PROJECT REPORT

On

COMPARATIVE ANALYSIS OF CELLULOSE NANOFIBER SYNTHESIS VIA CHEMICAL AND ECO-FRIENDLY METHODS: A SUSTAINABLE APPROACH TO MATERIAL SCIENCE

Submitted by

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(AM22CHE013)

In partial fulfillment for the award of the
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DEPARTMENT OF CHEMISTRY
AND
CENTRE FOR RESEARCH

ST. TERESA'S COLLEGE (AUTONOMOUS)
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ERNAKULAM

M.Sc. CHEMISTRY PROJECT REPORT

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Register Number : **AM22CHE013**
Year of Work : **2023-2024**

This is to certify that the project "**COMPARATIVE ANALYSIS OF CELLULOSE NANOFIBER SYNTHESIS VIA CHEMICAL AND ECO-FRIENDLY METHODS: A SUSTAINABLE APPROACH TO MATERIAL SCIENCE**" is the work done by **PREDITTA FERNANDEZ.**

Dr. Saritha Chandran A.
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Dr. Jaya T. Varkey
Staff-member in charge

Submitted to the Examination of Master's degree in Chemistry

Date: ……………………..…

Examiners:…………………………………………………………………..

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DEPARTMENT OF CHEMISTRY
AND
CENTRE FOR RESEARCH

ST. TERESA’S COLLEGE (AUTONOMOUS)
ERNAKULAM

CERTIFICATE

This is to certify that the project work titled “**COMPARATIVE ANALYSIS OF CELLULOSE NANOFIBER SYNTHESIS VIA CHEMICAL AND ECO-FRIENDLY METHODS: A SUSTAINABLE APPROACH TO MATERIAL SCIENCE**” is the work done by **Preditta Fernandez** under the guidance of **Dr. Jaya T. Varkey, Professor,** Department of Chemistry and Centre for Research, St. Teresa’s College, Ernakulam in partial fulfilment of the award of the Degree of Master of Science in Chemistry at St. Teresa’s College, Ernakulam affiliated to Mahatma Gandhi University, Kottayam.

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Project Guide	Head of the Department

DEPARTMENT OF CHEMISTRY
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CERTIFICATE

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Dr. Jaya T. Varkey
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DECLARATION

I hereby declare that the project work entitled "**COMPARATIVE ANALYSIS OF CELLULOSE NANOFIBER SYNTHESIS VIA CHEMICAL AND ECO-FRIENDLY METHODS: A SUSTAINABLE APPROACH TO MATERIAL SCIENCE**" submitted to Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous) affiliated to Mahatma Gandhi University, Kottayam, Kerala is a record of an original work done by me under the guidance of Dr. Jaya T. Varkey, Professor, Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous), Ernakulam. This project work is submitted in the partial fulfillment of the requirements for the award of the Degree of Master of Science in Chemistry.

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PREDITTA FERNANDEZ

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Abbreviation	
SCB	Sugarcane bagasse
AGU	Anhydro- D- glucopyranose units
SMCC	Silicified microcrystalline cellulose
MCC	Microcrystalline cellulose
HPC	Hydroxypropyl cellulose
HPMC	Hydroxyl propyl methylcellulose
EC	Ethylcellulose
SCMC	Sodium carboxymethyl cellulose
HEC	Hydroxyl ethyl cellulose
MC	Methylcellulose
CNF	Cellulose nanofiber
CNF – I	Cellulose nanofiber from chemical method
CNF - II	Cellulose nanofiber from alkaline H_2O_2 treatment
CNC	Cellulose nanocrystal
CS	Chitosan
PVA	Polyvinyl alcohol
E. coli	Escherichia coli
S.aureus	Staphylococcus aureus

Nanofiber film-I	Nanofilm from chemical method
Nanofiber film-II	Nanofilm from alkaline H_2O_2 treatment
AFM	Atomic force microscopy
SEM	Scanning electron microscopy
FESEM	Field emission scanning electron microscopy
TEM	Transmission electron microscopy
FTIR	Fourier transform infrared
XRD	X-ray diffraction
IR	Infra-red radiation
AHP	Alkaline hydrogen peroxide
LBMS	Ligno cellulosic biomass materials
FWHM	Full width at half maximum

Chapter 1

Introduction

The Brundtland Report of the International Commission on Environment and Development defines sustainable development as one that "meets the demands of the present without jeopardizing the ability of future generations to fulfill their requirements". According to this notion, current procedures and activities should not endanger societal norms or traditions. Due to differences in size, wealth, living standards, culture, and political and administrative systems, different countries have varying capacities for achieving sustainable development. Sustainable waste management includes making prudent use of material resources, minimizing waste creation, and disposing of garbage in a way that actively advances the social, environmental, and economic goals of sustainable development. The following methods of process planning can actively address sustainable waste management:

1. Optimum usage of building materials
2. Reducing the amount of waste generated.
3. Management of construction and demolition wastes.
4. Material requirements, such as the utilization of recycled and repurposed materials.
5. Provision of recycling space/facilities.

The Waste Management Hierarchy, which represents the relative sustainability of the various waste management alternatives, can be used to arrange them [1].

1.1CELLULOSE

Cellulose is an insoluble substance that is the basic building block of plant cell walls and fibers, including cotton. It is a naturally occurring polymer that is used in place of several different polymers. Cellulose is the main component of paper, cardboard, and textiles manufactured from cotton or other plant fibers. It is additionally used to make films and cellulose derivatives. It is tasteless, biodegradable, chiral, and odorless. The length, diameter, and source of natural cellulose all depend on the extraction process. There are crystalline and amorphous domains in natural cellulose. The source and the extraction techniques used can affect the crystallinity of the material. The amorphous region is less dense and more likely to interact with another chemical group than the crystalline region. However, the crystalline domains are more resistant to mechanical, chemical, and enzymatic treatments than the amorphous ones. A variety of cellulose forms, including cellulose I, II, III, and IV, can be produced based on the Vander Waals force, intra- and intermolecular interactions, isolation method, and treatment schedule[2]. Cellulose is the main building block of plants and the main element in the food chain. An extremely important substance and the most common biopolymer that occurs naturally is cellulose[3-4]. The main component of many natural fibers, such as cotton and higher plants is cellulose [5-6]. Long chains of anhydro-D-glucopyranose units (AGU) are present in it. Except for the terminal ends, each cellulose molecule possesses three hydroxyl groups per AGU.

1.2 SOURCES OF CELLULOSE AND WASTE MATERIAL SOURCE

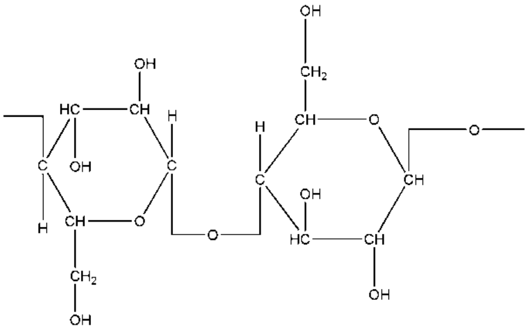


Fig 1: Structure of cellulose

Cellulose is a polymer composed of polysaccharides. The combination of polymer and pharmaceutical sciences led to the introduction of polymer in the design and development of drug delivery systems. Achieving controlled or sustained drug delivery is the primary objective of polymeric delivery systems. The primary source of cellulose used in industry is vascular plants. The majority of resources used in the construction industry, as well as textiles, come from the cotton plant. While wood pulp is used to make the majority of paper products, textile fibers are not separate from woody fibers. Although cotton fibers provide a biological source of nearly pure cellulose, they are typically used for other cellulose derivatives, pharmaceuticals, or chemical engineering applications such as chromatography, paints, and explosives rather than food grade cellulose. Additionally, Acetobacter xylinum, which ferments the glucose-fermenting substrates in corn syrup, has been used to create bacterial sources of cellulose. Different types of cellulose are used for different applications due to cost considerations. Typically, pulp and paper are made from wood. Many techniques for extracting cellulose have been developed; these techniques include esterification, etherification, micro-ionization, and oxidation, which convert prepared celluloses into cellulose derivatives. These derivatives include hydroxyethyl cellulose, silicified microcrystalline cellulose, microcrystalline cellulose, hydroxypropyl cellulose and hydroxyl propyl methylcellulose. Ethyl cellulose, sodium carboxymethyl cellulose, hydroxyl ethyl cellulose, oxycellulose, and methylcellulose are among other substances, in medicinal use. Depending on its concentration, cellulose can serve as a binder, diluents, or disintegrant. Because of its enormous value, cellulose is frequently used in pharmaceutical applications.

1.3 DIFFERENT SOURCES FOR CELLULOSE

1.3.1 NATURAL FIBERS

Natural fibers are made from plant, animal and mineral sources. Natural fibers can be classified according to their origin. This fiber can be further categorized into the following [5-6].

Category	Description	Examples	% of cellulose
Seed fibers	Fibers collected from seeds or seed cases	Cotton, kapok	90
Leaf fiber	Fibers collected from leaves	Sisal, fique, agave	33
Bast fiber	Fibers are collected from the skin or bast surrounding the	Flax, jute, kenaf, hemp, ramie, rattan and vine fibers	33
Skin fibers			

	stem of their respective plants		
Fruit fibers	Fibers collected from the fruits of the plants	Coconut (coir)fiber	30-50
Stalk fibers	Fibers are actually the stalks of the plants	Rice, barley, wheat straws, bamboo grass, tree wood	40-50

Table 1 : Different types of fibers and its corresponding percentage of cellulose

1.3.2 SYNTHETIC FIBERS

Man-made fibers are usually derived from synthetic materials such as petrochemicals. However, natural cellulose is used to make some varieties of synthetic fibers, such as rayon modal and the more recent Lyocell.

CELLULOSE FIBRES: Cellulose fibers are a type of synthetic fiber that is derived from natural cellulose. The cellulose comes from a variety of sources. Bamboo is used to make modal, and beach trees are used to seaweed is utilized to create sea cells, bamboo fiber, etc. a cellulose Bagasse is the name for fiber derived from sugar cane. Here, we make cellulose from sugarcane waste.

1.4 SUGARCANE BAGASSE - A SOURCE OF CELLULOSIC FIBER

Large amounts of sugarcane (Saccharum officinarum) are grown in tropical countries. In 2017, 1.84 billion tons of sugarcane were produced worldwide [7]. It's used in alcohol and sugar mills. Nevertheless, about 30% of the pulpy fibrous residue those mills produce is left over, making it impossible for them to use it all [8-9]. We refer to these byproducts as bagasse[10]. Bagasse is utilized for many different things, including the paper industry and as a feedstock for biofuel [11-12].Sugarcane bagasse is the term used to describe cellulosic material[13]. Although it may have some particular uses, it is usually a waste type. It has a significant amount of cellulose, which can be extracted and utilized for a number of purposes. The textile and civil engineering industries can also use fibrous materials as fiber, though they might require special handling before use. This bagasse can be used to reinforce composite materials to produce an entirely new kind of material[12]. The main benefit of using bagasse is that it is a pure waste product. Even with a few simple pretreatments, this product can be used in any application, and the process yields an extremely cost-effective final product that is definitely fully or partially biodegradable—a vital characteristic component of modern society. Furthermore, if the proper method is used, the extracted fiber may have reasonably good mechanical properties[12].In order to prepare CNF from plant biomass, the ultrasonication method is frequently utilized. As a more practical and environmentally friendly technology, it has emerged over time[14]. Mechanical techniques like high-pressure homogenization, high-pressure micro fluidization, grinding, and high-intensity ultrasonication are used to prepare CNFs[15-17].

1.5 APPLICATIONS

In the textile industry, fibrous materials are typically used most frequently. But when made from sugarcane bagasse, the fibers lack the necessary properties (fineness, crimp, tensile strength, etc.) [18]. The table above illustrates that sugarcane bagasse fibers are significantly weaker and coarser than other fibres, like cotton and flax, which are commonly utilized in items made of textiles. The only fibers that have shown some similarity are coir and sisal, and textile applications don't usually use these fibers [19]. Pre-treatment of the fibers included 10% H2SO4 and 1% NaOH. Increased 15.5% tensile strength, 45.4% impact strength, and 32.4% flexural rigidity were achieved with the addition of 20% fiber. In a polyester composite made with bagasse fiber reinforcement, Monteiro et al. discovered that the reinforcement increased the material's flexural rigidity [20].Oladele also discovered that adding bagasse fiber to a polyester composite increased its tensile strength.[19]. According to Moubarik et al., the mechanical characteristics of low-density polyethylene composite were considerably enhanced by the addition of pure sugarcane bagasse fiber [21].According to Monterio et al., the performance of a multi-layered epoxy composite reinforced with bagasse fiber may be comparable to that of a multi-layered sheet made of Kevlar [22].Nonetheless, artificial polymers such as polypropylene, polyethylene, and epoxy are employed. Composites composed of specific matrix types have limited biodegradation potential. Materials that are completely biodegradable have also been made using natural polymers, of which starch is one of the most common. Starch has also been used to modify textile fabrics [23].Excellent instances of starch composites reinforced with bagasse from sugarcane have been reported. Gilfillan et al. show that the crystallinity of the composite is increased by the addition of sugarcane bagasse fiber [24].According to Vallejos et al.'s study , the starch composite's tensile strength and water absorbency also increased with the accumulation of sugarcane bagasse fiber [25]. Jeefferie et al. developed a disposable food container composed of sugarcane bagasse reinforced starch composite [26].Our project involves providing an antibacterial coating to the CNF film by using chitosan and PVA blend.A popular technique for producing desirable polymeric materials with combination qualities appropriate for certain applications is polymer blending [27]. Research on chitosan blends containing artificial and natural macromolecules has drawn considerable attention [28]. A practical way to create synthetic biodegradable polymers with diverse qualities like improved mechanical characteristics and good water absorbance while retaining biodegradability is to blend chitosan with synthetic polymers [29].

1.6 ENVIRONMENTAL AND SUSTAINABILITY ASPECTS

A sustainable civilization cannot exist without the effective use of plant biomass resources for the production of materials and energy. A steady supply of renewable resources that don't interfere with the production of food can be obtained from wood biomass. The main components of plant cell walls are cellulose, hemicelluloses, and lignin. Wood-derived cellulose fibers are mostly used to make paper and board, as well as cellulose-related products including viscose rayon fibers and other cellulose derivatives. High strength, stability, and durability in plants are attributed to the hierarchical structure of wood and the nanocomposite structures generated between cellulose, hemicelluloses, and lignin. Crystalline and stable cellulose microfibrils, consisting of 20–40 completely extended cellulose chains, are found in the cellulose walls of plants. They have a width of around 3 nm 1, 2, 3. Thus, cellulose microfibrils are bio-based nanofibers that are most widely distributed on Earth and are continuously photosynthesized and stored in plants [30].

The emergence of nanocellulose derived from biomass satisfies the sustainability requirements (renewability, abundance, biodegradability, and recyclability) that are now placed on new materials, but were largely disregarded in the plastics era. Historically, cellulose nanofibers were obtained from wood products with the help of a well-established forestry infrastructure; but, throughout the past 15 years, the need for sustainable biomass has prompted researchers to look at non-wood sources. Non-wood sources, such as industrial wastes and agricultural residues, are a desirable substitute because of their quick creation, low initial cost, and abundance. Furthermore, there are several ways in which the manufacturing of cellulose nanofibers might be made more sustainable by using agricultural leftovers. The biochemical makeup of normal agricultural residue increases the fibrillation efficiency of cellulose bundles into nanoscale structures because it is richer in hemicellulose and lower in lignin than wood stems [31].

Products and byproducts of agriculture serve as the starting point for a wide range of technological applications across several industries. Nanofibers for usage in thermoplastic films, where they are mixed with polymeric matrix, and in biomedical applications, such tissue engineering, are prepared from agro-industrial wastes, like cotton and curaua fibers. By adding value to the manufacturing chains, the creation of products with nanofibers presents a viable substitute for the usage of agricultural resources. However, the introduction of novel nanotechnological goods necessitates an assessment of the risks they pose to the environment and public health. As a result, the field of nanotoxicology—which studies the toxicological properties of nanoscale materials—was born [32].

1.7 PHYSICAL STRUCTURE AND CHEMICAL COMPOSITION OF SUGARCANE BAGASSE FIBRE

Plant fibers called bast fibers come from the stalks or stems of plants [33].Because their leaves have net veins, the plants that provide jute, flax, ramie, and other bast fibers are categorized as dicotyledons [34].Sugarcane leaves are veined parallel to one another. This kind of fiber isn't considered bast fiber, unlike bast fiber, which has fiber bundles distributed throughout the fiber stem in a particular ring pattern [34].It is possible to separate the sugar cane stalk's outer rind from its interior pith. The outside of the rind contains longer, finer fiber bundles, whereas the inside contains shorter fiber bundles [33- 35].Whereas hemicellulose has an amorphous structure that includes components like xylose, glucose, etc., cellulose has a crystalline structure that ranges from 50 to 90%, depending on the source [13]. Because cellulose's anhydro-glucose units are joined by 1, 4 glycosidic linkages, it resembles a naturally occurring linear polymer more [36].Due to the presence of primary -OH groups in C-6 and secondary -OH groups in C-2 and C-3, it possesses three hydroxyl groups with differing levels of reactivity [36-37].These hydroxyl groups form potent hydrogen connections both between and within molecules [37].These cellulose polymers are scattered throughout fibrils that are covered in hemicellulose and lignin. Lignin functions as a glue between cellulose and hemicellulose, giving the substance its stiffness [13]. Alkyl-aryl, aryl-aryl, and alkyl-alkyl linkages bind the three distinct phenyl-propane precursor monomers—p-coumaryl, coniferyl, and sinapyl alcohol—together in this three-dimensional polymer[38]. These bagasse samples can yield 55-89% cellulose by lowering the contents with the help of different pre-treatments, such as hemi-cellulose, lignin, etc [39].

Name of the content	Percentage
Cellulose	26-47
Hemicellulose	19-33
Lignin	14-23
Ash	1-5

Table 2: Chemical composition of sugarcane bagasse

1.8 CHALLENGES AND FUTURE DIRECTONS

Since it is the most promising candidate feedstock for producing bio-based polymeric materials, cellulose has garnered a lot of attention. Significant advancements in the synthesis of biopolymers based on various cellulosic forms have been made throughout the last ten years. This overview addresses the breadth and uses of the three primary methods for the synthesis of cellulose-based biopolymers, highlighting the most recent advancements and breakthroughs in each. High-quality polymer composites and functional polymeric materials can be produced efficiently and sustainably by using cellulose fibers, nanocellulose, and cellulose derivatives as fillers or matrices in bio composite materials. In addition to offering practical alternatives to the majority of petroleum-based polymers, the use of cellulose-derived monomers (glucose and other platform chemicals) in the synthesis of sustainable biopolymers and functional polymeric materials also allowed [40].

The creation and use of bio composites represent noteworthy advancements in the replacement of traditional petroleum-based materials that are not biodegradable. Biobased reinforcing agents and biodegradable polymers, or biopolymers, are the main components of bio composites. Waste management issues and the lack of petroleum may be resolved by bio-based materials. As this chapter explains, these composites show better properties than the neat biopolymers. This study has provided a comprehensive review of bio composites by outlining their basic knowledge, manufacturing process, and impact on end qualities. Furthermore, bio nanocomposites—which are explained later—take use of the "nano" size. Particular attention has been paid to the synthesis of bio nanocomposites using wood-derived polymers, specifically lignin, hemicelluloses, and cellulose [41].

The successful use of biomass in the development of superior supercapacitors still requires the resolution of a few difficult problems. By taking into account a number of crucial factors, including lowering the temperature to produce excellent carbon for supercapacitors, standardizing the processes for preparing biomass-based membranes, allocating sufficient focus to the development of high-performance electrolytes from biomass, creating an easy-to-scale, cost-effective method for packaging materials, concentrating on techno-economic analysis, preparing compatible supercapacitors for use in various instruments, such as heart monitors, and resolving safety concerns [42].

1.9 CELLULOSE NANOFIBRE

Sugarcane bagasse (CNFs) is a possible source of nanocellulose, including cellulose nanocrystals (CNCs) and cellulose nanofibers. CNCs and CNFs are not the same in terms of composition, size, or form. In terms of strength, modulus, and aspect ratio, CNFs perform better than CNCs at the same concentration of nanocellulose[43-44].Owing to their remarkable attributes, such as low density, large surface area, and strong mechanical performance, isolated cellulose nanofibers and their byproducts find extensive use in paper packaging, sensors, water purification, textiles, and pharmaceutical delivery [39-45].Even with its many advantages that lead to a wide range of uses, the intricate structure of cellulose makes it challenging to consistently separate plant cells from fibers. Hemicellulose and lignin heavily bundle cellulose fibers, which can negatively impact mechanical separation [46]. Before the mechanical procedure, the fiber source is pre-treated to increase fibrillation. Physical, biological, chemical (Kraft soda, ionic liquids, acid and alkaline hydrolysis), and combination procedures (steam and ammonia fiber explosion) are among the several pre-treatment techniques documented for the delignification of biomass [47-53]. One of the main motivations behind the development of nanostructured materials has been the significance of selecting an appropriate synthesis pathway. In fact, this has ignited scientists' interest in developing flexible, generic synthetic procedures that are simple to alter for the creation of a wide range of nanostructured materials. One of the most potent instruments in the creation of nanostructured materials is ultrasonic, which has been used in many different ways and has been well studied over many years [55]. For a wide range of potential applications, including scaffolds for tissue engineering, protective coatings, barrier/separation membranes and filtration systems, organic solar cells, drug delivery, pharmaceuticals, transparent films, antimicrobial films, and scaffolds for tissue engineering, these nanomaterials have been thoroughly studied [56].Because of its intricate molecular structure and entwined chains of cellulose, hemicellulose, and lignin, lignocellulose needs to be pretreated in order to increase the yield of fermentable sugars, which may then be used to produce value-added molecules like ethanol at a reduced cost. The crystalline structure of lignocellulose molecules is often broken down by a physico-chemical pretreatment process, which can enhance the bioconversion of lignocellulosic feedstocks to biorefineries. Over the past thirty years, a number of pretreatment techniques have been developed [57].The optimization of xylose yields has been somewhat neglected in favor of maximizing cellulose availability in the majority of studies on lignocellulosic biomass conversion. Residues must undergo a pretreatment phase in order for the cellulose fraction to produce glucose, whether through the action of acids or enzymes. Pretreatment can eliminate hemicelluloses, decrease cellulose crystallinity, and increase the porosity of materials. It is acknowledged from an economic perspective as a crucial stage in the bioconversion process [58]. All methods, nevertheless, have advantages and disadvantages. Even though physical pre-treatments like grinding and milling require less chemicals, they still demand a lot of energy, especially when producing on a large scale [60].Although lignin and hemicellulose are preferentially broken down by the biological approach, the rate of hydrolysis is laborious and slow [15], [59-60].The chemical processes are extensively investigated, for example, the most used method in the paper and pulp sector Kraft process produces a huge number of hazardous compounds such as hydrogen sulphide [61].While hemicellulose can be effectively removed using diluted acid pre-treatment, this method corrodes equipment and pollutes the environment [62]. Compared to other chemical methods, alkali-based pre-treatment limits the degradation of carbohydrates and selectively removes lignin, making it more susceptible to enzymatic hydrolysis [63].This alkaline treatment consists of sodium hydroxide, lime, ammonia, and alkaline hydrogen peroxide (AHP)[63], [64].In the pulp and paper business, hydrogen peroxide is widely used for bleaching and enhancing pulp brightness. The dissolution of lignin and hemicellulose is caused by the hydroperoxyl anion that hydrogen peroxide produces at an alkaline pH (pH 11.5) [65].Numerous researchers have contributed to the invention of the AHP pre-treatment, which enhances the enzymatic hydrolysis of lignocellulose feedstocks [66-69].For instance, Su et al. separated lignin and hemicellulose from corncobs with removal ratios of 75.4% and 38.7%, respectively, following a 6-hour treatment with AHP [70].Following the pre-treatment, CNFs were prepared mechanically using techniques such as high-pressure homogenization, high-pressure micro fluidization, grinding, and high intensity ultrasonication. In a liquid media, ultrasonication causes both mechanical disintegration and chemical degradation. Acoustic cavitation causes high temperatures, pressures, and shear forces during the process; the ensuing harsh environment encourages a significant decrease in particle size [71-72].With time, the ultrasonication technique—also known as a "green process"—that is frequently employed in CNF preparations from plant biomass has proven to be a more practical strategy[15-17]. Feng et al. created a fine, thread-like individual structure of CNFs from pre-treated SCB using steam explosion, alkali-hydrothermal catalysis, 30% H2O2, and ultrasonication [52]. Like this, Khawas et al. separated crystalline CNFs from banana peels by ultrasonically treating them after pre-treating them with various chemicals like NaOH (20%), KOH (5%) NaClO3 (1%) and H2SO4 (1%) [73].All of the aforementioned research that isolated CNF took into account the eco-friendly ultrasonication-assisted approach, which simplified processing processes while using the least amount of chemicals. Growing interest is being shown in the synthesis of CNF from plant biomass.

1.10 FILM FABRICATION

Due to the high cost of accessible green solvents, such as ionic liquids, and the unavailability of common solvents for their dissolution, film production from CNF, MF, and mixed fiber remains a difficulty [74]. While some solvents, such as NaOH/urea, LiOH/urea, and ZnCl2 solutions, are thought to be simple ways to dissolve cellulose, they still need to be further purified, which could be dangerous for the environment and public health. Salvaggio et al., for instance, showed that high exposure to ZnCl2 in aquatic creatures (zebrafish) may have teratogenic effects [75].In contrast, a different technique for creating high-strength cellulose films or sheets from wet cellulose is compression molding in a hot press [76-77]. Pintiaux et al. created a smooth, plastic-like surface using compression-molding in a hot press using commercial alpha cellulose with a high tensile strength of 22.4 MPa without the need for any additives [78].Therefore, it is ideal to develop environmentally friendly methods for making use of and applying cellulose and its derivatives in a larger range of applications. Here, we describe a simple, efficient, and environmentally friendly process for using sugarcane bagasse with agro-residue to create CNF, MF, and mixed fiber films. To create the film, a cellulose suspension containing 1.5g of cellulose in solid (microfiber, nanofiber, or mixed fiber, respectively) was thoroughly mixed and then suctioned into a wet film.

OBJECTIVES

1. Sustainable waste management by utilizing bagasse.
2. Fabrication of Cellulose Nanofiber Film by chemical and environmentally friendly method.
3. Internal Antibacterial coating to the film.
4. Comparison of the exceptional properties including the
    - Mechanical strength
    - Hydro stability
5. Characterization and morphological analysis by XRD, FESEM, FTIR
6. Surface characterization by contact angle measurements and tensile strength.
7. Biodegradability study.
8. Fabrication of biodegradable straws and plates.

Chapter 2

Literature Survey

Y. R. Loh et al. 2013 from Curtin University Sarawak focused on Sugarcane Bagasse's (SCB) chemical composition and natural, biodegradable qualities, which have made it a highly promising source and adaptable element for composite materials. Due to their affordability and environmental friendliness, green materials with low pollutant indices were in high demand. Research has been done on the effects, capabilities, and uses of SCB in both its original and modified forms. Better results were obtained by treating with the right chemicals and/or methods, combining materials with different qualities, and adjusting manufacturing techniques[12].

Kullasatri Saelee et al. 2014 attended the 26th Annual Meeting of the Thai Society for Biotechnology and International Conference, examined the use of a steam explosion and xylanase-based environmentally appropriate pre-treatment to extract cellulose from SCB. The chemical analysis revealed that the extracted cellulose included less lignin and hemicellulose and more cellulose than the raw material. The results of the FTIR and SEM analyses demonstrated that lignin and hemicelluloses were removed at every stage of the extraction process. Furthermore, the xylanase treatment was effective in achieving a 23% reduction in chemical bleaching. Thus, an extremely promising environmentally friendly technology for cellulose extraction that can be used on an industrial scale was disclosed by the combination of the environmentally friendly approach, steam explosion, and xylanase pretreatment[39].

Zuluaga et al. 2009 from the University of Pontificia Bolivariana conducted a comparison study on the separation of cellulose microfibrils from banana rachis vascular bundles using four different alkaline treatments. The neutral sugar composition and attenuated total reflection of the isolated cellulose microfibrils were examined using high performance anion exchange chromatography. The cellulose microfibrils treated with potassium hydroxide in 5 weight percent, peroxide alkaline, or peroxide alkaline-hydrochloric acid had an estimated length of several micrometers and an average diameter of 3-5 nm, respectively. The cellulose microfibrils from banana rachis can be interpreted as either cellulose IV or cellulose I, according to the results of X-ray diffraction, 13C NMR, and ATR-FTIR. This is true even if their low crystallinity makes it challenging to determine their structure. Although the structure of the specimens treated with a stronger (18 wt %) KOH solution was changed to cellulose II, they were still micro fibrillated[54].

M Slavutsky et al. 2014 from the Universidad Nacional de Salta, Argentina created cellulose nanocrystals (CNC) using bagasse from sugarcane. We looked at the starch and starch/CNC sheets' water-barrier properties.The reinforced starch/CNC films have a lesser attraction to water molecules than starch films, according to tests for solubility, contact angle, and water sorption isotherms. The effects of the driving force and the water activity (aw) values on permeability were investigated on both sides of the film. Permeation, as determined by permeability, diffusivity, and solubility coefficients, is mostly controlled by water diffusion and is hence reliant on the winding track that the CNC integration creates. The connection between CNC and starch chain is facilitated by the two molecules' similar chemical compositions. CNC was made from CF, which was obtained by the alkaline hydrolysis of sugarcane bagasse. The inclusion of CNC improved the starch film matrix's water resistance and water barrier qualities. Robust attachment was made possible by the same chemical structures of the CNC and the polymer matrix, which allowed for hydrogen bonding. Measurements of contact angles and water sorption isotherms revealed that the attraction of water molecules to reinforced films is reduced [79].

Jeefferie Abd Razak et al.2011 from the Technical University of Malaysia examined the possibility of using sugar cane fiber cellulose (SCFC) as a sustainable material for food containers that are meant to be used once. To determine the fracture morphology as well as the mechanical and physical qualities. Different engineering properties for SCFC composites were investigated using optical microscope observation. The morphological view on the mechanical and physical tests cracked surfaces indicates the reinforcing role of varying fiber loading into the created composites' outcomes. The SCFC composite had poor adhesion, even though the results showed that composites performed well in terms of their mechanical and physical properties. Overall, the creation of this novel material for use in food packaging offers a tremendous prospective answer for an environmentally friendly and secure packaging medium, whether it be for food, consumers, or the environment [26].

Daehwan Kim et al. 2018 from the Laboratory of Renewable Resources Engineering, Department of Agricultural and Biological Engineering, Purdue University USA, conducted pretreatment procedures to enhance biomass surface area, decrease substrate particle sizes and crystallinecontent, and solubilize hemicellulose and/or ligninalter the lignocellulosic materials' chemical and physical makeup. These modifications enhance cellulose hydrolysis by increasing the accessibility of acids or enzymes to the cellulose surface [57].

Shady S Hassan et al. 2018 from the School of Food Science and Environmental Health, Dublin Institute of Technology, Cathal Brugha Street, Ireland used lignocellulosic biomass pretreatment methods, including chemical, physico-chemical, and biological ones. Current advancements in applied chemistry techniques used in non-traditional and extreme conditions have led to the development of potentially profitable solutions (such as high pressure homogenizers,microwaves, ultrasound machines, and hydrostatic pressure). These cutting-edge industrial technologies are competitive alternatives when it comes to environmentally friendly, sustainable pretreatment methods for using lignocellulosic biomass in large-scale biorefineries [15].

Maimunah Asem et al.2021 from the Biotechnology Engineering Department, Faculty of Engineering, International Islamic University isolatedMalaysia, isolated cellulose nanofibers (CNFs) from sugarcane bagasse by a combination of treatment techniques, such as ultrasonication, moderate acid hydrolysis, and alkaline treatment. In the beginning, cellulose nanofibers (CNFs) from sugarcane bagasse were extracted using a combination of treatment techniques, including alkaline treatment, moderate acid hydrolysis, and ultrasonication. The SCB fibers were defibrillated and dispersed using ultrasonication at 70% amplitude. After being treated with sodium hydroxide and aqueous hydrogen peroxide, a bleaching agent, the SCB fibers were first defibrillated and dispersed using ultrasonication at 70% amplitude before moderate acid hydrolysis. The focus of the study on acid hydrolysis was the cellulose fibers that were separated from SCB after being treated with mild sulfuric acid.In the FESEM images, the diameter and length of isolated CNFs varied from 20 to 30 nm to several micrometers, respectively. This result suggests that the combined treatment strategies have a good degree of efficacy in removing CNFs from the biomass of the plants. Furthermore, XRD analysis reveals the existence of cellulose type I, which exhibits peaks at 2h of 15.2 and 22, and a 42% crystallinity. However, the FTIR spectra show that non-cellulosic components were eliminated, enabling the successful isolation of each CNF. This result validates the removal of all amorphous materials, such as lignin and hemicellulose. Ultrasonication in conjunction with mild acid hydrolysis and alkaline treatment proved effective in removing CNFs from SCBs that had long entangled network fibrils[80].

Deepanjan Bhattacharya et al.2007 from the Eastman Chemical Company, Global Coatings Application Development, Kingsport, USA made use of three distinct procedures to extract the bagasse from the cellulose microfibers. Bagasse was initially pulped using a conventional method to get rid of lignin and hemicellulose. Following a two-stage homogenization process, the resultant entire cellulose fibers were mechanically divided into their parts and subsequently hydrolyzed with acid. The size of the resulting microfibers was influenced by the hydrolysis conditions. Persistent discoloration demonstrated that bagasse, a by-product of sugarcane, cellulose was far more resistant to hydrolysis than bacterial, tunicate, or even wood celluloses. By using controlled hydrolysis and mechanical shearing, the complete cellulose fibers were divided into components the size of microns. It was found that hydrolyzing the cellulose fibers for 2.5 hours at 60 degrees Celsius with 60% (w/v) sulfuric acid eliminated most of the amorphous domains with little to no damage to the crystal structure. The MFs' sizes varied widely, spanning from a few hundred nanometers to several microns. It was not possible to completely release each MF from the microfibrillar bundles under the previously described hydrolysis dispersion conditions. Extreme conditions led to considerable crystal deterioration in addition to reducing the aggregation phenomena between individual MF. Results from scanning electron microscopy (SEM) and atomic force microscopy (AFM) indicated that the transverse diameters of the particles varied from 200 nm to a few microns. Furthermore, solid-state NMR was employed to examine the morphological changes that were transpiring in cellulose. Solid-state NMR spectroscopy did show that during the pulping process, all of the lignin had been eliminated [81].

S. Panthapulakkal et al. 2012 from the Centre for Biocomposites and Biomaterials Processing, Faculty of Forestry, University of Toronto, Canada created polycarbonate composites reinforced with cellulose, nanofibril, and film using compression molding. Wood pulp fibers were mechanically defibrillated to produce nanofibers, which had a diameter distribution of 1-100 nm. The strength, crystallinity, and thermal properties of the nanofiber films that were made from the nanofibre suspensions were further investigated. The resulting nanofiber sheets are 11 GPa in modulus and 240 MPa in strength. The thermal properties of the sheets demonstrated that producing fiber sheets at high temperatures was suitable. The tensile properties of the films exposed to composite processing conditions demonstrated the thermal stability of the fiber films during the compression molding process. Variable fiber loading nanocomposites were produced by pressing-molding nanofiber sheets of different thicknesses between polycarbonate sheets under pressure and at 205°C. The fibers improved the strength and tensile modulus of the polycarbonate. The strength of the thermoplastic rose by 24% with 10% of the fibers and by up to 30% with 18% of the fibers. The tensile modulus of the polycarbonate demonstrated a significant improvement (about 100%) [77].

Xuezhu Xu et al. 2013 from the Department of Mechanical Engineering, North Dakota State University,Fargo, North Dakota ,United States studied in detail the interactions with the polyethylene oxide (PEO) matrix, morphologies, crystalline structures, dispersion properties, and ensuing reinforcing effects on the matrix polymer. Transparent PEO/CNC and PEO/CNF nanocomposites containing up to 10% nanofibers were made by solution casting. Scanning electron microscopy (SEM), transmission electron microscopy (TEM), wide-angle X-ray diffraction (WXRD), Fourier transform infrared spectroscopy (FTIR), dynamic mechanical analysis (DMA), and tensile testing were used to investigate the aforementioned characteristics of nanocellulose fibers and composites. CNFs generated materials with higher strength and modulus than CNCs at the same nanocellulose concentration because of their greater aspect ratio and fiber entanglement, but with lower strain-at-failure because of their relatively large fiber agglomerates. Using the Kardos and Ouali models to simulate the modulus of the composites, there were good matches between the expected and experimental values. Through the use of systematic comparison studies of this type, criteria for selecting the optimal nanocellulose as a biobased nano-reinforcement material in polymer nanocomposites can be devised [44].

Esam A. El-hefian*et al.* 2011 from the university of Malaya showed Different quantities of chitosan (CS) and polyvinyl alcohol (PVA) were combined to create blends, which were then tested using a universal mechanical tester, differential scanning calorimetry (DSC), and measurements of the contact angle. Analyzing the mechanical characteristics of the films revealed that while the elongation percentage at break of the blends was lower than that of the pure components, blending enhanced the tensile strength, which rose with increasing PVA content up to 40%. The DSC data revealed that there was a significant amount of interaction between PVA and chitosan. Measurements of the static water contact angle revealed an improvement in the films' wettability[82].

Amine Moubarik et al. 2013 from the University of Sultan Moulay Slimane used three distinct processes to extract the cellulose fibers from the Moroccan sugar cane bagasse. After removing hemicellulose from bagasse using hot water (70°C), an alkaline aqueous solution was applied. Lignin is removed with a 15% sodium hydroxide (NaOH) solution at 98°C, followed by a bleaching stage. FT-IR, 13 C NMR, and TG were three complementary techniques used to look at the cellulose fibers in sugar cane bagasse.. The study used low density polyethylene as a matrix to investigate the reinforcing ability of cellulose fibers made from sugar cane bagasse. The preparations made using cellulosic materials did not include any bound lignin. The molecular weight, average viscosity, and intrinsic viscosity were 511 ml/g, 1769 g/mol, and 286578 g/mol, respectively.It has been demonstrated that improved cellulose fiber-matrix adhesion enhances the mechanical properties of composites, leading to increases in flexural modulus of 85% and Young's modulus of 72%with a 25-weight percent addition of fiber[21].

Sirlene M Costa et al. 2013 from the University of São Paulo separated textile fibers utilizing cellulose derived from sugar cane straw and commercial sources. Following anthraquinone (AQ) and soda alkaline pulping, sugar cane straw pulps were generated. Hydrogen peroxide was used in chemical bleaching of the pulps to get rid of any lignin that remained. Bleached pulps were used to make fibers with Nmethylmorpholine-N-oxide (NMMO). The chemical properties of cellulose, polyoses, and lignin found in straw and pulps were determined. Examined were the fibers' maximal water absorption, swelling, weight loss, and mechanical properties. With the use of a scanning electron microscope (SEM), the microstructure was examined. The fibers' ability to absorb water ranged from 60 to 73%, and the pulping yield was 30%. The mass loss profile in 30 days was between 25 and 26 percent.Both commercial and straw cellulose fibers showed tenacity values ranging from 4.1 to 4.3 cN/tex, which are similar to commercial lyocell that is derived from wood pulp cellulose[83].

Chapter 3

Materials and Methods

3.1 LIGNOCELLULOSIC BIOMASS MATERIALS (LBMS)

SCB is washed with distilled water and dried in an oven at 60^0 C for 24 hours. The bagasse was then cut into small pieces of 1-2 cm pulverized into a fine powder and sieved through the mesh and stored in the air-tight container.

MATERIALS

- Sugarcane bagasse
- Sodium hydroxide (NaOH)
- Hydrogen peroxide (H_2O_2)
- Sulphuric acid (H_2SO_4)
- Distilled water

METHODS

3.2 CHEMICAL WAY OF EXTRACTION OF CELLULOSE NANOFIBRE FROM SUGARCANE BAGASSE AND FILM FABRICATION

(a) (b) (c)

(d) *(e)* *(f)*

Fig.2: (a) Granular form of sugarcane bagasse (b) Alkaline treatment (c) Bleaching and acid treatment (d) Probe sonication (e) Homogenization (f) cellulose nanofiber suspension

3.2.1 ALKALINE TREATMENT

Alkaline treatment involved the transfer of ground SCB to a 1L beaker filled with a 2 %(w/v) sodium hydroxide (NaOH) solution. Followed by heating the mixture in a water bath at 80^{0}C for 5 hours to produce white solid residues. Then the solid residues were filtered and washed with distilled water about 4 times during the filtration process using a vacuum pump to separate the white precipitate from the solution mixture. Repeat the process with 12 % (w/v) NaOH at 80^0 C for an hour.

3.2.2 BLEACHING AND HYDROLYSIS PROCESSES

200 mL of aqueous hydrogen peroxide (H_2O_2), a bleaching agent taken in a 1:1 ratio was used to dissolve the alkaline treated SCB at 75^{0}C for 1 hour. Then washed and filtered the bleached samples about three times using a vacuum pump to eliminate all impurities. With 1% (v/v) H2SO4 solution, the treated SCB fibers were further treated at 80^{0}C for an hour. Then the treated samples were

washed and filtered ten times to remove all impurities and stored in a refrigerator at 4^0C until further treatment.

3.2.3 PROBE SONICATION AND HOMOGENIZATION

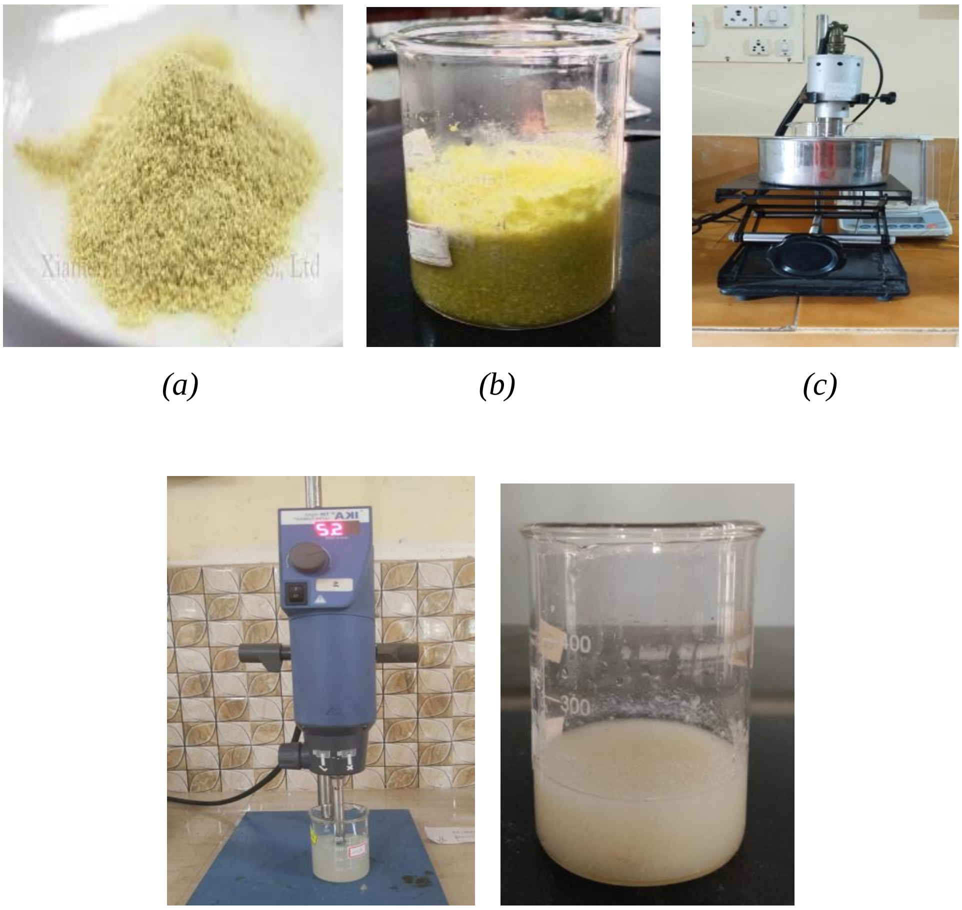
The treated samples went through a procedure of probe sonication and homogenization. The treated samples flasks were kept in an ice bath throughout the procedure to prevent overheating issues. The sample was homogenized to reduce the fibers into smaller particles. This propels the suspension of the MFs at extremely high speeds through two small orifices. By adjusting the pressure, the degree of mechanical breakdown of the fibers can be managed. The cellulose suspension samples were then separated from the pellets (solid structure) using a centrifuge at a rotational speed of 10,000 rpm for 15 min. They were then kept in a refrigerator at 4^0C pending further processing.

3.2.4 CELLULOSE NANOFIBER FILM FABRICATION

The cellulose nanofiber suspension was blended well, homogenized and vacuum filtrated into a wet film by using a funnel with 14 cm in diameter.

Fig.3: Cellulose Nanofiber film - I

3.3 ECO-FRIENDLY CELLULOSE NANOFIBRE EXTRACTION AND FILM FABRICATION


(a) (b) (c)

(d) (e)

Fig.4: (a) Granular form of sugarcane bagasse (b) Alkaline H_2O_2 treatment (c) Probe sonication (d) Homogenization (e) Cellulose nanofiber suspension

3.3.1 ALKALINE H_2O_2 TREATMENT

Alkaline H_2O_2 treatment involved the transfer of SCB to a 1-litre beaker filled with AHP solution (1 N NaOH in 3% H_2O_2 solution). It is then subjected to a water bath at 40^0C for 4 hours. After the pulping process, the pH of the mixture was adjusted to neutral by adding 2N H_2SO_4. It is then washed with distilled water and the insoluble residue is collected by vacuum filtration.

3.3.2 PROBE SONICATION AND HOMOGENIZATION

The treated samples went through a procedure of probe sonication and homogenization. The treated sample flasks were kept in an ice bath throughout the procedure to prevent overheating issues. It was homogenized to reduce the fibers into smaller particles. This propels the suspension of the MFs at extremely high speeds through two small orifices. By adjusting the pressure, the degree of mechanical breakdown of the fibers can be managed. The cellulose suspension samples were then separated from the pellets (solid structure) using a centrifuge at a rotational speed of 10,000 rpm for 15 min. They were then kept in a refrigerator at 4^0C pending further processing.

3.3.3 CELLULOSE NANOFIBRE FILM FABRICATION

The cellulose nanofibre suspension was blended well, homogenized and vacuum filtered into a wet film by using a funnel of 14 cm in diameter.

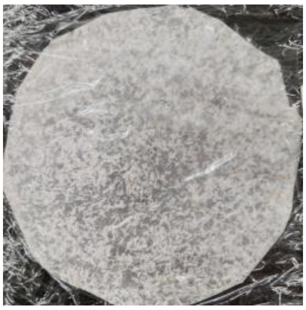


Fig.5: Cellulose nanofiber film - II

3.4CHARACTERISATION TECHNIQUES

3.4.1 FIELD EMISSION SCANNING ELECTRON MICROSCOPY(FESEM)

A sophisticated method known as field emission scanning electron microscopy (FESEM) is used to see the materials' microstructure. FESEM is typically carried out in a high vacuum because gas molecules tend to interfere with the electron beam and the secondary and backscattered electrons that are created and used for imaging[80]. Electrons are released from a field emission source and accelerate in the presence of a significant electrical field gradient. Focused and referred to as prime electrons, these produced a narrow scan beam that bombards the target after being refracted by electronic lenses inside the high vacuum column. Consequently, secondary electrons are released from every part of the object. The angle and velocity of these secondary electrons are connected to the surface structure of the object. A detector gathers the secondary electrons and uses them to produce an electrical signal. Once amplified, this signal creates a digital image that may be recorded and processed further, or a video scan image that can be watched on a monitor.

3.4.2 X-RAY DIFFRACTION (XRD)

The crystallinity of samples was characterized by X-ray diffraction (XRD) measurement on a D/max-III X-ray diffractometer, equipped with nickel-filtered Cu Kα radiation (λ = 0.15418). The diffraction angle (2θ) ranged from 3° to 90° and the step size was 0.01°. The d-spacings (d) of films were calculated with the Bragg equation,

$$D=\lambda 2\sin\theta$$

where λ is the wavelength of the X-ray source (0.15418 nm) and θ is the Bragg's angle corresponding to the plane.

The apparent crystallite size (D) of the reflection plane was obtained using the Scherrer equation.

$$L=K\lambda/\beta\cos\theta$$

where K is the Scherrer constant of 0.94 and β is the half-height width of the diffraction band.

3.4.3 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR).

This technique was used to manipulate structural changes on samples because of chemical modification by the identification of the functional groups. The changes in functional groups of the materials CNF films were investigated using FTIR spectroscopy using Nicolet, iS50, FTIR spectrophotometer. The FTIR spectra of the samples were recorded in the transmittance mode in the range of 4000 cm^{-1} to 500 cm^{-1}.

3.4.4 TENSILE STRENGTH

Tensile strength is a measure of the strength that results from bonding, fiber length, and strength. Maximum elongation, area reduction, ultimate tensile strength, and breaking strength are qualities that a tensile test is able to precisely evaluate. These observations can be used to compute strain-hardening properties, yield strength, Young's modulus, and Poisson's ratio.

3.4.5 CONTACT ANGLE

Wetting, or the interaction of water with a surface, is essential to daily life as well as numerous biological and industrial processes. The contact angle measures how likely it is that the surface will become wet with water. It is the angle at the interface where water, air, and solids meet. High contact-angle values represent the surface's propensity to repel water, whilst low contact-angle values show the tendency of the water to spread and cling to the surface. Sessile-drop goniometry is the most used technique for characterising surface-wetting since it is straightforward. The technique, which derives the contact angle from the droplet's shape, can be used with a wide range of materials, including biological surfaces, polymers, metals, ceramics, and more. The ultimate objective was to develop new areas to adapt the wetting characteristics of cellulose fibers. Cellulose-based surfaces interact with water as well as other liquids rely on the morphology of the surface and the intrinsic characteristics of the material[84].

Chapter 4

Results and discussion

4.1 X-RAY DIFFRACTION (XRD) ANALYSIS

The X-ray diffraction spectrum of the nanofiber suspension and the CNF films are shown below in the figures.

4.1.1 XRD ANALYSIS OF NANOFIBER FILM– I

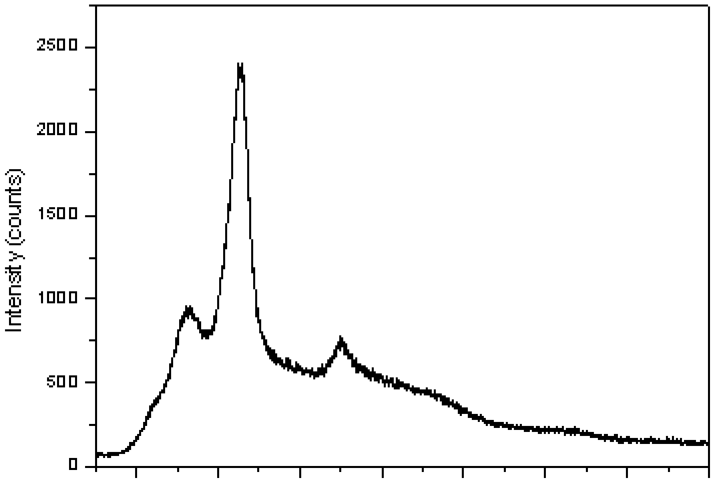


Fig.6: XRD spectrum of cellulose nanofiber film – I

In the XRD spectrum of the cellulose nanofiber film three clear peaks were obtained.

The average crystal size was determined using Debye Scherrer equation,

$$D=0.9\ \lambda/B\cos\theta$$

Analysis of peaks

Peak-1

$2\theta=16.148^0$

$\theta=8.074$ =0.1409 radian

B=0.817=0.01426 radian

$\lambda=1.54060 \times 10^{-10}$

From the Debye-Scherrer equation,

$D = 0.9\ \lambda/B\cos\theta = (0.9 \times 1.54060 \times 10^{-10})/0.01426 \times \cos(0.1409)$

= 9.906 nm

Peak-2

$2\theta=22.626^0$

$\theta=11.313$ =0.1974 radian

B=0.806 =0.01407 radian

$\lambda=1.54060 \times 10^{-10}$

From the Debye-Scherrer equation,

$D=0.9\ \lambda/B\cos\theta = (0.9 \times 1.54060 \times 10^{-10})/0.01407 \times \cos(0.1974)$

= 10.049 nm

Peak-3

$2\theta=34.934^0$

$\theta=17.467$ = 0.3049 radian

B=1.08 = 0.01885 radian

$\lambda=1.54060 \times 10^{-10}$

From the Debye-Scherrer equation,

$D=0.9\ \lambda/B \cos\theta = (0.9 \times 1.54060 \times 10^{-10})/0.01885 \times \cos(0.3049)$

$= 7.7113$ nm

2θ of the intense peak (deg)	**FWHM of intense peak**	**Size of particle, D (nm)**	**Average particle size (nm)**
16.148	0.817	9.906	9.222
22.626	0.806	10.049	
34.934	1.08	7.711	

Table.3: Average particle size of nanofiber film - I

The peaks were observed at 16.148^0 , 22.626^0 and the major peak at 34.934^0 which shows the corresponding average size of 9.222 nm for nanofiber film.

4.1.2 XRD ANALYSIS OF NANOFIBRE FILM – II

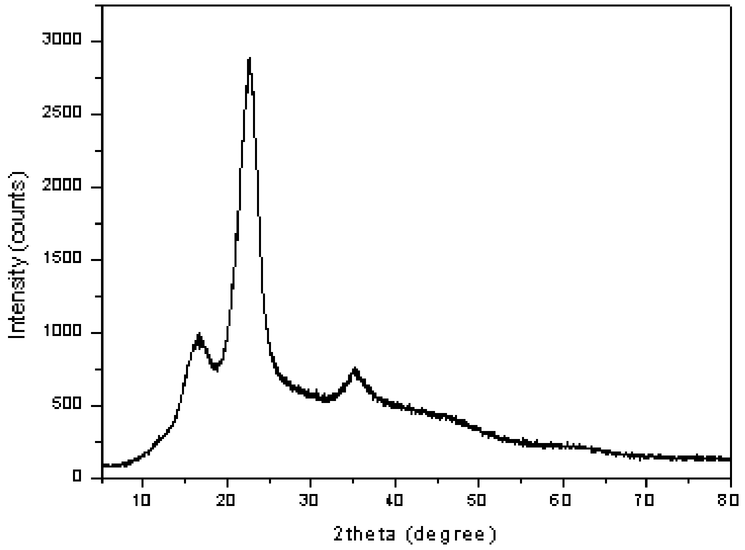


Fig.7: XRD spectrum of cellulose nanofiber film – II

In the XRD spectrum of cellulose nanofiber film, three clear peaks were observed. The average crystal size was determined using Deby Scherrer equation

$$D=0.9\ \lambda/B\cos\theta$$

Analysis of peaks

Peak-1

$2\theta=16.329^0$

$\theta=8.1645=0.1425$ radian

$B=0.818=0.01428$ radian

$\lambda=1.54060\times10^{-10}$

From the Debye-Scherrer equation,

$D = 0.9\ \lambda/B \cos\theta = (0.9 \times 1.54060 \times 10^{-10})/0.01428 \times \cos(0.1425)$

$= 9.809$ nm

Peak-2

$2\theta=22.538^0$

$\theta=11.269 = 0.1967$ radian

$B=0.773 = 0.01349$ radian

$\lambda=1.54060 \times 10^{-10}$

From the Debye-Scherrer equation,

$D=0.9\ \lambda/B \cos\theta = (0.9 \times 1.54060 \times 10^{-10})/0.01349 \times \cos(0.1967)$

$= 10.480$ nm

Peak-3

$2\theta=35.061^0\theta=17.5305= 0.306$ radian

$B=0.78=0.01361$ radian

$\lambda=1.54060 \times 10^{-10}$

From the Debye-Scherrer equation,

$D=0.9\ \lambda/B \cos\theta = (0.9 \times 1.54060 \times 10^{-10})/0.01361 \times \cos(0.306)$

$= 10.683$ nm

2θ of the intense peak (deg)	**FWHM of intense peak**	**Size of particle, D (nm)**	**Average particle size (nm)**
16.148	0.818	9.809	10.324
22.538	0.773	10.480	
35.061	0.78	10.683	

Table.4: Average particle size of cellulose nanofiber film – II

The peaks were observed at 16.148^0, $22.538^0$and the major peak at 35.061^0 which shows the corresponding average size of 10.324 nm for nanofiber film.

4.2 TENSILE STRENGTH

By calculating the tensile strength of the two films, we can study the mechanical strength of the respective films which is calculated directly from the graph by taking the highest point in the curve which is the ultimate stress. We performed tensile tests of the two films prepared with their stress-strain graph plotted below (Fig 8 and Fig 9).

4.2.1 TENSILE STRENGTH OF NANOFIBER FILM – I

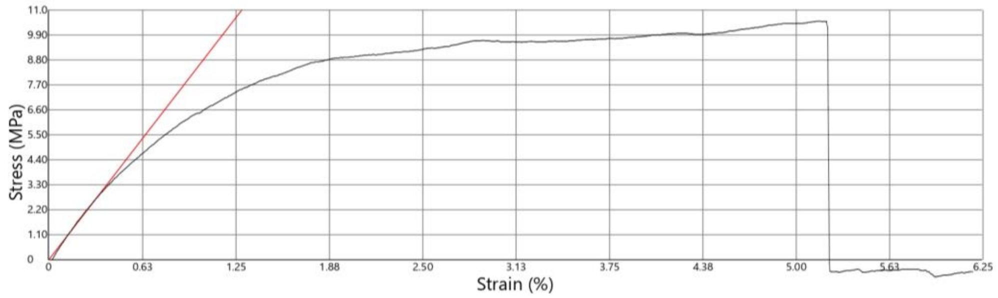


Fig.8: Tensile strength of cellulose nanofiber film – I

The Tensile strength of nanofiber film is found to be 10.5MPa as obtained from the above figure (Fig.8). It was obtained by finding out the ultimate stress from the stress-strain graph.

4.2.2 TENSILE STRENGTH OF NANOFIBER FILM – II

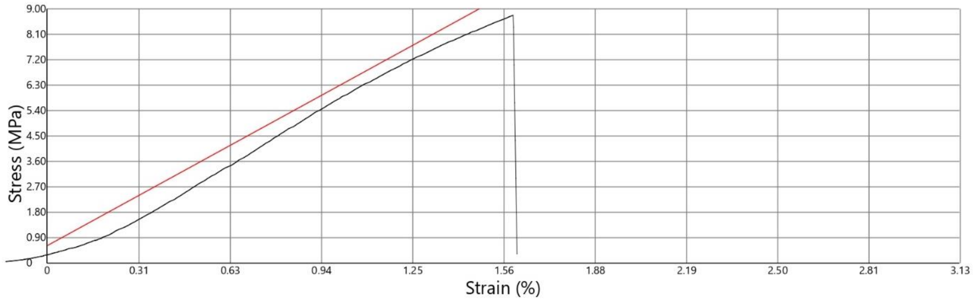


Fig.9: Tensille strength of cellulose nanofiber film - II

The Tensile strength of nanofiber film is found to be 8.77 MPa as obtained from the above figure (Fig 9). It was obtained by finding out the ultimate stress from the stress-strain graph.

4.3 CONTACT ANGLE

One popular method for determining a surface's or materials' wetting stability is to measure the contact angle. The study of how a liquid spreads out when it is put on a solid (or liquid) substrate is known as wetting. Wetness is measured by taking the contact angle that the liquid creates when it comes into touch with the solids or liquids. In addition to indicating whether the sample film is hydrophobic or hydrophilic, the contact angle of the two films can be used to determine the film's stability in water. Furthermore, the wetting tendency of the film—which is inversely related to the contact angle—tells us the hydro stability. Therefore, a bigger surface tension or contact angle will result in a lower wetting tendency and a higher hydro stability.

4.3.1 CONTACT ANGLE OF CELLULOSE NANOFIBER FILM - I

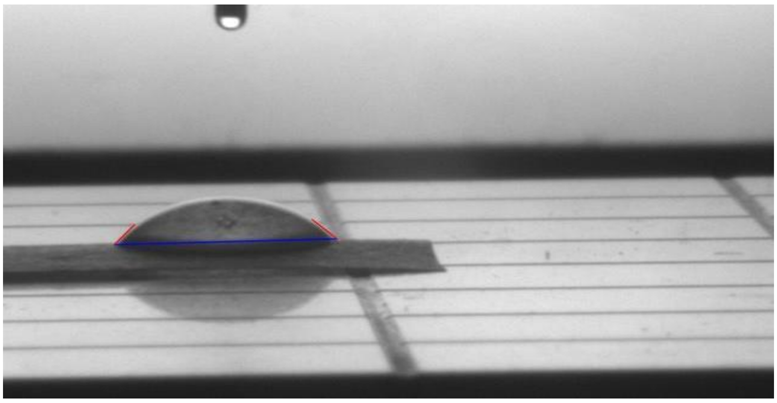


Fig.10: Contact angle of cellulose nanofiber film -I

The contact angle of nanofiber film is 78.71^0 which is calculated by finding out the angle between the surface of the film and the drop of water used.

4.3.2 CONTACT ANGLE OF CELLULOSE NANOFIBER FILM –

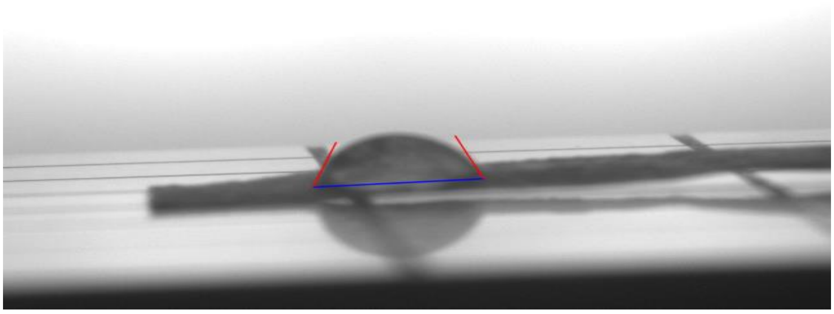


Fig.11: Contact angle of cellulose nanofiber film -II

The contact angle of nanofiber film is 66.85^0 which is calculated by finding out the angle between the surface of the film and the drop of water used.

4.4 COMPARATIVE STUDY OF THE TENSILE STRENGTH

Cellulose film	Tensile strength (MPa)	Fractional energy (MJ/mm^3)
CNF – I	10.55	4,27,415
CNF- II	8.77	70,908

Table.5: Comparative study of tensile strength

For a better understanding of the mechanical properties tensile strength of both films was performed. Cellulose nanofiber film - I with a stiffness of 10.5 MPa is ductile with a fracture strain of 5.21 %.

Cellulose nanofiber film – II is compliant with a stiffness of 8.77 MPa but brittle with a fracture strain of 1.60 %. Hence cellulose nanofiber film – II is sufficiently stiff leading to a desirable combination of both mechanical strength and deformability.

The area under the stress-strain curve measures the energy needed to fracture a material (fracture energy). It is evident that cellulose nanofiber film – I has a much higher fracture energy of 4,27,715 MJ/mm^3 (more tougher) than the cellulose nanofiber film – II with a fracture energy of 70,908 MJ/mm^3.

4.5 COMPARATIVE STUDY OF CONTACT ANGLE

Name of the film	**Contact angle (deg)**
CNF – I	78.71^0
CNF – II	66.85^0

Table.6: Comparative study of contact angle

To better understand the hydro stabilities of two films, we performed a contact angle test for two films. By measuring the contact angle of water on the film surface, we evaluated the hydrostabilty of the two different films. Due to the hydrophilic nature of cellulose, the contact angle of the cellulose nanofiber film – I is 78.71^0, showing an apparent wettability. A well-defined wettability is indicated by the cellulose nanofiber film – II, contact angle of 66.85^0. As a result, it is discovered that the nanofiber film – I have a lower wetting tendency and greater hydro stability than the nanofiber film – II.

In the cellulose nanofiber film – II, which is prepared from alkaline H_2O_2 treatment. It involves the usage of an AHP solution to modify the cellulose fibers. This can cause a change in the surface chemistry and morphology. This treatment can result in the removal of impurities and will generate nanofibrous structures. Therefore the film exhibits considerably stronger wetting and lower hydrostability.

4.6 FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY ANALYSIS OF CELLULOSE

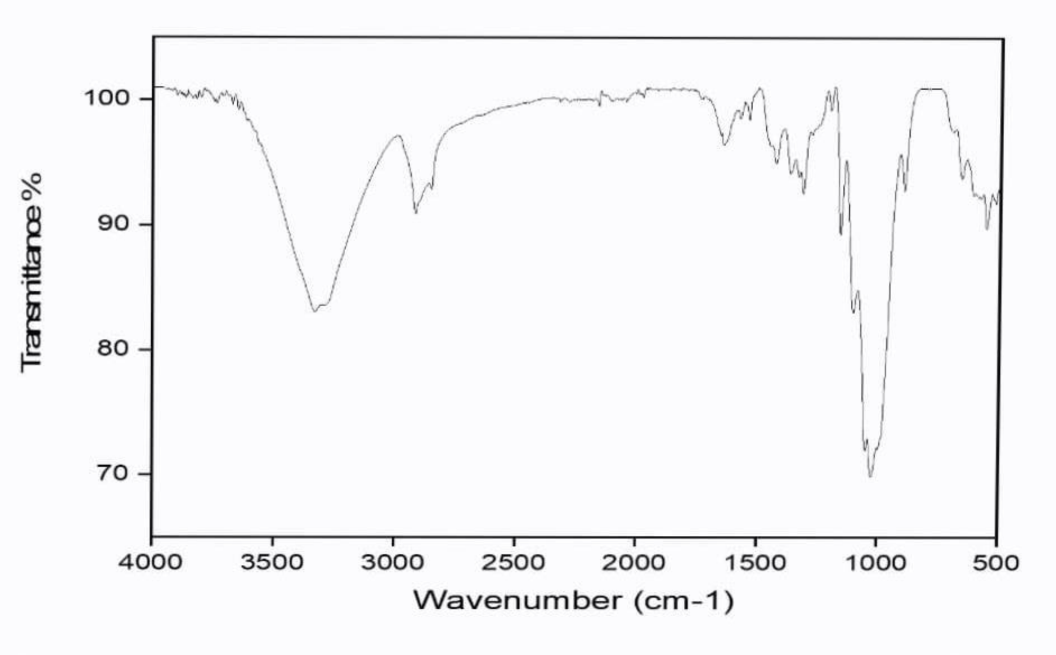


Fig.12: FTIR spectrum of Cellulose

The presence of functional groups in SCB was identified using FTIR analysis. The spectrum demonstrated in figure exhibits identical patterns but varied in the terms of peak sharpness. The FTIR bands at the range of 3200 and 3400 cm-1 shows the existence of OH stretching vibrations on the hydrogen bonding of the cellulose structure. The extending peak of OH bonds at 3300 cm-1 is less sharp, which indicates the elimination of non -cellulosic components from the SCB fiber. When it underwent alkaline and acid treatments, due to the rapid increase in the OH concentration, the FTIR peak became sharper. The peaks at the range 1500 and 1700cm-1 shows C=O broadening.

4.7 FIELD EMISSION SCANNING ELECTRON MICROSCOPE ANALYSIS

FESEM Analysis were conducted to examine the morphology of the prepared nanofiber films.

4.7.1 FESEM OF NANOFIBER FILM – I

The FESEM images of a nanofiber film made from sugarcane bagasse (SCB) is shown here. As shown in Figure, the film samples that underwent an alkaline treatment,acid hydrolysis, probe sonication, and homogenization formed fibers with a uniformly long rod-shaped structure. The generated fibers are uniformly dispersed and have dimensions that fall within the range of nanometers. This outcome shows that mild acid hydrolysis, alkaline treatment, and bleaching, followed by probe sonication and homogenization process, separated the microfibrils from the fiber bundles and produced a homogeneous distribution of fibers. The

CNFs' random entanglement makes it impossible to quantify the fiber lengths exactly, and because the ends are hidden, the length of the fibers can reach several micrometers.

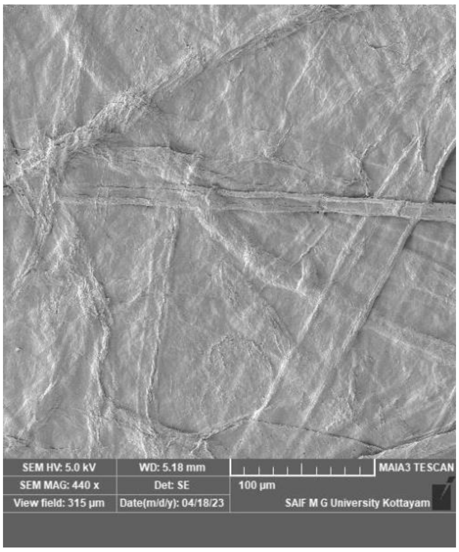


Fig.13: FESEM image of cellulose nanofiber – I

4.7.2 FESEM OF NANOFIBER FILM – II

The FESEM images of a nanofiber film made from sugarcane bagasse (SCB) are shown here. As shown in Figure, the film samples that underwent an alkaline H_2O_2 treatment, probe sonication, and homogenization caused the detachment of fibers from the bundle. This outcome shows that of alkaline H_2O_2 treatment followed by probe sonication and homogenization process produced an entangled structure. It may be due to the synergistic effect of H_2O_2 treatment. The highly entangled nanofibers are of approximately 6-100 nm size.

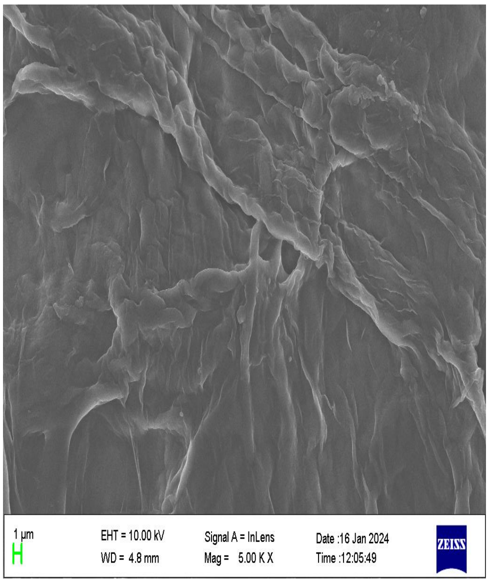


Fig.14: FESEM image of cellulose nanofiber film – II

4.8 APPLICATIONS

A popular technique for producing desired polymeric materials with combination qualities appropriate for certain applications is polymer blending. The study of chitosan blends containing artificial and natural macromolecules has garnered a lot of interest in recent years on a number of occasions. A practical way to create synthetic biodegradable polymers with diverse qualities like improved mechanical characteristics and good water absorbance while retaining biodegradability is to blend chitosan with synthetic polymers[82].

4.8.1 ANTIBACTERIAL INTERNAL COATING

Chitosan is blended with a water soluble synthetic polymer Polyvinyl alcohol (PVA) and use it as a coating in CNF films to give an antibacterial property.

4.8.1.1 CHITOSAN

The hard outer skeleton of shellfish, such as crab, lobster, and shrimp, is where chitosan is extracted. It is applied to medicine. Many of the benefits of chitosan, which include treating high blood pressure, cholesterol, obesity, wound healing, and other illnesses. Chitosan is used in pharmaceutical manufacture as a tablet filler, to enhance the solubility of some medications, and to cover up unpleasant flavors. It also aids in extending the shelf life of food.Commercial chitosan is made by deacetalizing chitin, the structural component of crustaceans' exoskeletons. The United States Food and Drug Administration has recognized chitosan as a naturally occurring antibacterial polymer. The most widely accepted mechanism for chitosan's antibacterial action involves attaching to the negatively charged bacterial cell wall and disrupting the cell, changing the permeability of the membrane. This is followed by attachment to DNA, which inhibits DNA replication and ultimately results in cell death.[85]

4.8.1.2 POLYVINYL ALCOHOL

Polyvinyl alcohol (PVA) is a water-soluble synthetic polymer, and we encounter it in various applications. PVA is approved safe by the FDA for food packaging and has biomedical and pharmaceutical applications as well [86]. Chitosan is a biopolymer, which is an amino polysaccharide derived from the N-deacetylation of chitin. It is a natural polymer because of the presence of degradable enzyme Chitosan. Chitosan is blended with synthetic polymer PVA with formaldehyde enhances the thermal stability.

4.8.2 PREPARATION OF CHITOSAN-PVA FILM

4.8.2.1 CHITOSAN PREPARATION

To prepare a 10 g L^{-1} chitosan solution, 500 mL of 0.1 M acetic acid was mixed with 5 g of chitosan, gently stirred, and heated to approximately 60°C or an entire night. To get rid of dust and other remnants of contaminants, the solution was filtered. After two hours at room temperature, air bubbles were removed from the solutions.

4.8.2.2 PVA PREPARATION

5 g of PVA were dissolved in 500 mL of hot ultrapure water to create a corresponding 10 g L^{-1} solution. Following a 2-hour stirring period, the solution was maintained at roughly 80°C.

4.8.2.3 PREPARATION OF THE BLENDED FILM

A chitosan solution was held on a magnetic stirrer at roughly 94°C, and the aqueous PVA solution was added drop by drop to the solution. The mixture was then stirred at a moderate speed for 30 minutes. The final volume ratio of chitosan and PVA (CS/PVA) taken was 45:5. The film was prepared by casting particular amount of solution to a OHP sheet, dried and peeled off.

4.8.3 DETERMINATION OF ANTIBACTERIAL ACTIVITY

4.8.3.1 PREPARATION OF NUTRIENT MEDIA

By dissolving 1.3 gm of nutrition broth in 100 millilitres of distilled water, nutrient broth was created. Five millilitres of nutritional broth were placed within test tubes, which were then autoclave-sterilized. In 100 millilitres of distilled water, 1.3 gm of nutrient broth and 2 gm of agar agar were combined to create nutrient agar media. Under aseptic conditions, 20 millilitres of the media were each autoclaved and then put into sterile petriplates.

4.8.3.2 PREPARATION OF MICROBIAL CULTURES

The test organisms; E-Coli and S- Aureus were inoculated into a 5ml of sterilized nutrient broth and kept for overnight incubation at 37^0C.

4.8.3.3 DISC DIFFUSION METHOD

0.6 cm diameter filter paper disc were punched out and sterilized by autoclave. It was then dipped with sample and used to tests the antibacterial sensitivity. The method used for antibacterial sensitivity was Kirby Bauer disc diffusion method. A lawn culture of each bacterium was prepared using sterilized cotton swabs. A sterilized swab was dipped into the bacterial suspension and moved side to side from top to bottom leaving no space uncovered. The plate is rotated to 90^0 and the same procedure was repeated so that the entire plate was coated with bacteria. Once the lawn had been prepared, the sterilized filter paper impregnated with the sample to be tested was placed on a plate. The antibacterial activity of the sample (a) Chitosan only (CS) and (b) Chitosan+ Polyvinyl alcohol (CS+PVA) were compared with the standard antibiotics available. This plate was incubated at 37^{0}C for 24 hrs. The radius of each zone was measured using a standard ruler in cm. If the compound is effective against bacteria at certain concentrations, no colonies will grow. This is the zone of inhibition which is a measure of the compound effectiveness, the larger the clear area around the filter paper, the more effective the compound.

4.8.3.4 KILLING AND DISPOSING

After the experiment, the bacteria are destroyed by autoclaving the plates for 20 minutes. All the glassware used for the experiment was also autoclaved to remove any bacteria if present.

4.8.4 ANTIBACTERIAL STUDY ON THE SPECIMENS

(a) *(b)* *(c)*

Fig.15: (a) Antibacterial property of CNF alone on E.Coli

(b) Antibacterial property of CS+PVA and CS on E.Coli

(c) Antibacterial property of CS+PVA and CS on S.aureus.

4.8.4.1 ZONE MEASUREMENT OF THE SAMPLE

Name of the organism	**Sample**			**Antibiotic Gentamicin**
	CS+PVA	**CS**	**CNF**	
E-Coli	0.9	1	No activity	2.3
Staphylococcus aureus	0.8	0.7	No activity	2.5

Table.7: Zone measurement of the sample.

For the analysis of antibacterial property of the samples, we performed Kirby Bauer disc diffusion method with two organism: E-Coli and S-Aureus. By measuring the zone of the samples, we evaluated the antibacterial property of the samples. The antibiotic Gentamicin which has been taken as the standard has the zone diameter of 2.3 cm on the E-Coli and 2.5 cm on the S-Aureus respectively. For the CNF alone there is no antibacterial property for the organisms. The zone diameter for CS+PVA is 0.9 cm and CS is 1 cm for E-Coli and the zone diameter for CS+PVA is 0.8 cm and CS is 0.7 cm for S-Aurues. From this we can conclude that CS is showing more antibacterial property with E-Coli than CS+PVA and CS+PVA showing more antibacterial property with S-Aureus than CS.

4.8.5 BIODEGRADABILITY TEST

The increasing amount of non-biodegradable materials accumulating in the environment is a major global hazard to biodiversity, human health, and ecosystems. As a result, there is rising interest in creating biodegradable substitutes that can lessen the negative effects of conventional non-biodegradable materials. Because biodegradable materials break down naturally and eventually return to the environment as benign byproducts, they have the potential to lessen pollution and the load on landfills. Wood pulp sheets are better for the environment because they decompose in a composting environment, but their production requires a lot of water, mass, energy, and trees. Sugarcane bagasse is a waste material which has no issues regarding the same mentioned above.

In this study, we are testing the biodegradability of the two CNF films, made by chemical method and the alkaline H_2O_2 treatment from sugar cane bagasse. Elucidating the biodegradation behavior of these materials helps the development of sustainable solutions for a wide range of industries, including packaging, agriculture, and biomedical applications.

The cellulose nanofibers are buried under the soil and left for a definite number of days and checked their weight every time between periods. After every time burying it, the new weight was compared to its initial weight. The formula used to measure the weight loss of the sample:

$$\text{Biodegradability } \% = (W_1 - W_2)/W_1 \times 100$$

W_1 = Initial weight of the sample
W_2 = Final weight of the sample

The table below shows the biodegradability rate of the CNF films over 1 month.

Sample	Burial period (days)	Initial weight (g)	Final weight (g)	Weight loss (g)	Biodegradability %
CNF - 1 film	0	5	5	0	0
	7	5	4	1	20
	14	4	3	1	25
	21	3	1.5	0.5	50
	28	1.5	0.5	1	66
	35	1	0	1	100
CNF - 2 film	0	5	5	0	0
	7	5	2	3	60
	14	2	0	2	100

Table 8: Biodegradability rates of the cellulose nanofiber films

The biodegradability test proved that both the CNF films are biodegradable. Among the two, Nanofilm - II is more biodegradable than the Nanofilm - I. Because Nanofilm - II degraded over 14 days while the Nanofilm - I film takes about 35 days for its complete degradation.

Chapter 5

Conclusion

The purpose of this study is to fabricate all-natural cellulose-based films composed of cellulose nanofibers from sugarcane bagasse following two methods; chemical and alkaline H_2O_2 treatment. The prepared films were characterized using a wide range of analytical and imaging techniques and compared their mechanical strength and hydro stability. To improve the properties of the prepared films an antibacterial internal coating using chitosan blended with polyvinyl alcohol to the nanofiber film that has been prepared.

Combination treatment methods like alkaline treatment and mild acid hydrolysis assisted with probe sonication and homogenization were used to extract cellulose nanofibers (CNFs) from sugarcane bagasse. The SCB fibers were first bleached with sodium hydroxide and aqueous hydrogen peroxide, then mild acid hydrolyzed. The alkaline-treated cellulose fibers isolated from SCB were treated with mild sulfuric acid on acid hydrolysis. The formation of cellulose nanofiber film was supported by the XRD, FTIR, and FESEM studies. The CNF film's FESEM images showed 20–30 nm diameters. This finding implied that the combined treatment approaches successfully separated CNFs from the plant biomass. The existence of peaks at 2θ of 16.148^0, 22.626^0 and the major peak at 34.934^0 of cellulose nanofiber film – I indicating cellulose-type structure was revealed by XRD analysis.

The second method involved the eco-friendly procedure where alkaline H_2O_2 treatment assisted with the sonication process helped to extract cellulose nanofiber from sugarcane bagasse. The SCB fibers underwent the alkaline treatment using an AHP solution followed by sonication processes. The production of cellulose nanofiber film - I was supported by the XRD, FTIR, and FESEM studies. The existence of peaks at 2θ of 16.148^0, $22.538^0$and the major peak at 35.061^0, indicates a cellulose-type structure and is supported by XRD analysis.

The FTIR spectra, however, demonstrated that the successful isolation of each unique CNF was made possible by the removal of non-cellulosic components. This outcome confirmed that all amorphous components, including lignin and hemicellulose were eliminated. By using a combination of alkaline treatment and mild acid hydrolysis assisted by sonication processes, CNFs with long entangled network fibrils were successfully recovered from SCB. The CNFs should be strong and have a high aspect ratio, making them ideal for use as reinforcement for creating nanocomposites.

Tensile strength makes the mechanical properties of the two films more understandable from a mechanistic perspective. Cellulose nanofiber film - I with a stiffness of 10.5 MPa is ductile with a fracture strain of 5.21 %. Cellulose nanofiber film – II is compliant with a stiffness of 8.77 MPa but brittle with a fracture strain of 1.60 %. Hence cellulose nanofiber film – II is sufficiently stiff leading to a desirable combination of both mechanical strength and deformability.

Hydro stability is another important performance metric of the films in addition to mechanical qualities. The two films' contact angles, which are inversely correlated with their hydro stability, were measured. Due to the hydrophilic nature of cellulose, the contact angle of the cellulose nanofiber film – I is 78.71^0, showing an apparent wettability. A well-defined wettability is indicated by the cellulose nanofiber film – II, contact angle of 66.85^0. As a result, it is proved that the nanofiber film – I have a lower wetting tendency and greater hydro stability than the nanofiber film – II. The cellulose nanofiber film – II, is prepared by alkaline H_2O_2 treatment. It involved the usage of an AHP solution to modify the cellulose fibers. This can cause a change in the surface chemistry and morphology. This treatment can result in the removal of impurities and will generate nanofibrous structures. Therefore, the film exhibits considerably stronger wetting and lower hydro stability.

The antibacterial studies revealed that the cellulose nanofiber film itself does not have an antibacterial property against E.coli and S. Aureus. The studies showed that by culturing the bacteria in the cellulose nanofiber film alone they have no antibacterial activity. Antibacterial internal coating to the film by using chitosan and polyvinyl alcohol showed activity and the zone was measured. The antibiotic Gentamicin which has been taken as the standard has a zone diameter of 2.3 cm on the E-Coli and 2.5 cm on the S-Aureus respectively. By the zone measurements, CS showed more antibacterial properties with E-Coli than CS+PVA, and CS+PVA showed more antibacterial properties with S-Aureus than CS.

The biodegradability test proved that both the CNF films are biodegradable. Among the two, Nanofilm - II is more biodegradable than the Nanofilm - I. Because Nanofilm - II degraded over 14 days while the Nanofilm - I film takes about 35 days for its complete degradation.

Furthermore, the raw materials required for the developed films were abundant on Earth and sustainable, offering the possibility of their mass production at minimal cost. Another long-needed property that holds promise to address the growing negative environmental impact of barely biodegradable plastic is the intrinsic degradability of the films. Therefore, cellulose films are a good contender to replace petroleum-derived plastics in packaging materials, straws, etc. along with different applications spanning from sensor, and water filtration to drug delivery due to excellent mechanical strength, hydro stability, and low-cost raw materials.

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