as lipases, cutinases, and esterases, have shown potential for surface functionalization of PET [8, 9]. Of the many enzymes suitable for textile applications, cellulases are one of the most important. Cellulases are used in biopolishing of cotton fabrics to improve their smoothness, softness, and wettability [10]. The extent of enzymatic treatment is governed by many factors such as accessibility of cellulosic substrate to cellulases enzymes, confirmation of the enzyme protein as well as enzyme activity, treatment conditions, i.e. enzyme dosage, pH, temperature, time, coexisting chemicals in the treatment bath, fabric's processing history, as well as mechanical action [11–13].

The biocatalytic method can be performed under mild reaction conditions for avoiding the use of large amounts of chemicals and energy for the finishing and dyeing processes. The enzymatic modifications are specific and can be limited to the fiber surface. Consequently, the bulk properties and mechanical stability of the fibers is not compromised and material savings and products of better quality or with new functionalities can be obtained where enzymatic treatment leads to an increase of free hydroxyl and carboxylic end groups changing the surface properties of the treated material [14]. This introduction of charged and functional groups directly leads to an increased hydrophilicity. Furthermore, the increased amount of hydroxyl and carboxylic groups facilitates the attachment of nanoparticles from sol-gel solutions [15].

This study discusses the possibility of applying enzymatic treatment for fabric surface activation that can facilitate the loading of ZnO NPs from solutions onto PET and PET/C blend fabrics and, thus, improve the laundering durability of their antimicrobial activity as well as the level of UPF factor. The addition of this technology to polyester finishers offers an environmentally friendly and mild alternative to the chemical and mechanical finishes currently being used in industry.

2. Experimental work

2.1 Materials

Polyester (PET) and polyester/cotton blend (PET/C 50/50) fabrics used throughout this study were in the form of filament woven fabric cloth made from filament yarns. They were kindly supplied by Misr polyester Co., Kafr EL-Dwar, Egypt. The fabrics were scoured at 80°C for 45 min. With solution containing 2 g/L nonionic detergent, washed with cooled water, squeezed, and finally air dried.

2.2 Enzyme

Acid cellulases used through this work: multifunctional acid cellulases enzyme formulations namely: Cellusoft ® L (Novo Nordisk).

2.3 Microorganisms

Gram-positive bacterium [*Bacillus mycoides* (*B.m*)], Gram-negative bacterium [*Escherichia coli* (*E.c*)], and nonfilamentous fungus [*Candida albicans* (*C.a*)] were selected for investigation of antimicrobial activity of parent and modified samples. Microorganisms were obtained from the culture collection of the Department of

Microbial Chemistry, Division of Genetic Engineering and Biotechnology, National Research Centre of Egypt.

2.4 Culture medium

Modified nutrient agar medium has been used and consists of the following components (g/L): peptone (10.0), beef extract (5.0), NaCl (5.0), and agar (20.0). The pH was adjusted to 6.8. The abovementioned medium was sterilized under pressure at 121°C for 20 min.

3. Methods

3.1 Preparation of ZnO NPs by sol-gel method

The typical procedure for synthesis of ZnO sol is based on the method described in the literature by [16]. Zinc acetate dihydrate was used as zinc oxide source. In a typical procedure, 0.01 mol of zinc acetate dihydrate was dissolved in 50 ml of methanol and heated at 50°C along with stirring for 30 min, thus making precursor solution A. Then, 0.02 mol of sodium hydroxide was dissolved in 50 ml of methanol and heated at 50°C for 60 min, making precursor solution B. In order to make ZnO nano-sol, solution B was added into solution a dropwise under constant stirring for 30 min and then mixture was heated at 50°C for further 30 min. Subsequently, after continuous stirring for 2 hours and cooling at room temperature, a homogenous and transparent sol was obtained.

3.2 Preparation of activated polyester fabrics

Two different methods were used to activate polyester fabrics:

A. Polyester fabrics treated by cellulases

The treatment of PET and PET/C blend fabrics with the cellulases was carried out using a high-temperature high-pressure laboratory dyeing machine. The required amounts of cellulases were placed in stainless-steel bowls (1 and 3%), the fabric samples were immersed in the solutions, its pH = 4.5 (with acetic acid), and the sealed bowls were rotated in a closed bath containing ethylene glycol at 45°C. The material:liquor ratio (M:L) was 1:15. The bath temperature increased at rate of 5°C/min. After 40 min, the enzymatic treatment was then terminated by raising the pH to 10 by using Na_2CO_3; the samples were removed from the bath, rinsed repeatedly with distilled hot and cold water, and then the treated fabric samples allowed to dry in the open air. The extent of biodegradation was estimated from the weight loss (WL) of the fabric samples based on the following equation:

$$WL\ (\%) = [W_1 - W_2/W_1] \times 100.$$

where W_1 and W_2 are the weights of the samples before and after enzymatic treatments.

B. Polyester fabrics treated by alkali before cellulase

The alkaline treatment of PET and PET/C blend fabrics was carried out according to the method described by [17] using a high-temperature, high-pressure

laboratory dyeing machine. Required amounts of alkali solutions were placed in stainless-steel bowls, fabric samples were immersed in the solutions (0.25 mol/L), and the sealed bowls were rotated in a closed bath containing ethylene glycol at 90°C. The liquor-to-fabrics ratio (M:L) was 1:50. The bath temperature increased at rate of 2°C/min. After the predetermined durations (60 min), the samples were removed from the bath, rinsed repeatedly with distilled water, neutralized with a solution of 1% hydrochloric acid, and rinsed. The samples were then dried at 100°C, cooled in a desiccator, and weighed. The weight loss is expressed as relative WL was calculated according to the equation:

$$WL\ (\%) = [W_1 - W_2/W_1] \times 100.$$

Where W_1 and W_2 are the weights of the samples before and after alkaline treatments, respectively.

The treatment of hydrolyzed polyester fabrics with cellulases was carried out according to the above mentioned method.

3.3 Preparation of polyester fabrics loaded by ZnO NPs

The activated PET and PET/C blend fabrics by cellulases and hydrolyzed fabrics before enzymatic treatment were immersed in the ZnO NPs dispersion, the samples were then squeezed to a pickup of 60% (wt/wt) of the solution, and dried in air at 22°C (laboratory temperature) for 24 hours, and finally cured in an oven at 150°C for 15 min. The modified polyester fabrics were rinsed five times to assess the adhesion of ZnO NPs to the fabrics by using the standard method AATCC test method (61-1989).

3.4 Analysis

Carboxylic content was calculated by using the analytical method described by Daul et al. [18].

3.5 Antimicrobial activity

Antimicrobial activity of PET and PET/C blend fabrics modified with ZnO NPS was measured using the technique below.

The antimicrobial efficacy by disk diffusion was calculated in this technique by measuring in millimeters the width of the growth inhibition area around the specimen according to the conventional test method of AATCC [19].

3.6 SEM and EDX

Surface structure and the morphology of all fabric samples characterized by a JEOL-Model JSM T20 scanning electron microscope (SEM) operating at 19 kV was used to obtain photomicrographs of fabrics surfaces.

3.7 FT-IR

The chemical composition was defined using the spectrometer Fourier transformation infrared (FT-IR), model NEXUS 670, NICOLET USA. Measurements ranged from 4000 to 500 cm^{-1} in spectral range. The method of measuring the percentage of reflection (R percent) was applied to all the specimens under investigation.

3.8 UPF factor

The ultraviolet protection factor (UPF) was estimated using the spectrophotometer UV-Shimadzu 3101 P C. It is a scheme of double beam direct measurement proportion. It comprises of the unit of the photometer and a P C. The UPF factor was determined by the technique outlined in AS/NZS 4399, Australian/New Zealand: 1996 [20].

4. Results and discussion

4.1 Polyester fabrics treated by cellulases

It is clear from **Table 1** that the PET fabrics had the lowest hydrolysis rate with 1.5% weight loss [21], while enzymatic treatment brings about a noticeable decrease in loss the weight of PET/C blend fabrics, 2.1 and 3.0% respectively, by increasing cellulases concentration from 1.0 to 3%. Cellulases have a higher specific activity toward cotton fibers than polyester; this explains the high weight loss with PET/C fabric. A higher specific activity correlates with higher weight loss % with PET/C fabric. This is a direct consequence of a partial enzymatic hydrolysis of the cellulosic fibers especially on the fabric surface and amorphous regions, yielding soluble products such as short-chain oligomers and glucose [22].This finding accompanied by an increase in the carboxylic content, and the extent of the ZnO NPs increase by increasing the amount of carboxyling groups. The atomic weight % of ZnO value is higher, the greater the loss in weight, regardless of the used polyester fabrics.

4.2 Polyester fabrics treated by alkali before cellulases

PET fabrics had the lowest degradation rate with less than 2.0% weight loss. This is a direct consequence of the chemical and physical structure of polyester fabrics and specific activity of the celluloses toward cellulosic fibers, (**Table 1**), so in order to

Fabrics	Weight loss %	Carboxylic content (meq/100 gr. Fabric)	Zn content (atomic %) estimated by EDX*
PET	0.0	3.30	0.0
PET+E	1.5	6.50	0.0
PET+E + ZnO			1.19
PET+H + E	3.4	10.8	0.0
PET+H + E + ZnO			1.53
PET/C	0.0	8.10	0.0
PET/C + E	4.2	13.3	0.0
PET/C + E + ZnO			1.33
PET/C + H + E	6.6	16.4	0.0
PET/C + H + E + ZnO			1.71

Enzymatic treatment conditions: (Cellulases): 3%, pH = 4.5, Time, 40 min, Temperature, 45°C, M:L, 1:15. Alkali treatment conditions: (NaOH), 0.25 mol/L, Time, 60 min, Temperature, 90°C, M:L, 1:50. Sol-gel treatment conditions: (ZnO), 0.4×10^{-1} mol/l; Curing Temperature, 150°C; Curing Time, 15 min. E, cellulases; H, alkali hydrolyzed.
**According to AATCC test method (61-1989).*
***According to Australia (AS)/New Zealand (NAS) Standard No. 4399 (1996).*

Table 1.
Effect of the cellulases treatment on the amount of carboxylic content and ZnO NPs loaded on PET and PET/C blend fabrics.

enhancement the bond ability of ZnO NPs loaded onto polyester fabrics, the surface textile modifications induced by cellulases treatment of PET and PET/C blend fabrics was enhanced by doing partial weight loss 3.4% with PET and 6.6% with PET/C using alkali hydrolysis before cellulases treatment. **Table 1** shows that increasing the oxygenated polar groups (OH and COOH) onto polyester fabrics able to enhance its ability to bind ZnO NPs in stable way on their surfaces where the atomic weight % of Zn loaded onto PET and PET/C were 1.53 and 1.71, respectively. This funding can be attributed to the action of NaOH on the surface of the polyester and short fibers by partial hydrolysis, which help the cellulases to be more effective, then the Zn ONPs is attached to the modified textile surfaces by exchange with the carboxylic groups.

4.3 Formation of ZnO NPs on polyester fabrics

The dispersion solution prepared as mentioned before was applied directly to the pre-activated PET and PET/C blend fabrics, and ZnO NPs is fixed during the thermal treatment. The preparation of ZnO NPs in the nanometer range can be effectively conducted through the hydrolysis and condensation of zinc alkoxide in aqueous media. The chemical reactions that occur during this synthesis are explained as follow:

$$M\,(OR)_n + H_2O \rightarrow M(OH)\,(OR)_{n-1} + ROH \quad (1)$$

$$M\,(OH)\,(OR)_{n-1} + M\,(OR)_n \rightarrow (RO)_{n-1}MOM\,(OR)_{n-1} + ROH \quad (2)$$

$$2\,M\,(OH)\,(OR)_{n-1} \rightarrow (RO)_{n-1}MOM\,(OR)_{n-1} + H_2O \quad (3)$$

Interaction of alkoxides with water yields precipitates of hydroxides, hydrates, and oxides. The precipitate particles usually range in size from 0.01 to 1 μm. So we can easily produce nanoparticles. Metal alkoxides undergo hydrolysis very easily; the hydroxyl metal alkoxide product can react by a further condensation reaction to form polymerizable species.

4.4 Characterization of polyester fabrics loaded with ZnO NPs

The verification of ZnO NPs on the surface of PET fabrics was confirmed by EDX analysis. EDX spectra of the PET fabrics loaded with ZnO NPs after five washing cycles are shown in **Figure 1**. It is noteworthy to conclude on the basis of these spectra that, the precipitated substance consists mainly of Zn and O_2. This demonstrates that ZnO is still available on the surface of the polyester fabrics (**Table 1**) even after five washing cycles (25 home washings). EDX measurements also reveal higher Zn content on hydrolyzed and treated polyester fabrics by cellulases more than treated fabrics by cellulases only (Zn atomic weight % was 1.19 increased to 1.33 in case of PET fabric, on the other hand 1.33 up to 1.71 with PET/C fabric). This means that ZnO NPs have sufficient adhesion toward the activated PET fabrics either by cellulases or by alkali treatment followed by cellulases.

4.5 Surface topography

4.5.1 Scan electron microscope (SEM)

In order to investigate the morphology of the modified polyester fabrics and loaded by ZnO NPs, SEM images of samples were recorded in **Figure 1**. **Figure 1** shows the

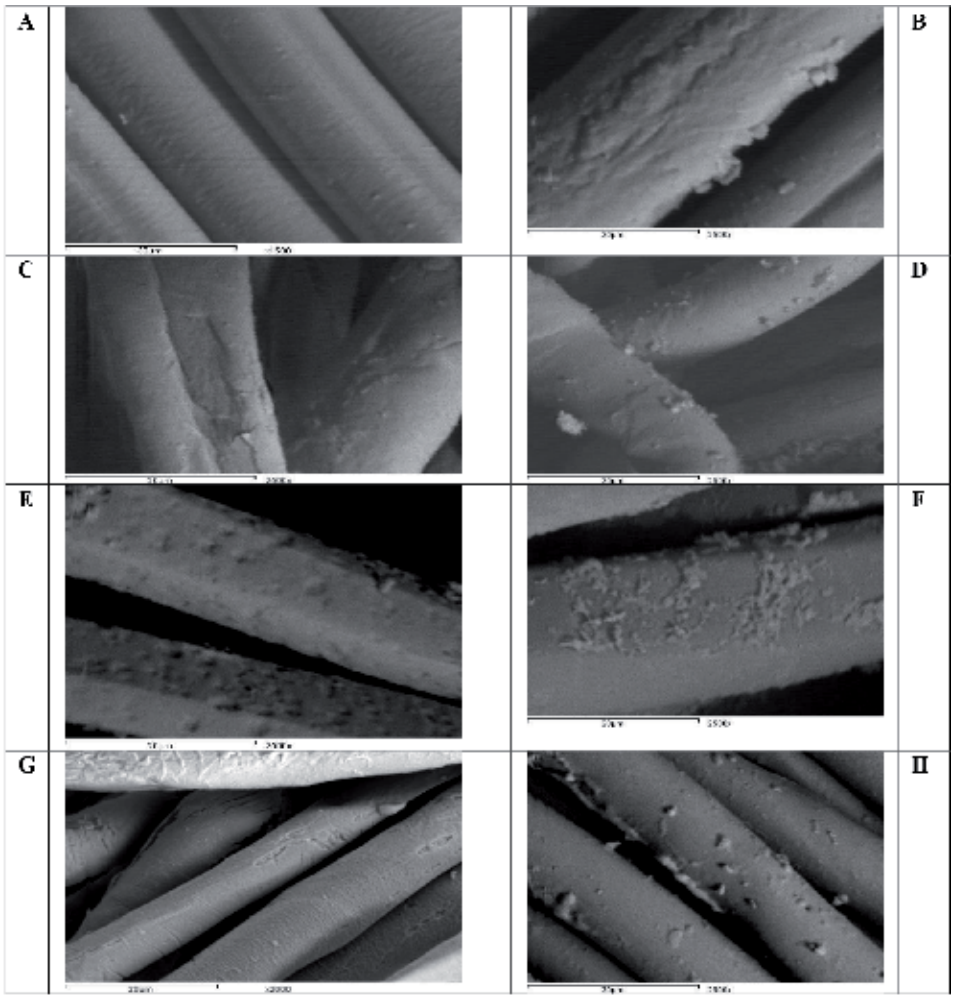

Figure 1.
SEM micrographs of activated PET and PET/C blend fabrics and loaded with ZnO NPs (1000×). (A) PET+E; (B) PET+ E + ZnO; (C) PET/C + E; (D) PET/C + E + ZnO; (E) PET+H + E; (F) PET+H+ E + ZnO; (G) PET/C + H + E; (H) PET/C + H + E + ZnO. *After five washing cycles according to AATCC test method (61-1989). E, cellulases; H, alkali hydrolyzed.*

images of the activated and treated fabrics followed by five washing cycles. **Figure 1A** and **C** show that the surfaces of treated PET and PET/C blend fabrics with cellulases are clean and smooth. After treatment by alkali before cellulases, a few pits were appeared on the surfaces of PET and PET/C, the latter have gained a roughness on fabric surfaces (**Figure 1E** and **G**). The treated polyester fabrics by sol-gel (**Figure 1B**, **D**, **F** and **H**) are covered by a thinner uniform surface layer; a continuous deposited material is shown clearly. Based on the images seen in **Figure 1,** the following can be concluded:

1. The surfaces of treated PET and PET/C fabrics by enzyme are clean and smooth (**Figure 2**). A partial hydrolysis by cellulases imparted the fabrics a smooth surface with improved resiliency and soft handle. This is due to the amount of weight reduction along with elimination of hairiness on the fabric surface, thereby minimizing stiffness and thickness as well as imparting a smooth surface.

2. PET and PET/C fabrics hydrolyzed with alkaline solutions before treatment with enzyme are characterized with pits and grooves. The treatment with ZnO

leads to blocking of these defects and formation of thin layer of active substrate on the fiber surface (**Figure 1**).

3. The treatment of the fabrics with ZnO leads to the formation of some deposits on the surface of treated fabrics. The shape and the size of such deposits vary according to the fabrics used during the enzymatic treatment.

4.5.2 EDX

The surface topography of PET and PET/C blend fabrics was investigated using EDX technique (**Figure 2**). Treatment of polyester fabrics with ZnO NPs activated with cellulases only or after alkali hydrolysis is also accompanied with the formation of precipitates (**Figure 1**). This is reflected on the amount of ZnO NPs percentage on the fiber's surface (**Table 1**). The above mentioned changes that took place on the surface topography of polyester fabrics loaded with ZnO NPs are a direct indication that ZnO NPs are directly attached to the fabrics surfaces.

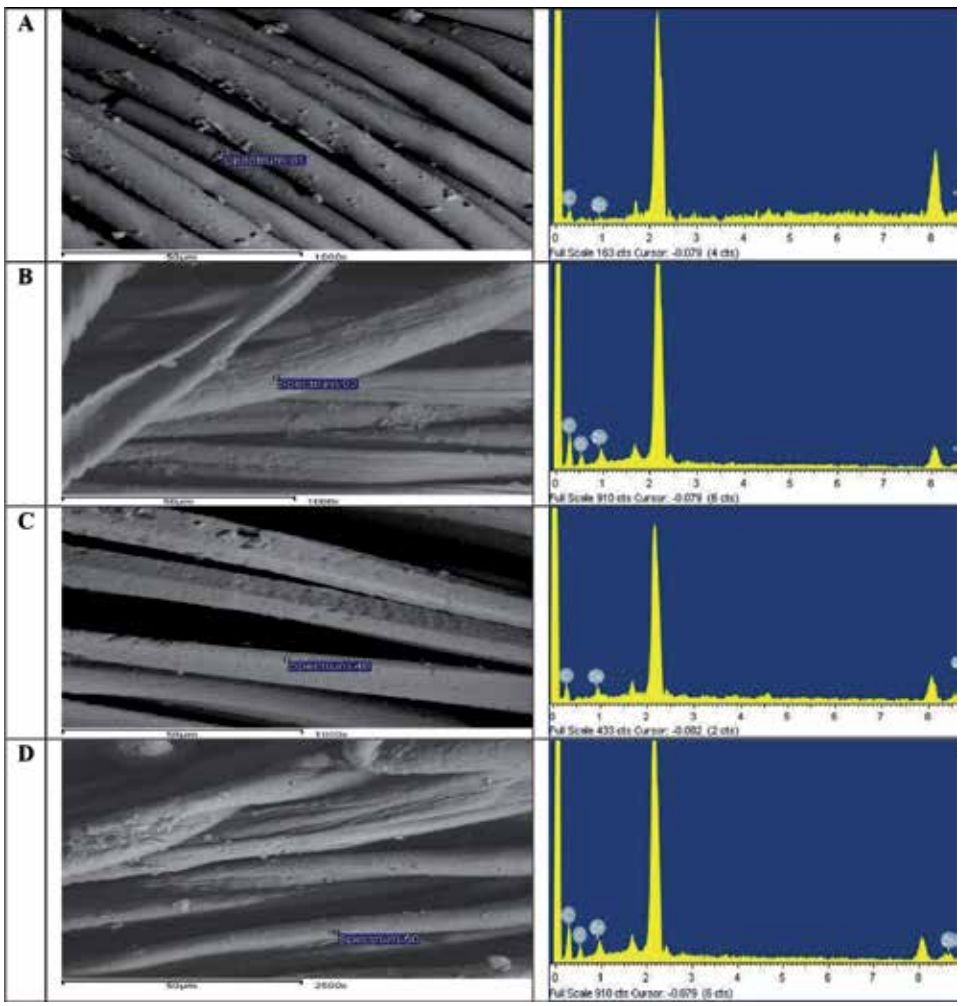

Figure 2.
EDX micrographs of activated PET and PET/C blend fabrics and loaded with ZnO NPs (1000×). (A) PET+ E + ZnO; (B) PET/C + E + ZnO; (C) PET+H+ E + ZnO; (D) PET/C + H + E + ZnO. *After five washing cycles according to AATCC test method (61-1989). E, cellulases; H, alkali hydrolyzed.*

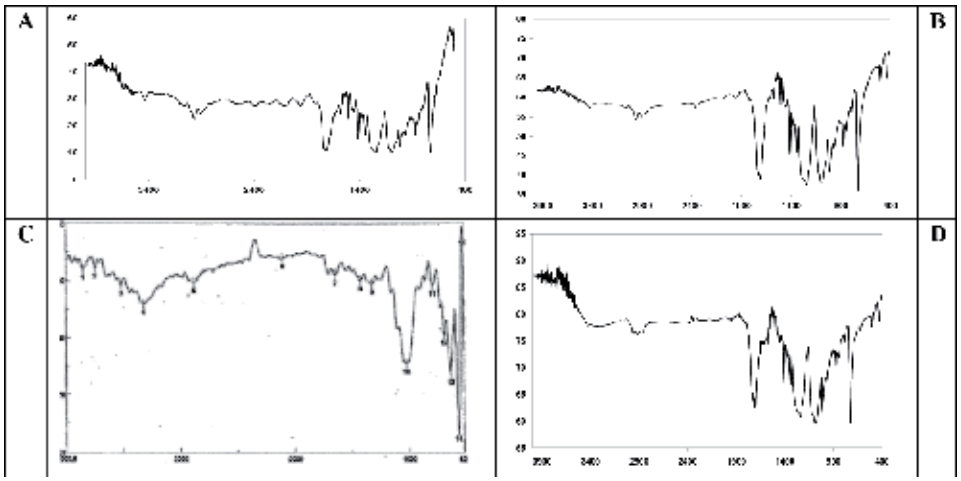

Figure 3.
FT-IR spectra of activated PET fabrics and loaded with ZnO NPs (1000×). (A) PET+ E; (B) PET/C + E + ZnO; (C) PET+H+ E; (D) PET/C + H + E + ZnO. *After five washing cycles according to AATCC test method (61-1989). E, cellulases; H, alkali hydrolyzed.*

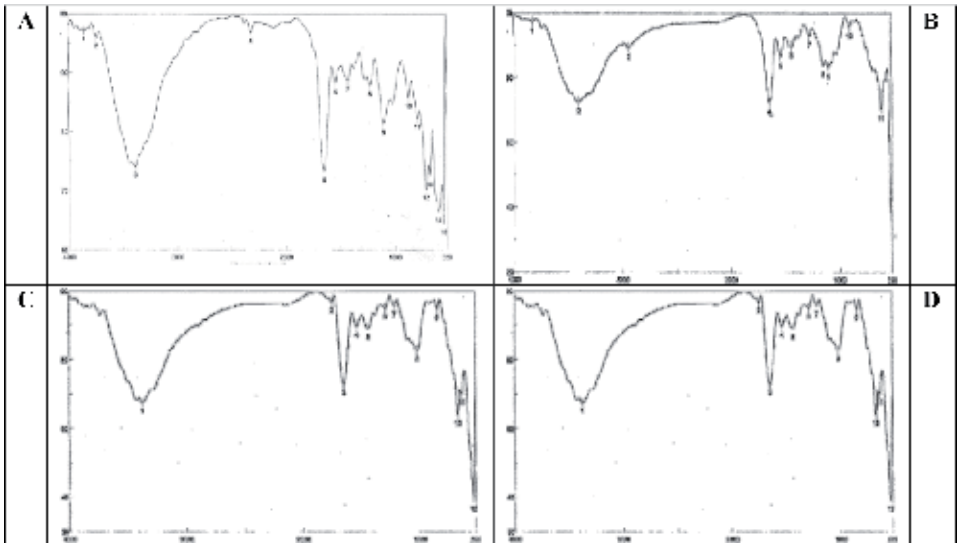

Figure 4.
FT-IR spectra of PET/C fabrics activated and loaded with ZnO NPs. (A) PET/C+ E; (B) PET/C + E + ZnO;(C) PET/C + H+ E;(D) PET/C + H + E + ZnO. *After five washing cycles according to AATCC test method (61-1989) E, cellulases; H, alkali hydrolyzed.*

4.5.3 FT-IR

Evidently, both enzymatic and alkali hydrolysis before activation led to a substantial shift in the chemical structure of the surfaces of polyester fabrics. The FTIR spectrum (**Figures 3** and **4**) of parent polyester fabric shows absorptions at 1649–1712, 3408–3388, and 2317 cm^{-1}, which are typical to those of C═O, OH, and CH stretching, respectively. New bands at 640 and 660 cm^{-1}, respectively, are observed in the spectrum PET and PET/C blend fabrics activated with cellulases and alkali hydrolyzed before activation, which can correspond to Zn-O of the new bonds PET + ZnO and PET/C blend + ZnO. The presence of this band can support

the ionic character of the new band formed due the addition of ZnO NPs to enzymatic and alkali hydrolyzed fabrics.

The FT-IR spectrum of activated PET and PET/C blend fabrics with enzyme and/or alkali hydrolysis and loaded by ZnO NPs (**Figures 3** and **4**) shows that new characteristic peaks are appeared and located at around 665 and 770 cm^{-1}, as well as 794 cm^{-1}, respectively. These peaks are corresponding to Zn-O bond. The similar finding was reported by Hong et al. [23]. During this study, we found that only activated surfaces were able to fix ZnO NPs from dispersion solutions.

4.5.4 Antimicrobial activity

The antimicrobial activity of PET and PET/C blend fabrics activated with cellulases on one hand, and with alkali hydrolysis followed by enzyme, on the other hand, and loaded with ZnO NPs, was investigated against *B. mycoides* (Gram-positive), *E. coli* (Gram-negative), and *C. albicans* (nonfilamentous fungus). The activity by diffusion is quantified by the measurement in millimeters of the width of the zone of inhibition around the sample. The antimicrobial efficacy of PET and PET/C fabrics modified with ZnO NPs after activation using distinct techniques is shown in **Table 2**. It is seen from the data listed in this **Table 2** that, all polyester fabrics showed, after five washing cycles, high antimicrobial activity against the previously mentioned three microorganisms. In fact, the inhibition zones for all tested polyester fabrics samples are significant, whereas no dedication is found for all untreated fabrics. The role of activation of polyester fabrics with cellulases after alkali hydrolysis before loading with Zn ONPs on the antimicrobial activity seems to be more significant as the samples were laundered repeatedly in Launder-Ometer. This demonstrates the validity of the enzymatic activation of PET and PET/C blend fabrics on its antimicrobial finishing with ZnO NPs.

4.5.5 Ultraviolet protection properties

The effect of activation of PET and PET/C blend fabrics either with cellulases or by alkali hydrolysis before enzymatic treatment and before loading with ZnO NPs on UV protection efficiency was investigated. The rate of UV protection was

Fabrics	Inhibition zone diameter (mm) in case of loaded polyester fabrics with ZnO NPs*		
	B.m	***E.c***	***C.a***
PET	−ve	−ve	−ve
PET+E + ZnO	18	20	18
PET+H + E+ ZnO	21	22	20
PET/C	−ve	−ve	−ve
PETC+E + ZnO	18	20	19
PET/C + H + E + ZnO	20	22	21

Enzymatic treatment conditions: (Cellulases), 3%, pH = 4.5, Time, 40 min, Temperature, 45°C, M:L, 1:15. Alkali treatment conditions: (NaOH), 0.25 mol/L, Time, 60 min, Temperature, 90°C, M:L, 1:50. Sol-gel treatment conditions: (ZnO), 0.4×10^{-1} mol/l; Curing temperature, 150°C; Curing time, 15 min. E, cellulases; H, alkali hydrolyzed.
**According to AATCC test method (61-1989).*
***According to Australia (AS)/New Zealand (NAS) Standard No. 4399 (1996).*

Table 2.
Effect of activation of PET and PET/C blend fabrics on its antimicrobial activity.

Fabrics	UPF values after no of washing cycles			
	1*		5*	
	UPF value	UPF** rating	UPF value	UPF** rating
PET	19.0	Good	9.6	Poor
PET+E	17.3	Good	11.3	Poor
PET+E + ZnO	28.2	V. Good	18.2	Good
PET+H + E	16.8	Good	15.2	Poor
PET+H + E + ZnO	31.2	V. Good	27.4	V. Good
PET/C	18.5	Good	12.8	Poor
PETC+E	20.1	Good	14.2	Poor
PET/C + E + ZnO	51.1	Excellent	35.4	V. Good
PET/C + H + E	19.2	Good	14.7	Poor
PET/C + H + E + ZnO	68.4	Excellent	55.2	Excellent

Enzymatic treatment conditions: (Cellulases), 3%, pH = 4.5, Time, 40 min, Temperature, 45°C, M:L, 1:15. Alkali treatment conditions: (NaOH), 0.25 mol/L, Time, 60 min, Temperature, 90°C, M:L, 1:50. Sol-gel treatment conditions: (ZnO), 0.4×10^{-1} mol/l; Curing temperature, 150°C; Curing time, 15 min. E, cellulases; H, alkali hydrolyzed.
**According to AATCC test method (61-1989).*
***According to Australia (AS)/New Zealand (NAS) Standard No. 4399 (1996).*

Table 3.
Effect of activation of PET and PET/C blend fabrics on its UPF values.

quantified and expressed via UPF values that are given in **Table 3**. It was found that the UPF factors for untreated PET, PET/C blend fabrics are equal to 9.6 and 12.8, respectively. Activation with cellulases followed by the ZnO NPs deposition onto the above mentioned polyester fabrics led to a significant increase in UPF factor to the level corresponding to UPF rating of 25+, which assigns the very good UV protection, after five washing cycles. These results imply good laundering durability of polyester fabrics and excellent laundering durability of polyester fabrics activated with enzyme and loaded with ZnO NPs. It was found that PET and PET/C blended fabrics activated with alkali hydrolysis before enzymatic treatment and loaded with ZnO NPs showed better UV protection efficiency compared to enzymatic treated ones 50+, which assigns the excellent UV protection. The UV protection efficiency of these fabrics is higher even after five washing cycles, indicating the excellent laundering durability.

5. Conclusions

The present study illustrates a simple method for improving the binding ability of ZnO NPs to PET and PET/C blend fabrics. This method is based on applying the biological activation method by cellulases before loading polyester fabrics with ZnO NPs by sol-gel method. These loaded fabrics were characterized by SEM, EDX, and FT-IR spectroscopy, which confirmed that ZnO NPs is chemically bonded to PET fabrics. The effect of surface activation method on antimicrobial activity and UV protection efficiency of polyester fabrics was evaluated. It was found that PET and PET/C fabrics activated with enzyme before its treatment with ZnO NPs showed better antimicrobial and UV protection properties compared to parent fabrics. Activated polyester fabrics even after five washing cycles showed excellent antimicrobial activity and UV protection effectiveness, revealing the excellent durability

of laundering. In general, the results obtained in this study show the possibility of implementing the technique of biological surface activation to attach the ZnO NPs to polyester fabrics. The addition of this technology to polyester finishers offers an environmental friendly and mild alternative to the chemical and mechanical finishes currently being used in industry

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Edited by Alejandro Rodríguez Pascual and María E. Eugenio Martín

Cellulosic fibers are becoming increasingly important in many industrial sectors. Indeed, their availability, low cost, and durability make them suitable for application in various fields. This book presents important information about the structure of cellulose as well as its uses and applications. Topics covered include: the dynamic modeling of cellulose industry systems for biofuels in the United States; the integration of alternative raw materials to wood in biorefinery processes; the influence of size classification on the properties of cellulose materials; the chemistry of cellulose, its extraction, and its properties; cellulases; and enzymatic treatments for producing surface activation on cellulosic fibers.

Published in London, UK

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ISBN 978-1-83968-058-8
9 781839 680588