MASTERS OF SCIENCE (BIOTECHNOLOGY)

TANYA SRIVASTAVA

2023

ISOLATION, QUANTIFICATION AND CHARACTERIZATION OF MICROCELLULOSE FROM AGRICULTURAL WASTES

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENTS OF THE REQUIREMENTS

FOR THE AWARD OF THE DEGREE

OF

MASTERS OF SCIENCE

IN

BIOTECHNOLOGY

Submitted by

Tanya Srivastava

2K21/MSCBIO/56

Under the supervision of

Professor Jaigopal Sharma

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana road, Delhi, 110042

June, 2023

1

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana road, Delhi, 110042

CANDIDATES'S DECLARATION

I, Tanya Srivastava, Roll Number: 2K21/MSCBIO/56, student of M.Sc. Biotechnology, hereby declare that the work which is presented in the Major Project entitled —**"Isolation, Quantification and Characterization of Microcellulose from Agricultural Wastes"** in partial fulfillment of the requirement for the award of the degree of Master of Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi, is an authentic record of my own carried out work during the period from January-May 2023, under the supervision of Prof. Jaigopal Sharma. The matter presented in this report has not been submitted by me for the award for any other degree of this or any other Institute/University.

My work has been accepted in Scopus indexed conference with the following details:

Title of the Paper: Utilizing agricultural waste derived nanoparticles for wastewater treatment: A synergistic step towards circularity and sustainability

Authors: Tanya Srivastava, Jyoti Sharma, Prof. Jaigopal Sharma

Name of the conference: 2nd International conference on "Advances in Water Treatment and Management" (ICAWTM-23)

Conference date: 11th- 12th march, 2023

Date of acceptance: 17th January, 2023

Status of Paper: In Proceedings

Tanya

Place: Delhi **TANYA SRIVASTAVA**

Date: 30/5/23 **2K21/MSCBIO/56**

2

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana road, Delhi, 110042

CERTIFICATE

I hereby certify that the Project Dissertation titled — **"Isolation, Quantification and Characterization of Microcellulose from Agricultural Wastes"** which is submitted by **Tanya Srivastava (2K21/MSCBIO/56)**, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science is recorded for the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any degree or any diploma to this university or elsewhere.

Place: Delhi

Date: 30/5/23

Prof. Jaigopal Sharma

(Supervisor)

Professor

Department of Biotechnology

Delhi Technological University

30/05/2023

Prof. Pravir Kumar

Head of the Department

Department of Biotechnology

Delhi Technological University

ACKNOWLEDGEMENT

Learning something new may be difficult, but at the same time the journey of this dissertation work- "**Isolation, Quantification and Characterization of Microcellulose from Agricultural Wastes**" and writing this thesis is attached with many good memories and at the same time has some flaws. Overcoming these times has provided me with lots of experiences and new learning. I would like to thank all the people who helped me in the completion of this project and supported me in this journey. First and the foremost, I would like to thank my supervisor, Professor Jaigopal Sharma for his mentorship and support. Sincere gratitude to Prof. pravir Kumar, Head of the Department of Biotechnology, DTU for providing required facilities for the timely completion of the project work. I would further like to extend a heartfelt gratitude to Ms. Neha Tiwari, Ms. Megha Bansal and Ms. Sweety for their wonderful advices, teaching and insights. The experiments would not have been completed without their knowledge and support. I am particularly grateful to the lab staff of the department of biotechnology for supplying the necessary paraphernalia, leading to the uninterrupted completion of the project. Lastly, I would like to thank my partner Jyoti Sharma, classmate Devansh Sharma and my sister Manya Srivastava for their continuous help and support.

Tanya

TANYA SRIVASTAVA

2K21/MSCBIO/56

ABSTRACT

With continuous progress in effective research, microcellulose is slowly emerging as a low cost, non-toxic and superior compound which bears the potential to be exploited in numerous industries. Microcellulose is primarily extracted from wood and woody residues, however, with growing ecological concerns, the focus has been shifted towards searching for more sustainable alternatives. Agricultural wastes appear to be the most suitable agents for facilitating the process of cellulose synthesis. India alone generates more than 350 million tonnes of agricultural waste every year, which generates additional burden on society and hampers development. Thus, the utilization of agricultural wastes for microcellulose synthesis can emerge as a two-way approach which converts wastes to resources and simultaneously facilitates the low cost synthesis of industrially useful compounds. The presented study elucidates the isolation of microcellulose from agricultural wastes i.e. rice husk and pomegranate peel and its subsequent quantification and characterization using FTIR analysis and particle-size distribution analysis. Cellulose could be isolated only from rice husk for which, the characterization studies confirmed the presence of abundant cellulose in the isolated sample along with significant removal of lignin and hemicellulose. Particle size distribution analysis revealed the size of particles to be around 5.3 micrometers, thus affirming the reduction of cellulose particles to micro size.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

S.no.	Abbreviation	Full form
1	FTIR	Fourier transform infrared spectroscopy
2	MCC	Microcrystalline cellulose
3	CNC	Cellulose nanocrystals

9

4	CNF	Cellulose nanofibrils
5	MFC	Microfibrillated cellulose
7	SEM	Scanning electron microscopy
8	XRD	X-ray diffraction
9	TEM	Transmission electron microscopy
10	TGA	Thermogravimetric analysis
11	OD	Optical Density

10

CHAPTER 1

INTRODUCTION

As the world population is increasing at an alarming rate, an incessant depletion of natural resources and fossil fuels is being witnessed as well. Considering the current scenario and growing environmental concerns, the global scientific community has shifted its focus towards the development of eco-friendly, renewable and sustainable biocomposites [1]. Cellulose is the most ancient and profouse biopolymer found on our planet and it has attracted the interest of researchers from all across the world due to its promising properties and potential applications. As a bioresource, it has utilizations in several industries such as textile, pharmaceutical, chemical etc [2]. Cellulose is thus emerging as a low cost, energy efficient, safe and non-toxic compound for multiple utilizations.

Microcellulose is obtained from cellulose by bringing down its particle size to nano-range. This is facilitated by treating the cellulose fibers with suitable chemicals and reagents. An additional homogenization and spray drying step can produce microcrystalline cellulose. The particles can be further treated to yield nanofibrils or cellulose nanocrystals [3]. Physical, chemical as well as enzymatic methods are exploited for the treatment of raw cellulose source to yield desired particles. Each step has advantages as well as limitations, and thus need to be selected cautiously [4].

Cellulose is traditionally obtained from wood and woody residues, however, with growing environmental awareness, a significant number of experiments have reported the successful extraction of cellulose from agricultural wastes such as wheat straw, coconut and rice husk, mango fibers, date palm etc. Several studies have also reported the profound exploitation of waste extracted cellulose for the manufacturing of automobiles, sports equipment, biofuels etc [5].

The presented study focuses on the extraction of cellulose from agricultural wastes i.e. rice husk and pomegranate peel. The process was initiated with the pretreatment of powdered rice husk and chopped pomegranate peel. The pretreatment was done in three steps, after

11

which fine concentrate of cellulose was obtained for rice husk. Pomegranate peels, however, got drastically degraded during the process and did not yield a significant amount of concentrate. Hence, they were not processed any further.

Quantification of obtained cellulose was done by anthrone test and the amount of cellulose present in it was compared with raw sample. The test displayed a considerable increase in the cellulose content of the extracted sample.

The isolate was finally characterized using FTIR and particle size distribution analysis. FTIR analysis confirmed the removal of a considerable percentage of lignin and hemicellulose along with the occurence of noteworthy cellulose content in the isolate. Particle size analysis revealed the size of cellulose particles to be around 5.3 micrometers, which suggested that microcellulose was successfully isolated from the sample.

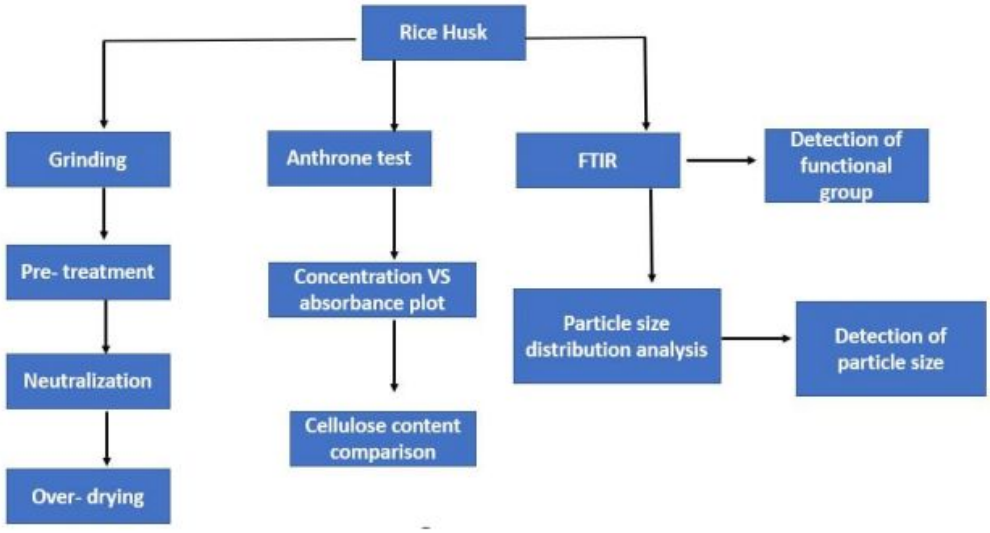


Fig 1.1: General workflow of the presented project

12

CHAPTER 2:

LITERATURE REVIEW

Cellulose is the most copious natural polymer found on earth, and also regarded as the oldest one [6]. It is biodegradable as well as renewable, and is found in a plethora of plants such as cotton, sugarcane, rice, straws etc. Cellulose is commonly exploited in multiple industries, and has several applications, including fiber processing, paper production etc. However, with ongoing research, scientists have found the application of cellulose for the processing of novel materials, wherein the most common application is the production of Microcellulose. Microcelulose is obtained from plant fibers through various physical, chemical and enzymatic methods. It is increasingly gaining popularity due to its superior characteristics such as large surface area, non-toxicity and low production cost. It also has high crystallinity, high tensile strength, low density, and high modulus. It also bears a highly reactive surface with significant water holding capacity [7]. Moreover, the polymer chain in it is found in optimum crystalline state, which provides strength and stiffness, making nanocellulose the strongest cell wall component. Owing to these unique properties, microcellulose is slowly emerging as the desirable material for the production of packaging, electronic devices, sensors and even cosmetics [5].

2.1 Cellulose: Structural Aspects

The molecular structure of cellulose can be elucidated as a linear polysaccharide that consists of repeating units of β-1, 4 anhydro D-glucopyranose molecules. The chemical formula of the polymer is $(C_6H_{10}O_5)_n$, wherein the value of n (the degree of polymerization) can vary between 10 to 15 thousand [8]. The glucopyranose structure bears six carbon atoms linked via acetal bond between C1 carbon of one pyranose unit and C4 carbon of the other. The C4 carbon bears a free alcohol group and denotes a non reducing end [9]. The cellulose building blocks are composed of elementary which contain segments having highly ordered as well as randomly distributed cellulose chains that are bundled together to form microfibrils. Crystalline cellulose is majorly water insoluble and displays

13

multiple structural conformations. At nanoscale, there are four major polymorphic forms of cellulose i.e. Cellulose I, II III and IV. They are obtained from different sources and through different extraction methods. The first two forms, however, are the most widely studied ones.

Cellulose I is found in natural sources and is therefore referred to as the native cellulose. It is thermodynamically metastable and highly crystalline. It exists as two polymorphs i.e. I α and Iβ [10].

Cellulose I is predominant in algae and bacteria. It comprises a triclinic unit cell wherein one cell bears one glucose unit. Through hydrothermal treatment in alkaline solution, it can be converted to Iβ. Cellulose II is mainly found in higher plants and consists of a monoclinic unit cell that bears two cellulose chains in every cell, that are present in antiparallel confirmation. Its allomorphs are comparatively more stable than that of cellulose I, but they rarely occur in nature. It can be generated from cellulose I through chemical processes such as mercerization. The arrangement of cellulose chains in both the forms is such that the β (1, 4) glycosidic bonds are oriented parallelly, but in the same direction [10]. The third form i.e. Cellulose III, exists in two subforms, Cellulose III_I and cellulose III_{II}. It is usually formed from cellulose I and II via ammonia treatment. The final polymorph, i.e. cellulose IV is obtained by the modification of cellulose III with glycerol [11].

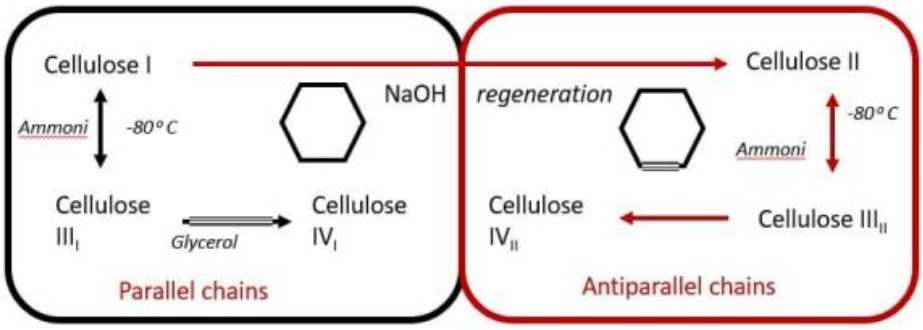


Fig 2.1: Methods for obtaining cellulose polymorphs

14

2.2 Sources of Cellulose

Cellulose is the most plentiful polymer found on earth and is obtained from various natural sources including wood residues, bacteria, tunicates and algae. Plants mainly contain a primary cell wall, which is surrounded by three secondary cell wall layers and a central lumen. The primary cell wall contains cellulose fibers organized in a random manner whereas the secondary cell wall has these fibers arranged in a helically oriented and hierarchically ordered manner. Herein, cellulose and hemicellulose molecules are arranged in a long chain, bound by lignin. Tunicates generally contain Iβ cellulose whereas algal genera such as *Chlorophyta, Rhodophyta, Phaeophyceae* etc. possess cellulose in Iα form. Bacterial cellulose is mainly obtained from *Acetobacter xylinum* and has higher elasticity, crystallinity and degree of polymerization [12].

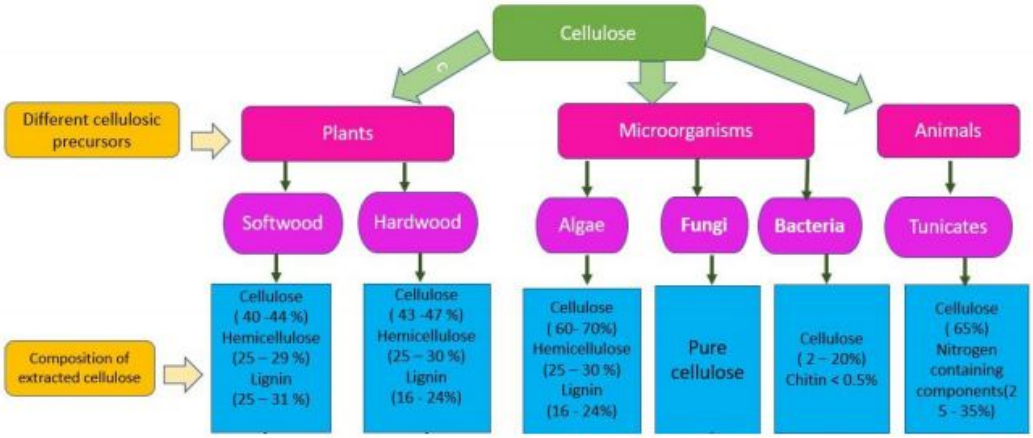


Fig 2.2: Composition of cellulose content derived from different sources

15

2.3 Microcellulose

2.3.1 Natural Fibers

The natural cellulose fibers obtained from different sources have remarkable difference sizes and shapes. For example, fibers obtained from plants like cotton and flax are as huge as several centimeters in size, whereas those obtained from woody sources are comparatively shorter, and have lengths in the range of 1-3 mm. Microcellulose is obtained from these fibers either as microcrystalline cellulose or as microcellulose powder after adequate pretreatment and bleaching of raw fibers, which facilitates the extraction of cellulose from cell wall along with the removal of lignin and hemicellulose. Subsequently, the size of cellulose particles is reduced down to micro range, i.e. 1 to 10 micrometers [13].

2.3.2 Microcrystalline Cellulose (MCC)

It is obtained after the treatment of cellulose fibers with dilute mineral acids. Industrially, MCC is produced by the hydrolysis of cellulose with hydrochloric acid followed by its neutralization [14]. The suspension hence obtained is homogenized and spray dried to yield MCC particles of size between 50 to 200 micrometers. An additional grinding step is inserted to bring down the particle size below 50 micrometers. The treatment conditions at each step may be varied to obtain MM of desired crystallinity, density or compatibility [15]. MCC possesses several unique properties, including small particle size, high crystallinity, low solubility and great purity.

2.3.3 Powdered Microcellulose

Powdered microcellulose is produced by dry grinding of raw cellulose source via different techniques such as ball mills, knife mills, centrifuge etc. It has white appearance and is tasteless and odorless. The produced powder is often screened for particle size to obtain homogeneous particle size.

16

2.3.4 Microfibrillated Cellulose (MFC)

Microfibrillated cellulose is derived from the treatment of thoroughly grinded cellulose with appropriate chemical reagents [16]. It has high viscosity, high yield stress as well as appreciable water holding capacity. An additional step of pressurized homogenization can further reduce the particle size and produce nanofibrillated cellulose, having particles of size within 1 to 100 nm.

2.4 Methods of Cellulose Pretreatment

Natural sources of cellulose, especially plants and wood residue contain a large percentage of lignin, hemicellulose and other polysaccharides along with cellulose. Hence it is essential to digest down the cell wall and remove the residual biomass for efficient cellulose extraction. This is primarily facilitated by the method of "Pretreatment" in which the lignocellulosic biomass is digested through biochemical processes [17]. Pretreatment procedures can be physical, chemical or biological depending on the requirements and conditions.

2.4.1 Physical Treatment

Physical methods include mechanical procedures which rely on the use of external force and shear stress to mediate the breakdown of cellular content. The commonly utilized techniques are sonication, microwave treatment, pyrolysis and mechanical processes such as grinding and milling. The mechanical methods aim at reducing the particle size to less than 0.2 mm, thereby conferring a uniform size to various particles. It is the simplest and most convenient method of biomass processing since it does not involve the use of toxic chemicals or harmful precursors. The process, however, is energy demanding and may be expensive [4]. The softer portions of the biomass are simply decomposed by shredding at high temperature (50-70 °C). It ruptures the fibers present in cellulose, thereby reducing the time required for further treatment [18]. Microwave treatment at radiation energy of 300-

17

700 W/m^3 reduces the stability of lignocellulose molecules by evaporating water and destroying hydrogen bonds. Sonication is performed by exposing the biomass to ultrasound, i.e. sound with frequency between 20-40 kHz, and results in the rupture of fibers along with the breakdown of internal hydrogen bonds [19]. Biomass treatment under gamma radiation is yet another method of pretreatment of biomass, which leads to the breakage of glycosidic bonds leading to the enhancement of its specific area and a decrease in its crystallinity. Gamma radiation is highly penetrating and is thus suitable for application on thicker materials as well. The final method i.e. pyrolysis involves the thermochemical decomposition of biomass. The process occurs at temperatures above 200 °C. High temperature results in intensive gasification, thereby liberating various pyrolytic gases, including hydrogen and carbon monoxide [20].

2.4.2 Biological and Enzymatic Treatment

Enzymatic methods rely on hydrolyzing simple sugars present in the biomass. The catalytic conversion of cellulose and hemicellulose is respectively mediated by cellulases and hemicellulases. Endoglucanases, exoglycanases and glycosylases mediate the hydrolysis of glycans. Two or more enzymes can also be added simultaneously to enhance the efficiency of hydrolysis. Several white rot fungi such as *Phanerochaete chrysosporium, Phlebia radiate, Dichmitus squalens, Rigidosporus lignosus and Jungua separabilima* can be used for the purpose as well. They act by releasing enzymes like lignin peroxidase and polyphenol oxidase along with specific chemicals, which lead to cell wall decomposition and wood degradation [21]. Brown rot fungi such as *Gloeophyllum trabeum* produce β-glucosidase, a thermophilic xylanase that can depolymerize amorphous cellulose by methylating lignin and oxidizing cellulose [22].

2.4.3 Chemical Treatment

Chemical treatment methods are the most widely adopted methods for cellulose purification. By using these methods, portions of lignin, wax, pectin and hemicellulose can be conveniently removed.

18

2.4.3.1 Acid Hydrolysis

Acid hydrolysis is considered to be the easiest and simplest method of cellulose pre-treatment. It increases the digestibility of sugar substrates. The most widely utilized acids are sulfuric acid and hydrochloric acid in the concentration of 5-10%. It is often performed at temperatures above 160 °C. However, if the concentration of acid is high, hydrolysis can occur efficiently at lower temperatures as well [23]. It damages lignin, dissolves hemicellulose and mediates the decomposition of cellulose to simple sugars. Acid hydrolysis proceeds via two steps, wherein the first step involves the dissolution of hemicellulose in relatively milder conditions. In the second step, cellulose hydrolysis occurs [23]. Nitric and phosphoric acid can also fulfill the purpose, particularly for leguminous plants and grasses.

2.4.3.2 Alkali Treatment

Alkali treatment decomposes various lignocellulosic components by penetrating into crystallites and destroying inter and intra molecular hydrogen bonds in cellulose molecules . Hydroxides of sodium, potassium, calcium and ammonium are the primarily employed reagents in the process. Alkali treatment is particularly effective at removing hemicellulose since it causes the loss of rigidity and integrity of fibers, thereby facilitating their rearrangement. It is also suitable for partial degradation of lignin [24]. Alkali treatment is a low cost and highly efficient technique which can be easily carried out at room temperature, thus making it less energy intensive [4].

2.4.3.3 Oxidation & Bleaching

Bleaching usually succeeds the alkali treatment process and mediates the complete removal of lignin. Common agents are Sodium chlorite ($NaClO_2$), sodium hypochlorite (NaClO), hydrogen peroxide (H_2O_2,), and sodium sulfite (Na_2SO_3), which oxidize lignin by attacking aromatic ring [25]. Bleaching usually produces harmful byproducts, hence several

19

researches have tried to use more environment friendly oxidizing agents such as hydrogen peroxide, peracetic acid and EDTA [26].

2.4.3.4 Treatment with Ionic Liquids

Ionic liquids are salt composed of ions with a melting point less than 100 °C. They are considered as "green solvents" and are popularly exploited for cellulose extraction [27]. The ions present in them solubilize the cellulose network structure by dissolving the inter and intra hydrogen bonds present between them.

2.5 Methods of Quantification

Cellulose content in a sample can be quantified through various dry weight and spectroscopic methods.

A commonly used method relies on the insolubility of cellulose in water and its degree of resistance to the attack of dilute acids and bases. In this test, the sample is degraded with a mixture of acids (mainly nitric acid and acetic acid). The solution is put in a boiling apparatus which is placed on a condenser and allowed to boil. The solution is then filtered and the residue is oven dried. The weight of the dried sample is measured to obtain an estimate of cellulose content. The method, however, provides only a superficial estimate of total content.

2.5.1 Anthrone Test for Detection of Cellulose Content

Anthrone test is a widely popular method utilized for the estimation of carbohydrates in a sample. It is, however, particularly popular for the detection of cellulose.

The principle of the test is based upon acid mediated hydrolysis of carbohydrates. If the sample contains carbohydrates in either free form or bound with glycoproteins, it gets broken down into component monosaccharide due to the action of concentrated acid. It is

20

followed by the dehydration of monosaccharides to yield furfural from pentoses or hydroxyl furfural from hexoses. The furfural thus formed goes on to condense with two molecules of naphthol present in the anthrone reagent to impart a blue green colored complex. The intensity of coloration is quantified by measuring the absorbance of the 620 nm wavelength through a spectrophotometer.

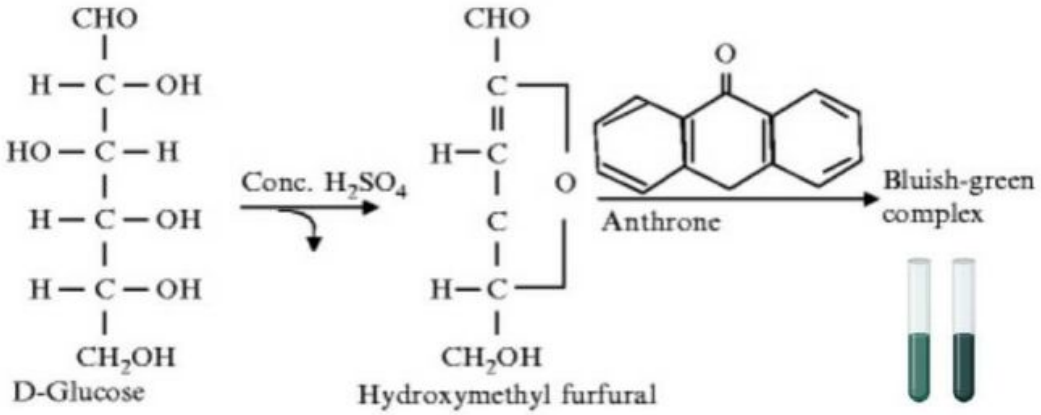


Fig. 2.3: Principle of anthrone test (Katoch, 2011)

21

2.6 Methods of Characterization

Once cellulose has been isolated, it needs to be analyzed through various techniques to find the morphological, electronic and other surface properties of the particles. Currently, a number of physicochemical methods are employed for cellulose characterization. Some common methods are as follows

2.6.1 Electron Microscopy

Electron microscopy involves the use of a focused beam of accelerated electrons in order to generate a highly magnified and highly resolved image of the target. As the electrons collide with the sample, an emission of various particles and ions is generated which gets both reflected as well as transmitted. The rate of acceleration of these particles and the differences in their energy is detected and incorporated into an image. Electron microscopy is generally performed to detect the shape, size, degree of aggregation on dispersion and morphology of the isolated particles.

2.6.2 Zeta Potential analysis

Since microcellulose particles contain charged groups on their surface, it is necessary to evaluate surface charge density while analyzing the sample. Zeta potential puts forward an assessment of surface potential and charge density. It is measured by determining the mobility of a particle under an electric field via electrophoretic light scattering or laser Doppler velocimetry. Electric mobility is then converted into zeta potential.

2.6.3 X-ray Diffraction

The X-ray diffraction process can be used for the analysis of all kinds of cellulosic materials, including microcellulose and nanocellulose. The technique involves the irradiation of the target sample with X-rays followed by the measurement of intensity and scattering angle of X-rays that leave the material. XRD is basically used to determine crystallinity of the sample along with factors that influence the transition and distribution of highly ordered to least ordered regions.

22

2.6.4 FT-IR Analysis

FT-IR stands for Fourier-transform infrared spectroscopy. The technique involves the irradiation of the target sample with infrared light. The light absorbed, transmitted or reflected by the sample is measured and reported as a function of wavenumber. The obtained values are plotted as a graph which displays specific absorption bands and peaks that correspond to a particular molecular vibrations, and can hence be used for the detection of specific functional groups and bonds present within the sample.

2.6.5 Thermogravimetric Analysis

It is done to obtain the information regarding reaction conditions and thermal decomposition behavior of material. It provides an idea regarding kinetics of decomposition and thermal stability. It also provides valuable insights regarding purity of cellulose and can be helpful in performing surface modification of cellulose particles.

2.7 Microcellulose and Cellulose Extraction from Agricultural Wastes

Numerous studies from all around the world have reported the extraction of cellulose and microcellulose from various agricultural wastes including wheat straw, sugarcane bagasse, date palm, corn cob etc.

Lorenzo-Santiago and Villalobos reported the isolation of microcellulose from the fibrous endocarp of waste mangoes. The presence of multiple biopolymers with large amounts of starch and cellulose facilitated convenient extraction. The alkaline method was employed for pretreatment and additional bleaching was performed to carry out whitening. Following, acid hydrolysis was performed using 52% sulfuric acid at 45 °C and the reaction was quenched using cold water. The acid treated sample was further subjected to sonication for disintegrating the fibers into microfibrils. The chemical characterization of isolated cellulose revealed that after pretreatment, the lignin content decreased from an initial 10.3% to 0.64%. Structural characterization of pre-treated samples was performed using SEM, TEM and FTIR. The micrographs revealed that cellulose fibers of size ranging between 40-

23

400 um were obtained post bleaching. FTIR analysis displayed a peak at 894 cm-1 which corresponds to the anomeric carbon present in cellulose. A narrow absorption band was obtained in the region between 1170 and 920 cm-1, representing vibrations of C-O, C-C and C-OH bonds, thus confirming the presence of cellulose. The FTIR analysis of native samples was also performed for comparison purpose and the results revealed peaks corresponding to stretched carboxylic acid groups and alkyl, aliphatic and aromatic rings which are actually present in hemicellulose and lignin. The analysis hence confirmed that significant destruction of lignin and hemicellulose occurred after pre-treatment. Further, the crystallinity of extracted cellulose and native fibers was also examined, wherein the crystallinity of native fibers was found to be lower 75.18% which was lower than that of extracted microcellulose (77.5%). It was reasoned that the crystallinity index in microcellulose increases due to the elimination of amorphous regions of cellulose which results in the release of individual crystals. The study thus proved that mango waste can be an excellent source for the isolation of natural polymers [28].

Another study reported the isolation of cellulose from wheat straw and regarded wheat straw as the most preferable material for cellulose isolation, given its abundance and availability throughout the year. Two different methods of pretreatment were used in the study: while the first one utilized acidified sodium chlorite, the second one relied on alkaline hydrogen peroxide. The obtained microfibrils were characterized using multiple techniques. Chemical composition of isolated fibers was analyzed and it was found that alkaline treatment of raw material led to an increase in cellulose content from 44% to 79% and decreased the content of hemicellulose and lignin from 36 % to 14% and from 17% to 2% respectively. On the other hand, the content of cellulose in acid treated fibers was enhanced from 44% to 81% whereas hemicellulose content decreased from 36% to 13% and lignin content decreased from 17% to 6% respectively. FTIR analysis of native sample showed peaks pertaining to acetyl group of hemicellulose and aryl-alkyl ether group of lignin. However, both of the treated samples showed peaks representing the -OH bending of absorbed water along with an asymmetric stretching ring of cellulose. The absence of peaks corresponding to lignin and hemicellulose confirmed the removal of these components from the isolated fibers. XRD analysis of the treated samples was also

24

performed which showed the existence of cellulose I polymorphs and affirmed that treatment procedures did not affect crystal polymorphism and high crystallinity was obtained due to effective removal of non-cellulosic content. SEM analysis of the samples revealed that cellulose obtained from alkaline treatment was irregularly shaped and missed some components whereas the cellulose obtained from acid treatment was in regular shape consisting of straight fibers connected with each other. The study concluded that alkaline treatment required higher temperature, generated irregular shaped fibers and was a more environment-friendly method as compared to the acid treatment method. The latter, however, required lower temperature, had better yield and generated fibers having regular shape and fine thermal stability [29].

In their research, Sun et al. reported the isolation of cellulose from sugarcane bagasse and characterized the isolated fibers using CP/MAS and C-NMR technique. Pretreatment of bagasse was performed using three separate chemicals i.e., acidified sodium chlorite, alkaline peroxide and acetic acid-nitric acid mixture. Primary isolation steps during alkaline peroxidase method involved initial dewaxing of bagasse by ultrasonic irradiation followed by treatment with distilled water at 55 C. This was succeeded by the addition of sodium hydroxide and peroxide, after which the filtrate was separated and the concentrate was neutralized to obtain cellulose. The other pathway used acidic sodium chlorite as the reagent for pretreatment. The initial delignification was performed by treating the bagasse sample with distilled water at high temperature. This was followed by the treatment of free sample with sodium hypochlorite and acetic acid. Alkali treatment was given using potassium hydroxide and sodium hydroxide to extract holocellulose and the suspension was filtered. Finally, ethanol washing of residue was done to obtain cellulosic fractions. The last techniques involved the treatment of bagasse with 80% aqueous acetic acid and 70% nitric acid. This was followed by filtration and ethanol washing of residue to yield extraction products. The cellulose yield of fibers obtained using each technique was determined and it was found that the alkaline peroxidase method yielded 44.7 to 45.9% cellulose and the fibers contained 6-6.2% hemicellulose and around 3.4 - 3.9 % of lignin. The acid chlorite treatment yielded around 44.2 to 44.7% cellulose that contained around 3-7% residual hemicellulose and 1.5% lignin. The mixed acid treatment pathway yielded 43.0

25

to 43.6% cellulose which had 3-4% residual hemicellulose and 0.2-0.6% associated lignin. Gas chromatographic analysis of cellulose preparation showed that bagasse contained glucose as the most abundant sugar, constituting around 92-98% of total sugars, followed by traces of galactose, arabinose and mannose, affirming that bagasse is indeed an excellent source of natural cellulose. FT-IR analysis was also performed for the native and treated samples to deduce the presence of functional groups. The peaks corresponding to lignin were not found in the treated sample, confirming the removal of lignin. Various peaks obtained corresponded to OH bending, C-C and C-O skeletal vibrations and OH in-plane bending cellulose. Another peak at 903 cm-1 corresponded to b-glycosidic linkages between glucose units in cellulose, validating the presence of a high amount of cellulose in the sample. C-NMR analysis showed that the treatment of bagasse with acid sodium chlorite decreased the crystallinity of cellulose to the greatest extent whereas it was least affected by mixed acid exposure [30].

Yu et al. utilized a two stage process for the isolation of high quality cellulose and lignin from wheat straw. The first stage was operated at temperature of 150 °C and acid concentrations of around 1% to facilitate the separation of hemicellulose and lignin from cellulose whereas the subsequent stage incorporated the use of high temperature of around 180 °C and high acid concentration for the treatment of residual liquid. The IR spectroscopic study of the solid samples revealed bands that corresponded to aliphatic esters of hemicellulose and C=C of lignin. Certain bands addressed the presence of β-1,4-glucosidic bonds, confirming the presence of cellulose. The content of hemicellulose and lignin was found to be drastically reduced in samples treated with 1% H2SO4 whereas the samples treated with 1.5% H2SO4 showed the complete removal of both the compounds. In the latter case, however, the content of cellulose was also slightly decreased. The first stage treatment yielded 55% cellulose with a recovery rate of 92.8%. A recovery rate of 86.6% was obtained for the samples after second stage treatment [31].

An interesting study describing the isolation of cellulose from date palm biomass waste was carried out by Galiwango et al. Three different parts of the date plant, i.e. rachis, leaflet and fibers were used in the process. The isolation process was facilitated through treatment of

26

raw biomass with hydrochloric acid at 100 °C. Following filtration, the concentrate was treated with NaOH at 100 °C. Bleaching was then performed by exposing the sample to acetic acid, hydrogen peroxide and sulfuric acid. The obtained cellulose was then characterized by different techniques to obtain its chemical composition and other characteristics. A high cellulose yield (>70%) was obtained for all three raw materials, validating the efficiency of acid-alkali treatment. The TGA curve showed that initial decomposition of cellulose begins after the temperature crosses 200-250 C, which suggests that the compound remains stable until this temperature is attained. FT-IR results displayed broad absorption bands between 3400 and 3500 cm^{-1}, which are respectively attributed to stretching vibrations of OH group and CH groups present in cellulose. Peaks corresponding to C-O-C group of pyranose ring, CH_2 stretching of aromatic ring and C-O stretching of ether link were obtained as well, affirming the presence of cellulose and nearly complete removal of lignin. The obtained band was also compared to the IR bands of commercial cellulose and a near similar result was obtained. Through SEM analysis, the difference in morphologies of cellulose obtained from each raw material was detected. The micrographs revealed that the isolated cellulose had rod-like microfibril structure with significant diameter and thickness. Rachis and fibers yielded cellulose with well defined uniform structure that were highly porous and had decent diameter. The leaflet, however, yielded aggregated structures whose diameter and length couldn't be measured. X-ray diffraction graphs of isolated cellulose showed highly crystalline structures. The study presented date palm waste as a highly suitable source for cellulose extraction [32].

Another research exploited the fibers of areca fruit bunch for obtaining cellulose. The initial dewaxing of raw areca fibers was done by ethyl alcohol treatment. Subsequently, pretreatment was done by the popular acid-alkali treatment method followed by bleaching to facilitate extensive delignification. Delignified fibers were washed repeatedly with distilled water. Finally, the pH of the set-up was adjusted using NaOH solution. Cellulose yield from delignified fibers was found to be close to 65%, whereas the content of α-cellulose in the sample was close to 93% Characterization of isolated fibers through FTIR spectral studies presented peaks attributable to to stretching vibrations of cellulose –OH and C-H groups. Peaks corresponding to β-glycosidic linkage were also observed.

27

Moreover, certain peaks corresponding to the C=O acetyl group of hemicellulose or carbonyl ester of lignin were obtained from raw fibers. These peaks were, however, absent from the isolated fibers, hence demonstrating the complete removal of lignin and hemicellulose. The XRD analysis suggests that raw areca fibers are composite of lignin and hemicellulose. However, the peaks of isolated fibers aligned perfectly with patterns obtained for commercial cellulose. The crystallinity index and crystallite size of isolated fibers was found to be 71% and 9.6 nm respectively, as opposed to 44% and 31.6 nm values of raw fibers. SEM micrographs of fibers presented their appearance as separate fibrils with crystallite parallel chains [33].

2.8 Commercial Applications of Microcellulose

Cellulose, along with being the most abundant biopolymer, is also a highly versatile compound that has multiple utilities in various industries, including textile, packaging, cosmetic wood and paper etc. With ongoing research, cellulose structures such as microcellulose, microcrystalline cellulose and nanocellulose are being developed, which have further enhanced the applicability of cellulose and extended its usage to pharmaceutical, adhesive and research industry as well. Ether derivatives and ester derivatives of cellulose are also being constructed, which have further increased its utility as a commercially significant compound. Cellulose fibers, micro and nano cellulose particles obtained from agricultural wastes have been prudently incorporated into organised systems that can be efficiently exploited for industrial purposes. Major industrial applications of cellulosic fibers obtained from agricultural wastes have been described.

Manufacturing of Furniture- Cellulosic fibers isolated from fruit and vegetable wastes have been used to create medium density fiber boards and fibers from flax plants have been used for furniture production [34], [35].

Automobile Industry- Cellulosic fibers have been used to design panels, doors, dashboards of vehicles. They are gaining large popularity since their incorporation into

28

automobile parts decreases the overall weight of the vehicle and also minimizes fuel consumption [36].

Manufacturing sports goods- Natural fibers have been utilized to manufacture sporting equipment and wearables. These goods often exhibit better stiffness and vibration absorbing capacity. Tennis rackets, golf equipments, sports bicycle have been manufactured with the help of flax fibers [37]

Manufacturing Electronics- Scientists are increasingly identifying the potential of nanocellulose for the production of electronic devices. It not only confers low cost and light weight but is also renewable and biodegradable. Most prominently, the electronic systems manufactured using cellulose are being integrated into biosensors.

Water Purification- Nanocellulose fibers have nanosize , high surface area and they are non-toxic as well. Thus, they are considered suitable for the construction of membranes for purification of water. Cellulose nanofibers have already been utilized for the removal of heavy metals, oil and organic pollutants from wastewater samples [38].

Food Packaging- Cellulose nanofibers are generally hydrophilic and water insoluble, thus they are considered appropriate agents for developing packaging materials and films. They can even be modified with compounds having antimicrobial, moisture and odor absorbing capacity. Hence, they can effectively prevent microbial growth and moisture transfer [39].

Medical Applications- Cellulose based materials have significant applications in the pharmaceutical industry. Cellulose ethers including hydroxypropyl cellulose and ethylcellulose are used as binders, integrants and controlled-release agents in tablets. MCC is used to enhance tablet compressibility and improve drug dissolution. Cellulose based scaffolds are also being increasingly used in tissue engineering and regeneration [40].

Energy Industry: Cellulose acts as vital feedstock for the production of biofuel and renewable chemicals. Hydrolysis and fermentation processes are synchronized for the

29

conversion of cellulose to bioethanol, which can be blended with gasoline or used as a standalone fuel. Biofuels reduce greenhouse emission and thus offer a sustainable alternative to petroleum fuels.

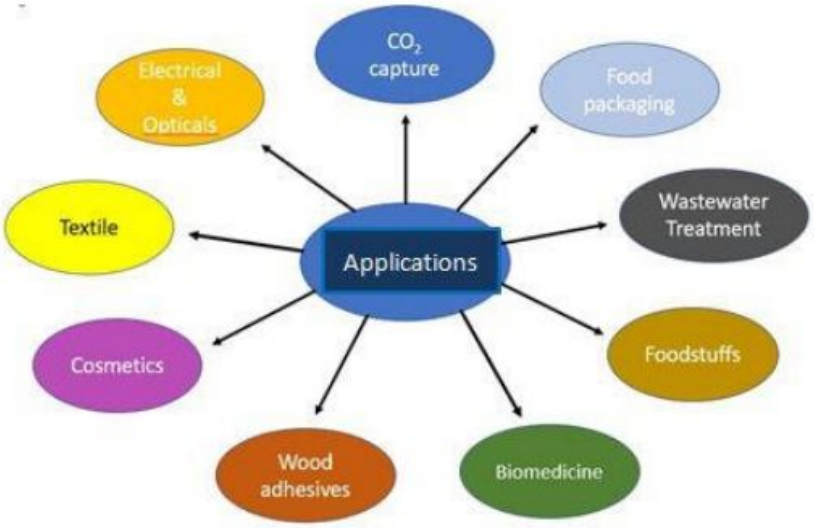


Fig. 2.4: Applications of Microcellulose

30

CHAPTER 3

METHODOLOGY

3.1 Sample Preparation

For microcellulose extraction, rice husk and pomegranate peel were sourced from local marketplaces. The Husk and peel sample was washed thoroughly and sundried for 3 to 4 weeks.

Fig. 3.1 a: Raw pomegranate peel **b: Dried rice husk**

After sufficient drying, the husk was grinded to form powder whereas the peel was chopped down into small pieces.

31

Fig. 3.2 a: Finely grinded rice husk b: Finely chopped pomegranate peel

3.2 Extraction Process

1. 500 ml of 10% NaOH was prepared by mixing 50 g of NaOH pellet in 200 ml distilled water and dissolved thoroughly. Distilled water was further added to make the final volume of 500 ml. Grinded rice husk and finely chopped pomegranate peel samples were subjected to pulping by mixing 25 g of each sample to 250 ml NaOH solution. The suspension was heated to 160 °C for 2 hours.

Fig. 3.3 Alkali treated rice husk sample

2. The pulped fibers were washed thoroughly and dried in open air

32

Fig 3.4 Air drying of pulped fibers

3. 200 ml of 20% H_2SO_4 was prepared by mixing 20 ml conc. H_2SO_4 in 180 ml distilled water
4. The solution was heated to 120 °C on the hot plate with continuous stirring

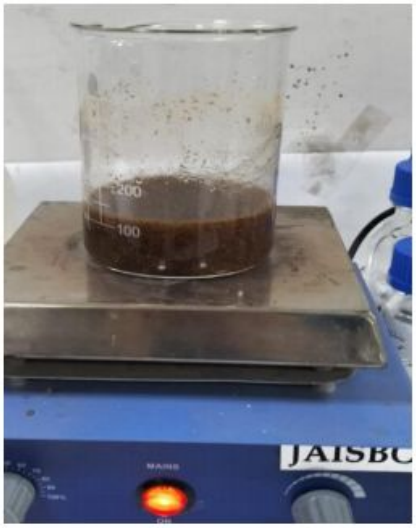


Fig 3.5 High temperature acid treatment of pulped fibers

5. After around 30 minutes (or after brownish paste-like texture showed up), 1 L frozen distilled water was added into the solution in order to quench the reaction.

33

6. After this, the suspension was passed through whatman filter paper and the filtrate and concentrate were separated.
7. The concentrate was transferred to falcon tubes and neutralized using 5% $NaHCO_3$ solution. The suspension, hence formed, was centrifuged at 6000 rpm for 15 minutes.
8. The supernatant obtained after centrifugation was decanted and replaced by deionized water. Subsequently, the tube was recentrifuged and pH was checked.

Fig 3.5: Rice husk sample after first round of centrifugation

9. The neutralization step was repeated until the final pH was obtained between 7 and 8.
10. After the required pH was attained, the supernatant was decanted and the fibers were collected in a glass petri plate.
11. The petri plate was put in a hot air oven at around 50 °C for around two hours in order to evaporate the water. Following this, the samples were taken out and finely dried microcellulose was obtained.

34

3.3 Quantification

The quantification of cellulose was done by performing the anthrone test.

3.3.1 Preparation of glucose standard

1. For preparing a working standard solution of glucose, an initial stock solution was prepared by mixing 0.01 g commercial glucose powder in 10 ml distilled water.
2. 10 µl solution was taken from the stock and added to a test tube. It was diluted with 990 µl distilled water to obtain glucose concentration of 10 µg/ml.
3. Following, 20 µl sample was taken from the stock and mixed with 980 µl distilled water to obtain final concentration of 20 µg/ml
4. The steps were repeated to obtain a series of concentrations, i.e. 40 g/ml, 60 g/ml, 80 g/ml upto 200 g/ml.

Table I: Glucose dilutions for standard curve plot

S.no.	Glucose concentration (µg)	Glucose volume (µl)	Distilled water (µl)	Anthrone reagent (ml)
1.	10	10	990	5
2.	20	20	980	5
3.	40	40	960	5
4.	60	60	940	5
5.	80	80	920	5
6.	100	100	900	5
7.	120	120	880	5
8.	140	140	860	5
9.	160	160	840	5

35

10.	180	180	820	5
11.	200	200	800	5

3.3.2 Preparation of Test Sample

The oven dried microcellulose samples were taken and weighed. They were then added to test tubes containing 1 ml distilled water. The tubes were labeled as follows:

RH-W1: 0.01 g sample (pH 7) /1 ml H_20
RH-W2: 0.05 g sample (pH 7) /1 ml H_20
RH-D1: 0.01 g sample (pH 7.8) /1 ml H_20
RH-D1: 0.05 g sample (pH 7.8) /1 ml H_20

The original powdered rice husk sample was taken and weighed for the purpose of comparison. The weighed samples were added to test tubes containing 1 ml distilled water. The tubes were labeled as follows:

RH-O1: 0.01 g husk /1 ml H_20
RH-O2: 0.05 g husk /1 ml H_20

3.3.3 Preparation of Anthrone Reagent

Anthrone reagent was prepared by dissolving 0.2 grams of antrone (powder, lab grade) in 100 ml conc. H2SO4.

36

3.3.4 Anthrone Test

1. In a test tube stand, the six tubes containing test samples were taken.
2. 5 ml anthrone reagent was added to each test tube and vortexed to mix the contents thoroughly.
3. The tubes were incubated in a water bath at the temperature of 90 °C.
4. The appearance of green color indicated the presence of cellulose
5. The tubes were allied to cool down to room temperature
6. The intensity of color in each tube was determined by measuring optical density of each solution at 620 nm while using the anthrone reagent as blank.
7. A calibration curve was constructed by plotting the value of glucose concentration at X axis and absorbance at Y axis in Microsoft excel.
8. The concentration of cellulose in test samples was measured from the calculation curve.
9. Percentage of cellulose in each test sample was calculated from the formula:
10. Cellulose %age = (conc of cellulose in test sample/ dry weight of test sample)*100
11. The percentage of cellulose present in original sample and pretreated sample was compared to confirm the efficacy of the extraction process

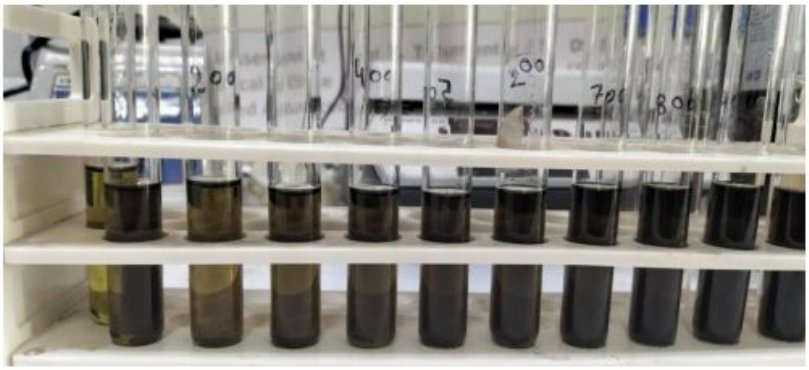


Image 3.6 a: Development of green colored gradient in glucose standard solution

37

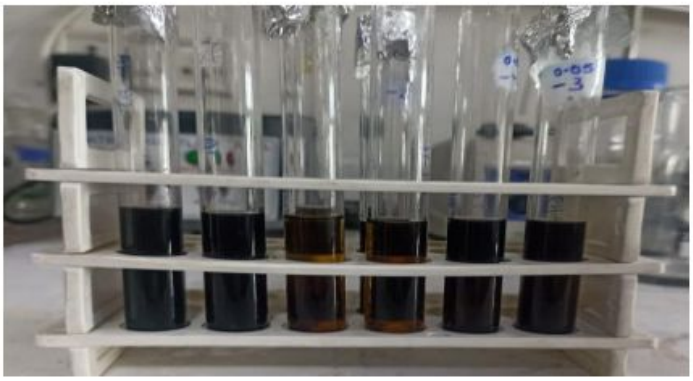


Fig 3.6 b: Development of greenish color in pretreated samples

Fig 3.6 c: Development of light green color in untreated (original) samples

3.4 Characterization

Characterization of the obtained microcellulose sample was done to assess their morphology, particle size and the functional groups present.

3.4.1 Particle Size Distribution

Particle size distribution analysis was performed to obtain the average diameter and size distribution of the samples. 0.05 g of dry sample was weighed and mixed with 10 ml deionized water before analysis.

38

3.4.2 FTIR Analysis

1. FTIR analysis was performed to determine the presence of various functional groups on the extracted sample. Considering that the sample was in powdered form, the KBr disc method was used for analysis.
2. KBr powder and sample was mixed and crushed with mortar and pestle. KBr pellets were prepared by placing the samples in a disc die to achieve flat, even surface. The die assembly was then placed into a hydraulic press and pressure was released slowly.
3. A separate pellet containing only KBr was prepared to be used as control or blank
4. FTIR spectrometry was then performed in the range between 4000 to 400 cm^{-1}.
5. The peaks obtained from the analysis were compared with the standard table taken from [41] to deduce the nature of functional groups present on the extracted sample.

Fiber component	Wave number (cm_1)	Functional group	Compounds
Cellulose	4,000–2,995	OH	Acid, methanol
	2,890	H–C–H	Alkyl, aliphatic
	1,640	Fiber–OH	Adsorbed water
	1,270–1,232	C–O–C	Aryl-alkyl ether
	1,170–1,082	C–O–C	Pyranose ring Skeletal
	1,108	OH	C–OH
Hemicellulose	4,000–2,995	OH	Acid, methanol
	2,890	H–C–H	Alkyl, aliphatic
	1,765–1,715	C=O	Ketone and carbonyl
	1,108	OH	C–OH
Lignin	4,000–2,995	OH	Acid, methanol
	2,890	H–C–H	Alkyl, aliphatic
	1,730–1,700		Aromatic
	1,632	C=C	Benzene stretching Ring
	1,613, 1,450	C=C	Aromtic skeletal mode
	1,430	O–CH_3	Methoxyl– O–CH_3
	1,270–1,232	C–O–C	Aryl-alkyl-ether
	1,215	C–O	Phenol
	1,108	OH	C–OH
	700–900	C–H	Aromatic hydrogen

Fig 3.7: Reference table for FTIR analysis

39

CHAPTER 4

RESULTS AND DISCUSSION

Microcellulose was successfully extracted from the rice husk sample. The pomegranate peel, however, underwent severe degradation due to highly acidic conditions and could not be retrieved after filtration. Fig 4.1 shows the physical appearance of the sample

Fig 4.1 a: Appearance of sample after final centrifugation

Fig 4.1 b: Appearance of oven-dried microcelllulose

40

4.1 Quantification of Cellulose

Quantification of cellulose was done by anthrone test. The optical density obtained for glucose standard, isolated cellulose and original rice husk sample are enlisted in Table

Table II: Spectrophotometric readings of glucose standard

S. no.	Glucose concentration (µg)	Optical Density
1	10	0.093
2	20	0.205
3	40	0.244
4	60	0.264
5	80	0.408
6	100	0.522
7	120	0.622
8	140	0.766
9	160	0.863
10	180	0.992
11	200	1.260

41

Table III: Optical density for rice husk samples

S. no.	Sample	Dry Weight (g)	Optical Density
1	RH-W1	0.01	0.901
2	RH-W2	0.05	1.37
3	RH-D1	0.01	0.429
5	RH-D2	0.05	1.718
5	RH-O1	0.01	0.084
6	RH-O2	0.05	1.044

The concentration VS absorbance graph was plotted in MS excel

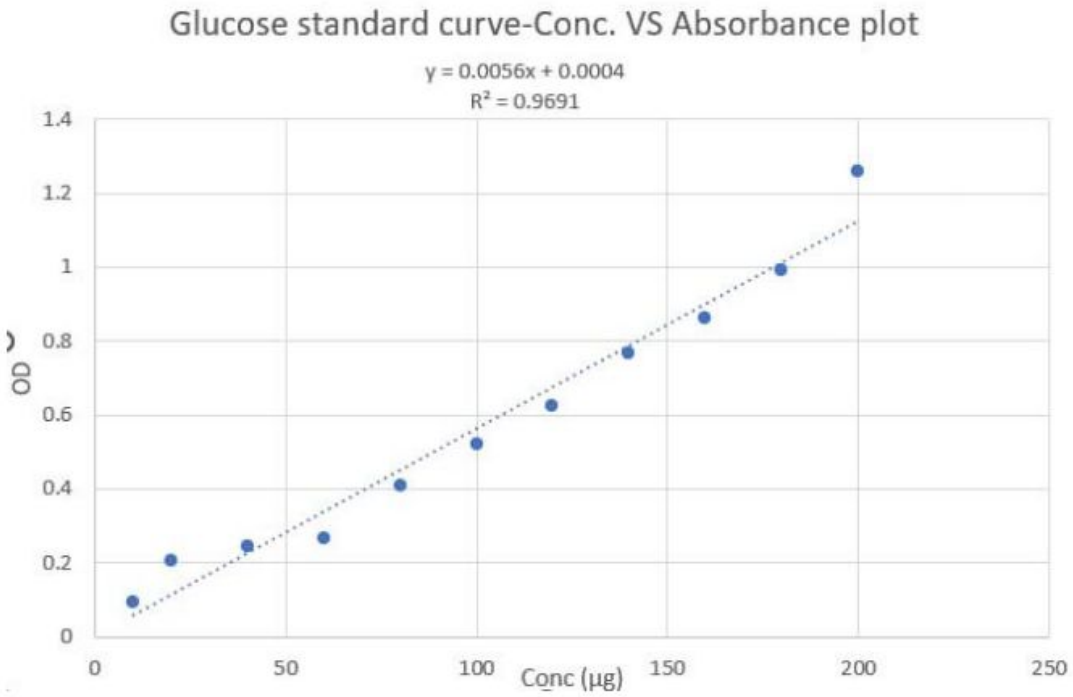


Fig. 4.2 Glucose standard curve

42

Trendline equation obtained from the graph: Y= mX + C

Y= 0.0056x + 0.0004

$R^2 = 0.9691$

From equation , the value of X was calculated using:

X= (Y-C)/X

From equation , the concentration of cellulose present in pretreated and original samples was obtained

Table IV: Concentration of cellulose and percentage cellulose in unknown samples

S. no.	Sample	Dry Weight (g)	Cellulose concentration (µg)	Percentage (%) of cellulose
1	RH-W1	0.001	60.3407	6.03
2	RH-W2	0.005	343.8395	6.87
3	RH-D1	0.001	76.307	7.63
5	RH-D2	0.005	305.796	6.11
5	RH-O1	0.001	14.885	1.48
6	RH-O2	0.005	161.765	3.23

The comparison of cellulose concentration in untreated rice husks and pretreated samples revealed that after pretreatment, the concentration of cellulose increased from an initial 1.5-3% to around 6 to 7.5%. This is indeed a significant elevation, which indicates the efficacy of the pretreatment process, thereby confirming that cellulose extraction has been successful.

43

4.2 Characterization

FTIR analysis of isolated cellulose fibers was performed to detect the nature of functional groups. Fig. 4.3 shows the results of FT-IR analysis.

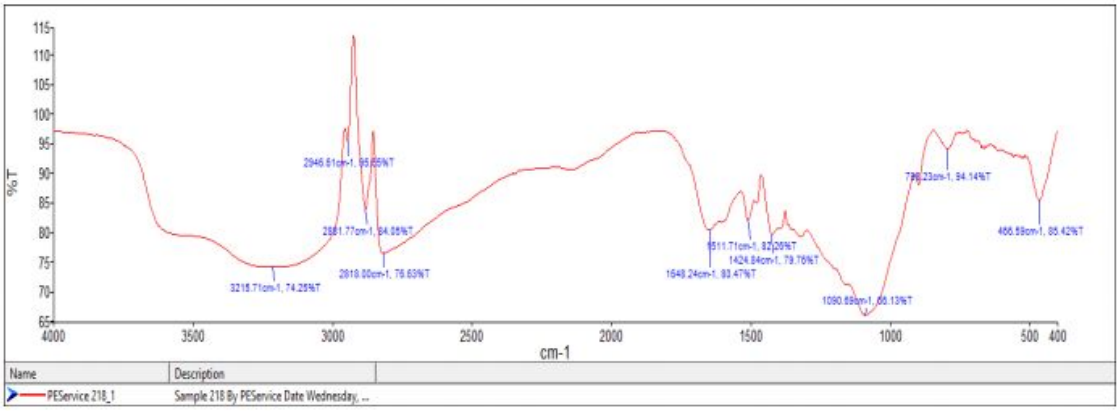


Fig 4.3 FTIR spectroscopy results of husk residues

As observed in the figure, the sample shows the main absorbance region. The first one at low range of 450 to 800, second one at a higher range between 1100 to1650. Another high range is observed between 2800 and 3200 wave number/cm. Peaks obtained at 2946 cm^{-1} and 3215 cm^{-1} correspond to stretching vibrations of hydroxyl groups found in cellulose. Peak obtained at 2881 cm^{-1} represents the H-C-H group found in cellulose as well as hemicellulose. Another peak formed at 1648 cm^{-1} represents fibrous -OH of cellulose. Peak obtained at 1090 cm^{-1} represents C-O-C group of cellulose. Another peak obtained at 1242 cm^{-1} may represent the O-CH3 group found in lignin. It was thus deduced that peaks corresponding to lignin and hemicellulose were lesser as compared to those of cellulose. Therefore, it can be concluded that pulping and pretreatment process succeeded in the removal of lignin and hemicellulose and profound isolation of cellulose occurred.

Particle size distribution analysis of cellulose particles was performed to determine the size of isolated fibers. Fig. 4.4 shows the results for the same.

44

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	5358	Peak 1:	4989	100.0	534.3
PdI:	0.147	Peak 2:	0.000	0.0	0.000
Intercept:	0.970	Peak 3:	0.000	0.0	0.000
Result quality	Refer to quality report				

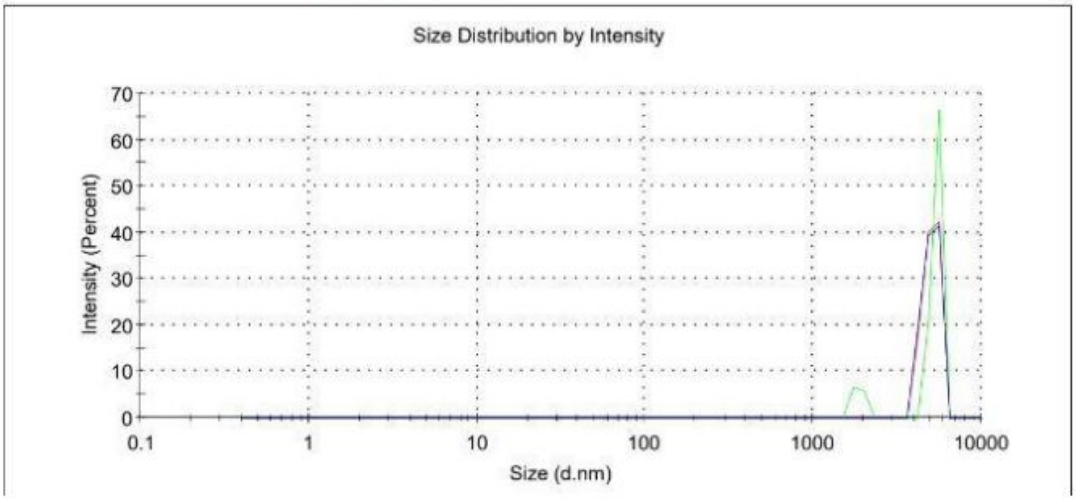


Fig 4.4 Particle size distribution analysis of isolated cellulose

As seen in the figure, a low peak is observed around 4900 nm and another sharp, narrow peak is observed beyond. The average diameter of cellulose particles was found to be around 5358 nm, which is close to 5.3 µm. The test confirms that the isolation process has managed to successfully reduce the size of cellulose particles and brought it down to micrometer range.

45

CHAPTER 5

CONCLUSION

For rice husk, the isolation technique utilized was found to be successful. It triumphantly removed a considerable portion of lignin and hemicellulose from the sample, thereby facilitating the extraction of microcellulose. However, desired results could not be obtained for the pomegranate peel sample, which were found to be extremely sensitive to acidic hydrolysis. The quantification and characterization studies confirmed the removal of lignocellulosic content and showed the presence of high amount of cellulose in the pretreated sample. The study concluded that rice husk is indeed a highly suitable sample for microcellulose extraction and can definitely provide novel insights in the field of material development, packaging, pharmaceutics etc.

46

REFERENCES

[1] S. P. Bangar and W. S. Whiteside, "Nano-cellulose reinforced starch bio composite films-A review on green composites," *Int. J. Biol. Macromol.*, vol. 185, pp. 849–860, Aug. 2021.

[2] R. Jagadeesan, I. Suyambulingam, R. Somasundaram, D. Divakaran, and S. Siengchin, "Isolation and characterization of novel microcellulose from Sesamum indicum agro-industrial residual waste oil cake: conversion of biowaste to wealth approach," *Biomass Conversion and Biorefinery*, vol. 13, no. 5, pp. 4427–4441, Jan. 2023.

[3] "Website."

[4] K. Kucharska, P. Rybarczyk, I. Hołowacz, R. Łukajtis, M. Glinka, and M. Kamiński, "Pretreatment of Lignocellulosic Materials as Substrates for Fermentation Processes," *Molecules*, vol. 23, no. 11, Nov. 2018, doi: 10.3390/molecules23112937.

[5] A. V. Samrot *et al.*, "Waste-Derived Cellulosic Fibers and Their Applications," *Advances in Materials Science and Engineering*, vol. 2022, Mar. 2022, doi: 10.1155/2022/7314694.

[6] M. Raza, B. Abu-Jdayil, F. Banat, and A. H. Al-Marzouqi, "Isolation and Characterization of Cellulose Nanocrystals from Date Palm Waste," *ACS Omega*, vol. 7, no. 29, pp. 25366–25379, Jul. 2022.

[7] A. A. B. Omran *et al.*, "Micro- and Nanocellulose in Polymer Composite Materials: A Review," *Polymers* , vol. 13, no. 2, Jan. 2021, doi: 10.3390/polym13020231.

[8] R. J. Moon, A. Martini, J. Nairn, J. Simonsen, and J. Youngblood, "Cellulose nanomaterials review: structure, properties and nanocomposites," *Chem. Soc. Rev.*, vol. 40, no. 7, pp. 3941–3994, Jul. 2011.

[9] N. Halib *et al.*, "Potential Applications of Nanocellulose-Containing Materials in the Biomedical Field," *Materials* , vol. 10, no. 8, Aug. 2017, doi: 10.3390/ma10080977.

[10] S. Rongpipi, D. Ye, E. D. Gomez, and E. W. Gomez, "Progress and Opportunities in the Characterization of Cellulose - An Important Regulator of Cell Wall Growth and Mechanics," *Front. Plant Sci.*, vol. 9, p. 1894, 2018.

[11] N. Lavoine, I. Desloges, A. Dufresne, and J. Bras, "Microfibrillated cellulose - its barrier properties and applications in cellulosic materials: a review," *Carbohydr. Polym.*, vol. 90, no. 2, pp. 735–764, Oct. 2012.

[12] A. Poulose *et al.*, "Nanocellulose: A Fundamental Material for Science and Technology Applications," *Molecules*, vol. 27, no. 22, Nov. 2022, doi: 10.3390/molecules27228032.

[13] I. H. Mondal, *Cellulose and Cellulose Derivatives: Synthesis, Modification and Applications*. Nova Science Publishers, 2015.

[14] "[No title]." https://doi.org/10.1021/ie50633a003 (accessed May 28, 2023).

[15] "Effect of batch variation and source of pulp on the properties of microcrystalline cellulose," *Int. J. Pharm.*, vol. 91, no. 2–3, pp. 133–141, Apr. 1993.

[16] V. Kumar *et al.*, "Comparison of nano- and microfibrillated cellulose films," *Cellulose*, vol. 21, no. 5, pp. 3443–3456, Oct. 2014.

[17] Y.-L. Loow *et al.*, "Recent Advances in the Application of Inorganic Salt Pretreatment for Transforming Lignocellulosic Biomass into Reducing Sugars," *J. Agric. Food Chem.*, vol. 63, no. 38, pp. 8349–8363, Sep. 2015.

[18] R. Martin-Sampedro, I. Filpponen, I. C. Hoeger, J. Y. Zhu, J. Laine, and O. J. Rojas, "Rapid and Complete Enzyme Hydrolysis of Lignocellulosic Nanofibrils," *ACS Macro Lett.*, vol. 1, no. 11, pp. 1321–1325, Nov. 2012.

[19] M. J. Bussemaker, F. Xu, and D. Zhang, "Manipulation of ultrasonic effects on lignocellulose by varying the frequency, particle size, loading and stirring," *Bioresour. Technol.*, vol. 148, pp. 15–23, Nov. 2013.

[20] Y. K N *et al.*, "Lignocellulosic biomass-based pyrolysis: A comprehensive review," *Chemosphere*, vol. 286, no. Pt 2, p. 131824, Jan. 2022.

[21] A. Leonowicz *et al.*, "Biodegradation of lignin by white rot fungi," *Fungal Genet. Biol.*, vol. 27, no. 2–3, pp. 175–185, Jul-Aug 1999.

[22] D. J. Yelle, J. Ralph, F. Lu, and K. E. Hammel, "Evidence for cleavage of lignin by a brown rot basidiomycete," *Environ. Microbiol.*, vol. 10, no. 7, pp. 1844–1849, Jul. 2008.

[23] A. T. W. M. Hendriks and G. Zeeman, "Pretreatments to enhance the digestibility of lignocellulosic biomass," *Bioresour. Technol.*, vol. 100, no. 1, pp. 10–18, Jan. 2009.

[24] R. Vijay *et al.*, "Characterization of raw and alkali treated new natural cellulosic fibers from Tridax procumbens," *Int. J. Biol. Macromol.*, vol. 125, pp. 99–108, Mar. 2019.

[25] Y. Wu, J. Wu, F. Yang, C. Tang, and Q. Huang, "Effect of HO Bleaching Treatment on the Properties of Finished Transparent Wood," *Polymers* , vol. 11, no. 5, May 2019, doi: 10.3390/polym11050776.

[26] L. Duan, W. Yu, and Z. Li, "Analysis of structural changes in jute fibers after peracetic acid treatment," *J. Eng. Fiber. Fabr.*, vol. 12, no. 1, p. 155892501701200, Mar. 2017.

48

[27] H. Wang, G. Gurau, and R. D. Rogers, "Ionic liquid processing of cellulose," *Chem. Soc. Rev.*, vol. 41, no. 4, pp. 1519–1537, Feb. 2012.

[28] M. A. Lorenzo-Santiago and R. Rendón-Villalobos, "Isolation and characterization of micro cellulose obtained from waste mango," *Polímeros*, vol. 30, no. 3, p. e2020036, Dec. 2020.

[29] "Website."

[30] "Website."

[31] B. Yu *et al.*, "Simultaneous isolation of cellulose and lignin from wheat straw and catalytic conversion to valuable chemical products," *Applied Biological Chemistry*, vol. 64, no. 1, pp. 1–13, Jan. 2021.

[32] E. Galiwango, N. S. Abdel Rahman, A. H. Al-Marzouqi, M. M. Abu-Omar, and A. A. Khaleel, "Isolation and characterization of cellulose and α-cellulose from date palm biomass waste," *Heliyon*, vol. 5, no. 12, p. e02937, Dec. 2019.

[33] "Website." DOI : http://dx.doi.org/10.13005/msri/160112

[34] "Website."

[35] M. S. Salit, M. Jawaid, N. B. Yusoff, and M. Enamul Hoque, *Manufacturing of Natural Fibre Reinforced Polymer Composites*. Springer, 2015.

[36] L. A. Elseify, M. Midani, A. El-Badawy, and M. Jawaid, *Manufacturing Automotive Components from Sustainable Natural Fiber Composites*. Springer Nature, 2021.

[37] "Mechanical and damping performances of flax fibre composites – A review," *Composites Part C: Open Access*, vol. 4, p. 100081, Mar. 2021.

[38] D. Kumar, *Nanocellulose and Its Composites for Water Treatment Applications*. CRC Press, 2021.

[39] S. Ahmed, *Bionanocomposites for Food Packaging Applications*. Woodhead Publishing, 2022.

[40] E. H. Fragal *et al.*, "Hybrid materials for bone tissue engineering from biomimetic growth of hydroxiapatite on cellulose nanowhiskers," *Carbohydr. Polym.*, vol. 152, pp. 734–746, Nov. 2016.

[41] "Extraction and characterization of nanocellulose from three types of palm residues," *Journal of Materials Research and Technology*, vol. 10, pp. 526–537, Jan. 2021.

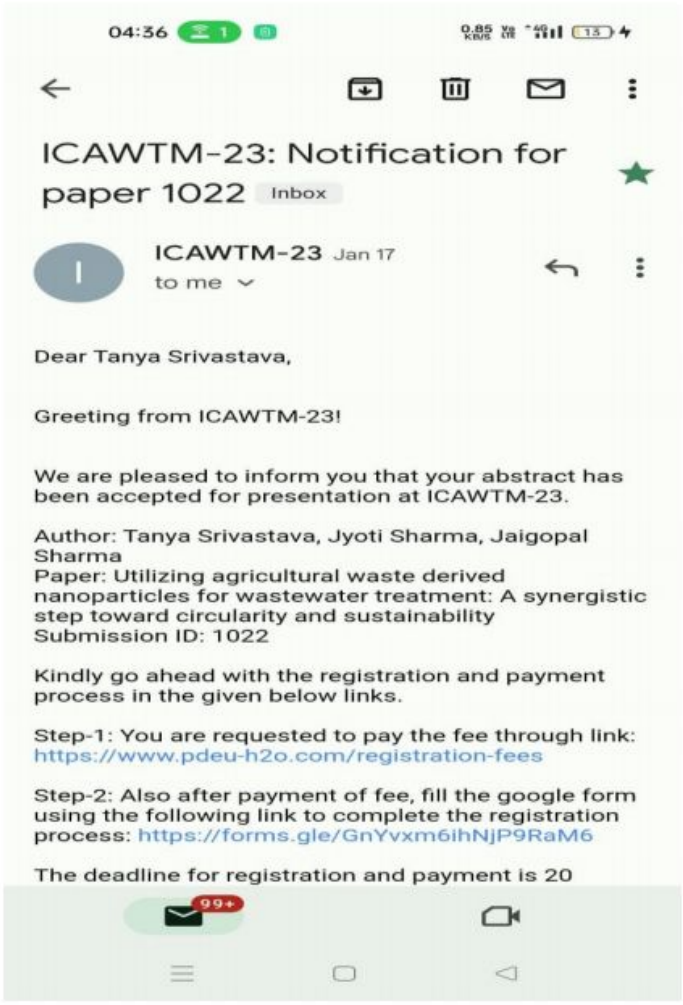
49

PROOF OF CONFERENCE

2nd International Conference on Advances in Water Treatment and Management-

"ICAWTM-23"

Mail of Acceptance



04:36

ICAWTM-23: Notification for paper 1022 Inbox

ICAWTM-23 Jan 17
to me

Dear Tanya Srivastava,

Greeting from ICAWTM-23!

We are pleased to inform you that your abstract has been accepted for presentation at ICAWTM-23.

Author: Tanya Srivastava, Jyoti Sharma, Jaigopal Sharma
Paper: Utilizing agricultural waste derived nanoparticles for wastewater treatment: A synergistic step toward circularity and sustainability
Submission ID: 1022

Kindly go ahead with the registration and payment process in the given below links.

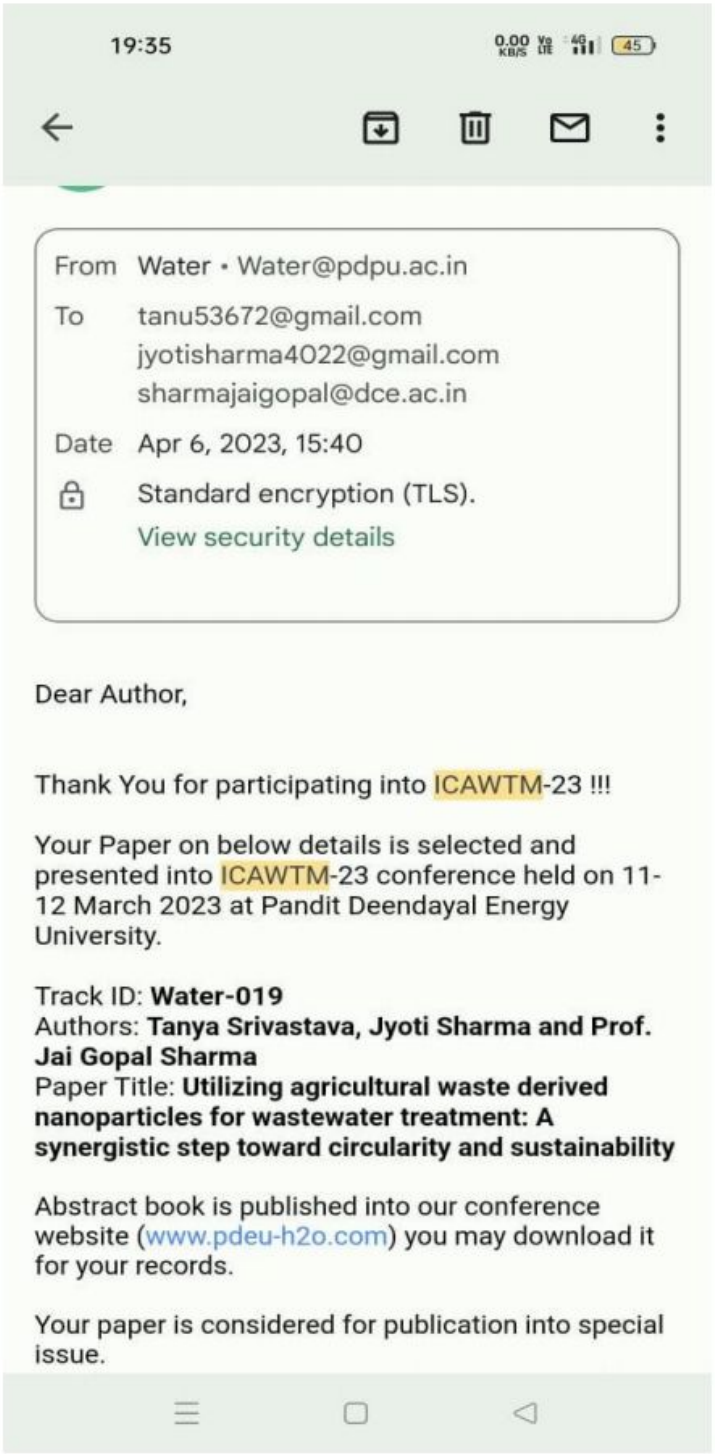
Step-1: You are requested to pay the fee through link: https://www.pdeu-h2o.com/registration-fees

Step-2: Also after payment of fee, fill the google form using the following link to complete the registration process: https://forms.gle/GnYvxm6ihNjP9RaM6

The deadline for registration and payment is 20

50

Mail of Paper Selection



19:35

From Water • Water@pdpu.ac.in

To tanu53672@gmail.com
jyotisharma4022@gmail.com
sharmajaigopal@dce.ac.in

Date Apr 6, 2023, 15:40

Standard encryption (TLS).
View security details

Dear Author,

Thank You for participating into ICAWTM-23 !!!

Your Paper on below details is selected and presented into ICAWTM-23 conference held on 11-12 March 2023 at Pandit Deendayal Energy University.

Track ID: **Water-019**
Authors: **Tanya Srivastava, Jyoti Sharma and Prof. Jai Gopal Sharma**
Paper Title: **Utilizing agricultural waste derived nanoparticles for wastewater treatment: A synergistic step toward circularity and sustainability**

Abstract book is published into our conference website (www.pdeu-h2o.com) you may download it for your records.

Your paper is considered for publication into special issue.

51

Portion of Abstract Book

and 68% Turbidity removal at optimum conditions. Adsorption experiments were done in batch mode.

***Keywords**: Polyaniline; Adsorption; CETP; TOC Removal, Colour Removal*

Utilizing agricultural waste derived nanoparticles for wastewater treatment: A synergistic step toward circularity and sustainability

Tanya Srivastava [a], Jyoti Sharma [a], Jai gopal Sharma [a]

[a] *Delhi Technological University, Shahbad Daulatpur main Bawana road, Delhi-110 042, India*

Abstract: Rapid population growth and urbanization, accompanied by haphazard industrial activities, incessantly contaminate water with landfill wastes, heavy metals and toxic chemicals, leading to a significant deterioration in its quality and availability. The scenario has attracted global scientific community towards the development and establishment of cost-effective, sustainable and energy efficient water treatment strategies. Over the last few years, nanotechnology has convincingly addressed a plethora of environmental issues and subsequently, nanoparticles have been prudently engineered and successfully used in wastewater treatment. With constant advancement, novel methodologies of nanoparticle synthesis are being designed out of which plant-mediated "Green synthesis" route has gained considerable attention as a fast, scalable, non-toxic, and low-cost procedure. The present review aims to highlight the potential of agricultural wastes as useful ingredients for implementation of "Green" route for the fabrication of nanoparticles. It further elucidates the process and mechanism of utilizing waste- derived NPs for efficacious removal of various pollutants, dyes and organic substances from wastewater. Lastly, it concludes by discussing the limitations and challenges associated with the practical applications of the presented approach, along with the ongoing research and prospective solutions to overcome them. On an overall basis, the review intends to pose this approach as a magnificent amalgamation which simultaneously tackles the problem of waste disposal as well as water remediation and can thus emerge as an appealing technique to accomplish circularity and sustainability.

***Keywords**: Agricultural Waste; Nanoparticles; Wastewater Treatment; Green Synthesis*

52

Conference Certificate

Certificate of Presentation

This is to certify that

Tanya Srivastava and Jyoti Sharma

has presented a paper entitled

Utilizing agricultural waste derived nanoparticles for wastewater treatment: A synergistic step toward circularity and sustainability

at 2nd International Conference on "Advances in Water Treatment and Management (ICAWTM-23)" on 11–12 March 2023

organized by Pandit Deendayal Energy University, Gandhinagar, Gujarat, INDIA

Prof. S. Sundar Manoharan, Director General, PDEU, Gujarat, India

Prof. Dhaval Pujara, Director, School of Technology, PDEU, Gujarat, India

Prof. Anurag Mudgal, Convener, ICAWTM-23, PDEU, Gujarat, India

Prof. Philip Davies, Co-Convener, ICAWTM-23, University of Birmingham, United Kingdom

53

PAPER NAME

thesis.pdf

WORD COUNT	CHARACTER COUNT
9747 Words	**54742 Characters**
PAGE COUNT	FILE SIZE
53 Pages	**1.6MB**
SUBMISSION DATE	REPORT DATE
May 29, 2023 12:19 PM GMT+5:30	**May 29, 2023 12:20 PM GMT+5:30**

17% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

- 12% Internet database
- 8% Publications database
- Crossref database
- Crossref Posted Content database
- 12% Submitted Works database

Excluded from Similarity Report

- Bibliographic material
- Small Matches (Less then 8 words)

17% Overall Similarity

Top sources found in the following databases:

- 12% Internet database
- Crossref database
- 12% Submitted Works database
- 8% Publications database
- Crossref Posted Content database

TOP SOURCES

The sources with the highest number of matches within the submission. Overlapping sources will not be displayed.

1	dspace.dtu.ac.in:8080 Internet	4%
2	mdpi.com Internet	1%
3	Sherif Mehanny, Ehab E. Abu-El Magd, Maha Ibrahim, Mahmoud Farag ... Crossref	<1%
4	microbenotes.com Internet	<1%
5	hindawi.com Internet	<1%
6	applbiolchem.springeropen.com Internet	<1%
7	etd.astu.edu.et Internet	<1%
8	open.library.ubc.ca Internet	<1%

Sources overview

9	scielo.br Internet	<1%
10	Nanyang Technological University on 2022-04-04 Submitted works	<1%
11	Queen's University of Belfast on 2017-01-24 Submitted works	<1%
12	University of Sydney on 2020-11-12 Submitted works	<1%
13	researchgate.net Internet	<1%
14	Miguel Angel Lorenzo-Santiago, Rodolfo Rendón-Villalobos. "Isolation ... Crossref	<1%
15	ipl.org Internet	<1%
16	Universiti Tenaga Nasional on 2020-09-06 Submitted works	<1%
17	ir.library.oregonstate.edu Internet	<1%
18	vdoc.pub Internet	<1%
19	Kwame Nkrumah University of Science and Technology on 2021-06-15 Submitted works	<1%
20	Nur-Azzah Afifah Binti Taib, Md Rezaur Rahman, Muhammad Khusairy ... Crossref	<1%

Sources overview

21	Zhila Honarmandrad, Karolina Kucharska, Jacek Gębicki. "Processing ... Crossref	<1%
22	SpringerBriefs in Molecular Science, 2016. Crossref	<1%
23	The Hong Kong Polytechnic University on 2022-04-14 Submitted works	<1%
24	Sun, J.. "Isolation and characterization of cellulose from sugarcane ba... Crossref	<1%
25	Universiti Teknologi Malaysia on 2023-03-10 Submitted works	<1%
26	open.bu.edu Internet	<1%
27	Institute of Technology, Tralee on 2020-04-16 Submitted works	<1%
28	RAGHU PATEL G. RANGANAGOWDA, SAKSHI SHANTHARAM KAMAT... Crossref	<1%
29	Trinity College Dublin on 2016-09-08 Submitted works	<1%
30	Imperial College of Science, Technology and Medicine on 2011-12-26 Submitted works	<1%
31	Kingshuk Dhali, Mehran Ghasemlou, Fugen Daver, Peter Cass, Benu Ad... Crossref	<1%
32	Moufida Beroual, Djalal Trache, Oussama Mehelli, Lokmane Boumaza, ... Crossref	<1%

Sources overview

33	Swinburne University of Technology on 2015-09-03 Submitted works	<1%
34	University of Abertay Dundee on 2009-04-30 Submitted works	<1%
35	University of KwaZulu-Natal on 2019-12-10 Submitted works	<1%
36	api.intechopen.com Internet	<1%
37	coek.info Internet	<1%
38	core.ac.uk Internet	<1%
39	etd.cput.ac.za Internet	<1%
40	repository.ar-raniry.ac.id Internet	<1%
41	tudr.thapar.edu:8080 Internet	<1%
42	"Bioremediation and Sustainable Technologies for Cleaner Environmen... Crossref	<1%
43	Brunel University on 2011-12-16 Submitted works	<1%
44	Farzad Seidi, Mohsen Khodadadi Yazdi, Maryam Jouyandeh, Sajjad Ha... Crossref	<1%

Sources overview

45	Flauzino Neto, Wilson Pires, Hudson Alves SilvÃ©rio, NoÃ©lio Oliveira ... Crossref	<1%
46	Hamid Essabir, Mohammed Ouadi Bensalah, Denis Rodrigue, Rachid B... Crossref	<1%
47	Indian Institute of Technology Guwahati on 2018-09-28 Submitted works	<1%
48	Indian Institute of Technology, Kharagpure on 2014-09-26 Submitted works	<1%
49	Universiti Malaysia Pahang on 2013-12-27 Submitted works	<1%
50	University of Birmingham on 2018-05-01 Submitted works	<1%
51	University of Birmingham on 2023-04-10 Submitted works	<1%
52	University of Stellenbosch, South Africa on 2010-07-13 Submitted works	<1%
53	liu.diva-portal.org Internet	<1%
54	noesis.uis.edu.co Internet	<1%
55	tandfonline.com Internet	<1%
56	"Cellulose-Based Superabsorbent Hydrogels", Springer Science and Bu... Crossref	<1%

Sources overview

57	C.D. Midhun Dominic, Vandita Raj, K.V. Neenu, P.M. Sabura Begum et a... Crossref	<1%
58	Clarkson University on 2021-04-26 Submitted works	<1%
59	Federation University on 2016-04-14 Submitted works	<1%
60	Junichi Goto, Fazel Shamsa, Toshio Nambara. "Studies on Steroids. CL... Crossref	<1%
61	University of the Aegean on 2023-04-07 Submitted works	<1%
62	cora.ucc.ie Internet	<1%
63	hal.univ-lorraine.fr Internet	<1%
64	link.springer.com Internet	<1%
65	pure.sruc.ac.uk Internet	<1%
66	"Sustainable Biotechnology- Enzymatic Resources of Renewable Energ... Crossref	<1%
67	Aalto Yliopisto on 2022-05-31 Submitted works	<1%
68	Baby Salini Jacob Rani, Sivakumar Venkatachalam. "Cleaner approach ... Crossref	<1%

Sources overview

69	Caledonian College of Engineering on 2020-08-17 Submitted works	<1%
70	Heriot-Watt University on 2022-04-25 Submitted works	<1%
71	King Fahd University for Petroleum and Minerals on 2022-01-28 Submitted works	<1%
72	Monash University on 2019-06-07 Submitted works	<1%
73	PSG Institute of Management Coimbatore on 2021-04-09 Submitted works	<1%
74	Taylor's Education Group on 2018-11-15 Submitted works	<1%
75	The Robert Gordon University on 2022-10-06 Submitted works	<1%
76	Universiti Putra Malaysia on 2012-11-29 Submitted works	<1%
77	Universiti Teknologi Petronas on 2020-11-19 Submitted works	<1%
78	University of Bath on 2018-05-23 Submitted works	<1%
79	University of Newcastle upon Tyne on 2023-05-24 Submitted works	<1%
80	University of Sheffield on 2020-05-13 Submitted works	<1%

Sources overview

81	kalaharijournals.com Internet	<1%
82	research.nottingham.edu.cn Internet	<1%
83	spel3.upm.edu.my Internet	<1%