PVA BIOCOMPOSITES WITH REINFORCED CELLULOSE MICROFIBERS FROM AGRICULTURAL RESIDUE

Thesis

Submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

by

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October, 2018

D E C L A R A T I O N

I hereby *declare* that the Research thesis entitled **“PVA biocomposites with reinforced cellulose microfibers from agricultural residue”** which is being submitted to the National Institute of Technology Karnataka, Surathkal in partial fulfillment of the requirements for the award of the Degree of **Doctor of Philosophy** in Chemical Engineering is a *bonafide report of the research work carried out by me*. The material contained in this Research Thesis has not been submitted to any University or Institution for the award of any degree.

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C E R T I F I C A T E

This is to *certify* that the Research Thesis entitled **"PVA biocomposites with reinforced cellulose microfibers from agricultural residue"** submitted by **Ms**. **Manjula P.** (Register Number: 112014CH11F05) as the record of the research work carried out by her, *is accepted as the Research Thesis submission* in partial fulfillment of the requirements for the award of degree of **Doctor of Philosophy**.

Research Guides

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Dedicated to my teachers

ACKNOWLEDGEMENT

Pursuing the path of this doctoral study has been a very insightful phase in the journey of my life. This wonderful experience would not have been completed without the contribution and support of several people, whom I shall remain indebted to.

It is with great honour and respect I thank my guide **Dr. G. Srinikethan.,** for guiding me in the right direction throughout the research work. His calm and composed aura, provided constructive ideas and support in shaping the research work throughout the years of research. No words will be adequate to quantify the support, motivation and cooperation received from my research co-guide, **Dr. Vidya Shetty K**. She stood as a role model throughout my life in NITK and will be forever. I am grateful for her incomparable patience, unconditional love, support, encouragement and extensive discussion in helping me remain focused and strive for excellence during the years of my doctoral research endeavour and her valuable inputs in shaping of the thesis. I thank her for imparting strength and invaluable knowledge to enable my transformation in the both professional and personal life front.

I profoundly thank the RPAC members **Dr. M B Saidutta**, Professor, Department of Chemical Engineering and **Dr. Kasturi V. Bangera**, Professor, Department of Physics, NITK, Surathkal for their invaluable advice and constructive criticisms that helped in the refinement of the research work. I also take this opportunity to thank the faculty and staff of the Department of Chemical Engineering for their kind support and encouragements.

I extend my humble gratitude to the Director, NITK, the former and present academic Deans, **Dr. Suman David**, **Dr. Katta Venkataramana** and **Dr. M B Saidutta**, the Head of the Department **Dr. Hari Mahalingam**., Associate Professor and Head, and former Heads of the Department **Dr. Raj Mohan B**, **Dr. Vidya Shetty K** and **Dr. M B Saidutta**, Department of Chemical Engineering, NITK, Surathkal for providing necessary facilities, funding and infrastructure for carrying out the research work.

I would like to thank **Dr. K P R Prasanna**, Professor, Seed Technology, GKVK, University of Agricultural Science, Bengaluru, for providing Jatropha seed shell and Pongamia seed hull for the research work. I'm thankful to **Late. Ms. Eeramma**, from

Pavagada, Tumkur for providing Finger millets straws from their fields for the research work.

I am also obliged to **Mr. Sadashiva,** for his support in handling laboratory equipments and chemicals, **Ms. Thrithila Shetty**, for her timely help and moral support throughout my research work, **Ms. Shashikala**, for maintaining all the official documentation required **Mr. Suresh,** for his valuable support and all technical and non-technical staff of the Department of Chemical Engineering for their whole hearted help during the course of my work.

I would like to thank **Ms. Rashmi,** for her unmatched support and patience in analysing SEM samples during the course of research. I would like to also thank my research colleague **Mr. Palaksha**, from Department of Materials and Metallurgy Engineering for his support in mechanical characterization of samples.

I thank all my friends from NITK **Ms. Swapna Mansani**, **Dr. Aparna, Dr. Ankita Khanna, Dr. Aishwarya Devadiga**, **Ms. Deekshitha, Ms. Amruth Shet, Ms. Shankramma** for their support, love and affection.

Words fall short to thank my friend **Mr. Shankar M**, for his motivating and uplifting words that helped me during the tough and challenging times faced in this journey. I owe my gratitude towards my supportive family for understanding and supporting me.

As they say, save the best for the last; I feel extremely grateful and thank to my husband **Mr. Sunil Honnurappa,** for his unconditional love, encouragement, patience and for being there in all the stages of my life. His continual support in all my academic endeavours has lead me through the past years and enabled me to complete this research work.

Above all, I owe it all to Almighty God for granting me the wisdom, health and strength to accomplish this research work.

Manjula Puttaswamy

ABSTRACT

Biocomposites are finding application in several fields such as medical, automobiles and packaging industries. The cellulose fiber isolated from natural plant sources have proven to be potential reinforcements in the manufacturing of biocomposites. In the present study, cellulose microfibers are isolated from underutilized and abundantly available biofuel industrial residues: Jatropha seed shell, Pongamia seed hull and Finger millet straw, an agricultural residue. The organosolv (Method O) treatment and combined alkaline and organosolv treatments (Method IO) were carried out to isolate cellulose fibers. The cellulose fibers thus isolated by methods O and IO were further subjected to ultrasonication or enzymatic treatment. The removal of matrix components such as lignin and hemicellulose along with the isolation and defibrillation of cellulose microfibers was confirmed by analysis of chemical, thermal and morphological characteristics of the untreated and isolated fibers. The combined alkaline, organosolv and ultrasonication treatment (IOU) was found to be most effective in isolating cellulose microfibers from Jatropha seed shell, Pongamia seed hull and Finger millet straw yielding cellulose micro fibers with higher cellulose content (90%, 85% and 93%) and smallest fiber size (194, 145 and 147nm) compared to other treatments. Ultrasonication has been found to play a major role in defibrillation of the microfibers. Poly vinyl alcohol (PVA) based biocomposites with cellulose microfibers as reinforcement were prepared by solution casting. Fiber reinforcement has resulted in biocomposites with increased tensile strength and tensile modulus. The transmittance of the biocomposites film was found to be reduced as compared to that of neat PVA, which proves that the films provide protection against UV light and sunlight induced photo degradation. The cellulose fiber reinforced PVA biocomposites were found to be biodegradable in garden soil and Municipal waste dump yard soil with complete degradation being achieved in 2 weeks. Further, the biocomposites exhibited low oxygen transfer rates. Good tensile and thermal properties along with lower affinity for oxygen transfer makes these biocomposites as ideal choice in the field of food packaging. These biodegradable composites prepared from the cellulose fibers isolated from industry and agricultural residues can serve as economical

and eco-friendly replacements for the conventional composites.

Key words: cellulose microfibers; PVA; ultrasonication; biocomposites; food packaging.

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LIST OF ABBREVIATIONS

AR	Analytical Reagent
ASTM	American Society for Testing and Materials
CI	Crystallinity Index
DLS	Dynamic Light Scattering
DSC	Differential Scanning Calorimetry
E	Enzymatic treatment
ET	Enzymatic Treatment
FTIR	Fourier Transform Infrared
H	Hemicellulose
IO	Inorganic Chemical treatment with Organosolv treatment
IOE	Inorganic Chemical treatment with Organosolv treatment and Enzymatic treatment
IOU	Inorganic Chemical treatment with Organosolv treatment and Ultrasonication treatment
L	Lignin
MFC	MicroFibrillated Cellulose
MWDY	Municipal Waste Dump Yard
NMR	Nuclear Magnetic Resonance
O	Organosolv treatment
OE	Organosolv treatment with Enzymatic treatment
OTR	Oxygen Transfer Rate
OU	Organosolv treatment with Ultrasonication
PE	PolyEthylene
PHAs	PolyHydroxyAlkanoate
PHB	PolyHydroxybutyrate-Valerate
PLA	Poly Lactic Acid
PP	PolyPropylene
PS	Polystyrene
PVA	Poly Vinyl Alcohol
PVC	PolyVinyl chloride
SEM	Scanning Electron Microscopy

T	Temperature
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl
TGA	Thermogravimetric Analyser
TSS	Total Safety Solution
U	Ultrasonication treatment
UV/ VIS	Ultraviolet-Visible
XRD	X-ray Diffraction
^{13}C-NMR	Carbon-13 (C13) Nuclear Magnetic Resonance
α-C	Alpha Cellulose

NOMENCLATURE

%	percentage
μm	micrometer
δ	Solubility parameter
^{0}C	Degree Celsius
cm^{-1}	per centimeter
D	diameter of cellulose microfiber
E	enzyme concentration
FR	Untreated fibers of Finger millet straw
g	gram
GPa	Giga pascal
h	hour
I	intensity
JR	Untreated fibers of Jatropha seed shell
kg	kilogram
kJ/mol	kilojoule per mole
kW	Kilowatt
min	minutes
MPa	Mega pascal
nm	nanometer
PR	Untreated fibers of Pongamia seed hull
rpm	revolutions per minute
t	acid treatment time
t_u	ultrasonication time
v/v	volume/volume percent
w/w	weight/weight percent
wt %	weight percentage
w/v	weight/volume percent

CHAPTER 1

INTRODUCTION

Fiber-reinforced plastic composites began with cellulose fiber in phenolics in 1908, later extending to urea and melamine, and reaching commodity status in the 1940s with glass fiber in unsaturated polyesters. From guitars, tennis racquets and cars to microlight aircrafts, electronic components and artificial joints, composites are finding use in diverse fields. Because of increasing environmental consciousness and demands of legislative authorities, the manufacture, use and disposal of traditional composite structures, usually made of glass, carbon or aramid fibers being reinforced with epoxy, unsaturated polyester resins, polyurethanes, or phenolics, are considered critically. Carbon fibers have remarkable properties with a tensile strength of 3.2 GPa and a tensile modulus of 230 GPa (Liu and Satish kumar, 2012). The disadvantages of carbon fibers are their high cost and brittle nature. Aramid fibers (e.g., Kevlar 49 by DuPont) possess good properties but are also expensive. Glass fibers are the most widely used fiber for general reinforcement of polymers. The most important disadvantage of such composite materials is the problem of convenient removal after the end of life time, as the components are closely interconnected, relatively stable and therefore difficult to separate and recycle (Menges et al. 1992). These composites are difficult to recycle as the separation of the components are is tedious process (Henshawet al. 1996; Pickering, 2006; Conroy et al. 2006). Therefore, these composites are often disposed in unsatisfactory ways such as landfills or incineration which causes a vast environmental impact (Ramamoorthy et al. 2015).

In the modern polymer technology, it is a great demand that every material should especially be adapted to the environment. To successfully meet the environmental and recycling problems, a renewed interest has been created in natural fibrils which could be used as reinforcements or fillers in the composites and are thus referred to as “ecocomposites” or “biocomposites” (Mohanty et al. 2000). Biofibers are the natural fibers derived from plant, animal or bacteria and often serve as promising reinforcements or fillers for composites.

Advantages of biofibers over traditional reinforcing materials such as glass fibers, talc and mica are (Karnani et al. 1997): low cost, low density, no abrasiveness, combustibility, nontoxic, high toughness, high surface area-to-volume ratio, high

Young's modulus, high tensile strength, low coefficient of thermal expansion, acceptable specific strength properties, reduced tool wear, chemical stability, reduced dermal and respiratory irritation, good thermal properties, ease of separation, enhanced energy recovery and biodegradability (Samir et al. 2005; Beecher, 2007; Eichhorn et al. 2010; Habibi et al. 2010; Siro and Plackett, 2010).

The main drawback of biofibers is their hydrophilic nature which lowers the compatibility with hydrophobic polymeric matrix during composite fabrications. The other disadvantage is the relatively low processing temperature required due to possibility of fiber degradation and/or the possibility of volatile emissions that could affect composite properties. The processing temperatures for most of the biofibers are thus limited to about 200^{0}C, although it is possible to use higher temperatures for short periods (Sanadi et al. 1996).

Among these natural fibrils, cellulose nanofibers with complete biological degradability and renewability are extensively researched. Cellulose fibers have shown great potential in several applications, including biomedical (Czaja et al. 2006), bioimaging (Dong et al. 2007), nanocomposites (Juntaro et al. 2008; Liu et al. 2010; Siqueira et al. 2008), gas barrier films (Fukuzumi et al. 2009), and optically transparent functional materials (Nogi et al. 2009; Nogi and Yano, 2008; Yano et al. 2005).

Although cellulose fibers can be extracted from algae, tunicates (Iwamoto et al. 2009; Berg et al. 2007), and bacteria (Hirai et al. 2009; Roman and Winter, 2004), the main source of cellulose fibers is found in natural plant cell walls (Klemm et al. 2005). Considering that the plant fibers are derived from renewable, abundant sources of low cost, and can be extracted into fibers thinner than the fibers from bacterial cellulose and tunicates (Saito et al. 2006). Many researchers have extensively studied the extraction of nanofibers from natural plant fibers. Cellulose is nature's most lavishly available polymer. Highly-purified cellulose fibers have been isolated from several plant sources, such as wood (Abe et al. 2007), bamboo (Abe and Yano, 2010), cotton (de Morais Teixeira et al. 2010), soy hulls (Alemdar et al. 2008), hemp (Wang et al. 2007), sisal

(Mora´n et al. 2008; Ramires et al 2010), branch-barks of mulberry (Li et al. 2009), pineapple leaf fibres (Cherian et al. 2010; Mangal et al. 2003), pea hull fibre (Chen et al. 2009), coconut husk fibers (Rosa et al. 2010), banana rachis (Zuluaga et al. 2009; Et Meligy et al. 2010) and sugar beet (Dinand et al. 1999; Dufresne et al. 1997), wheat straw (Daniel et al. 2010), hemp (Pickering et al. 2007; Santulli et al. 2009; Hepworth et al. 2000), jute (Ray et al. 2001; Sarkar et al. 2004; Wang et al. 2010) ,Branch-barks of mulberry (Li et al. 2009), Pea hull fiber (Chen et al. 2009), Palm leaf sheath (Maheshwari et al. 2012), *Arundo donax* L stem (Fiore et al. 2014), Cotton stalk (Hou et al. 2014), Rice husk (Das et al. 2016), Astragalus gummifer (fabaceae) trunk (Kaya et al. 2016), Rice straw (Boonterm et al. 2016), Jerusalem artichoke stem (Li et al. 2016), Arecanut husk fiber (Chandra et al. 2016), Eucalyptus sawdust (Vallejos et al. 2016), Cotton stalk bark (Miao et al. 2016), and Grape fruit peel (Karatas et al. 2016).

In view of better utilization of renewable resources, there is a need to explore other renewable sources, which can be utilized in developing high strength light weight bio-composites for high-end applications.

In India and SouthEast Asia, *Pongamia pinnata* (Pongamia/Honge/Karanja) seed and *Jatropha curcas* (Jatropha) seed are used as significant fuel sources (Demirbas et al. 2009). Biofuel production using these seeds has resulted in large scale cultivation of these trees (Shwetha et al. 2014). Oil is the most important product of these trees in some parts of the world. 2 kg of mature pods of Pongamia tree yield about 1 kg of husked kernels and seed shell. The pods containing seed husk are discarded as waste. Carbonaceous porous solids (active carbons, chars and composites) are produced from waste seed husks and pods. The biofuel processing fallouts results in significant amount of residual Pongamia seed hull. Pongamia seed shell has been explored for the preparation of activated carbon (Warhurst et al. 1997, Martins, 2007, Jambulingam et al. 2007).

When producing bio-oil from the Jatropha seeds, tons of seeds are needed and in turn tons of shells become available. The shell is mechanically removed from the fruit as the first step during oil extraction. Raw Jatropha seed shell is toxic (Palanivel et al. 2012) and demands appropriate treatment in order to be not harmful to human and the environment.

About one tonne of shell material can be obtained from one hectare. The shells have been used as an energy source (Singh et al 2008), for the production of hydrogen-rich gas and liquid oil by pyrolysis and have been demonstrated for its possible use in the production of high quality charcoal (Kratzeisen et al. 2009) and porous activated carbon (Kratzeisen et al. 2009; Raphael et al. 2010).

Cellulose percentage in Pongamia seed hull is approximately 40 % and is similar as in shelly wood (Subbarao et al. 2010, Nadeem et al. 2009). The chemical analysis of Jatropha seed shell has shown that it is made up of 34 % cellulose, 10 % hemicellulose and 12 % lignin, respectively (Singh et al. 2008, Abreu, 2009). The cellulose content in Pongamia seed hull and Jatropha seed shell are in line with that of many other agricultural residues (coir 32-45 %, wheat straw 38-45 %, soft wood 40-44 %, hard wood-43-47 %, rye straw-37.9 %, oat straw-38.5 %, barley straw-34.8 % (Diego et al. 2012)) which have been used for cellulose production, and hence they can be used as potential sources for cellulose fibers isolation and for possible production of biocomposites.

Eleusine coracana L. (Finger millet) is the third most important millet in India (locally called as *Ragi*), next to sorghum and pearl millet. In Karnataka, it is grown in an area of 0.8 million hectares with an annual production of 1.34 million tonnes. Its grain tastes good and is nutritionally rich (compared to cassava, plantain, polished rice and maize meal) as it contains high levels of calcium, iron and manganese. The millet straw is also an important livestock feed, building material and fuel (Apoorva et al. 2010). Like other cereal straws, finger millet straw is highly fibrous but is poorly digested by the ruminants mainly due to presence of lignocellulose bond which is much resistant to enzymatic digestion. Several researchers have tried to improve nutritive value of poor quality roughages by urea ammonisation (Sundasto et al. 1978; Dasilva et al. 1986; Subbarao et al. 1989). Cellulose fibers of wheat straw, rice straw, barley straw, oat straw and rey straw have been used for composite preparation by several researchers (Sun et al. 1998; Kaushik et al. 2010; Almender et al. 2008a, b; Liu et al. 2006; Xio et al. 2001; Lam et al. 2001; Fang et al. 2000 etc). Similarly, finger millet straw can also be used to extract

cellulose fibers for composite application, since they come under the category of cereal straw (Sun et al. 2010).

With the view to effectively utilize the large quantities of biofuel industrial residues and the agricultural wastes with considerable amount of cellulose content, in the present study Jatropha seed shell, Pongamia seed hull and Finger millet straw have been used as the source for the isolation of cellulose for the production of biocomposites.

Isolation of cellulose fibers are customarily carried out by mechanical treatments such as homogenisation (Du et al. 2016; George et al. 2016), sonication, (Sheltami et al. 2012; Saurab et al. 2016), steam explosion (Saelee et al. 2014) etc; chemical treatments such as acid hydrolysis (Abidin et al. 2001), TEMPO oxidation (Du et al. 2016), chlorination and alkaline treatments (Sheltami et al. 2012; Johar et al. 2012; Maheshwari et al. 2012) etc; enzymatic treatments (Saelee et al. 2014) and conjointly with combination of two or more of the aforementioned processes. Chemical treatments usually act upon the binding material of the fibril structure enabling the fibers to individualize (Johar et al. 2012). Chlorination treatment being a chemical treatment is a well-established treatment which assists isolation of high quality pure cellulose fibers by bleaching and delignifies the cellulose material; while, alkali treatment dissolves the wax, pectin and hemicellulose ensuring efficient isolation of cellulose microfibers. Organosolv treatment is also one such chemical treatment method in which cellulose are extracted using organic solvents such as acetic acid in presence of nitric acid as a catalyst. These chemical methods are used in combination to isolate cellulose fibers from different sources (Espino et al. 2014; Johar et al. 2012; Sheltami et al. 2012; Chakrabarty et al. 2011; and Moran et al. 2008) and are also found to be efficient and economical when compared to high energy consuming mechanical methods (Motaung et al. 2015). The mechanical treatments, include cryocrushing (Chakraborty et al. 2005), grinding (Abe et al. 2007; Iwamoto et al. 2007; Iwamoto et al. 2008), high-pressure homogenization (Herrick et al. 1983; Nakagaito and Yano, 2004, 2005; Turbak et al. 1983), ultrasonication (Cheng et al. 2007; Tischer et al. 2010), and electrospinning methods (Frenot et al. 2007; Peresin et al. 2010), as well as combination of two or more of the treatments. All these methods lead to

different types of fibrillar materials, depending on the cellulose raw material and its pretreatment, and the disintegration process itself (Chen et al. 2011). In the present work, the well-known chemical methods such as organosolv treatment, Inorganic method involving bleaching and alkaline treatment are combined with ultrasonication treatment and enzymatic treatment to isolate high concentration of cellulose microfibers from Jatropha seed shell, Pongamia seed hull and Finger millet straw and to defibrillate the isolated fibers. Various process parameters are involved in the isolation process and these process parameters can influence the cellulose concentration and size of the isolated fibers. The present work is focused on optimizing various process parameters involved in the isolation of cellulose fibers from Jatropha seed shell, Pongamia seed hull and Finger millet straw in order to maximize the cellulose content and for efficient defibrillation to reduce the size of the fibrils. The best method involving the combination of the above said methods has been chosen for the isolation of cellulose fibers from each of these sources based on the maximum cellulose content and the size of the fibers.

From past two decades, the cellulose fibers are being used as filler material in composites preparation and have gained prodigious attention (Hubbe et al. 2008). Cellulose due to its high crystalline nature when used as filler in the production of biocomposites result in improved mechanical properties (Xiao et al. 2016). Thus, in the present study, the cellulose fibers isolated by the chosen method has been used as a filler material in the preparation of biocomposites in a polymeric matrix by solution casting method. Further these biocomposites have been assessed for their suitability for food packaging application in terms of their properties.

CHAPTER 2

LITERATURE REVIEW

2.1 Background

Plastic, the synthetic polymer revolutionized industrial sector in 1940s (Saleem et al. 2009). Plastic is inevitable in modern society as they are highly flexible, non-corrosive and economical in production. These factors have made them an obvious choice in many applications. High demand for the synthetic polymer has resulted in larger production and in turn led to alarming situation. The plastic wastes are non-degradable and difficult to recycle as the process itself is tedious. Presence of these synthetic polymers in nature for innumerable years has affected environment to very large extent (Rosa et al. 2002; César et al. 2009; Takasu et al. 2002). To be specific, Reddy et al, (2003) have reported the estimated plastic waste make up to 100,000 tons per year in the ocean and 14 million tons of municipal solid waste per year. Major problem in recycling the plastic is screening of different grades of plastic as this could lead to difficulties in reforming the plastic due to ununiformed composition (Saleem et al. 2018).

Generally, composites are made of one or more components one of them being continuous phase which is termed as matrix and other being discontinuous phase termed as reinforcement. Reinforcing component contribute to the stiffness and hardness of the composites as they are usually stronger, harder and stiffer than the matrix component. The composite formed possess the properties of both its components ultimately resulting in superior properties over the individual materials. The stiffness and high strength combined with low density of the composite allows for weight reduction of final assembled paraphernalia. The materials such as carbon fiber, glass, Kevlar, polyethylene and aramid reinforced in thermoset matrix polyimide or epoxy resin revolutionised the composite industry (Kalia et al. 2011). These reinforcements specifically glass fiber has been well established in many fields such as aerospace, automobiles and thermal insulations which has made the plastic waste to raise exponentially. The increase of non-biodegradable composite wastes and the arising environmental issues has led to surge of inclination towards bio reinforcement or biofiller materials from renewable resources, which are influenced by several factors, including the growing ecological, social and economic awareness and for producing materials by sustainable methods associated with

lighter-weight structures of lower environmental impact. In order to tackle the environmental issues, depletion of fossil fuels and also to replace the traditional plastic, biodegradable polymer composites consisting of components derived from renewable resources have been developed recently (Vroman and Tighzert, 2009).

One of the versatile natural polymer is “Cellulose” and it is a linear semi-crystalline polysaccharide synthesized by living species for all in the vegetable kingdom, but also by other species such as bacteria and the sea-animal tunicate. In nature, the load bearing component in plant cell walls is cellulose (Höfte et al. 2012). This polymer available in abundance has been proved to be a potential source as reinforcement in most of the composite materials. Since cellulose features most of the properties of the synthetic reinforcement they are the indispensable replacement for the synthetic counterparts. The composites consisting of any of one of their components derived from natural substances are termed as Biocomposites. These biocomposites are reported to be biodegradable polymers which can decompose by composting (Netravali, 2005).

Thus, this century has witnessed a pursuit for environment compatible, sustainable, renewable, biodegradable, economical, green materials which can serve as replacements for depleting, non-renewable petroleum resources and leading to biocomposites era (Gurunathan et al. 2015), a recent development in the field of composites.

Cellulose fibers are abundantly accessible natural polymer which are framework of the cell wall in plant material which account to annual production of approximately 1.5×10^{12} tonnes (Klemm et al. 2005; Cao et al. 2009). Cellulose fibers are naturally designed to be reinforcement factor in plant cell wall, this makes them an apparent choice for reinforcement in modern composites too. Cellulose was first noted as such in 1838 (Dufresne et al. 2000).

Plant structure is morphologically complex with lignin, hemicellulose, cellulose and pectin being intimately associated with each other as presented in Figure 2.1. Cellulose is the building material of long fibrous cells made up of long glycan chains with repeating

(1-4)-β-glucopyranose units, lignin is made up of phenyl propane, hemicelluloses consists of arabinose, galactose, rhamonose, mannose, glucose and xylose sugars which link cellulosic and non- cellulosic polymers and finally pectins are highly hydrophilic polysaccharides which are amorphous in nature (Mustata et al. 1997).

Typically plant fibers cell consists of primary and secondary (S1, S2 and S3 layer) cell walls. The composition of primary cell wall is mainly 90 % of carbohydrates (mostly pentose and hexose units) which are comprised in 9-25% of cellulose microfibrils, 25-50% matrix of hemicellulose and 10-35% pectin. The cellulose fibers present in primary cell wall are composed of randomly arranged sugar units and the molecules are further bound with each other (Bhatnagar, 2005).

Primary cell wall composition has cellulose fibers bound together by molecules made of sugar units and are random. Secondary wall is richer in cellulose (40-80 %) content than the primary wall (Brett et al 1996) and contains 10-40 % hemicellulose and 5-25 % lignin. Cellulose has a complex architecture distributed on many levels. Cellulose fibers are prominent in secondary wall consisting 13,000-16,000 glucose units which is comparatively higher than that of cellulose (6000 glucose units) present in primary cell wall (Liu, 2010).

Cellulose is available in amorphous and crystalline form. In amorphous cellulose, hydroxyl groups are linked by hydrogen bonding at positions C-3 and C-2, whereas in case of crystalline cellulose hydrogen bonding is linked at the C-6 position (Moritz et al. 2009). Amorphous chains are not straight and do not have preferred orientation as in that of crystalline form. Crystalline cellulose is present as I, II, III, VI, V and VI polymorphs but in nature they exist in native cellulose I form (O'Sullivan, 1997; Dufresne, 2012).

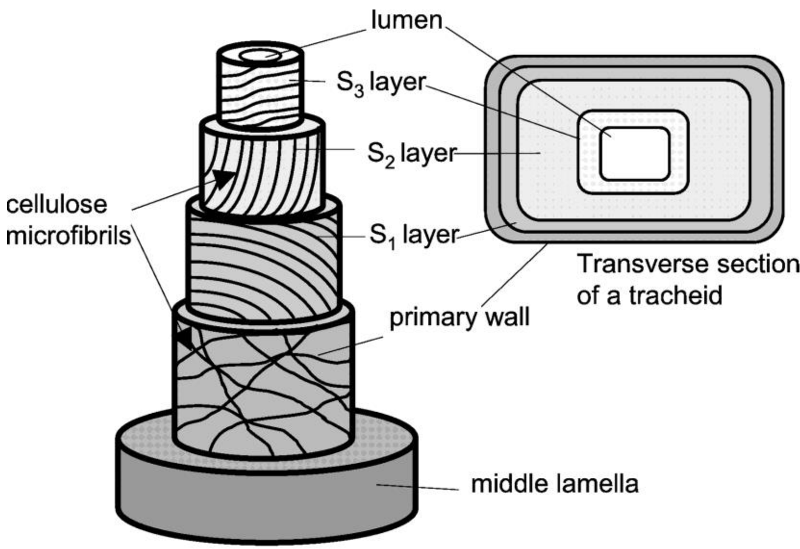


Figure 2.1. Composition of plant cell wall (adopted from Plomion et al. 2001)

Numerous plants such as jute, kenaf, sisal, and hemp were primarily subjected to cellulose isolation however in few plants, by-products such are coir, oil palm and pineapple are considered as cellulose source (Faruk et al. 2012). Similarly, cellulose fibers were isolated from wood basically, but past few decades has seen extensive exploitation of non-wood plant materials which include stem, trunk, straw, bast, seed, fruit and leaf (Khalil et al. 2010; Guedes et al. 2016). The chemical composition of these lignocellulosic components varies from lower to higher plant structure, growth environment, plant species, nature of growth and maturity of the components (Jawaid et al. 2017). Some of the major natural fibers exploited for cellulose fibers are presented in Table 2.1.

The cellulose fibers are hydrophobic in nature due to strong hydrogen bond network between the molecules. Thus, the cellulose fibers are considered stable polymer as they do not dissolve in common solvents (Gray et al. 1996; Khazraji and Robert, 2013). In nature, the cellulose fibers impart the flexibility and elasticity in the plants (Dalena et al. 2017) and are more flexible and elastic compared to that of carbon and glass fibers (Yeasmin, 2012).

The cellulose microfibers and nanofibers possess high stiffness as they majorly contain crystalline cellulose which makes them an excellent choice for reinforcement in composites (Merkel et al. 2014). Thus, cellulose fiber reinforced composite materials have found to have potential application in many fields such as electrical and electronical, paper, medical, construction, cosmetic and packaging, textile industries (Hubbe et al. 2008, Frone et al. 2011).

Noticeable factor of cellulose is that cellulose fibers isolated from any source is reported to be a potential source of reinforcement in composites. This has led to explore abundant, natural, locally and effortlessly available resources and also effective utilization of many underutilized plant materials which are usually byproducts of processing industries. The biofuel industrial residues Jatropha seed shell and Pongamia seed hull and agricultural residue Finger millet straw is in line with the requirements for potential source for cellulose fibers. These residues are underutilized and are usually found in dumping site or landfills.

The lignocellulosic composition of their residues is in line with many of the sources exploited for cellulose fibers. Thus, these industrial and agricultural residues can find a potential application in the field of biocomposites as they could contribute as potential source for isolation of cellulose fibers.

Table 2.1: Chemical composition of natural fibers exploited for cellulose fibers (Faruk et al. 2012; Uddin et al. 2013; Mohammed et al. 2015; Kalia, 2016; Dufresne, 2017; Ansari et al. 2017)

Fibre	Cellulose (wt %)	Hemicellulose (wt %)	Lignin (wt %)	Waxes (wt %)
Bagasse	55.2	16.8	25.3	–
Bamboo	26–43	30	21–31	–
Flax	71	18.6–20.6	2.2	1.5
Kenaf	72	20.3	9	–
Jute	61–71	14–20	12–13	0.5
Hemp	68	15	10	0.8
Ramie	68.6–76.2	13–16	0.6–0.7	0.3
Abaca	56–63	20–25	7–9	3
Sisal	65	12	9.9	2
Coir	32–43	0.15–0.25	40–45	–
Oil palm	65	–	29	–

Table 2.1 continued

Fibre	Cellulose (wt %)	Hemicellulose (wt %)	Lignin (wt %)	Waxes (wt %)
Pineapple	81	–	12.7	–
Curaua	73.6	9.9	7.5	–
Wheat straw	38–45	15–31	12–20	–
Rice husk	35–45	19–25	20	14–17
Rice straw	41–57	33	8–19	8–38
Cotton	82.7	5.7	–	0.6
Soft wood	40-44	25-29	25-31	–
Hard wood	43-47	25-35	16-24	–

2.2 Isolation of Cellulose microfibers

It is well known that in lignocellulosic materials, cellulose is embedded in a gel matrix composing of hemicellulose, lignin, and other carbohydrate polymers. The complexity of cell wall structure in plants has been a challenge in isolating cellulose fibers with high purity from the lignocellulosic matrix. The cell wall is invaded layer by layer, wherein the waxes are first dissolved from the matrix followed by delignification which dissolves the matrix or to the maximum loosen the matrix such that the holocellulose (cellulose and hemicellulose bond together) is exposed for chemical, mechanical and enzymatic effects. Later the hemicellulose is hydrolysed and dissolved in order to end up with cellulose fibers. This basic strategy of breaking lignocellulosic

components for the removal of matrix components lignin, hemicellulose, and non-cellulosic components have steered to several treatments which are in general categorised in terms of chemical, mechanical and biological treatments. Thus, the isolation of cellulose fibers involves three steps (Tibolla et al. 2014)

i) Pretreatments to remove waxes, pectin and other non-cellulosic components
ii) Degradation of lignin and hydrolysis of hemicellulose.
iii) Mechanical disintegration of isolated cellulose fibers

Acidified sodium chlorite bleaching is a well-established and effective delignifying process which is usually the initial step in isolation of cellulose fibers from woody materials (Loader et al. 1997). Alkali extraction to dissolve hemicellulose before or after delignification is the common method (Sun et al. 2004). The treatment of the lignocellulosic materials with chlorite can remove almost all of the lignin, followed by isolation of cellulose with alkali extraction which can be performed at room temperature. These have been applied to isolate cellulose from woody materials for analysis for more than a century.

Fibers with high amounts of lignin are coarse, stiff, and have a brownish colour which affect the structure and properties of the cellulose fibers. Therefore, it is challenging to obtain fibers that are relatively free of bound lignin. However, many improved and simplified techniques have been proposed for isolating cellulose with high purity. Several mechanical treatments are also incorporated in isolation of cellulose fibers. A major obstacle is the high energy consumption connected to the mechanical disintegration of the fibers into cellulose micro/nano fibers, which values around 20,000–30,000 kW/tonne. Even higher values reaching 70,000 kW/tonne have also been reported (Eriksen et al. 2008).

By combining the mechanical treatment with certain pre-treatments (e.g., chemical or enzymatic treatments it is possible to decrease the energy consumption significantly to the level of 1,000 kW/ tonne (Ankerfors et al. 2007). The combination of the chemical and the mechanical treatments is necessary for the dissolution of lignin, hemicellulose, and other non-cellulosic substances.

Before mechanical processing, a number of researchers have applied alkaline treatment of fibers in order to disrupt the lignin structure and to separate the structural linkages between lignin and carbohydrates (Dufresne et al. 1997; Wang et al. 2007, a, b, c). Saito, (2006) introduced an oxidation pretreatment of cellulose, applying 2, 2, 6, 6-tetramethylpiperidine- 1-oxyl (TEMPO) radicals before mechanical treatment in a Waring-blender.

Enzymatic pre-treatments have also shown potentiality in isolation of cellulose fibrils with significantly reduced energy consumption (Pääkkö et al. 2007). Enzymatic treatment with endo glucanase has been carried out before mechanical treatments. Such enzymatic hydrolysis is less aggressive than acid hydrolysis, and it allows for selective hydrolysis of the non-crystalline cellulose, which facilitates the mechanical disintegration. Organic acid treatments such as formic acid (Baeza et al. 1991) and acetic acid (Vazquez et al. 2000) pretreatment have proven to be effective in removal of lignin.

The different methods which are incorporated for isolation of cellulose are discussed in detail in the following sections.

2.2.1 Chemical treatment

Alkali extraction

Delignification and extraction using alkali is considered as the most efficient method for separating cellulose from matrix components viz. lignin and hemicellulose. In particular, most of the lignin can be removed in a delignification step using chlorite. During alkali treatment, hydrolysable glycosidic bonds of carbohydrates and α- and β-aryl ethers linkages of lignin are cleaved which further leads to dissolution of lower alkali stability lignin and carbohydrates (Lia, 1991).

From holocellulose, hemicellulose and cellulose can be separated by using alkali at room temperature. Hoije et al. (2005) and Sun et al. (1998) extracted cellulose from wheat straw holocellulose using 24 % KOH and 2 % boric acid at 20°C for 2 h and obtained 41.8- 43.0 % of cellulose. In paper industry, soda process is the main pulping method for straws because most of the lignin and hemicellulose are dissolved in alkaline solution. Xiao et al. (2001) isolated cellulose from dewaxed maize stems, rice straw, and

rye straw by treatment with 1 M NaOH for 18 h at 30°C, which resulted in dissolution of 72, 84.6 and 72.6 % original hemicelluloses respectively. Sun et al. (1998) isolated cellulose from dewaxed wheat straw after alkali extraction, followed by delignification and alkali extraction.

The yields of cellulose ranged 38.0-39.0 %. Parameters such as concentration of the alkali, treatment time and temperature influence delignifying process Chen et al. 2013 have the subjected corn stover to alkali pretreatment (NaOH) where in the fiber to alkali loading was studied for its efficiency in removal of matrix components. Alkali loading (gNaOH/ g dry corn stover) was varied from 0.052g to 0.088g. Thus, the alkali concentration, treatment temperature and time play a major role.

Alkaline peroxide extraction

Hydrogen peroxide under alkaline conditions forms hydroperoxide anion (HOO^-) which is known to react with coloured carbonyl-containing structures in lignin and has been widely used for many years to bleach high-lignin wood pulps. On decomposition, hydrogen peroxide forms molecular oxygen and more active radicals such as the superoxide anion radicals (O_2^-) and hydroxyl radicals (OH^-), which in turn react with lignin resulting in delignification by both degradation and dissolution (Sun et al. 1998; Xiao et al. 2001).

Sun et al. (2003) found that the extraction of the dewaxed wheat straw using 2 % H_2O_2, 2 % NaOH for 45°C and 50°C at 5 h resulted in dissolution of 86 % of the original lignin and 76% of the original hemicelluloses, respectively, and left 53 % cellulose. Fang et al. (2000) compared the extraction of water-treated rye straw with alkali and alkaline peroxide to isolate cellulose and hemicelluloses. Dilute alkaline solution treatment resulted in lower removal of hemicellulose and lignin compared to that of extraction with 2% H_2O_2.

Acid hydrolysis

The treatment involves breakdown of polysaccharides such as cellulosic, starch, or hemicellulosic materials to simple sugars using acid solutions. The hetero polysaacaride hemicellulose is generally present in the form of hexoses and pentose in the lignocellulosic fibers and account to a total of 20% and 40% of total lignocellulosic content. These sugars can be obtained as monomers by acid hydrolysis. Since hemicellulose is amorphous unlike the cellulose, rapid oxidation and degradation of hemicellulose is favoured by acid hydrolysis.

Pectin occurs in a small degree in the middle lamella, especially in the pith and young tissue and consists of poly galacturonic acid, naturally soluble in aqueous media. Bhatnagar et al. (2004) reported removal of hemicellulose and pectin from the pulp using 1 M hydrochloric acid solution at high temperature (80°C). Zhou et al. (2012) treated 5 g microcrystalline cellulose with 45 mL sulfuric acid (64 wt %) and the mixture was hydrolyzed at 45°C for 120 min with continuous stirring (500 rpm) for removal of hemicellulose and pectin.

Wu et al. (2013) conducted sulfuric acid hydrolysis of hemicellulose by adding six grams of switch grass cellulose (or cotton cellulose) powders into 90 ml sulfuric acid (60 wt %) and allowed for hydrolysis at 45°C for 45 min. Yu et al. (2011) treated cotton using 64% sulfuric acid aqueous solution with cotton-to-acid weight ratio of 1 to 10 at 45°C temperature to hydrolyse hemicellulose and to form cotton nanocrystals.

Similarly, other plant sources such as Switchgrass and cotton (Wu et al. 2013), *Agave tequilana* (Espino et al. 2014), Barley bagasse husk (Tibolla et al. 2014), Banana peel bran (Rosa et al. 2010), Coconut fibers (Haafiz et al. 2014) and Oil palm empty fruit bunch (Fahma et al. 2010) are also treated with acid.

Organic solvent extraction

The processes currently used for commercial pulping using inorganic reagents achieve high cellulose extraction efficiency only at the expense of the hemicellulosic fraction, which undergoes hydrolysis and degradation. These processes not only

underexploit the lignin but also cause serious environmental problems. For these reasons, intensive research is being carried out on the development of environmentally friendly approaches, which generally involve the use of organic solvents for efficient separation of the cell wall components.

Lignocellulosic resources treated with aqueous organic solvent with or without the presence of catalyst results in reduced energy consumption and impact on environment compared to that of treatments involving inorganic reagents. Acetic acid and formic acid are mainly used organic acids for pulping of wood and non-wood plant materials (Muurinen et al. 2000), reason being that the acetic acid and formic acid have Hildebrand's solubility parameter (δ) value around 10.1 and 12.1 respectively, which is greater than the δ value (11) required for a lignin soluble solvent (Pan et al. 1999).
Pan et al. (1999) has reported that the acetic acid pulping is an effective method to delignify and fractionate straws. Lam et al. (2001) studied rice straw pulping using formic acid and reported ~85 % of delignification with a yield of 44.4% of cellulose pulp under relatively mild cooking temperature of 100°C, time of 1h and 90% formic acid solution. Recently, one of the developments in acetic acid pulping is the FORMACELL process. The process which involves mixture of 5-10 % of formic acid with aqueous acetic acid, has resulted in improved selectivity of delignification (Lehen et al. 2002). Besides the role of delignification, organic acids actively take part in the hydrolysis of hemicelluloses. Protocol described by Crampton et al. (1938) and Brendel et al. (2000) which involves treatment of lignocellulosic materials with organic solvent, acetic acid (80 %) and nitric acid (70 %) as catalyst taken in 10:1(v/v) is one of the recommended method. Similar procedure with acetic acid (80 %) and nitric acid as a catalyst (2.0-8.0 % (w/w)) was followed by Xu et al. (2005, 2006) group and significant degradation of hemicellulose and lignin, increase in degree of crystallinity of cellulose with the slight acetylation of cellulose was reported. Sun et al. (2004) found that wheat straw lignin and hemicelluloses were degraded in the medium containing 80% acetic acid and 0.92-13.5 % nitric acid. With increase in nitric acid concentration from 0.95 to 8.5 %, more than 96 %

original hemicelluloses and approximately 98 % original lignin were degraded and yielded the cellulose approaching 96 % purity. The same combination of acetic acid and nitric acid treatment was applied by several research group to isolate cellulose fibers from different lignocellulosic sources such as rice straw, poplar wood, barley straw, maize stems, oil plam frond and rye straw (Sun et al. 2005). Liu et al. (2010) and group have also isolated cellulose fibers with least amount of bound hemicellulose (2.3 -3.2 %) and lignin (0.4-0.6 %), from cereal straw by treating with acetic acid (80%) and nitric acid (70%) taken in 10:1 v/v ratio.

Literature review suggests that the organic acid (acetic acid) treatment in presence of catalyst (nitric acid) which is generally referred as organosolv process is an effective method for isolation of cellulose microfibers. Organic acid concentration, process time and temperature play a major role in removal of matrix components. However, due to their corrosive nature organic solvent treatments may lead to challenges in terms of selection of materials of construction for process equipment. Thus, it is preferable if the organic solvent treatment is combined with inorganic pretreatments.

2.2.2 Enzyme treatment

Enzymatic hydrolysis is one of the greener approach in isolation of cellulose fibers from the lignocellulosic sources. Enzymes which hydrolyse hemicellulose and cellulose are usually incorporated in isolation process. However, single enzymes available in nature cannot act upon the cellulose and they are effective when grouped and these are called cellulases. Cellulases are classified into to type A and B cellulase which are also termed as cellobiohydrolases. These cellulase readily act upon the crystalline cellulose. Whereas endoglucanases or type C and D cellulase require some disorder in the structure in order to degrade the cellulose (Henriksson et al. 1999, 2005). Cellulases are also reported to modify rather than degrade the cellulose (Henriksson et al. 2004). Henriksson et al. (2007) and Pääkkö et al. (2007) research group have also reported that endoglucanase pretreatment facilitates not only in hydrolysis but also in disintegration of cellulosic wood fiber pulp into cellulose fibrils/ nanofibers with less damage to cellulose

fiber structure compared to that of strong acid hydrolysis. Pang et al. (2016) also used cellulase and ß-glucosidase for hydrolysis cellulose of Aspen fibers. Tibolla et al. (2014) have presented the protocol for hydrolysis of amorphous components (hemicellulose) using hydrolytic enzymes “Xylanases”. The enzyme concentration and the hydrolysis time are the factors which effect the defibrillation on the cellulose fibers.

2.2.3 Mechanical treatment

Recently several mechanical treatments such as refiner and homogenizer have been used elaborately in order to produce finer cellulose fibrills (Stenstad et al. 2008; Pääkkoo et al. 2007; Nakagaito and Yano, 2005). A purely mechanical process can produce more refined, finer fibrils of several micrometres long and between 50 to 1000 nm in diameter. Taniguchi and Okamura (1998) processed micro fibrillated materials from natural fibers such as wood pulp fiber, cotton fiber, tunicin cellulose, chitosan, silk fibers and collagen by a super–grinding method. The cellulose fibers are subjected to shearing stress on the longitudinal axis. However recently mechanical methods such as high pressure homogenising and refining process steps are generally incorporated in isolation of cellulose fibers (Stenstad et al. 2008; Pääkkoo et al. 2007; Nakagaito and Yano, 2005). Few of the established mechanical treatments employed in fibrillation of cellulose fibers are Refining and high-pressure homogenization where in the fibers are subjected to cyclic stresses by passing them between surfaces fitted with grooves which results in irreversible changes due to modification in their morphology and size (Nakagaito et al. 2004). In case of homogenizer both shearing, and impact forces is forced upon the fibers exposed as a result of which the cellulose fibers are defibrillated resulting in micro fibrils (Nakagaito et al. 2004). Zimmermann et al. (2004) and Lo´pez- Rubio et al. (2007) groups have reported mechanical fibrillation of pulp fibers of diameter in range 20-100 nm using a microfluidizer in the homogenization step. However, Henriksson et al. (2007) have reported that the refining treatment of fibers reduce the degree of crystallinity and molar mass of cellulose fibers due to damage caused in the microfibril structure of the fibers. The refining process may not result in disintegration of the cellulose fibers but can

loosen up the fiber wall which could help in the further process of homogenization treatment (Nakagaito and Yano, 2004, 2005).

Cryocrusing is a mechanical process where the fibers are frozen by dispersing them in liquid nitrogen and high shear stress is applied on them (Chakraborty et al. 2005) which leads to rupture of cell walls thus leading to defibrillation (Wang and Sain, 2007a). Janardhnan et al. (2006) and Alemdar and Sain (2008 a), have incorporated cryocrushing in order to defibrillate soy hulls and wheat straw fibers and have observed that 60 % cellulose fibers were defibrillated to nanofibers of diameter 30–40 nm. Similary, Bhatnagar and Sain (2005) chemically treated rutabaga, hemp and flax fibers subjected to crycrushing were defibrillated to nanofibers with diameter ranging between 5–80 nm.

Grinding is the one of the mechanical process used in order to fibrillate fibers by applying shear force on the multilayers of nanofibers in the cell wall, which results in breaking of hydrogen bonds between them and thus leading to fibrillation. Taniguchi and Okamura (1998) have fibrillated microfibrillated cellulose fibers with diameters around 20–90 nm using super-grinding process.

Steam explosion is a high temperature treatment which leads to auto hydrolysis of acetyl group present in hemicellulose leading to formation of acetic acid. The acetic acid formed catalysis the hydrolysis of β-O-4 ether bonds in lignin leading to depolymerisation of lignin and partial hydrolysis of glycosidic linkages in hemicelluloses to produce water soluble phenolic compounds and sugars (Josefsson et al. 2001). Liu (2010) has reported that the cellulose also undergoes depolymerisation, defibrillation and also reduction in its crystallinity. However, the hemicellulose and the lignin separated by the steam exploitation process has to be further treated by chemical treatments such as alkaline extraction in order to dissolve them and isolate cellulose fibers (Sun et al. 2003).

Electrospinning process is based on the uniaxial stretching of viscoelastic solution by electrostatic forces. When the electric field reaches a critical value that overcomes the surface tension of polymer solution, the strong electrostatic forces leads to stretching of fibers in the solution towards the collector as fibrous mat (Fang et al. 2008; Teo et al.

2006; Doshi et al. 1995; Reneker et al. 1996; Gibson et al.2001). Zhang et al. (2003), Li and Xia (2004) and Walther et al. (2011) have incorporated electrospinning for fibrillation of cellulose fibers.

Ultrasonic technique is one of the mechanical process which has been reported to be efficient enough to fibrillate the cellulose fibers (Cheng et al. 2007, 2009, 2010). The cellulose solution generally consisting of cellulose fibers in water are subjected to sonic energy under cooled condition. The fibers exposed to ultrasonic energy are confronted by cavitation (hydrodynamic forces) which refers to the formation, growth, and violent collapse of cavities in water (Abramov, 1998). The ultrasonic energy produced in a probe type sonicator is approximately 10 to 100 kJ/mol, which is sufficient enough to break the hydrogen bond between the cellulose nano and microfibers resulting in fibrillation of cellulose fibers to few microns and even to nano scale (Zhao et al. 2007; Zhang et al. 2007; Wang and Cheng, 2009; Tischer et al. 2010; Urruzola et al. 2012). The ultrasonication method can be a clean and powerful method of defibrillation of cellulose fibers (Frone et al. 2011). Wang and Cheng, (2009) and Cheng et al. (2010a, 2011) have reported defibrillation of cellulose fibers from microcrystalline cellulose, pulp fiber, regenerated cellulose fiber and pure cellulose fiber.

Several investigators have applied ultrasonication after chemical treatments of cellulose, to fibrillate the nano cellulose fibers (Dujardin et al. 2003; Andanedo et al. 2005). Research by Dufresne and Vignon, (1998), Bhatnagar’s project (2004) and Choi and Simonsen, (2006) also suggested a method to extract the nanofibers from agricultural sources by chemo-mechanical treatments. The above mentioned mechanical treatments consume high amount of energy (Lavoine et al. 2012). Eriksen et al. (2008) have reported that homogenizer consumes as high as 70,000 kWh/t. Rojas et al. (2011) and Hubbe et al. (2011) have also reported highest consumption of energy to produce microfibrillated cellulose fibers from bleached and unbleached kraft hardwood pulps using grinder, microfludizer and homogenizer. Thus, it becomes very important to choose defibrillation processes which are less energy consuming. In order to meet this condition combination of mechanical, chemical and enzymatic treatments have been practiced in recent years.

Bhatnagar et al. 2003 and Alemdar et al. 2008 have reported defibrillation and isolation of cellulose fiber from soy hulls and wheat straw by combination of alkali treatment and cryocrushing. Similarly, Alemdar and Sain, (2008) have isolated cellulose nanofibers from wheat straw by chemical treatment alkali and acid treatment and mechanical treatments; cryocrushing, disintegration, and defibrillation. However, the list of different sources treated by chemo-mechanical methods and other combination of treatments for isolation and defibrillation of cellulose has been listed in Table 2.2.

Table 2.2. Different sources treated by chemo-mechanical methods and other combination of treatments for isolation of cellulose.

Sl. no	Source	Type	size	Isolation method	Crystallinity %	Onset degradation temperature °C	Reference
1	Wood powder	powder	5-20 nm	Bleached, alkaline, ultra-sonication	69	210 -335	Yu et al. (2011)
2	Wheat	straw	10–50 nm	alkali steam explosion coupled with high shear homogenization	57.43-80.05	239.5-276.2-283.2	Kaushik and Singh (2011)
3	Semi-chemical kraft bleached eucalyptus pulp	pulp	0.58 μm	Sonication			Urruzola et al. (2012)
4	Dry softwood pulp	Bleached softwood pulp	30 nm	high shear homogenization	73.2-77.2 78.1-79.5		Zhao et al. (2013)
5	Switchgrass and cotton		200 - 35 nm 140 -50 nm	Bleached, sulfuric acid hydrolysis, dialysis	69- 72		Wu et al. (2013)
6	Agave tequilana and barley	Bagasse husk	44.8 ± 4.3 - 6.5 ± 2.9 27.7 ± 8.4 μm	Acid hydrolysis Dialysis, Homogenisation, ultrasonication		224 217	Espino et al. (2014)

Table 2.2. continued…

Sl. no	Source	Type	size	Isolation method	Crystallinity %	Onset degradation temperature °C	Reference
7	Banana peel bran	bran	10.9-7.6 nm	alkaline treatment, bleaching, and acid hydrolysis) and enzymatic treatment (ETD Alkaline treatment and hydrolysis with xylanase	5.0, 58.6,49.2		Tibolla et al. (2014)
8	Poplar wood		5- 20 nm	Bleached, alkaline, ultrasonication	69	210 -335	Chen et al. (2011)
9	Coconut fibers	husk	5 nm	Bleached, acid hydrolysis	38.9 ± 0.3 62.5 ± 0.4 64.1 ± 0.4	275	Rosa et al. (2010)
10	Oil palm	biomass	10 nm 20nm	Acid hydrolysis, sonication	87 88 84	275 329 125	Haafiz et al. (2014)
11	Kapok fiber		4.5-8.5 µm	Bleached, alkaline,			Draman et al. (2014)
12	Alfa fibers		5 -10 nm	Alkaline, bleaching, acid hydrolysis	59 - 81	299.13 -335.67	Trache et al. (2014)
13	Corn husk			Alkali, bleaching, Acid hydrolysis, ultrasonic	81 - 87.3	260-193	Mendes et al (2015)
14	Bagasse		200 nm	Acid Hydrolysis alkali treatment bleached			Bhattacharya et al. (2008)
15	Rice	husk	6 to 14 nm	alkali treatment ultrasonic H_2O_2 -TAED Acid hydrolyse	67 -79		Rosa et al. (2012)

Table 2.2. continued…

Sl. no	Source	Type	size	Isolation method	Crystallinity %	Onset degradation temperature °C	Reference
16	Orange	peel	microcrystalline cellulose	Alkali- EDTA treatment bleached			Bicu and Mustata (2013)
17	De-pectinated sugar beet pulp	pulp	10–70 nm	alkali treatment and bleaching, high pressure homogenization	35.67-69.62	47.3 -71.7	Li et al. (2014)
18	Norway spruce	bark	2.8 nm	bleached Acid hydrolysis dialyzed	80	190	Normand et al. (2014)
19	Bamboo fibers	pulp	10-50 µm	dialysis ultrasonic			Zhang et al. (2014)
20	Posidonia oceanica	leaves and balls	7-8 nm	Alkali, bleaching, Hydrolysis ultrasonic treatment	41 -62 54 - 64		Bettaieb et al. (2014)
21	Helicteres isora plant	barks	10 µm	alkaline treatment, bleaching, acidic steam treatment and homogenization	38 -90	260	Chirayil et al. (2014)
22	Natural Pine	needle	30 - 70 nm	ultrasonic	66.19	221 - 267	Xiao et al. (2015)
23	Tomato	peels	42 nm	acidified sodium chlorite chlorine-free alkaline peroxide dialyzed	69.0 -80.8	275	Jiang and Hsieh (2015)
24	Posidonia oceanica	balls and leaves	7nm	sulfuric acid hydrolysis ultrasonic		250	Bettaieb et al. (2015)

Table 2.2. continued…

Sl. no	Source	Type	size	Isolation method	Crystallinity %	Onset degradation temperature °C	Reference
25	Oil palm	trunk	7.67 nm - 7.97 nm	Bleached, alkaline, sonicated, homogenised, acid hydrolysis	47.18-68.07	300	Lamaming et al. (2015)
26	Banana	pseudo-stem		Bleached, liquefaction, alkali	52.22-81.26	29.35-276.80	Li et al. (2015)
27	Energycane	bagasse	12±5 µm	alkali, Bleached	58.2- 68.8		Yue et al. (2015)
28	Cotton	stalks	3–15 nm	acid hydrolysis, TEMPO mediated oxidation, alkaline, Bleached, Ultrasonic		215 - 280	Soni et al. (2015)
32	Ushar (calotropis procera)	seed	14–24 nm 10–20 nm	acid hydrolysis and TEMPO-mediated oxidation	70 -59	240 -200	Oun and Rhim (2016)
33	Wheat	straw pulp		Ultrasonic-bleaching processes	35.4- 32.0	280	Xing et al. (2017)

Thus, it is very clear that combination of chemical, mechanical and enzymatic treatments has resulted in isolation of cellulose fibers into micro and nano fibers with reduction in chemical usage, energy consumption and processing time as compared to sole chemical, mechanical or enzymatic processes.

2.3 Characterization of cellulose fibers

In order to understand the properties of the isolated cellulose fibers and also to understand the effect of isolation process on the lignocellulosic composition, the cellulose fibers were subjected to several characterization techniques. Due to the complex structure of the lignocellulosic components, difficulties arise in the study of single cellulose fiber.

Thus, the combination of several characterization techniques is favourable as it provides partial but complementary information.
Characterization techniques such as Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), X-ray diffraction (XRD), Thermogravimetric (TG) and NMR spectroscopy analysis have been used majorly to study the structure of cellulose fibers.

Scanning electron spectroscopy assists in analysis of morphological changes observed in the fibers through the various treatment techniques. The presence and removal of lignocellulosic components mainly waxes, matrix components such as lignin and hemicellulose and also the cellulose fibers can be observed through SEM images (Movva and Kommineni, 2017; Kaczmar et al. 2011). The defibrillation of the cellulose fibers and their sizes can be derived from SEM analysis. This is one of the effective tool reportedly used in order to study the morphology of lignocellulosic components extensively in all the studies available in literature.

The FTIR spectrum of lignocellulosic components exhibit specific intensity of absorption bands which is an effective tool in analysing the changes in the lignocelluosic composition through the various stages of treatment (Rosa et al. 2010; Sun et al. 2004; Elanthikkal et al. 2010; Tibolla et al. 2014, Qiao et al. 2016; Shin et al. 2012; Kalita et al. 2015; Sun et al. 2004; Kaushik et al. 2011).
In general, the spectrum for cellulose possess mainly specific absorption bands at 2900 cm^{-1} which relates to the CH_2 and CH stretching, 1372 cm^{-1} to O-H bending, 893 cm^{-1} to glycosidic C_1-H β-glycosidic linkages between glucose in cellulose, 1426 cm^{-1} to CH_2 symmetric bending, 750 and 710 cm^{-1} to Iα and Iβ phases (R Zuluaga et al 2007; Kaushik & Singh et al. 2011; Bono et al. 2009; Draman et al. 2013; Juby et al. 2012; Sun et al. 2004; Kaushik et al. 2011; Elanthikkal et al. 2010; Haafiz et al. 2013; Obi Reddy et al. 2012; Shin et al. 2012; Zhong et al. 2013; Haafiz et al. 2014; Shin et al. 2012). Presence of peaks at these adsorption bands helps in understanding the presence of cellulose and

also the removal of matrix components in the analysed samples (Kalita et al. 2015; Rosa et al. 2012; Oun et al. 2016).

Table 2.3. Assignment of Peaks (ppm) for spectra of cellulose fibers.

Peaks at (ppm)	C-atom assignment
104.5	C-1
74.7	C-2
76.1	C-3
79.8	C-4
76.3	C-5
61.5	C-6

Similarly, the **^{13}C-NMR** analysis provides spectrum of lignocellulosic components consisting of specific carbon peaks. The spectrum of cellulose typically consists of six signals as represented in Table 2.3 for the six-carbon atom associated with the glucose units (Zang et al. 2005; Liu 2010).

Thus, the information obtained by the NMR and FTIR spectra enables in understanding of the presence and removal of lignocellulosic components in the fibers (Lu et al. 2003).

XRD analysis is a most reliable method frequently used for the determination of crystallinity of cellulose (Liu 2010; Sumari et al. 2013; Barbash et al. 2017). In general, the X Ray diffractograms of lignocellulosic components consists of crystalline peaks at 14.8°, 16.8°, and 22.6° corresponding to the (110), ($1\bar{1}0$), and (002) planes of crystals,

and weak crystalline peaks at 34.7° to the (004) plane (Focher et al. 2001; Meenatchi et al. 2017). The crystallinity is calculated by three methods considering the intensity data available in the diffractograms (Focher et al. 2001). However, the empirical method used for analysing the crystallinity of native cellulose is generally incorporated in number of studies. In this method, the crystallinity index (CI%) is calculated from the intensities of the ***2 0 0*** peak (I_{200}, 2θ = 22.6º) and the intensity minimum between the ***2 0 0*** and ***1 1 0*** peaks (I_{am}, 2θ =18º) by the Segal method (Nam et al. 2016) using Eq. (1).

$$CI\% = \left(1 - \frac{I_{am}}{I_{200}}\right) x\ 100 \quad \text{........(1)}$$

Where, I_{200} accounts for the intensities due to both crystalline and amorphous material, whereas I_{am} accounts for the intensity of amorphous material.

Thermogravimetric analysis helps in analysing the changes in thermal stability of the lignocellulosic fibers through the treatment processes. Generally, the thermograms show two step degradation curve which is related to degradation of lignocellulose components at specific temperature. The onset degradation temperature represents the stability of the fibers depending upon the crystalline cellulose concentration (Rosa et al. 2012; Jawid et al. 2017). The thermal stability of the fibers decreases as the concentration of amorphous components decreases (Reddy et al. 2012; Oushabi et al. 2017). Thus, the thermogravimetric analysis again provides information about the removal of matrix components from the treated fibers compared to that of untreated fibers.

2.4 Biocomposites

Cellulose fibers as reinforcement in polymer matrix is being extensively studied in recent years by many investigators in preparation of biocomposites. In line with this, several plant sources have been exploited for the isolation of cellulose fibers in micro and nano forms as discussed in Section 2.2. The strength, stability, low weight and specifically biodegradability of these cellulose fibers have grabbed attention for their application as reinforcement in the composites processing. More often, polymer matrices used are of petroleum based such as PVA (poly vinyl alcohol), Polypropylene (PP), PVC (polyvinyl

chloride), polystyrene (PS) and polyethylene (PE) (Faruk et al. 2012). Biobased plastic such as starch, poly lactic acid (PLA), polyhydroxybutyrate-valerate (PHB) and polyhydroxyalkanoate (PHAs) have also been used as matrix polymer (Faruk et al. 2012). Most of the polymers incorporated as matrix in biocomposites preparation have issues concerned with the dispersion and bonding of cellulose to the hydrophobic polymer as cellulose fibers are hydrophilic in nature (Kalia et al. 2011).

The high density of –OH groups on the surface of the cellulose leads to the bonding with adjacent hydroxyl group by hydrogen bonding which results is agglomeration. Thus, water is generally used as carrier for dispersion of cellulose fibers than non-polar solvents. Several surface modification treatments for cellulose fibers such as mercerization (Ray et al. 2001, Sreekala et al. 2000), silane treatment (Joseph et al. 2000; Gousse et al. 2004), acetylation (Bledzki et al. 2004, Mohanty, 2004) and Benzoylation (Manikandan, 1996; Sreekala et al. 2000) have been reported in literature in order to achieve better compatibility with the hydrophobic polymer matrices. However, PVA (poly vinyl alcohol) which is polar in nature when used as matrix helps in dispersion of the cellulose fibers (Bhatnagar 2004) and thus incorporated as matrix in several biocomposites studies. Sain et al. (2008) have also reported that the PVA reinforced with cellulose fibers isolated from hemp, rutabaga and flax have shown higher strength and better stiffness compared to that of neat PVA.

Polyvinyl alcohol is a fully hydrolysed, medium viscosity grade of polymer (Fig 2.2) (Wang 2007).

n

OH

Figure 2.2: Chemical structure of Poly Vinyl alcohol (PVA)

The PVA dispersed in water and on evaporation of water results in transparent films which possess high tensile properties and are resistance to tear (Kline, 1961). The poly

vinyl alcohol offers excellent binding and film forming characteristics which broadens their application in many fields. Geisari et al. (2008), Niu et al. (2015), Won et al. (2015), Tan et al. (2015), Heidarian et al. (2017) and many others have reported the preparation of biocomposites with PVA matrix and cellulose fibers as reinforcement.

Composites preparation

Several researchers have used solution casting (Alias et al. 2017; Mitra 2014; Chen et al. 2012; Lakshmeshwar et al. 2012; Geisari et al. 2008; Andresen et al. 2006, 2007; Saito et al. 2006; Dufresn et al. 1997) to form composites films of the polymer matrix and cellulose fiber dispersed in a solution. Solution casting involves the casting solution containing polymer dissolved in a solvent with cellulose fibers dispersed. This solution is poured onto the petri plates and generally kept at room temperature for removal of solvent in order to get composite films (Zhou et al. 2017).

However, there are several other methods such as rotational molding, compression molding, injection molding and extrusion (Throne et al. 1979; Crawford et al. 1992; Hensen et al. 1997) for preparation of composites which involve high energy consumption compared to that of solution casting. Methods used in preparing biocomposites and their characteristics are listed in Table 2.4.

It can be observed in Table 2.4, that solution casting is being extensively reported by several researchers. According to Oksman et al. (2016) around 45% of the papers published on composite preparation have adopted solution casting method, indicating that casting method is more favourable for biocomposites preparation.

Characterization of biocomposites

The characterization of the biocomposites including mechanical properties, morphological characteristics and thermal properties are generally carried out to find their suitability for potential applications.

Table 2.4: Methods used for cellulose reinforced composite preparation and the mechanical properties of the composites (adopted from SirÓ and Plackett 2010) and further modified.

Matrix	Procedure	Fiber content (%)	Max. Stress (MPa)	Modulus of elasticity (GPa)	Strain at break (%)	References
PVA poly (vinylacetate), Acrylic,Epoxy	Resin impregnation; Solution casting	33–67	55.7–145.1[b]	ND	ND	Bruce et al. (2005)
Melamine formaldehyde	Hot pressing of resin impregnated MFC mats	87–95	108–142	15.7–16.6	0.81–1.4	Henriksson and Berglund (2007)
Acrylic resin	Resin impregnation	73–88	~80–100[a]	7.2–8.2[a]	~2–6[a]	Iwamoto et al. (2007)
Poly(styrene-co-butyl acrylate)	Solution casting	0–10	(0.41)/0.75–4.9	(0.55)/0.99–34.59$10^{-3}$	(3,634)/216–2,353	Malainine et al. (2005)
		6	(0.18)/6.3	(0.2)/114 10^{-3}	(>3,000)/32	Samir et al. (2004)
Polyurethane	Film stacking	7.5–16.5	(5)/5–28	(25)/93–725 10^{-3}	ND	Seydibeyoglu and Oksman(2008)
Polyethylene	Solid-phase melt mixing	0–10	(11.4)/12.2–14.2	(0.21)/0.23–0.34	(235.5)/212–226	Wang and Sain (2007b)
Polypropylene fibers	Compression molding	0–20	(28)/34–35	(0.7)/1.4–1.6	ND	Cheng et al. (2007)

Table 2.4 contd…….

Matrix	Procedure	Fiber content (%)	Max. Stress (MPa)	Modulus of elasticity (GPa)	Strain at break (%)	References
Poly(lactide)	Extrusion by liquid pumping	5	(58)/58	(2)/2.6	(4.2)/2.8	Mathew et al. (2006)
	Compounding and injection molding	5	(65.5)/71.1	(2.7)/2.9	ND	Wang and Sain (2007c)
	Premixing, kneading	3–20	(50)/55–75[a]	4.7	(4.2)/ 1.6–3.0[a]	Iwatake et al. (2008)
			(57.7)/61.4–71.2)	(3.3) 3.8–5.7	(6.8)/1.7–2.7	Suryanegara et al. (2009
	Mixing in Waring Blender, filtering and hot-pressing	0–90	35–180[a]	5–13[a]	1–3.3[a]	Nakagaito et al. (2009)
Poly(caprolactone)	Solution casting	0–12	(25.5)/18–25[a]	(0.26)/0.4–0.6[a]	(680)/ 600–20	Siqueira et al. (2009)
Poly(vinyl alcohol)	Solution casting	10	(69)/76–178	(2.3)/6.1–10.1	ND	Bhatnagar and Sain (2005)
		5–10	(64.8)/ 102.6–108	(2.3)/6.2–6.6	(1.3)/ 1.7–2.1	Wang and Sain (2007a, b)
		50	(30.2)/145.1	(0.46)/8.49	ND	Bruce et al. (2005)
		0–90	(17)/61–84	(0.25)/5.3–7.7	(22.7)/ 1.6–2.0	Leitner et al. (2007)
		0–15	(38)/43–62	(3.8)/4–5.2	ND	Lu et al. (2008b)
Poly(vinyl alcohol)	impregnation	80		0.6- 6.0 31 -125		Wang and Li (2015)
Potato starch Solution	casting	0–50	ND	(0.1–2)/ 0.5–7.0[c]	ND	Dufresne et al. (2000)

Table 2.4 contd…….

Matrix	Procedure	Fiber content (%)	Max. Stress (MPa)	Modulus of elasticity (GPa)	Strain at break (%)	References
Thermoplastic starch	Solution casting	0–10	ND	(0.11)/ 0.15–0.27	ND	Alemdar and Sain (2008b)
		0–20	(2.5/4.5)/ 5–14	(0.02)/ 0.025–0.14[d]	(38/55)/ 70–32	Mondrago´n et al. (2008)
Amylopectin	Solution casting	0–10	(1.8)/4.1–38.8	(0.024)/0.14–1.8	(120.4)/ 1.9–33.6	Lo´pez-Rubio et al. (2007)
		0–70	(0.35)/5–160	(0.002)/0.18–13	(80)/ 8.1–25	Svagan et al. (2007)

Note: [a] Values estimated from charts presented in the original reference

[b] Depending on matrix nature and fiber content

[c] Depending on plasticizer content (0–30 wt%) and conditioning RH (25–75%)

Tensile and bending tests are normally used to measure the mechanical properties of composites as they aid in understanding the fiber distribution in polymer matrix and the contribution in overall strengthening of composites (Bhatnagar et al. 2005; Cheng et al. 2007a, 2009b; Choi et al. 2006; Taniguchi et al. 1998; Zimmermann et al. 2005; Nakagaito et al. 2005). For morphological characterizations of biocomposites, SEM and TEM are common tools (Cheng et al. 2009a; Kvien et al. 2005; Pu et al. 2007; Taniguchi et al. 1998; Zimmermann et al. 2004; 2005). Morphological characteristics of composites gives a clear picture of fiber distribution in polymer matrix which directly influence the other potential properties of the composites. Thermal properties are very important for biocomposites due to their applications in higher temperatures. Differential scanning calorimetry (DSC) and Thermogravimetric analyser (TGA) have been used to evaluate thermal properties (Ljungberg et al. 2006; Orts et al. 2005; Samir et al. 2006; Wang et al. 2010). Transmittance of composite films plays a major role in food packaging industry.

Lower transmittance of light through the packaging composite films prevents photo degradation of food material. Further, oxygen transfer rate through these biocomposites films specifically when used in food packaging, lower values are preferred to protect the food material from oxidation. The biocomposites stand apart from the tradition composites for their excellent biodegradability characteristics. The biodegradability of the composites is usually carried out by soil burial test. Where in the composites are buried in the moist soil and left over certain time period and checked for weight loss in regular intervals to account for the degradation (Othman et al. 2011; Tan et al. 2016).

Application of biocomposites

Cellulose fibers have found application as

- reinforcement in biocomposites (Dufresne et al. 2010),
- barrier films for food-packaging (Lindström et al. 2010),
- coating on papers to study release of caffeine and chlorhexidine (Lavoine et al, 2014, 2016).
- light and porous aerogels (Saito et al. 2014),
- non-leaching antibacterial surfaces (Saini et al. 2016).
- Insoluble matrix for drug delivery (Kolakovic et al. 2012; Valo et al. 2013) and many more as such.

Cellulose fibers in their different forms such as micro, nano fibrills and crystals are reinforced to form biocomposites which have widely found application in several fields (Duran et al. 2012; Siro et al. 2010). These biocomposites are mainly used in automobile and packaging industries (Singh and Gupta, 2005). However, they have also found to be potential in many other fields such as medical field (medical devices and blood bags), electronic field (displays for cellular phones, cameras, watch dials, and integrated circuits) and also in paper industry (Duran et al. 2012; Rebouillat and Pla, 2013).

The cellulose fiber reinforced in polymer matrix have also found application in food packaging field mainly due to the enhanced properties of biocomposites such as mechanical strength, thermal stability, optical clarity, barrier properties, chemical stability, biodegradability, recyclability, antifungal and antimicrobial surfaces and signaling and sensing biochemical and microbiological changes (Rhim et al. 2013). The main advantage of biocomposites used in packaging field is generation of less packaging waste as most of them are biodegradable. Several review articles have reported application of nanocomposite in food packaging field containing both thermoplastic and thermoset polymers as matrix. However, application of biocomposites in food packaging application are still under development. The efficient applicability of these composites is not yet being advanced. This creates an urge to exploit the nature for its potential as cellulose fibers mainly concentrating on the residual sources so that the pollution is at bay.

2.5 Scope of the present study

Biocomposites are to be developed in order to tackle the environmental impacts caused by traditional composites (plastics). The natural filler component; cellulose is a potential reinforcement in biocomposites preparation. Several researchers have reported different kinds of sources for isolation of cellulose fibers. However, most of them are food for human and animals and are economically important resources. Thus, sources which are residues, non-food, underutilized, abundantly available and possess disposal problems are to be a chosen for isolation of cellulose fibers. Fabrication of biocomposites without compromising with their mechanical and thermal properties, require cellulose microfibers with high cellulose concentration, lesser matrix components specifically lignin and smaller cellulose fiber size (nm). As per the literature reports, the chemical composition of the cellulose source and the treatments used for isolation of fibers influence the cellulose concentration and fiber size of the isolated cellulose fibers. Challenges in choosing effective isolation methods which involve chemicals with lower toxicity, consume less energy, and economical in isolating cellulose rich and micro sized cellulose fibers, have to be confronted. Chemical treatments have been extensively used for the

isolation and defibrillation of cellulose fibers, however chemical consumption can be reduced by combining with biological (enzymatic) and mechanical methods. The extent of isolation and defibrillation varies with the methods adopted for isolation and the conditions used during isolation. For any given set of conditions adopted, the extent of isolation and defibrillation can vary with the cellulose source as it is governed by the composition and structure of the matrix. When a new cellulose source is used for cellulose isolation, the process conditions need to be optimized in order to isolate defibrillated cellulose fibers with high cellulose content and small diameters.

Cellulose fibers reinforced in polymer matrix are associated with challenges such as dispersion and adhesion of fibers in matrix, due to their hydrophilic nature. By choosing a suitable polymer matrix and processing method these issues can be addressed. Packaging industry compel high strength and thermally stable composites. In specific, food packaging industry demands for transparent composites with lower oxygen transfer rate and are also biodegradable. Studies have reported enhanced thermal, mechanical properties and degradation of the polymer matrix on reinforcing the cellulose fibers and also the fiber loading plays a major role in enhancing the properties of the composites as a whole. Thus, the effect of fiber loading on the polymer properties is essential.

In order to effectively utilize the biofuel industrial and agricultural residues by channeling them towards cellulose isolation for production of environmentally friendlier, biodegradable polymer composites and to address the challenges in terms of isolation of cellulose fibers, the following objectives were formulated

2.6 Objectives of the research work

The main objective of the present work is to isolate cellulose microfibers from Jatropha (*Jatropha curcas L*) seed shell, Pongamia (*Pongamia pinnata*) seed hull and Finger millet (*Eleusine coracana L.*) straw for the preparation of cellulose fiber-reinforced/PVA, biocomposites.

The specific objectives are:

- To study the potential of Jatropha seed shell, Pongamia seed hull and Finger millet straw as the resource for production of cellulose fibers
- To optimize the parameters in various processes adopted for cellulose isolation and choose an effective method for isolation of cellulose rich and defibrillated, small diameter fibers.
- To characterise the isolated cellulose fibers.
- To prepare biocomposites using isolated cellulose fibers and evaluate its suitability for packaging applications in terms of their properties.

2.7 Organization of the thesis

The thesis comprises five chapters:

Chapter 1 presents the **Introduction.**

This chapter covers the discussion on the background of the research, need for the study and problem statement. The scope and objectives of the present research work are presented at the end of this chapter.

Chapter 2 presents the detailed **Literature review.**

This chapter encapsulates the related literature review carried out during the current study

Chapter 3 on **Material and Methods** lists the materials used, followed by description of the experimental methodologies and the analytical procedures adopted to achieve the stated objectives.

Chapter 4 is on **Result and Discussion.** The results obtained through the studies performed according to the methodologies presented in Chapter 3 are reported in this chapter. It includes result and discussion on isolation of cellulose fibers by different methods and optimization of process parameters. Comparison of different methods and the combination of methods to choose the effective isolation method and characterization of cellulose fibers are presented in this chapter. Characterization of biocomposites are presented at the end of the chapter.

Chapter 5 presents the **Summary and Conclusions** of the present research work along with the future scope for research**.**

CHAPTER 3

MATERIALS AND METHODS

This chapter furnishes details of materials used for the execution of experiments, analytical techniques and methodologies adopted to attain the objectives set forth in this research work.

3.1 Materials

All the chemicals used in the treatments for isolation of cellulose fibers in the present research work were of analytical (AR) grade. Enzymes Novozyme 476 were procured from Sigma Aldrich, USA. Acetic acid (glacial) (99-100%), sodium acetate buffer (99.5%), sodium hydroxide (98%), benzene (99%) and benzyl chloride (98.5%) were purchased from Merck India Pvt. Ltd., Mumbai, India. Nitric acid (70% assay), sodium acetate buffer (99.10%) and potassium hydroxide (85%) were purchased from Nice chemicals Pvt. Ltd., Kerala, India. Ethanol (99.9%) was purchased from analytical CS reagent, Mumbai, India. Acetone (99%) and sodium bisulphate (99%) were purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Toluene (99%) was purchased from Molychem, Mumbai, India. Sodium chlorite (80%), Tris (hydroxymethyl) amino methane were purchased from Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore, India. PVA (Poly Vinly Alcohol) - cold water soluble was purchased from HiMedia Laboratories Pvt. Ltd., Nashik, India.

3.2 Selection of lignocellulosic resources

The following lignocellulosic resources were selected and processed for isolation of cellulose microfibers

- Jatropha (*Jatropha curcas L*) seed shell.
- Pongamia (*Pongamia pinnata*) seed hull
- Finger millet (*Eleusine coracana L*) plant straw.

Jatropha (*Jatropha curcas L*) seed shell and Pongamia (*Pongamia pinnata*) seed hull, the residues from biofuel production unit were collected from “SEEDS” research centre, University of Agricultural Sciences, Bengaluru, Karnataka. These trees are grown in the

University campus and the seeds are being utilised in production of biofuel. The Jatropha seed shell and Pongamia seed hull which are separated from these seeds before the production of biofuel were collected and used as the resource for isolation of cellulose fibers. The straw of Finger millet (*Eleusine coracana L*) plant after the harvest were collected from the fields of Pavagada town, Tumkur district, Karnataka and were also used as the resource for isolation of cellulose fibers.

3.3 Preparation of lignocellulosic biomass for isolation of cellulose microfibers

The lignocellulosic biomass, Jatropha seed shell, Pongamia seed hull and Finger Millet straw, as collected from the respective sites were initially cleaned by washing extensively using water, to ensure that the material was free of dust, mud, and other plant materials. The material was dried under sunlight for two days. Further, the lignocellulosic biomass was ground in to a fine powder in a domestic blade mixer. The powder was sieved and material that passed through 0.25 mm mesh size (TSS Mesh Number 60) (ASTM E 11-09) screen was taken for further processing. The powder was dried in hot air oven for 8h until the weight remained constant to ensure that the fibers are moisture free. The powder thus obtained is referred hereinafter as untreated fibers. These untreated fibers were stored in air tight pouches.

The natural fibers contain waxes, fats, resins, oils, plus tannins and certain other ether-insoluble components which are not generally considered part of the wood polymeric structure (ASTM D 1105–96). These components were extracted using ethanol-toluene and benzene-ethanol as solvent by adopting the standard dewaxing method (ASTM D 1105– 96). Jatropha seed shell, Pongamia seed hull and Finger Millet straw fibers were dewaxed by treating with ethanol-toluene (2:1 by volume) solvent taken in Soxhlet apparatus and heating for 6 to 8 hours. The fiber to solvent ratio of 1:50 (w/v) was used for dewaxing the fibers. The temperature was set so as to achieve minimum of four siphons per hour and heated until the solvent in the siphoning tube was colourless, which indicates the removal of the extractives. After the treatment, the excess solvent in the dewaxed lignocellulosic biomass was removed by suction and was washed with distilled

water, finally dried in hot air oven at 105°C. The powder thus obtained is referred hereinafter as dewaxed fibers.

3.4 Isolation of cellulose fibers

Various treatments such as chemical, mechanical and biological treatments are reported in literature for isolation of cellulose fibers from different lignocellulosic sources and for defibrillation of the fibers as discussed in Section 2.4.

In the present work, chemical methods have been combined with mechanical or biological treatments such as ultrasonication and enzymatic treatment to examine the efficiency of combination of treatments on isolation of cellulose fibers from the chosen lignocellulosic biomass. The following methods were used for isolation and defibrillation of cellulose fibers

The chemical methods used were:

Method O which refers to organosolv (acetic acid as solvent and nitric acid as catalyst) treatment

Method IO which refers to inorganic chemical (sodium chlorite and sodium hydroxide) treatment followed by organosolv (acetic acid as solvent and nitric acid as catalyst) treatment

The fibers obtained by the above chemical methods were further subjected to either Ultrasonication treatment (Mechanical Treatment) or enzymatic treatment (Biological Treatment).

Method OU refers to the combination of organosolv treatment with ultrasonication

Method IOU refers to the combination of inorganic chemical treatment with organosolv treatment and ultrasonication

Method OE refers to the combination of organosolv treatment with enzymatic treatment

Method IOE refers to the combination of inorganic chemical treatment with organosolv treatment and enzymatic treatment.

3.4.1 Isolation of cellulose microfibers by method O

The Organosolv treatment method has been adopted from Brendel et al. (2000) with further modification in the process parameters. 50 mg of dewaxed fibers were taken in 10 mL Pyrex tubes, to which 2.0 mL of acetic acid (80 %; v/v) and 0.2 mL of concentrated nitric acid (69 %; v/v) were added. The lignocellulosic biomass was then suspended uniformly in the solution by careful vortexing and tubes were sealed using screw-caps fitted with Teflon liners and placed into a hot water bath pre-heated to the required temperature (100°C, 120°C or 130°C). Acid treatment was carried out for a required time (15, 20 or 25 min). Later the solution was cooled and 2.5 mL of ethanol (99 %; v/v) was added followed by centrifugation for 5 min at 2000 rpm. The supernatant was decanted, and the pellet was washed sequentially by centrifuging two-times with 2.5 mL ethanol, to remove extraction breakdown products followed by washing twice with 2.5 mL deionized water, to remove traces of nitric acid. Further washing was performed twice with 2.5 mL ethanol and finally with 2.5 mL acetone twice. Ethanol was filtered from the isolated cellulose fibers and were finally dried in hot air oven at 105°C. The effect of acid treatment time and temperature on cellulose isolation was studied. The optimum acid treatment time and temperature were chosen based on maximum cellulose isolation and defibrillation of the fibers.

3.4.2 Isolation of cellulose microfibers by method IO

In the present work, the inorganic chemical treatment has been combined with the organosolv treatment in order to improve the cellulose isolation from the lignocellulosic biomass and defibrillation of the fibers. The method IO includes a combination of sodium chlorite bleaching, NaOH and organosolv (acetic acid and nitric acid) treatment for the isolation of cellulose microfibers from Jatropha seed shell, Pongamia seed hull and Finger millet straw fibers. In the inorganic chemical treatment, the fibers were initially bleached with sodium chlorite solution which helps in delignification followed by sodium hydroxide and Organosolv treatment both of which result in hydrolysis of hemicellulose and lignin, thus leading to isolation of cellulose fibers.

The inorganic chemical treatment method for isolation of cellulose microfibers was adopted from Sun et al. (2004) with further modification in the process parameters. A known volume of sodium chlorite solution (0.7% w/v) was added to 5g of dewaxed fibers in order to obtain the fiber to liquor ratio (w/v) as desired (1:50 and 1:30). The pH of the solution was adjusted to pH 4 - 4.2 by the addition of acetic acid and sodium acetate buffer. The treatment was carried out under reflux conditions at 100°C for 2 h in a round bottomed flask mounted on a heating mantle. The fibers were then separated by filtration and extensively washed with 2% sodium bisulphate, distilled water and ethanol followed by drying at 105°C. The fibers were then treated with 100mL of 17.5% (w/v) NaOH solution at 20°C in cold water bath for 45 min, filtered, washed with 10% acetic acid and then with distilled water, later dried at 105°C in an oven. The fibers were then treated with 80% by volume acetic acid solution in distilled water and 70% by volume nitric acid in distilled water taken in 10:1, 15:1 and 20:1 volume ratio (acetic acid: nitric acid) in a round bottomed flask mounted on a heating mantle at 120°C for 15min under reflux conditions. Later the mixture was cooled and then filtered to separate the fibers. The isolated cellulose fibers were washed sequentially with 95% (v/v) ethanol in water, filtered and finally dried at 105°C in an oven until it reached a constant weight. The effect of variation in fiber to sodium chlorite ratio and acetic acid to nitric acid ratio on the cellulose content and size of the isolated fibre was studied.

3.5. Ultrasonication treatment of cellulose fibers isolated by chemical methods (Method OU and Method IOU)

The cellulose microfibers isolated from the lignocellulosic biomass by chemical method **O** and method **IO** were further subjected to Ultrasonication, a mechanical treatment in order to improve the extent of isolation and defibrillation of the isolated cellulose microfibers.

Ultrasonication treatment method for isolated cellulose fibers was adopted from Chen et al. (2011). In this process, the cellulose fibers obtained after chemical treatment method O or method IO, were soaked in distilled water such that the fiber concentration

was ~0.5 % by weight, to avoid hydrogen bonding between the fibers due to removal of the matrix i.e. lignin. About 120 mL of solution containing the cellulose fibers isolated by chemical method was then subjected to ultrasonication at 20–25 kHz frequency and 25% amplitude with cylindrical titanium alloy sonicator probe tip of 1.5 cm in diameter equipped with ultrasonication generator of 500 W power. The probe was dipped in the solution up to 2 cm of its total length. The ultrasonication was conducted for varying ultrasonication time of 20, 25 or 30 minutes with corresponding energy of 300 kJ, 375 kJ and 450 kJ respectively, to isolate the cellulose microfibers. The ultrasonic treatment was carried out in an ice water bath, to remove the heat produced during ultrasonication.

After ultrasonication the fiber suspension was filtered, the fibers were dried in oven at 105°C until it reached a constant weight. The effect of ultrasonication time on cellulose fiber isolation and the fiber size were studied.

3.6 Enzymatic treatment of cellulose fibers isolated by chemical methods (Method OE and Method IOE)

Enzyme treatment was adopted from Henriksson et al. (2007) in which Novozyme 476 enzyme which is endoglucanase, facilitates the disintegration of cellulosic wood fiber pulp into cellulose fibers. 10g of cellulose fibers isolated from the lignocelluosic biomass by method **O** or **IO** were suspended in water to a total weight of 75g such that the fiber content in the suspension was 13.3 %. These cellulose fibers were first mechanically beaten in a mortar and pestle for 30min to increase the swelling of cellulose fibers in water and make the cellulose more accessible for the enzyme. Further the beaten fiber suspension was diluted in distilled water to contain 3 wt % of fiber. Required quantity of Novozyme 476 enzyme was added to achieve desired concentration of the enzyme (0.5 wt % or 0.02 wt % of enzyme). Then 50 mM tris(hydroxymethyl) amino methane /HCl buffer was added to the suspension to adjust the solution pH at 7. The suspension was incubated at 50°C for 2 h to activate the enzyme and later filtered and washed on a Bϋchner funnel to remove the enzyme and buffer. The cleaned cellulose fibers were again incubated at 80°C for 30 min to stop the activity of the remnant enzymes and then

washed with water. Finally, these cellulose fibers were beaten again using mortar and pestle for 30 min and dried in oven at 105°C until it reached a constant weight. The effect of enzyme concentration on cellulose fiber isolation and the fiber size were studied.

The cellulose microfibers isolated using method O and IO were further defibrillated by method U or method E. Favourable method was chosen on the basis of isolation of highly defibrillated cellulose fibers with maximum cellulose content and smallest fiber diameter.

3.7 Characterization of cellulose microfibers

The untreated fibers of Jatropha seed shell, Pongamia seed hull and Finger millet straw were analysed for their chemical composition in terms of lignin, holocelluolse, α-Cellulose and ash content using ASTM standard methods. The cellulose fibers isolated from these lignocellulosic biomasses by different methods were also analysed for their chemical composition in terms of lignin, holocelluolse and α-Cellulose content using methods prescribed by ASTM standard. The chemical composition was presented in terms of lignin, hemicellulose and α-Cellulose content. Hemicellulose content was obtained by subtracting α-Cellulose content from holocelluolse content. These analyses were done to quantify the removal of matrix components from the cellulose fibers by isolation.

The fibers isolated from the lignocellulosic biomass by different methods were also analysed for their morphological characteristics and fiber diameter using SEM. The fibers isolated by the chosen method were also subjected to NMR and FTIR to qualitatively analyse the removal of matrix components and the changes in chemical characteristics by isolation process. Further the fibers were subjected to TG, XRD, and DLS analysis, to understand the changes in thermal characteristics, crystallinity and dimension of the fibers by isolation process. The details of the methods used for characterization are presented in the following sections.

Analysis of chemical composition of fibers

The chemical composition of Jatropha seed shell, Pongamia seed hull, and Finger Millet straw fibers and cellulose microfibers isolated from them by different methods were determined according to the following methods prescribed by ASTM standards.

- Lignin content by ASTM D 1106-56
- Holocelluolse (α-cellulose + hemicellulose) content by ASTM D 1104-56
- α-Cellulose content by ASTM D 1103-60
- Ash content by ASTM D1102-84

Hemicellulose content was determined by subtracting α-Cellulose content from holocelluolse content.

Scanning electron microscopy (SEM)

Scanning electron microscope (JSM-6380LA, Jeol, EVISA), was used to study the morphology of the untreated fibers and isolated cellulose microfibers. The fibers were gold coated prior to recording the micrographs and the acceleration voltage was set at 20 kV.

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra were recorded on a Fourier transform infrared (FTIR) spectrometer (Jasco 4200, Jasco analytical instruments, USA). The untreated fibers and isolated cellulose microfibers were dispersed in the form of powdered fiber samples in KBr pellets and spectra were recorded in 400-5000 cm^{-1} region at a resolution of 4 cm^{-1}.

X-ray diffraction analysis

The X-ray diffraction (XRD) patterns were measured with an X-ray Diffractometer (D/max 2200, Rigaku, Japan) using Ni-filtered Cu Kα radiation (λ= 1.5406A˚) at 40 kV and 15 mA. Scattered radiation was detected in the range of 2θ= 10 - 30º at a scan rate of 4º /min. The Crystallinity Index (CI) was calculated from the intensities of the *2 0 0* peak (I_{200}, 2θ = 22.6º) and the intensity minimum between the *2 0 0* and *1 1 0* peaks (I_{am}, 2θ

=18°) by the Segal method (Nam et al. 2016) using Eq. (1). I_{200} accounts for the intensities due to both crystalline and amorphous material, whereas I_{am} accounts for the intensity of amorphous material.

$$CI\% = \left(1 - \frac{I_{am}}{I_{200}}\right) x\ 100 \qquad \text{Eq. (1)}$$

Thermogravimetric analysis

The thermal stability of untreated source and extracted cellulose microfibers was established using a thermogravimetric analyser. The thermal stability of each sample was determined by obtaining the thermograms using a thermogravimetric analyser (Extra 6000, TG/DTA6300, SII nano technology Inc., Japan) with all the measurements performed under a nitrogen atmosphere with a gas flow of 100 mL min^{-1} with heating from 40 to 700°C at a rate of 10°C min^{-1}.

^{13}C NMR (CP/MAS)

The fibres were spun at 7.5 kHz spinning rate with filled 5mm rotor at room temperature. The NMR spectra of untreated fibers and isolated cellulose microfibers were obtained on solid-state NMR spectrometer (Bruker DSX 300MHz). Operating frequency was fixed at 75.46 MHz for ^{13}C nuclei.

Dynamic light scattering (DLS)

The dimension of isolated cellulose fiber dispersed in distilled water, was measured by dynamic light scattering instrument (DLS, nano particle analyzer, HORIBA Scientific, nano partica SZ-100, Japan).

3.8. Preparation of cellulose microfiber reinforced PVA biocomposites

The cellulose microfibers isolated from the lignocellulosic biomass: Jatropha seed shell, Pongamia seed hull and Finger millet straw were reinforced in PVA matrix to form the biocomposites. The biocomposite films reinforced with 5, 10 or 20 wt% of cellulose

microfiber loading were prepared by solution casting method. PVA solution in water was prepared under mechanical stirring for 24 h at room temperature. A known quantity of isolated cellulose microfibers which would lead to 5, 10 or 20 wt% fibers in PVA solution on addition, were dispersed in water by mechanical stirring at room temperature for 4 h. PVA solution and cellulose fiber dispersed suspension were mixed by further stirring for 4 hours and finally ultrasonicated for 5min to remove air bubbles in the solution. The filler-matrix mixture was then cast onto a Petri dish and was dried at room temperature (25°C) until water was removed from the film (approx. 3 days).

3.9 Characterization of cellulose microfiber reinforced PVA biocomposites

The biocomposites were also characterized by SEM and TGA for morphological and thermal characteristics as described in Section 3.7.2 and 3.7.5 respectively. The biocomposites were analysed for their light transmittance, mechanical, oxygen transfer properties and biodegradability as described in the following sections.

Transmittance analysis

Transparency of the biocomposites film and neat PVA was determined by measuring the percentage transmittance in the wavelength range from 200 nm to 800 nm using a UV/ VIS spectrophotometer (Hitachi, Hitachi high- technologies global, Japan).

Mechanical properties of the biocomposites

Tensile properties (Tensile strength and Tensile modulus) of the cellulose microfiber reinforced PVA biocomposites were measured at room temperature (25°C) using universal testing machine (AG-X plus, Shimadzu, Japan) with 100 kN load and 15-20mm/min cross head speed. The specimen dimension was 40 mm x 22 mm x 0.1mm and at least 5 sample specimens for each set were tested as per ASTM D882-02 to get the average value.

Oxygen transfer rate analysis of the biocomposites

Oxygen transfer rates of the biocomposite films were measured using the oxygen permeation tester (8001 Oxygen Permeation Analyser, Illinois Instruments Co., USA). The flow rates of oxygen and nitrogen were 20 and 10 cm^3/min, respectively. The Oxygen transfer rate of the test samples were measured at the temperature of 23°C and a relative humidity of 50 ± 5 % according to ASTM D 3985 (ASTM 2010).

Biodegradability of the biocomposites using Soil burial test

Biodegradability of biocomposites were tested in garden soil and Municipal waste dump yard (MWDY) soil and the soil burial test methodology proposed by Othman et al (2011) and Tan et al (2016) was adopted. Soil samples were taken from a garden of nursery at Surathkal, Mangalore, India, and municipal dump yard at Vamanjoor, Mangalore, India. The garden soil contained the vermicompost mixed into it. The soil was sifted through a 2-mm mesh sieve and was transferred to pots of 17cm height. The soil was filled up to 14cm of the pot. The biocomposite film peeled from the petri plate was cut into four equal quadrants of approximately same weight and their weights were noted (C_1). Two quadrants were placed in each type of soil as duplicates. The films were buried in the soil in pot at a depth of 7cm. Four such pots were kept with duplicate samples for each soil type for determining the weight loss after week 1, week 2, week 3 and week 4. The pots were placed in the laboratory, and the moisture of the soil was maintained by sprinkling water at regular time intervals. The degradation of the samples was determined at regular time intervals (7 days) by carefully removing the sample designated for the corresponding week from the soil and drying in oven at 50ºC until consistent weight (C_2) was attained. The residual biocomposite films collected from the soil contained attached mud and sand particles. So, these films were then charred in muffle furnace at 600ºC for 30 min, the residue consisting of mud, stone and ash of the composites was measured (C_3) after cooling. Two quadrants of the biocomposite film samples taken for the test (before biodegradation) were separately charred in muffle furnace at 600ºC, in order to

get the weight of the ash in the composite (C_4). The weight of the sand and mud particles retained in the biocomposite film (C_5) remained after soil burial test was obtained by subtracting C_4 from C_3. The final weight of biocomposite film after biodegradation test was obtained by subtracting C_5 from C_2. The weight loss percentage of the sample over time was measured and reported as percentage biodegradation (Eq. 2) for biodegradability of the biocomposites.

$$Percentage\ biodegradation\ of\ the\ biocompiosite\ film = \frac{(C_1\text{-}(C_2\text{-}C_5\ )}{C_1} \times 100$$

Eq. (2)

CHAPTER 4

RESULTS AND DISCUSSION

Jatropha (*Jatropha Curcas L*) seed shell, Pongamia (*Pongamia pinnata*) seed hull and Finger millet (*Eleusine coracana L.*) straw are agricultural residues which are composed of lignocellulosic components; lignin, hemicellulose and cellulose mainly. Recently there have been considerable interest in reinforcement of natural fillers in polymer matrix. It is known that cellulose fibers are potential resource for reinforcement in the matrix of composites. Isolation of cellulose fibers from the matrix components, *viz.* lignin and hemicellulose is the first step towards the preparation of reinforcement. Generally, it has been reported that cellulose microfibers are isolated from lignin, hemicellulose and pectin by chemical, mechanical and biological treatments. Well established pure inorganic chemical methods involve the use of large quantity of hazardous and toxic chemicals, mechanical methods consume large amount of energy and biological process are comparatively slower. To overcome these drawbacks inorganic chemical treatments are combined with organic chemical, mechanical and /or biological treatments. Many researchers have also reported higher cellulose content in the isolated fibers when two or more of the mechanical, chemical and biological treatments are combined.

In the present work, cellulose microfibers are isolated and defibrillated from the lignocellulosic sources by the following methods:

I. Chemical methods:

Method O: Organosolv (Acetic acid and Nitric acid (catalyst)) treatment,

Method IO: Alkaline (sodium hydroxide and sodium chlorite) and organosolv (acetic acid and nitric acid (catalyst)) treatment,

II. Mechanical method:

Method U: Ultrasonication

III. Enzymatic method:

Method E: Enzymatic (endoglucanase) treatment

Further, these methods were combined as **Method OU; IOU; OE and IOE,** in order to effectively isolate and defibrillate the cellulose microfibers from Jatropha (*Jatropha Curcas L*) seed shell, Pongamia (*Pongamia pinnata*) seed hull and Finger millet (*Eleusine coracana L.*) straw. This chapter presents the experimental results and observation in the form of Tables and Figures. The interpretations and discussions of the results of the present study is compared with those reported in the literature. The observations presented are the average of the results obtained in duplicates.

4.1 Chemical composition of untreated fibers

The lignocellulosic sources: Jatropha seed shell, Pongamia seed hull, and Finger millet straw as collected from the respective sites were subjected to cleaning, grinding, sieving and drying before the isolation of cellulose microfibers to ensure that the samples are free from dust, mud, and other plant materials and the fibers are of uniform size distribution and moisture free. The fibers thus obtained after the preliminary treatment were stored in sealed polythene bags and used when required. These fibers are referred hereinafter as untreated fibers. Figure 4.1 shows the source material as obtained and the untreated fibers.

Plant materials are generally composed of extractives, holocellulose (hemicellulose and cellulose), lignin and inorganics (ash) (Rowell and Rowell, 1996). The chemical composition of untreated and isolated cellulose fibers in the present study are presented in terms of the concentration of alpha-cellulose, hemicellulose and lignin. The chemical composition of the untreated fibers was determined by methods prescribed by ASTM standards. The extractive free fibers of Jatropha seed shell, Pongamia seed hull and Finger millet straw were prepared according to ASTM D1105-96 method and the chemical composition of these untreated fibers in terms of acid insoluble lignin, hemicellulose, alpha-cellulose and ash percentage were analysed by ASTM standard methods and the mean values are presented in Table 4.1.

(a) (b) (c)

(d) (e) (f)

Figure 4.1: The lignocellulosic sources as collected from the site: a) Jatropha seed shell b) Pongamia seed hull c) Finger millet straw and the untreated fibers of d) Jatropha seed shell e) Pongamia seed shell f) Finger millet straw.

Table 4.1. Chemical composition (dry basis) of the untreated fibers of Jatropha seed shell, Pongamia seed hull and Finger millet straw.

Fiber (Untreated)	Composition in weight percentage in dry basis (%)			
	Lignin(L)	**Hemicellulose(H)**	**α-Cellulose (α-C)**	**Ash (A)**
Jatropha seed shell	21±0.91	22±1.11	41±0.76	8.0±0.64
Pongamia seed hull	24±0.64	21±0.64	42±0.76	6.6±0.37
Finger millet straw	12±0.50	26±0.64	50±0.86	7.3±0.22

Finger millet straw fibers are composed of higher composition of cellulose (50 wt%) and hemicellulose (26 wt%), whereas Jatropha seed shell and Pongamia seed hull have almost similar composition of the holocellulose (cellulose and hemicellulose). The lignin content in Finger millet straw fibers is the lowest and only half of that in the untreated fibers of Jatropha seed shell and Pongamia seed hull. The presence of lesser amount of lignin in Finger millet straw would lead to isolation of high concentration of cellulose fibers compared to Jatropha and Pongamia fibers. In general, the chemical composition varies from plant to plant, within different parts of plant and also varies with geographical, climate, soil and age conditions (Perez et al. 2002). Further the chemical composition of Jatropha seed shell, Pongamia seed hull and Finger millet straw are of similar range as many other agricultural residues presented in Table 2.1. in Section 2 which have been exploited for cellulose microfibers. This justifies the selection of lignocellulosic sources in the present study for exploiting their potential in isolation of cellulose microfibers which are further used for reinforcement in composites.

These untreated fibers of Jatropha seed shell, Pongamia seed hull and Finger millet straw were observed under Scanning electron microscopic (SEM) in order to ensure the presence of lignocellulosic components and also to confirm isolation of cellulose microfibers by comparing SEM images of fibers obtained after several chemical,

mechanical and enzymatic treatments in the whole isolation processes. Morphological features of the untreated fibers of Jatropha seed shell, Pongamia seed hull and Finger millet straw are presented in the SEM images shown in Figure 4.2. The images of untreated fibers of Jatropha seed shell, Pongamia seed hull and Finger millet straw showed irregular surface. Normand et al. (2014) have reported similar observation from images obtained for untreated fibers of bark of Norway spruce. Lamaming et al. (2015) have also reported irregularities in the untreated oil palm trunk fibers on observation under SEM. This irregular surface of all the lignocellulosic sources depicts that the cellulose as filler material is encrusted within hemicellulose, waxes, pectin and lignin (Kaliyan and Morey, 2009; Li et al. 2014; Ali et al. 2016). Untreated fibers of Jatropha seed shell and Pongamia seed hull show flaky, rough structures present on the surface, which could be due to the effect of grinding action (Tibolla et al. 2014) during pretreatment. However, untreated Finger millet straw fibers have not shown such a feature. The surface morphology of the untreated fibers shows that the cellulose fibers are bound by natural binder components such as lignin, waxes, pectin and hemicellulose and thus, they affect the processeability of the cellulose fibers (Reddy and Yang, 2004). Hence the goal of the isolation process is to extract the waxes and pectin, break down the lignin and hemicellulose structure and remove them, so that the chemicals, enzymes and ultrasonic energy can easily act on the cellulose resulting in isolation of cellulose microfibers with high cellulose concentration and also defibrillation of the cellulose fibers. As an initial step in isolation of cellulose microfibers, the untreated fibers were subjected to dewaxing using toluene-ethanol or benzene- toluene mixture as the solvents. It is a known fact that all-natural fibers contain waxes, fats, resins, oils, tannins and certain other ether-insoluble components which are not generally considered as part of the plant polymeric structure (ASTM D1105-96).

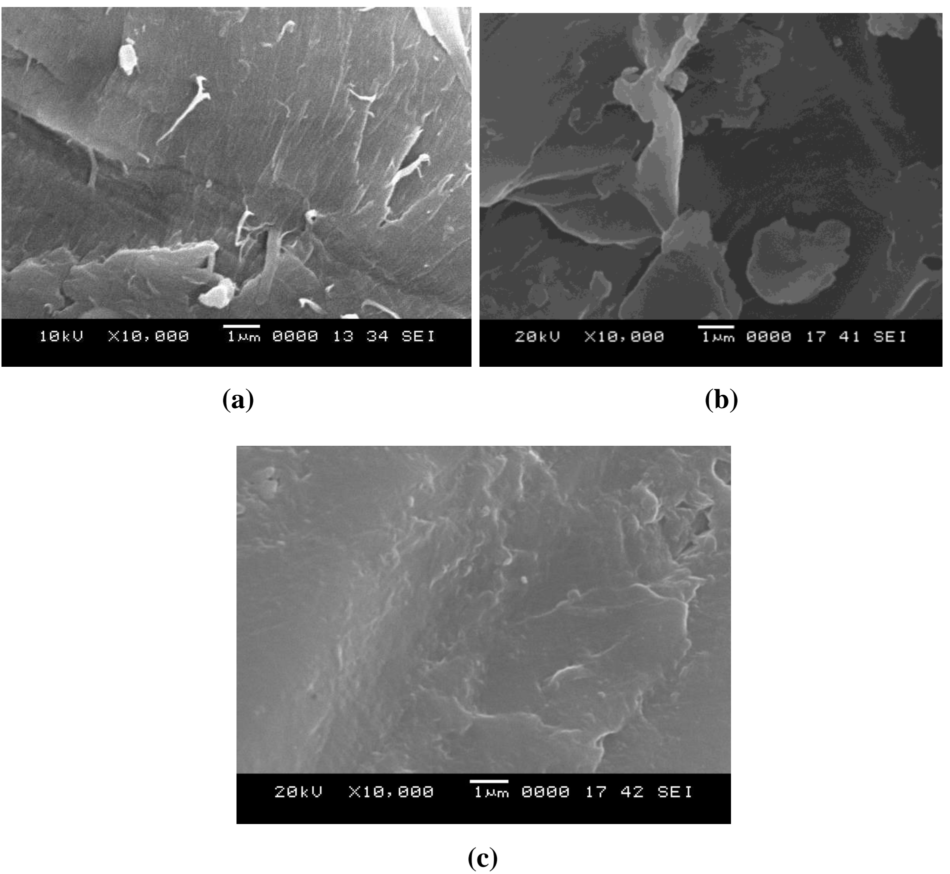


Figure.4.2: Scanning electron microscope images of untreated fibers of a) Jatropha seed shell b) Pongamia seed hull and c) Finger millet straw.

Extraction of waxes was carried out as described in Section 3. The images of dewaxed Jatropha seed shell, Pongamia seed hull and Finger millet straw fibers are presented in Figure 4.3. On comparison of the morphology of untreated Jatropha seed shell, Pongamia seed hull and Finger millet straw fibers as in Figure 4.2 with that of the dewaxed fibers in Figure 4.3, the surface of dewaxed fibers appeared to be peeled of the rough surface and it significantly differed from the surface of the untreated fibers. The removal of waxes has resulted in the surface roughness (Bismarck et al. 2001; Mani et al. 2012). This initial dewaxing treatment is essential in order to expose the hydroxyl groups of lignin, hemicellulose and cellulose to different chemical, mechanical and enzymatic treatments (Kalia et al. 2011) to be adopted further in the isolation process of cellulose microfibers

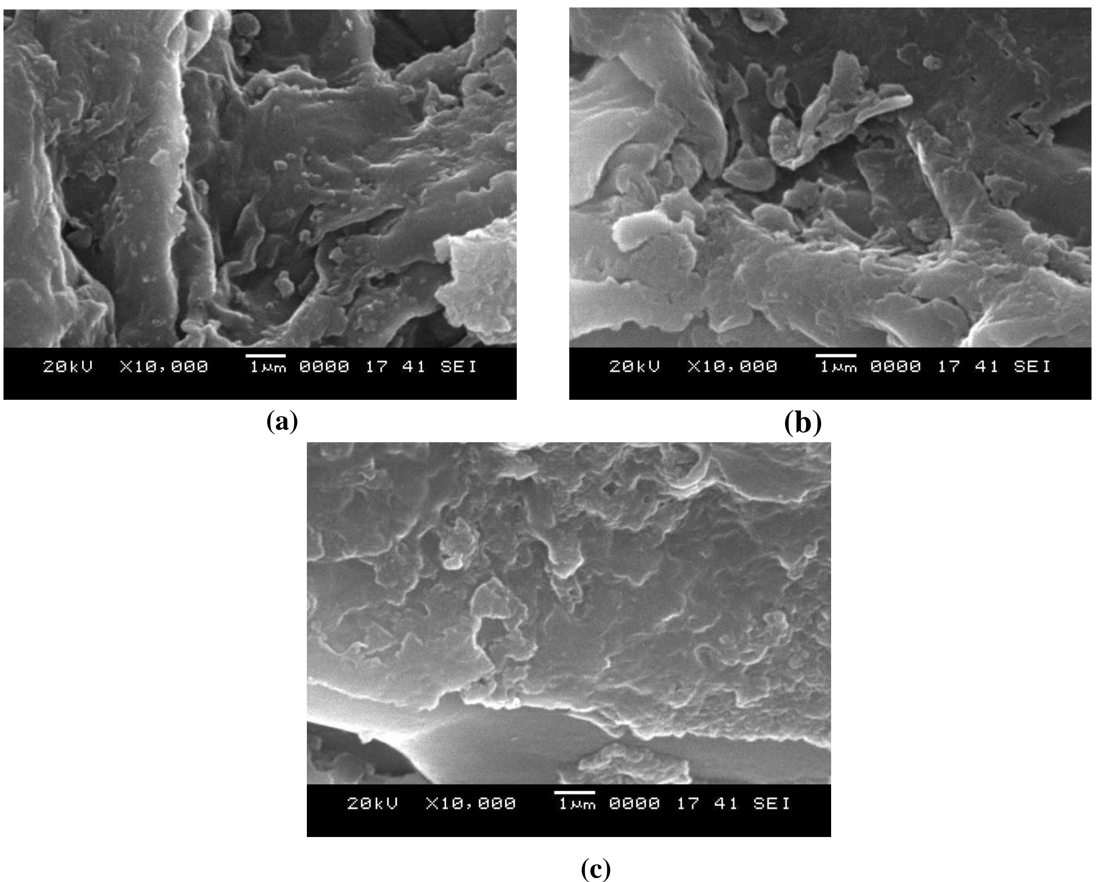


Figure.4.3. Scanning electron microscope images of dewaxed a) Jatropha seed shell b) Pongamia seed hull and c) Finger millet straw fibers.

4.2 Isolation of cellulose microfibers

As discussed earlier, lignin and hemicellulose bind the cellulose within the matrix by intra and inter molecular bonds. Removal of encrusting substances (lignin and hemicellulose) would ensure the isolation of high concentration cellulose fibers with rough surface (Normand et al. 2014; Reddy and Yang, 2015). The isolated cellulose fibers in turn self-assemble and aggregate to form microfibril structure, composing of crystalline and amorphous domains which are stabilized by hydrogen bonding between the hydroxyl groups (Eichhorn, 2010; Fernandes et al. 2011; Nishiyama, 2009). In order

to disintegrate the matrix components and isolate the cellulose microfibers, Jatropha seed shell, Pongamia seed hull and Finger millet straw were subjected to different isolation methods: O, IO, OU, IOU, OE, and IOE. The effect of various parameters in the isolation of cellulose microfibers was studied.

4.2.1 Isolation of cellulose microfibers by Organosolv treatment (O)

In the organosolv treatment, the temperature and time of acid treatment were considered to play a role in the cellulose isolation efficiency. The optimum temperature and time were chosen based on maximum concentration of cellulose which could be achieved in the isolated cellulose microfiber. The effect of acid treatment time in the range of 15 to 25 minutes and temperatures in the range of 100°C to 130°C, on the morphology and composition of cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by method **O** are presented in Table 4.2. –4.4 and Figure 4.4–4.6 respectively.

4.2.1.1 Effect of Acid treatment time on isolation of cellulose microfiber

It is clear from Tables 4.2. and 4.4 that the cellulose microfiber concentration has increased and matrix component (lignin and hemicellulose) concentration has decreased with increase in acid treatment time for cellulose microfibers isolated from Jatropha seed shell and Finger millet straw. However, the cellulose concentration of cellulose fibers isolated from Pongamia seed hull as presented in Table 4.3, has increased with increase in acid treatment time up to 20 minutes and further increase in acid treatment time has resulted in decreased cellulose concentration. The reduction in lignin and hemicellulose concentration and an increase in cellulose concentration in cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw with increase in acid treatment time can be accounted to the effect of longer exposure of fibers to acetic acid which promotes higher solvation of lignin (Young and Davis, 1991; Davis and Young, 1991a).

It has been reported by Aittamaa and Sundquist, (2000) that more of higher molecular weight lignin can be separated using high concentration acetic acid (70-85%) as compared to that using low concentration acetic acid (33-43%). This implies that the

acetic acid concentration of 80% incorporated in isolation of lignin in the present study does not result in separation of low molecular weight lignin. Strong acid and alkalis are potential enough to disturb the strong hydrogen bonding between the cellulose molecules and also in dissolution of amorphous region of the cellulose chain (Owalabi et al. 2016).

Table 4.2: The chemical composition (lignin (L), Hemicellulose (H) and alpha cellulose (α-C)) of the isolated cellulose microfibers from Jatropha seed shell fibers after treatment by Method O, at different acid treatment time (t) and temperature (T).

t (min)	T (°C)	Composition of cellulose fiber in wt % (dry basis)		
		L	H	α-C
15	100	12 ±0.99	10 ±0.24	75 ±0.49
	120	12 ±1.24	10 ±0.49	75 ±0.99
	130	12 ±0.49	10 ±0.75	73 ±0.75
20	100	10 ±1.24	11 ±1.24	78 ±0.99
	120	10 ±0.49	8 ±0.99	79 ±1.24
	130	10 ±0.99	11 ±0.49	78 ±1.24

Table 4.2 contd….

t (min)	T (°C)	Composition of cellulose fiber in wt % (dry basis)		
		L	H	α-C
25	100	10 ±0.49	9 ±1.24	80 ±0.99
	120	**8** ±0.75	**8** ±0.24	**82** ±0.49
	130	8 ±0.24	9 ±1.24	80 ±0.75

Table 4.3: The chemical composition (lignin (L), Hemicellulose (H) and alpha cellulose (α-C)) of the isolated cellulose microfibers from Pongamia seed hull fibers after treatment by Method O, at different acid treatment time (t) and temperature (T).

t (min)	T (°C)	Composition of cellulose fiber in dry wt %		
		L	H	α-C
15	100	6±0.49	10±1.24	81±0.99
	120	5±0.75	9±0.99	82±1.24
	130	5±0.24	10±0.49	80±1.24

Table 4.3 contd….

t (min)	**T (°C)**	**Composition of cellulose fiber in wt % (dry basis)**		
		L	**H**	**α-C**
20	100	4±1.24	10±0.75	83±0.24
	120	**4**±0.99	**9**±0.49	**85**±0.99
	130	5±1.24	10±0.99	81±075
25	100	5±0.49	10±0.24	80±0.99
	120	5±0.24	10±1.24	82±0.49
	130	5±0.75	11±0.49	80±1.24

In the present study 80% acetic acid and 70% nitric acid have been used. The hydronium ions released during the interaction of acetic acid in water, disturb the chain in amorphous region by penetrating through them and leading to hydrolytic cleavage of glyosidic bonds and isolation of crystalline cellulose (Tischer et al. 2010). Acetic acid dissolves all of the isolated lignin and partial amount of hemicellulose. However, cellulose is partially degraded but not dissolved in the acid (Hult et al. 2000). Though increase in treatment time attributes to increase in removal of lignin and hemicellulose, it may also adversely result in increase in cellulose degradation.

Table 4.4 The chemical composition (lignin (L), Hemicellulose (H) and alpha cellulose (α-C)) of the isolated cellulose microfibers from Finger millet straw fibers after treatment by Method O, at different acid treatment time (t) and temperature (T).

t , (min)	T(°C)	Composition of cellulose fiber in dry wt %		
		L	H	α-C
15	100	8 ±0.75	10 ±0.99	78 ±0.49
	120	8 ±1.24	10 ±0.24	78 ±0.99
	130	8 ±0.49	11 ±1.24	78 ±0.75
20	100	7 ±0.99	10 ±0.49	80 ±0.24
	120	6 ±0.49	10 ±0.99	81 ±1.24
	130	6 ±1.24	10 ±0.49	80 ±0.75
25	100	4 ±0.24	10 ±0.99	82 ±0.49
	120	**2** ±0.75	**9** ±1.24	**84** ±0.99
	130	3 ±0.49	10 ±0.75	83 ±0.24

Xu et al. (2005) reported that the isolated cellulose when exposed to acid for long exposure time leads to cellulose degradation of 15%. Thus, there exist an optimum treatment time up to which the delignification effect dominates and after which the cellulose degradation effect dominates. Thus, the optimum acid treatment time is 25 min for cellulose fiber isolation from Jatropha seed shells and Finger millet straw fibers, whereas the optimum time for cellulose fiber isolation from Pongamia seed hulls is 20 min at all the treatment temperatures studied.

4.2.1.2 Effect of acid treatment temperature on isolation of cellulose microfiber

Table 4.2 to 4.4 also show the effect of treatment temperature on the isolated fiber composition. The cellulose content has marginally increased as the treatment temperature increased from 100°C to 120°C due to increase in the rate of removal of lignin and hemicellulose with increase in temperature. However, further increase in temperature to 130°C results in increase in simultaneous cellulose degradation rate, thus leading to lowering of cellulose content at 130°C. Sun et al. (2004) in their studies on isolation of cellulose fibers from wheat straw, have reported that the acetic acid (80%) and nitric acid (70%) in the organosolv process at a temperature of 120°C and 15 min, leads to degradation of original cellulose in the range 3.1-5.4%. Singh et al. (2000) have reported that nitric acid not only inhibits oxidation of cellulose but also aid in partial degradation of cellulose. Similarly, Gert et al. (2009) have studied the effect of nitric acid on cellulose fibers and have concluded that with nitric acid (68.5%) treatment time of 1h and temperature of 20°C, depolymerisation of amorphous cellulose takes place due to hydrolysing, nitrating and oxidizing effect of nitric acid accompanied with slow degradation of cellulose. The effect of bleaching action of nitric acid on cellulose fibers can also be playing a vital role in reduction of cellulose (Xu et al. 2005). These reports support the observation made in the present study on cellulose degradation by the acids. The increase in temperature has dual effects of increase in the rate of cellulose isolation and the rate of cellulose degradation. If the increase in temperature, increases the rate of cellulose isolation to a greater extent than the increase in rate of cellulose degradation, then the net result would be the increase in cellulose concentration in the isolated fiber

with increase in temperature. But under conditions when increase in the rate of degradation of cellulose with temperature is higher than the increase in the rate of isolation, then the cellulose concentration decreases with increase in temperature. Thus, the optimum temperature is governed by these two rates. It is observed that the cellulose concentration has been reduced in the range of 0-4% with increase in temperature of acid treatment from 120°C to 130°C. Thus, 120°C can be considered as the optimum temperature for the isolation of cellulose fibres from all the three lignocellulose sources.

The chemical composition of isolated cellulose fibers from Jatropha seed shell, Pongamia seed hull and Finger millet straw at optimized acid treatment time and temperature are presented in Table 4.5. The maximum amount of cellulose concentration in cellulose microfibers from Jatropha seed shell is 82% and from Finger millet straw is 84% at the optimum acid treatment time of 25 minutes and optimum temperature of 120°C; whereas the cellulose concentration in cellulose microfibers isolated from Pongamia seed hull was found to be 85% at the optimum acid treatment time of 20 minutes and optimum temperature of 120°C. Further the cellulose composition of cellulose microfiber in the present work has been increased by around 40-50% as compared to that in untreated fibers by the organosolv process under optimum conditions. As this is a one-step method, the loss of cellulose may be limited (Brendel et al. 2000) and thus may lead to higher yield of cellulose.

It is found that the optimum temperature and acid treatment time may vary with the type of lignocellulosic source used for the isolation of cellulose microfibres. The acetic acid treatment time and temperature play a vital role in isolation of cellulose microfibers. The information from the earlier literature reports on cellulose isolation by organosolv method, suggest that optimum acid treatment time and temperatures depend on the type of lignocellulosic sources used for the isolation. For hard wood, the acid treatment temperature has been reported as maximum of 110-220°C and acid treatment time of 360-60 min (Vazquez et al. 1992; Young et al. 1986) and for soft wood the values are 119°C-124°C and 15-20 min (Anchukaitis, 2007; Brendel et al. 2000; Sun et al. 2004),

respectively. The optimum temperature and acid treatment time obtained in the present work falls in the range of the optimum values reported for the soft wood. From these studies, it can be concluded that the acid treatment time and temperature influence the efficiency of cellulose isolation by organosolv process.

Table 4.5 The chemical composition of the isolated cellulose microfibers from Jatropha seed shell Pongamia seed hull and Finger millet straw fibers after treatment by Method O, at optimized acid treatment time (t), temperature (T).

Lignocellulosic source	**t (min)**	**T (°C)**	**Composition of cellulose fiber in dry wt %**		
			L	**H**	**α-C**
Jatropha seed shell	25	**120**	**8** ±0.75	**8** ±0.24	**82** ±0.49
Pongamia seed hull	20		**4**±0.99	**9**±0.49	**85**±0.99
Finger millet straw	25		**2** ±0.75	**9** ±1.24	**84** ±0.99

4.2.1.3 Effect of acid treatment time and temperature on the morphology of the cellulose microfibers

The SEM images of isolated cellulose microfibers from Jatropha seed shell, Pongamia seed hull and Finger millet straw treated by method O at different acid treatment time and acid treatment temperature are presented in Figure 4.4- 4.6.

On comparison of the SEM images of untreated fibers presented in Figure 4.2 with the images presented in Figure 4.4- 4.6, it is observed that the isolated cellulose microfibers have undergone morphological changes showing prominent cellulose fiber bundles

emerging out from the matrix as the acid treatment time and temperature increases. It confirms the isolation of cellulose microfibers from all the three-lignocellulosic source by organosolv treatment, which has aided in removal of most of the binding substances such as lignin and hemicellulose from the original untreated fibers (Pan et al. 1999a, 1999b).

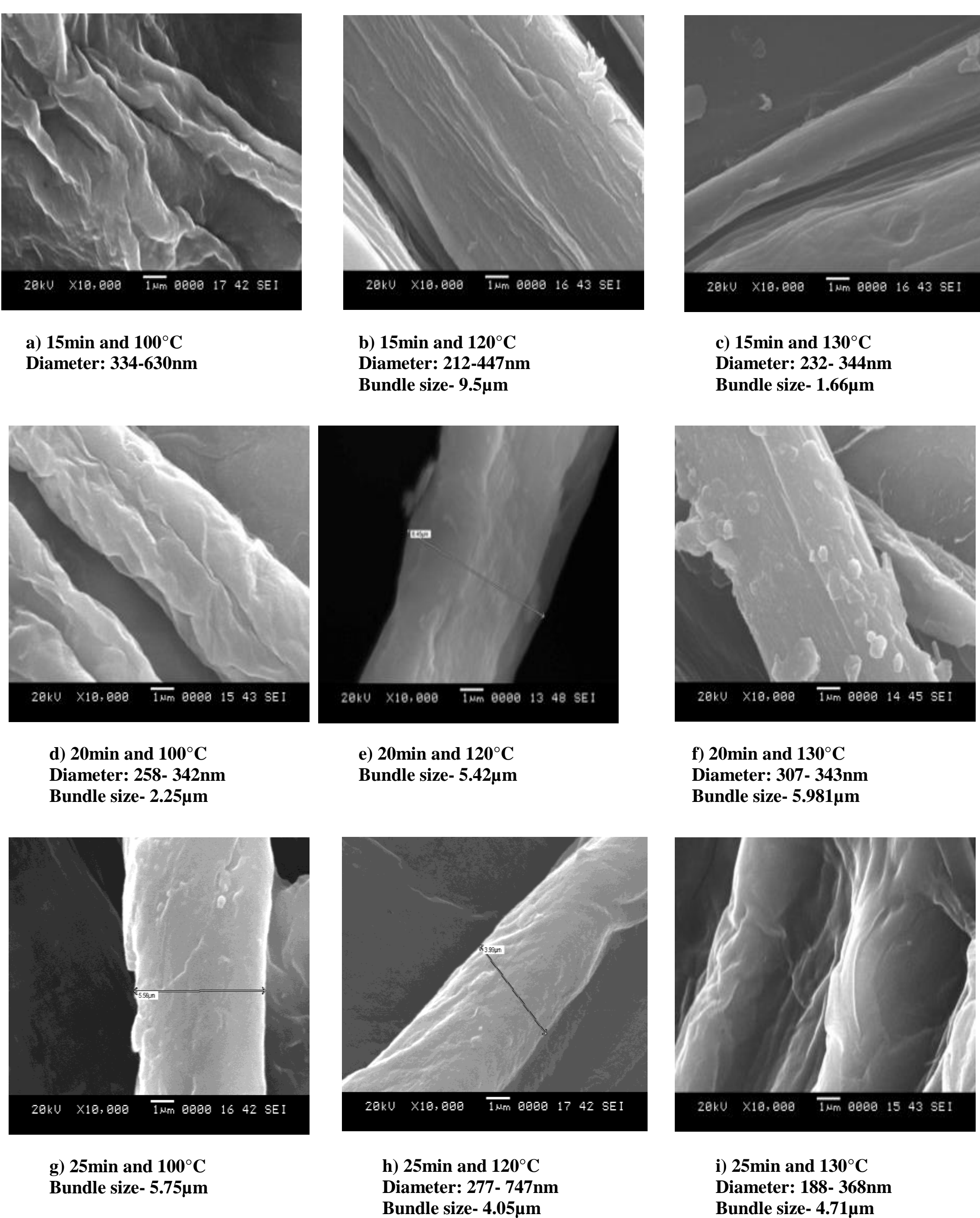
The comparison of SEM images of untreated fibers in Fig.4.2, with those of the isolated fibers from Jatropha seed shell, Pongamia seed hull and Finger millet straw treated by method **O** as shown in Fig. 4.4-4.6, show that the cellulose microfibers with the lowest lignin and hemicellulose concentration are of smallest diameter. The isolated cellulose microfibers from Jatropha seed shell, Pongamia seed hull and Finger millet straw at optimum acid treatment temperature and time consist of small diameter fibers of size 277nm, 319nm and 329nm respectively. In case of cellulose microfibers isolated from Jatropha seed shell and Pongamia seed hull, these small microfibers have further agglomerated to form microfiber bundles of 4.05 µm and 9.24 µm respectively. In case of cellulose microfibers isolated from Finger millet straw, defined bundle of microfibrils are not apparent. However, they show the emergence of small microfibrils from the matrix. High concentration of cellulose, hydrophilic nature and propensity of cellulose fibers to agglomerate has resulted in microfibrils bundle which are made up of smaller size microfibers (Frone et al. 2011; Peng et al. 2011; Agustin et al. 2014). These smaller size fibers are bound by hydrogen bonds between themselves and result in larger size cellulose microfiber aggregate (Liu et al. 2010; Nasri-Nasrabadi et al. 2014).

In case of cellulose fibers isolated from Jatropha seed shell (Table 4.2), it is observed that with acid treatment time of 15min, the variation in cellulose concentration with temperature in the range of 100°C to 130°C, is marginal. But the morphology of the cellulose fibers (as shown in Figure 4.4) varied from one another. At acid treatment temperature of 100°C, cellulose microfibers have gained rough surface with prominent cellulose microfibers having diameter ranging from 334- 630 nm, whereas at 120°C cellulose fibers were seen to be agglomerated to form a bundle of diameter around 9.5 µm with a few prominent cellulose microfibers of diameter ranging from 212-447 nm

protruding at the surface of cellulose fiber bundle. At acid treatment temperature of 130°C, prominent cellulose fiber bundle of 1.66 μm diameter is clearly observed. However, a few distinct cellulose fibers with diameter of 232-344 nm are seen emerging, which implies that they have been isolated.

For acid treatment time of 20 min and temperature 100°C, 120°C and 130°C the cellulose fibers have been observed to be bundles of size 2.25, 5.42 and 5.981 μm respectively. The presence of cellulose microfibers on the surface of cellulose fiber bundles is not clearly observed for acid treatment temperature of 120°C and 130°C. But the cellulose fibers were seen compactly bound along the fiber bundle with smallest cellulose fiber of size 258-342 nm and 307-343 nm for acid treatment temperature of 100°C and 130°C respectively. Further for acid treatment time of 25 min and acid treatment temperatures of 100, 120 and 130°C, the cellulose fiber bundle of size 5.75, 4.05 and 4.71 μm were prominent enough with smallest cellulose fibers having diameter of 277 and 188 nm for acid treatment temperatures of 120 and 130°C respectively. The size of isolated cellulose fiber bundle.

Considering the morphological changes in cellulose fibers isolated from Pongamia seed hull as presented in the Figure 4.5, it's clear that cellulose microfibers are seen running through the length of the fiber surface. The cellulose microfibers are observed to be prominent and the bundles have reduced to size of 9.24 μm as the treatment time increased from 15 min to the optimized treatment time of 20 min at the temperature of 120°C. However, with the same acid treatment time of 20min when the treatment was done at 130°C, the cellulose fibers are isolated as large fibers of size around 2.84μm.



a) 15min and 100°C
Diameter: 334-630nm

b) 15min and 120°C
Diameter: 212-447nm
Bundle size- 9.5μm

c) 15min and 130°C
Diameter: 232- 344nm
Bundle size- 1.66μm

d) 20min and 100°C
Diameter: 258- 342nm
Bundle size- 2.25μm

e) 20min and 120°C
Bundle size- 5.42μm

f) 20min and 130°C
Diameter: 307- 343nm
Bundle size- 5.981μm

g) 25min and 100°C
Bundle size- 5.75μm

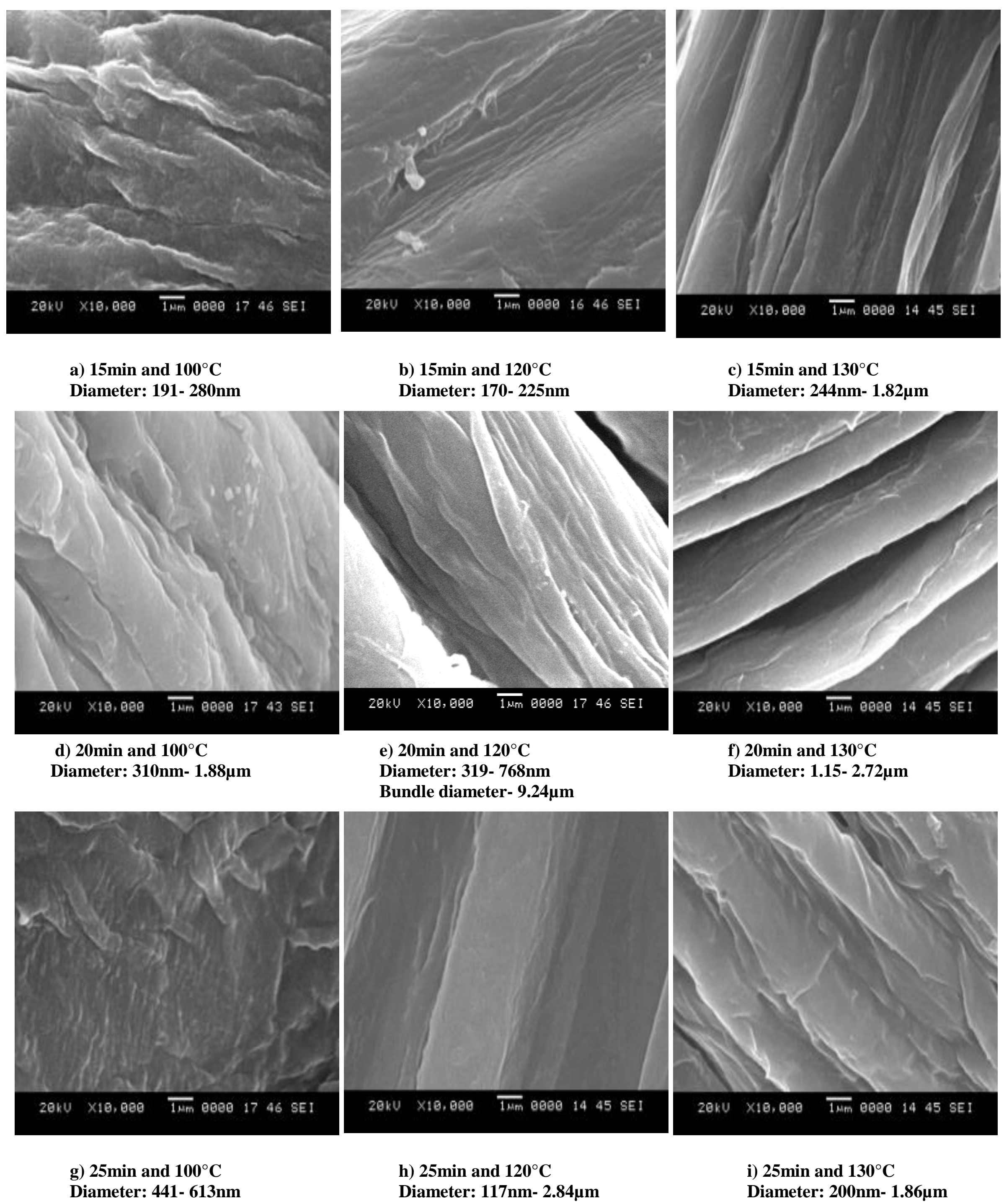
h) 25min and 120°C
Diameter: 277- 747nm
Bundle size- 4.05μm

i) 25min and 130°C
Diameter: 188- 368nm
Bundle size- 4.71μm

Figure 4.4 Scanning electron microscope images of cellulose fibers isolated from Jatropha seed shell after treatment by Method O, at different acid treatment time and temperature.

Further, it is observed that cellulose microfibers have agglomerated when the acid treatment time was increased to 25min irrespective of the acid treatment temperature adopted. The SEM images have shown that the smallest cellulose microfibers obtained with acid treatment time of 25 min and temperatures of 100°C, 120°C and 130°C are of diameter 441 nm, 117 nm and 200 nm respectively. However, for optimum treatment time of 20min and temperature 120°C the diameter of the smallest cellulose fiber was observed to be 319m with prominent cellulose fiber bundles emerging from the surface.

In case of cellulose microfibers isolated from Finger millet straw by organosolv treatment the morphological changes observed for different acid treatment time and temperature are presented in Figure 4.6. As the acid treatment time increases the cellulose microfibers appear to become more distinct and predominant. For acid treatment time of 15min and temperature of 100, 120 and 130°C, the cellulose microfibers are seen agglomerated into bundles with smallest cellulose microfibers having diameter of 179, 235 and 212 nm respectively. The cellulose microfibers are comparatively more visible, almost uniform with their boundaries seen isolated from the matrix more clearly in the images obtained for acid treatment time of 20min and temperature of 100, 120 and 130°C. The size of the smallest diameter cellulose microfibers increased with increase in acid treatment temperature i.e., 150, 118 and 385 nm for treatment temperatures of 100, 120 and 130°C respectively. Further, increase in treatment time to 25min has led to the formation of the distinct cellulose microfibers of smallest diameters 391, 329 and 221 nm on isolation at treatment temperature of 100, 120 and 130°C respectively. The cellulose fibers are of non-uniform structure with rough surface compared to that obtained with treatment time of 20 min. These morphological changes observed in cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by organosolv treatment method (O) indicate that organosolv used in the isolation process along with the treatment conditions such as acid treatment time and temperature have an influence on final morphologies of the cellulose microfibers. It is also clear that the strong intermolecular bond and hydrophilic interaction between the cellulose chains plays a vital role in bringing about the morphological changes (Frone et al. 2011; Xiao et al. 2015).



a) 15min and 100°C
Diameter: 191- 280nm

b) 15min and 120°C
Diameter: 170- 225nm

c) 15min and 130°C
Diameter: 244nm- 1.82µm

d) 20min and 100°C
Diameter: 310nm- 1.88µm

e) 20min and 120°C
Diameter: 319- 768nm
Bundle diameter- 9.24µm

f) 20min and 130°C
Diameter: 1.15- 2.72µm

g) 25min and 100°C
Diameter: 441- 613nm

h) 25min and 120°C
Diameter: 117nm- 2.84µm

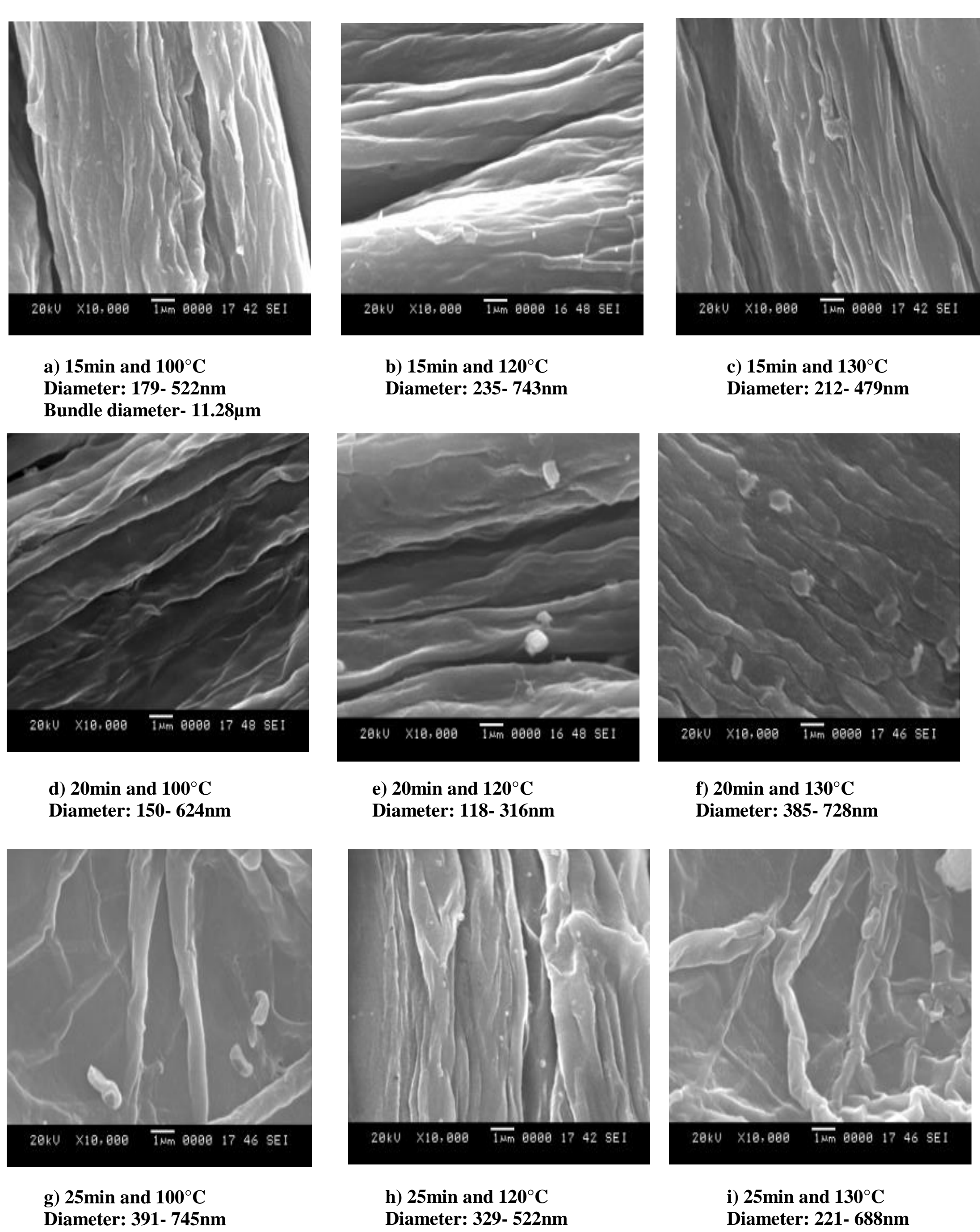
i) 25min and 130°C
Diameter: 200nm- 1.86µm

Figure 4.5 Scanning electron microscope images of cellulose fibers isolated from Pongamia seed hull after treatment by Method O, at different acid treatment time and temperature.

Cellulose chains agglomerate by both inter and intra molecular bonds. The cellulose chains are arranged in parallel by OH-O hydrogen bond between the cellulose molecule to form sheets of cellulose chains, these sheets are further stacked up by weak CH-O hydrogen bonds (Nishiyama et al. 2013). When cellulose microfibers are dispersed in water, the weak CH-O bonds are broken due to the effect of electron cloud resulting from water molecules at the surface of glucosidic bonds of cellulose. This results in polarization and weak hydrogen bonds (CH-O) are broken enabling dispersion of cellulose chain in water (Khazraji and Robert, 2013). The hydroxyl group present at the equatorial positions of glucopyranose rings in the cellulose molecule are the reason for the hydrophilic behaviour of cellulose (Yamane et al. 2006).

As the acid treatment temperature increases, it is observed that the morphology of cellulose microfibers changes drastically even though the composition of the cellulose microfibers is almost similar. This could be due to the effect of acid treatment at higher temperatures which disrupt the lignin, hemicellulose and also cellulose structure. As observed from the Tables 4.1- 4.5 the cellulose concentration of isolated cellulose microfibres from Jatropha seed shell, Pongamia seed hull and Finger millet straw has increased from 41, 42 and 50 % to 82, 85 and 84% respectively after organosolv treatment.

From the SEM images in Figure 4.4 – 4.6 its observed that the cellulose fiber bundles isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by treatment method **O** consists of smaller cellulose microfibers which are bonded with each other. Further fibrillation and reduction in size of these cellulose microfiber have been reported to be beneficial in fiber-matrix adhesion in biocomposites, as the available surface area increases for fiber matrix interaction (Petersson and Oksman, 2006; Lee, 2006; Wang and Sain, 2007; Dong and Roman, 2007; Hubbe et al. 2008; Kamel, 2009; Frone et al. 2011). The reduction in size and fibrillation of cellulose microfibers can be achieved by breaking the glyosidic bonds by ultrasonication and enzymatic treatment (Filson and Dawson-Andoh, 2009; Pandey et al. 2009; Sumari et al. 2013; Hafiz et al. 2014; Khalil et al. 2014; Tang et al. 2015; Santucci et al. 2016).



a) 15min and 100°C
Diameter: 179- 522nm
Bundle diameter- 11.28µm

b) 15min and 120°C
Diameter: 235- 743nm

c) 15min and 130°C
Diameter: 212- 479nm

d) 20min and 100°C
Diameter: 150- 624nm

e) 20min and 120°C
Diameter: 118- 316nm

f) 20min and 130°C
Diameter: 385- 728nm

g) 25min and 100°C
Diameter: 391- 745nm

h) 25min and 120°C
Diameter: 329- 522nm

i) 25min and 130°C
Diameter: 221- 688nm

Figure 4.6 Scanning electron microscope images of cellulose fibers isolated from Finger millet straw after treatment by Method O, at different acid treatment time and temperature.

In case of ultrasonic treatment (Method **U**) the hydrogen bond between the cellulose microfibers is confronted by cavitation energy (10–100 kJ/mol) generated by the ultrasound energy which leads to loosening of the cellulose surface by reducing the cohesion between the microfibrils (Cheng et al. 2007, 2009, 2010; Zhao et al. 2007; Wang and Cheng, 2009; Westfahl and Tischer, 2010; Li et al. 2011; Chen et al. 2011). Similarly, the enzyme Endoglucanase**,** is the most active enzyme which breaks glucosidic bonds of cellulose microfibers to weaken the microfibrils surface assisting microfibrils to become thinner (Manley et al.1964; White et al. 1981; Liu et al. 2009). Thus, the cellulose microfibers isolated by method **O** are further subjected to ultrasonication treatment method **U** and enzymatic treatment Method **E** for defibrillation of the cellulose microfiber. The two step fibrillation methods with combination of organosolv treatment method **O** with ultrasonication or enzymatic treatment are referred herein after as method **OU** and method **OE** respectively.

4.2.2 Isolation by cellulose microfiber by Method OU

The fibers isolated by Method O with different acid treatment time and treatment temperature were further subjected to ultrasonication treatment. The effect of ultrasonication time on the morphology and composition of cellulose microfibers isolated by method **OU** from Jatropha seed shell, Pongamia seed hull and Finger millet straw are presented in Table 4.6–4.8 respectively. The SEM images of the cellulose microfibers isolated by method **OU** at the optimum ultrasonication treatment time with respect to the highest cellulose concentration least lignin and hemicellulose concentration under each acid treatment time and temperature, are also presented in Table 4.6 – 4.8.

4.2.2.1 Effect of ultrasonication on isolation of cellulose microfiber

From Table 4.6-4.8, it is observed that the cellulose microfibers isolated from Jatropha seed shell fibers, Pongamia seed hull and Finger millet straw by treatment method **O** when subjected to ultrasonication treatment (Method **U**) with 300 kJ, 375 kJ and 450 kJ energy for 20, 25 and 30 minutes of ultrasonication time, further isolation of cellulose

microfibers has been brought about with maximum increase in cellulose composition by 3%. Rincon et al. (2016) and Fernández-Cegrí et al. (2012) have also reported increase in cellulose concentration during isolation of cellulose from olive mill solid waste and sunflower oil cake when subjected to ultrasonication treatment. This is due to disruption of bonds between hemicellulose, lignin and cellulose by ultrasonic energy (Bussemaker et al. 2013; Sulman et al. 2011).

At a given condition, different lignocellulosic sources react differently to the ultrasonic treatment as the structure and digestion ceiling of lignocellulosic sources vary with the variation in their composition (Madeleine and Dongke 2013). The pressure produced by ultrasonic waves in the solution accelerate both mechanoacoustic and sonochemical processes. Both of these physical and chemical processes result in isolation, depolymerisation and also degradation of cellulose and hemicellulose (Pawongrat et al. 2016). However, release of loosely bound hemicellulose and amorphous cellulose from the surface of crystalline cellulose by the effect of ultrasonic waves would also result in changes in cellulose concentration.

The microfibers which were isolated by Method O under optimum acid treatment time and temperature when subjected to ultrasonication yielded the maximum cellulose concentration and are presented in Table 4.9. In the isolation of cellulose microfibers from Jatropha seed shell, highest concentration of cellulose has been observed to be 84% when the fibers were subjected to method OU with the optimum acid treatment time of 25 min, temperature of 120°C and ultrasonication time of 20min, with highest reduction in hemicellulose and lignin by 2% and increase in cellulose concentration by 2% on the basis of those isolated by Method O. For cellulose microfibers isolated from Pongamia seed hull, the optimum acid treatment time of 20min, acid treatment temperature of 120°C and ultrasonication time of 20min has resulted in increase in cellulose concentration to 86% and reduction in hemicellulose and lignin concentration.

Similarly, for cellulose microfibers isolated from Finger millet straw fibers have highest cellulose content of 86% with no change in hemicellulose and cellulose concentration for optimum acid treatment time of 25 min, acid treatment temperature of 120°C and ultrasonication time of 25 min.

4.2.2.2 Effect of ultrasonication on morphology of cellulose microfiber

The SEM images of cellulose microfibers isolated from Jatropha seed shell fibers, Pongamia seed hull and Finger millet straw by treatment method **OU** are presented in Tables 4.6- 4.8. The isolated cellulose microfibers by method **O** when further subjected to ultrasonic treatment, (Method **OU),** have shown prominent morphological changes with the cellulose microfibers of smaller size emerging out from the matrix component with rough surface, but arranged in similar orientation. In the images presented in Tables 4.6–4.8, it is clear that the microfiber bundles are defibrillated/ reduced in size when compared to images of the fibers isolated by only Method O presented in Figures 4.4-4.6. This is due to the action of cavitation which results in collision of high velocity micro gas bubbles at the cellulose fiber surface leading to reduction of cohesion between the microfibrils (Li et al. 2011). The diameter of smallest cellulose microfiber isolated from the Jatropha seed shell fibers, Pongamia seed hull and Finger millet straw under different acid treatment time and acid treatment temperature conditions and with the optimum ultrasonication time are presented in Tables 4.6-4.8 respectively. The optimum ultrasonication time is taken as that which yielded highest cellulose concentration and the lowest lignin and hemicellulose content.

Table 4.6. The chemical composition of the isolated cellulose microfibers from Jatropha seed shell fibers after treatment by Method OU, at different acid treatment time (t), temperature (T), and ultrasonication time (t_u) with SEM images for optimum sonication time and range of diameter of cellulose microfiber (D)(nm) defibrillated with SEM images and increase in percentage of cellulose concentration for optimum sonication time.

t (min)	T (°C)	t_u (min)	Composition of cellulose fiber in dry basis wt %			SEM images and D (nm)	Increase in α-C%
			L	H	α-C		
		20	**11 ±0.24**	**9 ±0.99**	**77 ±0.49**		
	100	25	11 ±0.99	9 ±075	77 ±0.24		2
		30	10 ±1.24	9 ±0.49	77 ±0.75	255-693	
		20	**10 ±0.49**	**9 ±0.24**	**78 ±1.24**		
15	120	25	10 ±0.75	9 ±1.24	77 ±0.99		3
		30	10 ±0.99	9 ±0.75	78 ±0.49	219-829	
		20	10 ±1.24	7 ±0.49	75 ±0.24		
	130	**25**	**10 ±0.49**	**6 ±0.99**	**75 ±0.75**		2
		30	10 ±0.24	6 ±1.24	76 ±.99	219-757	

Table 4.6. contd…..

t (min)	T (°C)	t_u (min)	Composition of cellulose fiber in dry basis wt %			SEM images and D (nm)	Increase in α-C%
			L	H	α-C		
20	100	20	9 ±0.75	9 ±0.24	79 ±1.24	198-414	2
		25	**9 ±0.49**	**8 ±0.99**	**80 ±0.24**		
		30	9 ±0.99	8 ±0.24	79 ±0.75		
	120	**20**	**9 ±1.24**	**8 ±0.75**	**79 ±0.49**	140-336	0
		25	9 ±0.24	8 ±0.49	79 ±1.24		
		30	9 ±0.75	8 ±1.24	79 ±0.99		
	130	**20**	**9 ±0.99**	**10 ±0.24**	**78 ±1.24**	301-1.68μm	0
		25	9 ±0.49	10 ±0.75	78 ±0.24		
		30	9 ±0.24	10 ±0.49	78 ±0.99		

Table 4.6. contd…..

t (min)	T (˚C)	t_u (min)	Composition of cellulose fiber in dry basis wt %			SEM images and D (nm)	Increase in α-C%
			L	H	α-C		
25	100	20	8 ±1.24	7 ±0.99	80 ±0.75	122-347	3
		25	7 ±0.75	8 ±1.24	82 ±0.49		
		30	**7 ±0.24**	**7 ±0.49**	**83 ±0.99**		
	120	**20**	**6** ±0.75	**6** ±1.24	**84** ±0.49	231-468	2
		25	6 ±0.49	7 ±0.99	84 ±1.24		
		30	6 ±1.24	6 ±0.75	83 ±0.24		
	130	**20**	**8 ±0.99**	**6 ±0.24**	**80 ±0.75**	413-448	0
		25	7 ±0.49	7 ±0.75	80 ±1.24		
		30	7 ±0.24	6 ±0.99	79 ±0.49		

Table 4.7. The chemical composition and SEM images of the isolated cellulose microfibers from Pongamia seed hull fibers after treatment by Method OU, at different acid treatment time (t), temperature (T), and ultrasonication time (t_u) with SEM images for optimum sonication time (D)(nm) defibrillated with SEM images and increase in percentage of cellulose concentration for optimum sonication time (I α-C%).

t (min)	T (°C)	t_u (min)	Composition of cellulose fiber in dry basis wt %			SEM images and D (nm)	Increase in α-C%
			L	H	α-C		
15	100	20	5 ±0.99	9 ±0.24	83±1.24	425-447	3
		25	**5 ±0.75**	**8 ±0.49**	**84 ±0.24**		
		30	5 ±0.49	9 ±1.24	84 ±0.99		
	120	20	4 ±1.24	9 ±0.24	84 ±0.75	207-859	3
		25	4 ±0.24	9 ±0.75	84 ±0.49		
		30	**4 ±0.99**	**8 ±0.24**	**85 ±0.75**		
	130	**20**	**4±0.24**	**8±0.49**	**84±1.24**	334-845	3
		25	4 ±1.24	8 ±0.75	84 ±0.99		
		30	4 ±0.49	8 ±1.24	84 ±0.24		

Table 4.7. contd…..

t (min)	T (˚C)	t_u (min)	Composition of cellulose fiber in dry basis wt %			SEM images and D (nm)	Increase in α-C%
			L	H	α-C		
20	100	20	4±0.75	8 ±0.99	83 ±0.49	314-1.36 µm	3
		25	**4 ±0.99**	**8 ±0.24**	**84 ±075**		
		30	4 ±0.49	8 ±0.75	84 ±1.49		
	120	**20**	**3** ±0.75	**8** ±0.99	**86** ±0.49	251-614	3
		25	3 ±0.24	8 ±1.24	86 ±0.99		
		30	3 ±1.24	8 ±0.49	86 ±0.24		
	130	20	4 ±0.75	10 ±0.99	81 ±0.49	619-1.93 µm	3
		25	4 ±0.49	10 ±0.75	81 ±0.24		
		30	**4 ±1.24**	**10 ±0.49**	**81 ±0.75**		

Table 4.7. contd…..

t (min)	T (˚C)	t_u (min)	Composition of cellulose fiber in dry basis wt %			SEM images and D (nm)	Increase in α-C%
			L	H	α-C		
25	100	20	**3 ±0.24**	**8 ±1.24**	**80 ±0.99**	352-588	3
		25	3 ±0.99	8 ±0.24	8 0±1.24		
		30	4 ±0.49	8 ±0.75	70±0.24		
	120	**20**	**3 ±0.24**	**8 ±0.99**	**84 ±0.75**	430-2.5 µm	3
		25	3 ±0.75	7 ±1.24	82 ±0.49		
		30	3 ±0.99	8 ±0.24	84±1.24		
	130	20	**4 ±1.24**	**8 ±0.49**	**81 ±0.99**	181-884	3
		25	4 ±0.99	8 ±0.24	80 ±0.49		
		30	4 ±0.49	8 ±0.75	80 ±1.24		

Table 4.8. The chemical composition and SEM images of the isolated cellulose microfibers from Finger millet straw fibers after treatment by Method OU, at different acid treatment time (t), temperature (T), and ultrasonication time (t_u) with SEM images for optimum sonication time (D)(nm) defibrillated with SEM images and increase in percentage of cellulose concentration for optimum sonication time (I α-C%).

t (min)	T (°C)	t_u (min)	Composition of cellulose fiber in dry basis wt %			SEM images and D (nm)	Increase in α-C%
			L	H	α-C		
15	100	20	8 ±0.99	10 ±0.24	78 ±0.75	186-449	2
		25	**8 ±1.24**	**9 ±0.49**	**80 ±0.99**		
		30	8 ±0.24	9 ±0.75	79 ±1.24		
	120	**20**	**7 ±0.75**	**9 ±0.99**	**80 ±0.49**	137-424	2
		25	7 ±0.49	9 ±1.24	80 ±0.75		
		30	7 ±0.99	10 ±0.24	79 ±1.24		
	130	20	8 ±0.49	10 ±0.99	79 ±0.24	161-361	2
		25	**8 ±0.24**	**9 ±0.75**	**80 ±0.49**		
		30	8 ±0.75	9 ±1.24	78 ±0.99		

Table 4.8. contd…..

t (min)	T (°C)	t_u (min)	Composition of cellulose fiber in dry basis wt %			SEM images and D (nm)	Increase in α-C%
			L	H	α-C		
20	100	20	6 ±1.24	10 ±0.49	81 ±0.75	142-503	3
		25	6 ±0.49	9 ±1.24	80 ±0.99		
		30	**6 ±0.24**	**8 ±0.49**	**83 ±0.75**		
	120	20	5 ±0.99	10 ±0.75	81 ±1.24	131-297	2
		25	**5 ±1.24**	**9 ±0.24**	**83 ±0.49**		
		30	5 ±0.75	8 ±0.99	83 ±0.24		
	130	20	6 ±0.99	10 ±0.75	81 ±0.49	123-294	2
		25	**6 ±0.24**	**9 ±0.49**	**82 ±1.24**		
		30	6 ±0.75	10 ±1.24	81 ±0.99		
		25	3 ±0.99	10 ±0.49	82 ±0.24		
		30	3 ±1.24	10 ±0.75	81 ±0.99		

Table 4.8. contd…..

t (min)	T (°C)	t_u (min)	Composition of cellulose fiber in dry basis wt %			SEM images and D (nm)	Increase in α-C%
			L	H	α-C		
25	100	20	4 ±1.24	10 ±0.99	83 ±0.24	127-423	2
		25	**4 ±0.49**	**9 ±0.24**	**84 ±0.75**		
		30	4 ±0.99	10 ±0.49	83 ±0.24		
	120	20	3 ±1.24	9 ±0.75	85 ±0.49	153-359	2
		25	**2** ±0.75	**9** ±0.24	**86**±1.24		
		30	2 ±0.49	9 ±1.24	86 ±0.99		
	130	**20**	**3 ±0.24**	**10 ±0.99**	**83 ±0.75**	317-555	0
		25	3 ±0.99	10 ±0.49	82 ±0.24		
		30	3 ±1.24	10 ±0.75	81 ±0.99		

Table 4.9. The chemical composition of the isolated cellulose microfibers from Jatropha seed shell Pongamia seed hull and Finger millet straw fibers after treatment by Method OU, at optimized acid treatment time (t), temperature(T) and ultrasonication time (t_u).

Source	t (min)	T(°C)	Composition of cellulose fiber in dry wt % by method O			t_u (min)	Composition of cellulose fiber in dry wt % by method OU		
			L	H	α-C		L	H	α-C
Jatropha seed shell	25	**120**	**8** ±0.75	**8** ±0.24	**82** ±0.49	**20**	**6** ±0.75	**6** ±1.24	**84** ±0.49
Pongamia seed hull	20		**4**±0.99	**9**±0.49	**85**±0.99	**20**	**3** ±0.75	**8** ±0.99	**86** ±0.49
Finger millet straw	25		**2** ±0.75	**9** ±1.24	**84** ±0.99	**25**	**2** ±0.75	**9** ±0.24	**86**±1.24

It has to be noted that the cellulose microfibers isolated from Jatropha seed shell and Finger millet straw have more defibrillated appearance and the cellulose microfibers are still bonded to each other. However, in case of cellulose microfibers of Pongamia seed hull when subjected to organosolv treatment and ultrasonication, web like arrangements have been observed for lower acid treatment time in the entire range of the acid treatment temperatures studied. But as the acid treatment time and temperature increased, the cellulose microfibers are still found to be bonded with each other to form aggregates even after a sonication time of 30min. This is due their hydrophilic nature which leads to bond formation between cellulose components (Klemm et al. 1998; Hubbe et al. 2008; Wang et al. 2015). Similar agglomerations of the fibers have been reported by Wang et al. (2015) when nano cellulose fibers were isolated from residues of Moso bamboo by

ultrasonication with sonication time of 10 to 70 min operated under 500 W power. Agglomeration of microfibrils and presence of large sized unfibrillated cellulose microfibers are considered to be common in case of all mechanical treatments (Zimmermann et al. 2004; Cheng et al. 2009; Zang et al. 2015). The optimum sonication time which has resulted in the defibrillation and highest isolation of cellulose fiber has increased with increase in acid treatment time for cellulose isolation from Jatropha seed shell and Finger millet straw. The optimum operating condition for isolation of cellulose microfibers by Method OU has been found to be the acid treatment time of 25 min, acid treatment temperature of 120 ºC and sonication time of 20 min for Jatropha seed shell and acid treatment time of 25 min, acid treatment temperature of 120 ºC and sonication time of 25 min for Finger millet straw. Under these conditions, cellulose microfibers isolated from Jatropha seed shell and Finger millet straw are defibrillated to smallest cellulose microfiber of 231 nm and 153 nm diameter respectively. However, in case of cellulose microfibers isolated from Pongamia seed hull, the acid treatment time of 20 minutes, temperature of 120ºC and sonication time of 20min has been considered as the optimum with cellulose microfiber of diameter 251 nm being defibrillated.

Urruzola et al. (2012) have stated that higher defibrillation of cellulose fibers results with increase in ultrasonication time because fibers are exposed to sonic waves for longer time. But, Li et al. (2012) have reported that lower sonication time of 5 min was efficient in defibrillation of high crystalline cellulose in their studies on the effect of sonication time in the range of 5-15 min. Similarly, 10 min sonication was considered to be effective in defibrillation of cellulose microfibers by Frone et al, (2011) as compared to 20 min of sonication. For bleached fibers, sonication time of 30min was considered for defibrillation of cellulose fibers (Chen et al. 2011; Hu et al. 2015). Though, the ultrasonic impact can gradually lead to defibrillation of the cellulose fibers (Chen et al. 2011), the sonication energy required for defibrillation may change with the structure of the plant fibers. The complicated multi-layered structure of plant fibers and the interfibrillar hydrogen bonds (Manley, 1964; Somerville et al. 2004) lead to aggregation of

microfibers (Cheng et al. 2009). Thus, the extent of defibrillation of cellulose microfibers that can be achieved differs with sonication time.

From the above observation, it is clear that the isolation of cellulose microfibers enhances with the introduction of ultrasonic waves to the surface of cellulose fibers leading to reduction in the size of cellulose microfiber bundle, distribution of the microfibers with uniform arrangement as well as marginal reduction in size of the smallest isolated fibers as compared to those isolated by Method O (Figure 4.4.-4.6). Thus, the cavitation energy of ultrasonication treatment has been found to be favourable to break the linkages between cellulose, hemicellulose and lignin and also to disrupt the structure of cellulose microfibers.

4.2.3 Isolation of cellulose microfibers by method OE

The cellulose microfibers isolated from all the three lignocellulosic sources by treatment method **O** were further subjected to enzymatic treatment method **E**. The fibers isolated by organosolv method at optimum acid treatment temperature under each of the acid treatment time were subjected to enzymatic hydrolysis by using endoglucanase enzyme. The effect of enzyme concentration on cellulose composition and defibrillation was studied. Table 4.10- 4.12 present the chemical composition and SEM images of cellulose fibers isolated by method **OE.**

4.2.3.1 Effect of enzymatic hydrolysis on isolation of cellulose microfiber

The cellulose concentration of isolated cellulose microfibers has increased only marginally (≤2%) after treatment with enzymes as observed in Tables 4.10-4.12. This is due to the activity of endoglucanase enzymes which increases cell wall swelling (Henriksson et al. 2007), break glucosidic bonds to weaken microfibrils surface (Manley et al. 1964; White et al. 1981) and degrade amorphous region of cellulose microfibers (Tang et al. 2015).

Table 4.10. The chemical composition of the cellulose microfibers isolated from Jatropha seed shell fibers after treatment by Method OE, at different acid treatment time (t), temperature (T) and enzyme concentration (E) with SEM images for optimum enzyme concentration (wt%) and diameter range of defibrillated cellulose microfiber (D) (nm).

t (min)	T (^{0}C)	Composition of cellulose fiber dry wt % by method O			SEM images and D (nm)	E, (wt %)	Composition of cellulose fiber in dry wt % by method OE			SEM images and D (nm)
		L	H	α-C			L	H	α-C	
15	100	12 ±0.99	10 ±0.24	75 ±0.49	**334-630**	**0.02**	**11±0.49**	**9±0.24**	**77±0.75**	200-584
						0.5	11±0.75	10±0.49	76±1.24	
20	120	10 ±0.49	8 ±0.99	79 ±1.24	5.42 µm	**0.02**	**8±0.24**	**6±0.99**	**80±0.49**	379-559
						0.5	9±0.99	8±1.24	79±0.24	
25	120	8 ±0.75	8 ±0.24	82 ±0.49	277-747	**0.02**	**8±1.24**	**7±0.75**	**83±0.99**	248-452
						0.5	8±0.49	7±0.24	83±0.49	

Table 4.11. The chemical composition of the cellulose microfibers isolated from Pongamia seed hull fibers after treatment by Method OE, at different acid treatment time (t), temperature(T) and enzyme concentration (E) with SEM images for optimum enzyme concentration (wt%) and diameter range of defibrillated cellulose microfiber (D) (nm).

t (min)	T (^{0}C)	Composition of cellulose fiber dry wt % by method O			SEM images and D (nm)	E,(wt %)	Composition of cellulose fiber in dry wt % by method OE			SEM images and D (nm)
		L	H	α-C			L	H	α-C	
15	120	5±0.75	9±0.99	82±1.24	**170-225**	**0.02**	**5±0.49**	**8±0.99**	**83±0.24**	248-450
						0.5	5±0.75	9±0.24	82±0.49	
20	120	4±0.99	9±0.49	85±0.99	319-768	**0.02**	**4±0.24**	**6±1.24**	**86±0.75**	274-757
						0.5	4±1.24	7±0.49	85±0.99	
25	120	5±0.24	10±1.24	82±0.49	117-2.84 µm	**0.02**	**5±0.99**	**9±0.75**	**83±1.24**	186-519
						0.5	5±0.75	9±0.24	82±0.49	

Table 4.12. The chemical composition of the cellulose microfibers isolated from Finger millet straw fibers after treatment by Method OE, at different acid treatment time (t), temperature (T) and enzyme concentration (E) with SEM images for optimum enzyme concentration (wt%) and range of defibrillated cellulose microfiber (D) (nm).

t (min)	T (^{0}C)	Composition of cellulose fiber dry wt % by method O			SEM images and D (nm)	E (wt %)	Composition of cellulose fiber in dry wt % by method OE			SEM images and D (nm)
		L	H	α-C			L	H	α-C	
15	100	8± 0.75	10±0.99	78±0.49	179-225	**0.02**	**8±0.24**	**9±0.75**	**79±0.49**	219-406
						0.5	8±0.49	9±0.99	79±0.24	
20	120	6±0.49	10±0.99	81±1.24	118-316	**0.02**	**6±0.99**	**9±0.24**	**83±0.49**	222-447
						0.5	6±1.24	9±0.49	82±0.24	
25	120	2±0.75	9±1.24	84±0.99	329-522	**0.02**	**3±0.75**	**8±1.24**	**86±0.75**	178-389
						0.5	3±0.49	9±0.99	84±0.24	

However, the enzyme accessibility to cellulose surface depends on factors such as distribution of hemicellulose and lignin, concentration of matrix components, porosity of the cell wall, size of the fibers (Park et al, 2010) and also the fact that cellulases absorption to lignin is rapid compared to hydrolysis of cellulose microfibers (Zhang and Lynd, 2004; Hector and Leza, 2011). The maximum cellulose concentration was obtained by optimized enzymatic treatment are tabulated in Table 4.13 and it is observed that for

all the lignocellulosic sources, the lower concentration of 0.02% (by wt.) of enzymes has proven to be marginally better in isolation of cellulose microfibers as compared to that with 0.5% of enzyme concentration. There exists a saturation enzyme concentration which is just enough to bind to all the functional groups relevant to hydrolysis causing breakage of glucosidic bonds. The glyosidic bonds being present both in cellulose and hemicellulose, enzymes lead to separation of hemicellulose in the microfibers and also lead to defibrillation of cellulose microfibers. This would have led to increase in cellulose concentration in the microfibrils when treated with 0.02% (by wt.) of enzymes. However, the enzymes may also degrade the amorphous region of cellulose microfibers. The higher concentrations of enzymes above the saturation level, may not contribute to increase in cellulose concentration, but may lead to degradation of cellulose. 0.5% (by wt) of enzyme concentration may be the oversaturation level (Zhang and Lynd 2004). Thus, it is observed that with 0.5% (by wt.) of enzymes there is either no change or less increase in the cellulose concentration from the O method, in comparison to the case of treatment with 0.2% (by wt.) of enzymes. The optimum condition for OE treatment were chosen based on maximum cellulose concentration obtained.

Table 4.13. The chemical composition and diameter range of the cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw fibers by Method O, OU and OE at optimized acid treatment time (t), temperature (T), sonication time (t_u)and enzyme concentration (E).

Lignocellulosic source	t (min)	T (°C)	t_u (min)	E (%)	α-C (dry wt %)			D (nm)		
					O	OU	OE	O	OU	OE
Jatropha seed shell	25	120	20	0.02	82 ±0.49	**84** ±0.49	83±0.99	277-747	122-377	248-452
Pongamia seed hull	20		20		85±0.99	**86** ±0.49	86±0.75	319-768	251-614	274-757
Finger millet straw	25		25		84 ±0.99	**86**±1.24	86±0.75	329-522	153-359	178-389

4.2.3.2 Effect of enzymatic hydrolysis on morphology of isolated cellulose microfiber

The cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw, by method O on subjecting to enzymatic treatment showed morphological changes when observed under SEM and the images are presented in Table 4.10-4.12. The optimum conditions for OE treatment of all the three lignocellulosic sources are presented in Table 4.13.

The enzyme treatment has brought about defibrillation as observed from the SEM images and the decrease in fibers diameter as presented in table 4.10.The lignin content in cellulose microfibers isolated from Jatropha seed shell is high after treatment by Method O for initial acid treatment time of 15 min or 20 min which has resulted in lesser defibrillation process during enzymatic treatment, whereas the lignin and hemicellulose concentration in the cellulose fibers isolated by Method O with 25min of acid treatment time was less and thus enzyme hydrolysis was effective resulting in the fiber size reduction from microfiber of size 277 nm to smaller cellulose microfiber of diameter 248 nm.

The cellulose fibers isolated by method O from Pongamia seed shell contained almost similar percentage of lignin irrespective of the acid treatment time involved. But the surface morphology of the cellulose microfibers has changed after treatment by method E with more defibrillation being observed. The cellulose fibers obtained after treatment by method O with 20 min acid treatment time, when subjected to further treatment by Method E has resulted in reduction in smallest fiber diameters by 45 nm. The cellulose microfibers which have undergone treatment by Method O with acid treatment for 25min on subjecting to Method E, have shown maximum reduction in size as the size range of fibers are drop down from 117nm-2.84µm to 186-519 nm even though the changes in cellulose concentration is less. These results indicate that that enzymatic hydrolysis is more effective in defibrillation of isolated cellulose microfibers of Pongamia seed hulls, rather than acting upon the linkages between matrix component and cellulose.

In case of cellulose microfibers isolated from Finger millet straw, the images show uniform distribution of cellulose microfibers. The size of cellulose smallest microfiber

has reduced by 151 nm compared to cellulose microfibers isolated by method O for acid treatment time of 25 min. The defibrillation of the cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by enzymatic treatment can be accounted to the activity of endoglucanase, which reduces microfibrils to become thinner (Liu et al. 2009). It has been observed in the case of Jatropha seed shell and Finger millet straw that the reduction in size of cellulose fibers is higher when the concentration of cellulose is high. This shows that defibrillation by enzymatic treatment is favoured when cellulose concentration is higher. The accessibility of the enzyme onto cellulose for defibrillation increases when the cellulose concentration is higher.

The enzyme concentration of 0.02% has been found to be favourable owing to higher increase in cellulose content and extent of defibrillation or by being economical owing to less requirement of enzymes. Hassan et al. (2014) have also reported that lower concentration of enzymes is effective in defibrillation of cellulose fibers. Henriksson et al. (2007) have also reported 0.02% of enzyme concentration as the optimum for defibrillation of cellulose fibers from bleached wood sulphite pulps owing to easy disintegration and limited fiber shortening. They have observed that the cellulose fibers isolated by enzymatic treatment with 0.5 to 3% of enzyme concentration have led to shortening of the fibers.

Thus, from all the discussions over the isolation methods O, OU and OE it is clear that Method O has mainly resulted in isolation of cellulose microfibers from Jatropha seed shell, Pongamia seed hull and Finger millet straw by increasing the concentration of the cellulose from 41% to 82%; 42% to 85% and 50% to 84% respectively. Further subjecting the isolated fibers to either ultrasonication or enzymatic treatment has not resulted in considerable change in the cellulose concentration. However, these additional treatments have led to defibrillation of the cellulose bundles of the isolated cellulose fibers and the reduction in the fiber diameters. Thus, isolation of lignocellulosic components is achieved by organosolv treatment and defibrillation has been assisted by further ultrasonication and enzymatic treatment. This proves that organosolv treatment with combination of ultrasonication and enzymatic treatment results in isolation of

cellulose microfibers by increasing the cellulose concentration and also defibrillation to form smallest cellulose microfibers.

To enhance the isolation of cellulose microfibers and to check the effect of inorganic chemical treatment on isolation of cellulose microfibers from Jatropha seed shell, Pongamia seed hull and Finger millet straw, combination of inorganic chemical treatment with organosolv treatment has been carried out which is hereinafter referred as method IO.

4.2.4 Isolation of cellulose microfibers by method IO

Several researchers have reported inorganic and organic chemical treatments in combination with mechanical and enzymatic treatment to achieve higher isolation of cellulose fibers (Chen et al. 2011, Maheswari et al. 2012, Espino et al. 2014, Xie et al. 2016). The method IO includes a combination of sodium chlorite bleaching, NaOH and organosolv (acetic acid: nitric acid) treatment for the isolation of cellulose microfibers from Jatropha seed shell, Pongamia seed hull and Finger millet straw fibers. Table 4.14-4.16 present the cellulose composition of isolated cellulose microfibers by this method. Bleaching process was carried out at fiber to sodium chlorite solution ratio (w/v) of 1:30 and 1:50, which helps only in delignification and has no effect on hemicellulose removal due to its selective effect (Draman et al. 2014, Lazic et al. 2017). However, sodium hydroxide and Organosolv treatment result in hydrolysis of hemicellulose and lignin, thus leading to isolation of cellulose fibers (Maheswari et al. 2012). For the organosolv treatment acetic acid with nitric acid ratio (v/v) of 10:1, 15:1 and 20:1were used.

4.2.4.1 Effect of cellulose fiber to Sodium chlorite ratio and acetic acid to nitric acid ratio on isolation of cellulose microfiber

The fiber to sodium chlorite ratio and acetic acid concentration has influenced the isolation of cellulose fibers to a large extent. With fiber to liquor ratio of 1:30, the maximum content of cellulose in the isolated microfibers were 74%, 79% and 81% for Jatropha seed shell, Pongamia seed hull and Finger millet straw respectively which were achieved with acetic acid to nitric acid ratio of 20:1. With the increase of sodium chlorite

concentration (fiber to liquor ratio 1:50) there was an appreciable increase in the cellulose content. The maximum cellulose content of 89% and 92% were achieved with acetic acid to nitric acid ratio of 10:1 for fiber isolation from Jatropha seed shell and Finger millet straw respectively. For fiber isolation from Pongamia seed shell, the maximum cellulose content of 84% was achieved with acetic acid to nitric acid ratio of 15:1. These results show that increase in the amount of sodium chlorite leads to enhanced delignification of fibers, thus increasing the cellulose content and decreasing the lignin content in the isolated fibers.

The liquor to fibres ratio of 50:1 and acetic acid to nitric acid ratio of 10:1 is considered to be the optimum for cellulose isolation from Jatropha seed shell and Finger millet straw fibers. Liquor to fibres ratio of 50:1 and acetic acid to nitric acid ratio of 15:1 is considered to be the optimum for isolation of cellulose fibers from Pongamia seed hull. The concentration of acetic acid required for maximum isolation of cellulose fibers from Pongamia seed hull fibers is higher than that for Jatropha seed shell and Finger millet straw fibers. This could be accounted to high lignin content in the untreated Pongamia seed hull compared to that in Jatropha seed shell and Finger millet straw fibers. Further, it is noticed that the cellulose concentration drops down when treated with acetic acid to nitric acid ratio of 15:1 for Jatropha seed shell and Finger millet straw fibers and acetic acid to nitric acid ratio of 20:1 for Pongamia seed hull fibers. This reduction in cellulose content as compared to lower ratios can be attributed to increase in degradation of cellulose owing to the use of higher concentrations of acetic acid. As discussed in section 4.2.1, both acetic acid and nitric acid can act on the isolated cellulose and degrade it. The alkaline treatment can also lead to reduction in cellulose content. Treatment with alkali easily depolymerizes the alpha bonds in amorphous region of the cellulose than the beta bonds in crystalline region which is generally difficult to depolymerize (Peng et al 2012, Lima et al. 2014). Similarly, acetic acid and nitic acid also act upon the degradation of cellulose.

Table 4.14. The composition of the cellulose microfibers isolated from Jatropha seed shell fibers after treatment by Method IO, at different fiber to liquor (sodium chlorite) ratio (2 cycles of bleaching) and acetic acid to nitric acid ratio.

Fiber to liquor per cycle	**Acetic acid: nitric acid**	**Composition of cellulose fiber in dry basis wt %**		
		L	**H**	**α-C**
1:30	10:1	17±0.49	11±0.99	71±0.75
	15:1	15±0.24	14±0.75	70±0.49
	20:1	12±0.99	10±1.24	74±1.24
1:50	**10:1**	**2±0.75**	**6±0.49**	**89±0.24**
	15:1	3±1.24	6±0.24	87±0.99
	20:1	3±0.99	6±0.49	87±0.75

Table 4.15. The composition of the cellulose microfibers isolated from Pongamia seed hull fibers after treatment by Method IO, at different fiber to liquor (sodium chlorite) ratio (2 cycles of bleaching) and acetic acid to nitric acid ratio.

Fiber to liquor per cycle	Acetic acid: nitric acid	Composition of cellulose fiber in dry basis wt %		
		L	H	α-C

1:30	10:1	12±0.24	14±0.99	71±0.75
	15:1	11±0.75	12±0.24	74±0.99
	20:1	6±0.49	11±0.75	79±1.24
1:50	10:1	3±1.24	12±0.49	83±0.24
	15:1	**3±0.99**	**10±1.24**	**84±0.49**
	20:1	3±0.49	11±0.75	82±0.24

Table 4.16. The composition of the cellulose microfibers isolated from Finger millet seed hull fibers after treatment by Method IO, at different fiber to liquor (sodium chlorite) ratio (1.5 cycles of bleaching) and acetic acid to nitric acid ratio.

Fiber to liquor per cycle	Acetic acid : nitric acid	Composition of cellulose fiber in dry basis wt %

		L	**H**	**α-C**
1:30	10:1	8±0.99	12±0.75	74±0.24
	15:1	6±0.24	11±0.49	77±1.24
	20:1	3±0.75	9±1.24	81±0.99
1:50	**10:1**	**1±0.49**	**3±0.24**	**92±0.75**
	15:1	1±0.24	4±0.99	90±0.49
	20:1	1±0.75	4±0.49	90±0.24

Generally, bleaching step is repeated for 3 to 5 times until the fibers turn out to be white in colour (Chen et al 2012, Fortunati et al. 2013). This suggests that amount of sodium chlorite required for delignification of fibers is high. However, in the present work two cycles of 2h each, utilizing totally 100mL of the liquor per gram of fiber were sufficient enough to achieve the specified bleached state in case of isolation from Jatropha and Pongamia; whereas 1.5 cycles (3h) utilizing totally 100mL were sufficient for isolation from Finger millet. This can be accounted to the presence of higher content of lignin in untreated Jatropha seed shell (21%) and Pongamia seed hull (24%) which has resulted in

longer time of treatment (4h) than that for case of Finger millet straw (3h) which contained only 12 wt% lignin before the treatment. The decolourization of cellulose fibers can also be accounted to the treatment with sodium chlorite, which aids in breakdown of phenolic molecules with chromophoric groups in lignin (Tibolla et al. 2014). The 0.7% sodium chlorite was used in the present work. Lie et al. (2014) have reported bleaching treatment using 0.9% sodium chlorite taken in fiber to liquor ratio of 1:32 for 3h for delignification of sugar beet pulp. Le Normand et al. (2015) have reported bleaching of bark of Norway spruce by 1% sodium chlorite solution for 4h repeated for four times adding to the total of 16h with fiber to liquor ratio of 1:25. Hoop pine veneers were bleached by 1 or 2% sodium chlorite aqueous solution taken at fiber to liquor ratio of 1:30 for a total of 12h (Yano et al. 2001). At the same concentration of sodium chlorite and fiber to liquor ratio Sitka spruce, Oil palm empty fruit bunch and Coconut husk were bleached by sodium chlorite for 5 h (Iwamoto et al. 2008), 4 h (Fahma et al. 2010) and 4 h (Fahma et al. 2011) until the fibers turned out white in colour.

On comparison of cellulose microfiber isolation by Method O and Method IO in terms of the composition under optimized condition as shown in 4.17 it is inferred the lignin concentration in the cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by Method IO contain 2%, 3% and 1% of final lignin concentration and those isolated by Method O contain 8%, 4% and 2% of final lignin concentration respectively. Thus, lignin content in the fibers isolated by the combined treatment Method IO is much lower than that in the fibers isolated by Method O. This can be accounted to the bleaching process with chlorites involved in IO method, by which lignin is swiftly oxidised to form carboxylic, carbonyl, and hydroxyl groups, these groups further help in solubilisation of lignin in alkaline solution and finally increase in cellulose concentration (Dufresne et al. 1997). It is observed that with increase in sodium chlorite concentration, the removal of lignin has enhanced. This can be accounted to the significant effect of sodium chlorite on strong chain of C-C bonds and aromatic groups of lignin (Wang et al. 2003). The hemicellulose concentration in isolated cellulose microfibers from Jatropha seed shell, Pongamia seed hull and Finger millet straw by

method O has been reported to be 8, 9, 9 % respectively, whereas by method IO the hemicellulose concentration has dropped down to 6 and 3% respectively in cellulose microfibers isolated from Jatropha seed shell and Finger millet straw. Hemicellulose concentration has not much varied in the cellulose microfibers isolated by Pongamia seed hull. The enhanced reduction in hemicellulose by method IO can be accounted to the alkaline treatment using NaOH, which is known to disrupt the cell wall and dissolve lignin, hemicellulose, and swelling of cellulose (Jackson 1977; Bledzki and Gassan 1999; Ludueña et al. 2011; Lazic et al. 2017). The combination of chlorites, alkaline and organosolv process have resulted in higher removal of hemicellulose and lignin by Method IO as compared to method O.

As observed in Table 4.17, the maximum cellulose concentration obtained in the isolated cellulose microfiber after treatment by method IO is 89%, 84% and 92% for Jatropha seed shell, Pongamia seed hull and Finger millet straw respectively and is higher than that obtained by Method O. Method IO is proven to be better compared to method O, since the involvement of chlorites, alkali and organosolv in removal of matrix components from Jatropha seed shell and Finger millet straw fibers have resulted in maximum amount of cellulose concentration in isolated cellulose microfiber. However, there is only a marginal change observed in cellulose concentration in cellulose microfibers isolated from Pongamia seed hull by method IO as compared to that by method O. This can be accounted to the presence of high amount of lignin and hemicellulose in untreated Pongamia seed hull and their structural linkage may be such that it is not easily broken in spite of the incorporation of bleaching and alkaline treatment along with organosolv method.

Lignocellulosic source	t (min)	T (°C)	Composition of cellulose fiber in dry wt % by method O	Fiber to liquor	Acetic acid to nitric acid ratio	Composition of cellulose fiber in dry wt % by method IO

			L	H	α-C			L	H	α-C
Jatropha seed shell	25	**120**	**8 ±0.75**	**8 ±0.24**	**82 ±0.49**	1:50	10:1	2±0.75	6±0.49	89±0.24
Pongamia seed hull	20		**4±0.99**	**9±0.49**	**85±0.99**		15:1	3±0.99	10±1.24	84±0.49
Finger millet straw	25		**2 ±0.75**	**9 ±1.24**	**84 ±0.99**		10:1	1±0.49	3±0.24	92±0.75

Table 4.17. The chemical composition of the isolated cellulose microfibers from Jatropha seed shell Pongamia seed hull and Finger millet straw fibers after treatment by Method O and IO, at optimized fiber to liquor ratio and acetic acid to nitric acid ratio.

4.2.4.2 Effect of cellulose fiber to sodium chlorite ratio and acetic acid to nitric acid ratio on the morphology of cellulose microfiber

The morphological changes observed in cellulose microfibers isolated by treatment method IO from Jatropha seed shell, Pongamia seed hull and Finger millet straw are presented as SEM images in Figures 4.7- 4.9. As observed in Fig. 4.7 to 4.9, the surface of isolated cellulose microfiber looks rough and defined cellulose microfibers run through the length of the fiber bundle. Considering the cellulose microfibers isolated from Jatropha seed hull (Figure 4.7), it is observed that the cellulose fiber bundle has reduced in size as the fiber to liquor ratio is increased from 1:30 to 1:50. For example, with acetic acid to nitric acid ratio of 15:1, the fiber bundle is wider with size of 6.16µm in case of treatment with 1:30 ratio of fiber to liquor and has reduced to a size of 2.2µm on treatment with 1:50 fiber to liquor ratio. However, these fiber bundles are made of small cellulose microfibers and the smallest of these fibers have shown reduction in fiber diameter from 281nm and 235nm as the ratio was increased from 1:30 to 1:50

respectively. Thus, reduction in the cellulose microfiber diameter and the reduction in bundle size have been observed when the fiber to liquor ratio is changed from 1:30 to 1:50. Further in Figure 4.7, it is clear that the fiber bundles are reduced in size and also show cellulose microfibers running through them, but they are bonded to form aggregates. The smallest cellulose microfiber isolated from Jatropha seed hull for optimum condition i.e. 1:50 fiber to liquor ratio and 10:1 acetic acid to nitric acid ratio is 225nm.

In case of cellulose microfibers isolated from Pongamia seed hull as shown in Figure 4.8, large fiber bundles containing defibrillated fibers are observed in case of fibers obtained by 1:30 fiber to liquor ratio. However, the size of the fiber bundles in case of 1:50 fiber to liquor ratio, appears to be smaller than those obtained with 1:30 fiber to liquor ratio. The extent of defibrillation is also better in case of 1:50 fiber to liquor ratio, as smaller diameter fibers running through the fiber bundles are observed. Thus, the diameter of cellulose microfibers has reduced as the amount of sodium chlorite is increased. On comparison of the cellulose microfibers with maximum cellulose concentrations obtained with both 1:50 and 1:30 fiber to liquor ratio (corresponding to optimum acetic acid to nitric acid ratios), it is observed that the cellulose microfiber diameter obtained has decreased from 230nm to175nm respectively. In isolation of cellulose fibers from Pongamia seed hull using IO method, the smallest of the fibers has been obtained with bleaching action carried out using 1:50 fiber to liquor ratio and organosolv treatment with acetic acid to nitric acid ratio of 15:1.

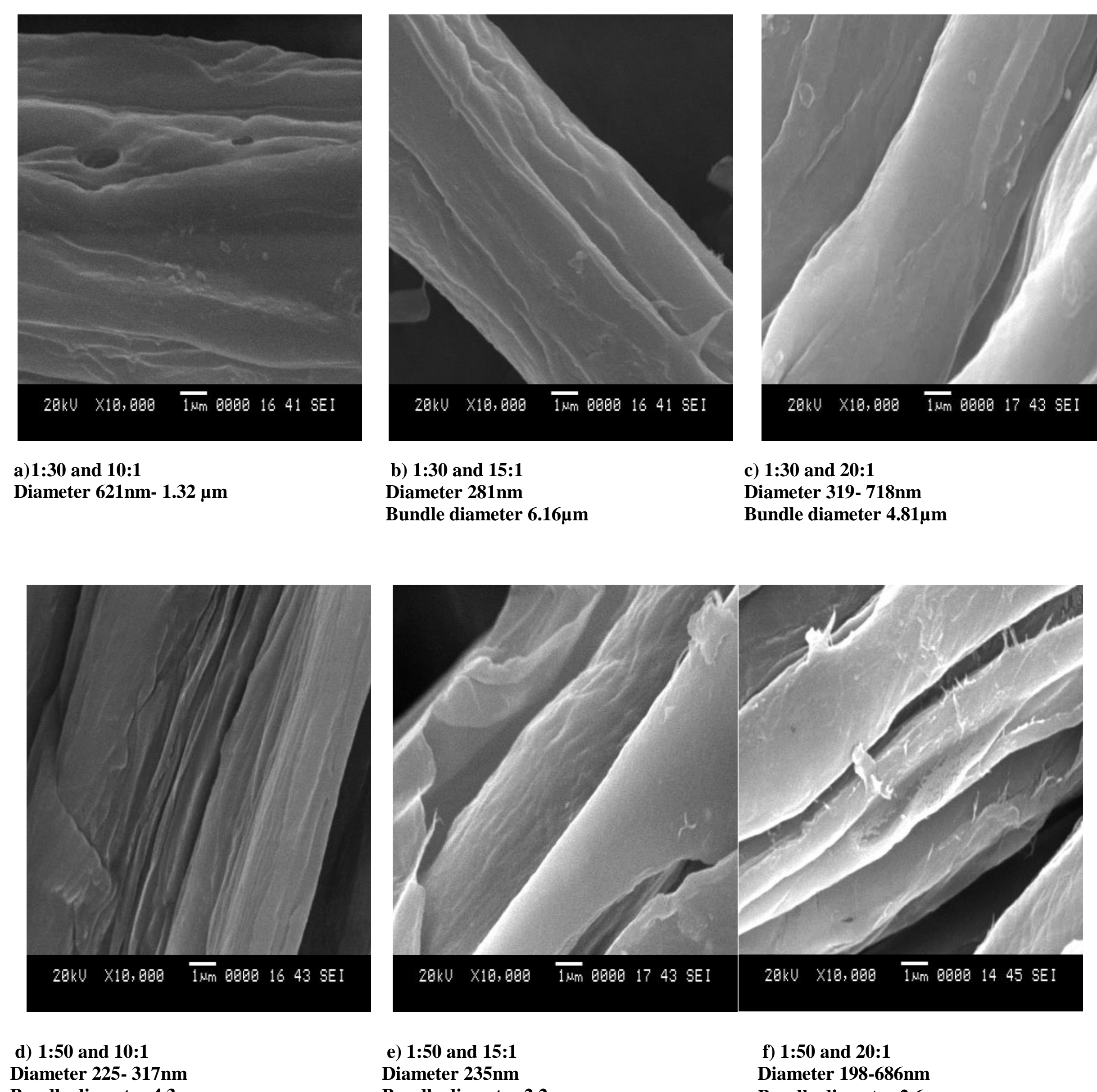
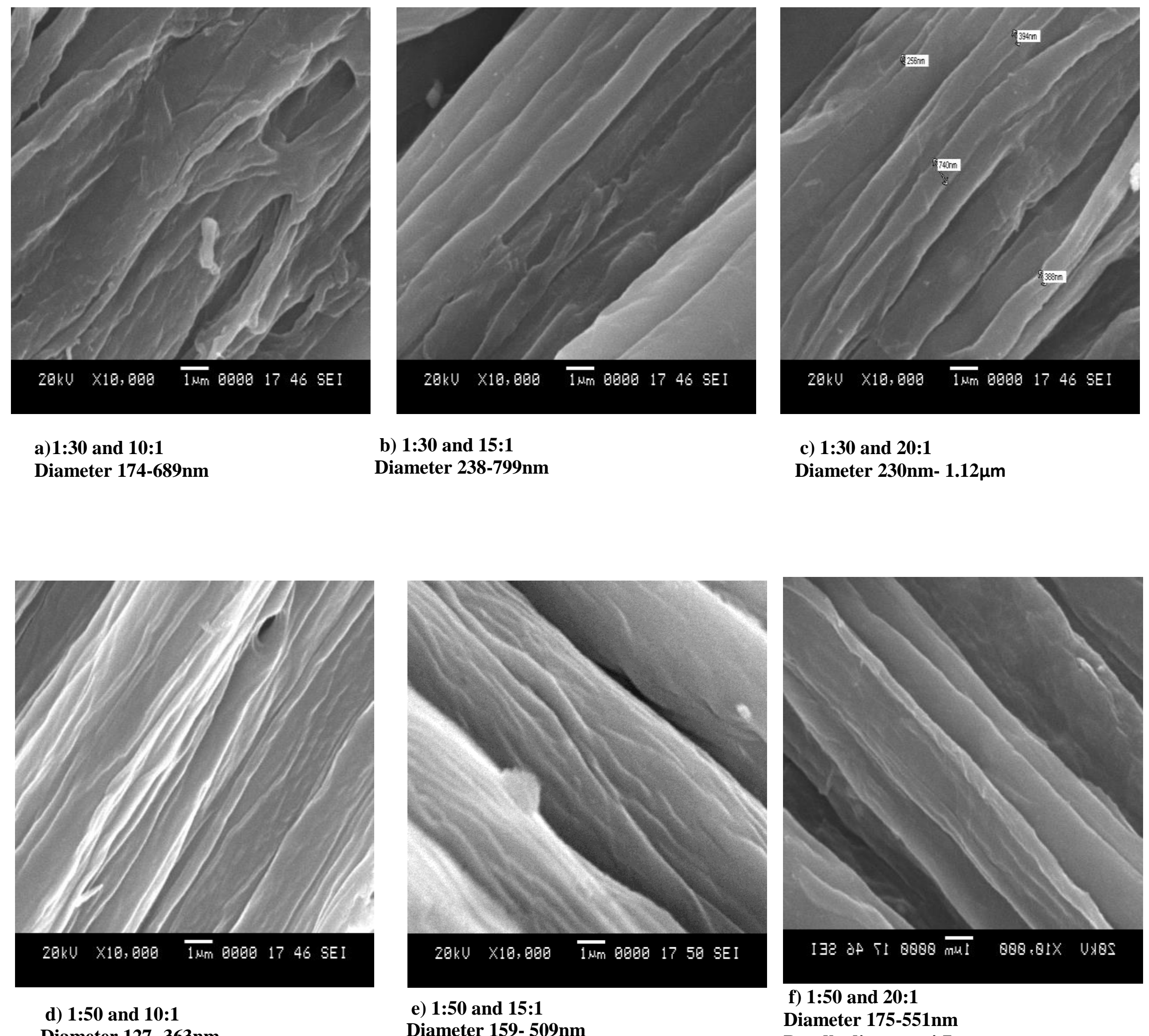


Figure 4.7. Scanning electron microscope images of cellulose fibers isolated from Jatropha seed shell after treatment by Method IO, at different fiber to liquor ratio and acetic acid to nitric acid ratio.



a)1:30 and 10:1
Diameter 174-689nm

b) 1:30 and 15:1
Diameter 238-799nm

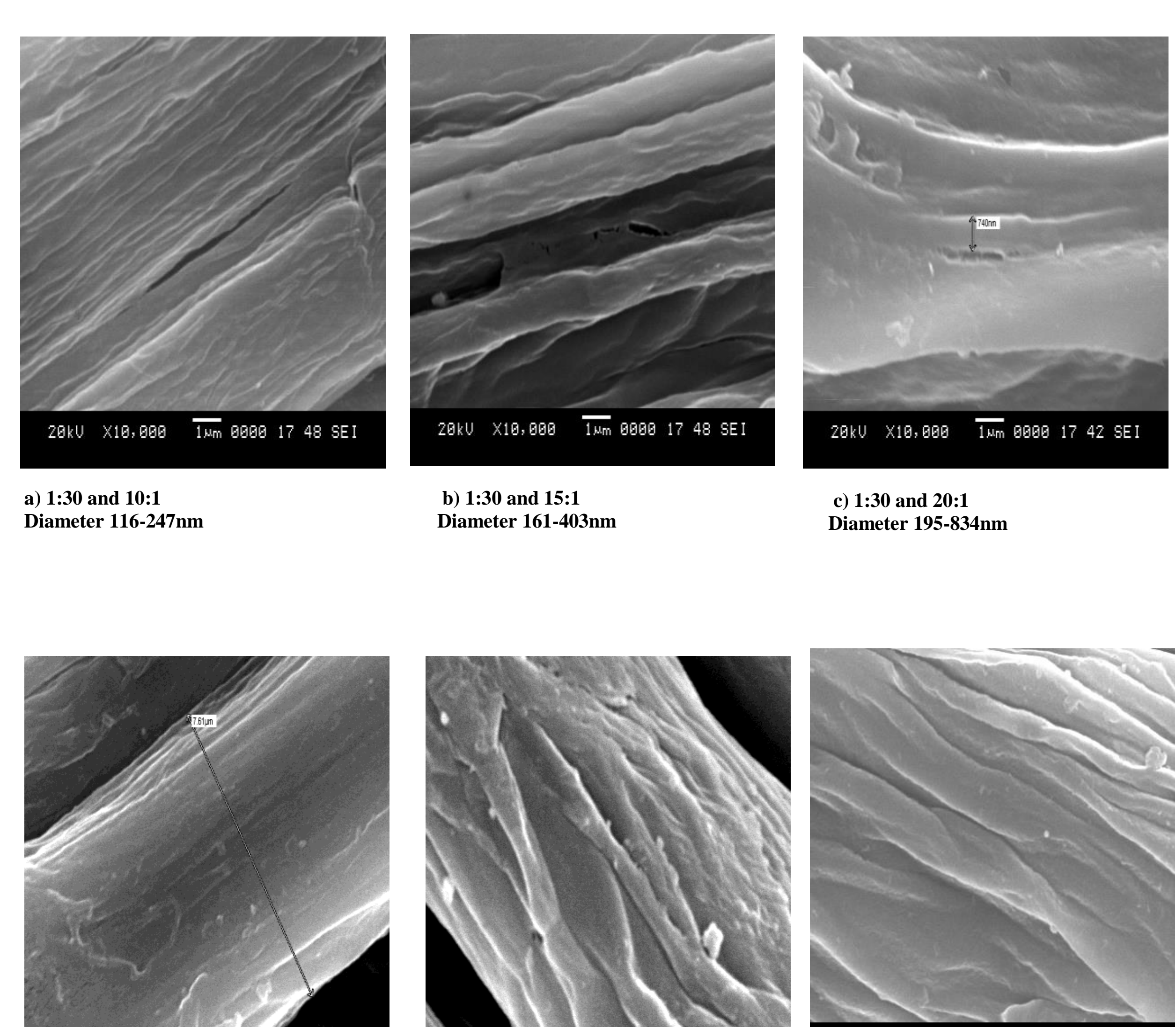
c) 1:30 and 20:1
Diameter 230nm- 1.12µm

d) 1:50 and 10:1
Diameter 127- 363nm

e) 1:50 and 15:1
Diameter 159- 509nm
Bundle diameter 4.1µm

f) 1:50 and 20:1
Diameter 175-551nm
Bundle diameter 4.7 µm

Figure 4.8. Scanning electron microscope images of cellulose fibers isolated from Pongamia seed hull fibers after treatment by Method IO, at different fiber to liquor ratio and acetic acid to nitric acid ratio.



a) 1:30 and 10:1
Diameter 116-247nm

b) 1:30 and 15:1
Diameter 161-403nm

c) 1:30 and 20:1
Diameter 195-834nm

d) 1:50 and 10:1
Diameter 153-458nm
Bundle diameter 7.58μm

e) 1:50 and 15:1
Diameter 181-878nm
Bundle diameter 10.04μm

f) 1:50 and 20:1
Diameter 341-857nm

Figure 4.9. Scanning electron microscope images of cellulose fibers isolated from Finger millet straw fibers after treatment by Method IO, at different fiber to liquor ratio and acetic acid to nitric acid ratio.

The cellulose microfibers isolated from Finger millet straw by treatment method IO are aggregated and the bundle size has reduced as the fiber to sodium chlorite liquor ratio was varied from 1:30 to 1:50. The SEM images presented in Figure 4.9, show the presence of larger diameter cellulose microfiber bundles for 1:30 fiber to sodium chlorite liquor ratio as compared to those obtained with the ratio of 1:50. The fibers appear to be more defibrillated with prominent cellulose microfibers on their surface as the amount of sodium chlorite liquor is increased. The diameters of the smallest cellulose microfibers corresponding to the fibers with maximum cellulose content in case of both 1:30 and 1:50 fiber to sodium chlorite liquor ratio, show reduction in diameter from 195nm to 153nm respectively. The smallest of the cellulose microfibers isolated from Finger millet straw was obtained under the optimum condition of 1:50 fiber to liquor ratio and 10:1 acetic acid to nitric acid ratio and the diameter of the fiber is 153nm.

From the SEM images for fibers isolated by Method O (Figure 4.4 h) and by Method IO (Figure 4.7 d), it is observed that the fibers isolated by Method IO are dominantly visible on the surface of fiber bundles and the fibers isolated by Method O are merged within the bundle surface. The smaller diameter cellulose microfiber isolated from Jatropha seed shell by treatment method O has reduced by 52 nm after treatment by method IO. In case of cellulose microfiber isolated from Finger millet straw, the smallest fiber isolated by treatment method IO has the diameter of 153nm (Figure 4.9d) which is much lesser with a reduction of size by 176 nm compared to that of cellulose microfiber isolated by treatment method O which has smallest cellulose microfiber of diameter 329 nm (Figure 4.6h). The cellulose microfiber isolated from Pongamia seed hull fibers by treatment method IO have almost similar cellulose composition as that of cellulose microfiber isolated by treatment method O. But the diameter of the smallest cellulose microfiber isolated by treatment method IO (159 nm) is very much smaller than the smallest cellulose microfiber isolated by treatment method O (319 nm) for the optimum operating conditions as tabulated in Figure 4.5e and 4.8e. From all the above observation, it is very clear that the cellulose microfibers isolated by IO method are generally of higher

cellulose content and are also of smaller diameters as compared to those isolated by Method O.

Inorganic chemical treatment method is a well-established treatment and has been followed for centuries in paper, pulp, and textile industries for delignification and isolation of cellulose fibers. However, the drawbacks related to the toxicity and handling of these chemicals are considered to be significant. In order to tackle this issue, the usage of these inorganic chemicals was reduced by incorporating organic chemicals and mechanical methods for the existing inorganic treatments (Sun et al 2004, Maheswari et al 2012). By combining inorganic and organosolv treatments we have observed isolation of cellulose microfibers with higher cellulose content and also smaller cellulose microfibers. As mentioned before in section 4.2.2, the chemical treatment methods when combined with ultrasonication process helps in defibrillation of isolated cellulose microfibers. Thus, the cellulose microfiber isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by treatment method IO are further subjected to ultrasonication treatment (IOU).

4.2.5 Isolation of cellulose microfiber by method IOU

The cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by treatment method IO were further subjected to ultrasonication for 20, 25 and 30 minutes in order to assist isolation and defibrillation of cellulose microfibers. Tables 4.18 to 4.20 present the cellulose composition and the SEM images of the isolated fibers from Jatropha seed shell, Pongamia seed hull and Finger millet straw after IOU treatment.

4.2.5.1 Effect of ultrasonication on isolation of cellulose microfiber

The maximum increase in cellulose concentration in the microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by Method IOU in comparison to cellulose concentration in the microfibers isolated by treatment method IO is 5 or 6%. The maximum increase in cellulose concentration from Method IO to Method IOU is also higher compared to the maximum increase (3%) (Table 4.6-4.8) achieved from Method O to Method OU. This shows that the effect of ultrasonication on cellulose

isolation is more effective with Method IO as compared to that with Method O. From Table 4.18 to Table 4.20, it is also observed that as the fiber to sodium chlorite liquor ratio was changed from 1:30 to 1:50, increase in percentage of cellulose concentration of the cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull fibers and Finger millet straw by method IOU in comparison to method IO, has decreased. It is also evident that lower the cellulose concentration, higher is the increase in percentage of cellulose concentration by Method IOU in comparison to that obtained by Method IO. This is due to the effect of sonication which is oriented towards breakage of linkages between matrix components and cellulose at lower concentration of cellulose, whereas the effect of sonication energy is concentrated on defibrillation of cellulose microfibers at higher concentration of cellulose (Sumari et al. 2013).

4.2.5.1 Effect of ultrasonication on morphology of cellulose microfiber

The SEM images and the range of diameter of the cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw which were subjected to treatment method IOU are presented in Tables 4.18 to 4.20. In the cellulose fibers isolated from Jatropha seed shell at fiber to liquor ratio of 1:30, acetic acid to nitric acid ratio of 20:1 and sonication time of 30min, the maximum cellulose content of 79% and smallest cellulose microfiber of diameter 421 nm is obtained. Under 1:50 fiber to liquor ratio with optimum acetic acid to nitric acid ratio of 10:1 and sonication time of 20min resulted in maximum cellulose content of 90% and the smallest cellulose fiber of diameter of 194 nm which is half the diameter obtained under optimum conditions for 1:30 fiber to liquor ratio. However, on comparison of the fiber size obtained under the optimum conditions by treatment method IO and IOU presented in Table 4.21, the smallest cellulose microfiber isolated by IOU method is lower by ~50nm than the cellulose microfibers isolated by treatment method IO. As observed from the SEM Images presented in Figure 4.7 and Table 4.18, the cellulose microfibers isolated by treatment method IOU show reduction in bundle size and also defibrillation of cellulose microfibers compared to cellulose microfibers isolated by method IO.

Table 4.18. The composition of the cellulose microfibers isolated from Jatropha seed shell fibers after treatment by Method IOU, at different fiber to liquor (sodium chlorite) ratio and acetic acid to nitric acid ratio and ultrasonication time (t_u)with SEM images, diameter range of cellulose microfiber (D) and increase in percentage of cellulose concentration for optimum sonication time.

Fiber to liquor ratio per cycle	Acetic acid to nitric acid ratio	t_u (min)	Composition of cellulose fiber in dry basis wt %			SEM images and D (nm)	Increase in α-C (%)
			L	H	α-C		
1:30	10:1	20	13±0.49	9±0.75	74±0.99	342-561	5
		25	12±0.99	10±0.24	75±0.49		
		30	**12±0.24**	**9±0.49**	**76±1.24**		
	15:1	**20**	**10±1.24**	**11±0.99**	**75±0.75**	350-665 bundle 3.498 μm	5
		25	10±0.75	12±1.24	74±0.24		
		30	10±0.99	12±0.49	75±0.75		
	20:1	20	9±0.24	10±0.99	78±0.49	421 bundle 2.33 μm	5
		25	9±0.75	9±1.24	78±0.24		
		30	**9±0.49**	**9±0.24**	**79±1.24**		

Table 4.18. contd…….

Fiber to liquor ratio per cycle	Acetic acid to nitric acid ratio	t_u (min)	Composition of cellulose fiber in dry basis wt %			SEM images and D (nm)	Increase in α-C (%)
			L	**H**	**α-C**		
1:50	10:1	**20**	**3±1.24**	**5±0.75**	**90 ±0.99**	194-477	2
		25	3±0.24	6±0.99	89±0.75		
		30	3±0.99	6±0.24	88±0.49		
	15:1	20	2±0.49	6±0.75	86±1.24	272-707	2
		25	**2±0.24**	**5±0.49**	**86±0.75**		
		30	2±0.75	6±1.24	85±0.24		
	20:1	20	3±0.49	6±0.99	84±0.75	271-666	1
		25	**3±0.99**	**6±0.24**	**85±0.49**		
		30	3±0.24	6±0.49	85±1.24		

Table 4.19. The composition of the cellulose microfibers isolated from Pongamia seed hull fibers after treatment by Method IOU, at different fiber to liquor (sodium chlorite) ratio and acetic acid to nitric acid ratio and ultrasonication time (t_u)with SEM images, diameter range of cellulose microfiber (D)and increase in percentage of cellulose concentration for optimum sonication time.

Fiber to liquor ratio per cycle	**Acetic acid to nitric acid ratio**	**t_u (min)**	**Composition of cellulose fiber in dry basis wt %**			**SEM images and D (nm)**	**Increase in α-C (%)**
			L	**H**	**α-C**		
1:30	10:1	20	10±0.49	12±0.24	74±0.75	336-675	5
		25	**10±1.24**	**11±0.75**	**76±0.49**		
		30	11±0.24	11±0.49	74±1.24		
	15:1	20	10±0.75	12±1.24	74±0.49	306-604	2
		25	**9±0.99**	**10±0.99**	**76±0.24**		
		30	9±0.75	11±0.24	75±0.99		
	20:1	20	5±0.24	10±0.49	80±0.75	277-466	2
		25	**5±0.49**	**10±1.24**	**81±0.24**		
		30	5±1.24	10±0.99	81±0.49		

Table 4.19. contd…..

Fiber to liquor ratio per cycle	Acetic acid to nitric acid ratio	t_u (min)	Composition of cellulose fiber in dry basis wt %			SEM images and D (nm)	Increase in α-C (%)
			L	H	α-C		
1:50	10:1	**20**	**3±0.99**	**9±0.75**	**84±1.24**	210-357	1
		25	3±0.24	9±0.49	84±0.99		
		30	3±0.99	9±0.75	84±0.24		
	15:1	20	3±075	9±1.24	84±0.49	145-404	1
		25	**3±1.24**	**9±0.99**	**85±0.75**		
		30	3±0.49	9±0.24	85±1.24		
	20:1	**20**	**3±0.75**	**10±0.49**	**82±0.99**	204-355	0
		25	3±0.24	10±0.99	82±0.75		
		30	3±0.49	10±0.24	82±1.24		

Table 4.20. The composition of the cellulose microfibers isolated from Finger millet straw fibers after treatment by Method IOU, at different fiber to liquor (sodium chlorite) ratio and acetic acid to nitric acid ratio and ultrasonication time (t_u) with SEM images, diameter range of cellulose microfiber (D) and increase in percentage of cellulose concentration for optimum sonication time.

Fiber to liquor ratio per cycle	Acetic acid to nitric acid ratio	t_u (min)	Composition of cellulose fiber in dry basis wt %			SEM images and D (nm)	Increase in α-C (%)
			L	H	α-C		
1:30	10:1	20	8±0.49	10±1.24	78±0.75	226-1034	6
		25	8±0.24	10±0.99	78±1.24		
		30	**6±0.75**	**9±0.49**	**80±0.24**		
	15:1	20	6±0.99	9±0.24	81±0.49	228-644	5
		25	7±1.24	9±0.75	81±0.99		
		30	**6±0.49**	**9±0.99**	**82±0.75**		
	20:1	20	5±075	8±0.24	83±0.49	200-295	4
		25	6±1.24	8±0.49	83±0.24		
		30	**5±0.24**	**7±1.24**	**85±0.99**		

Table 4.20. contd…..

Fiber to liquor ratio per cycle	Acetic acid to nitric acid ratio	t_u (min)	Composition of cellulose fiber in dry basis wt %			SEM images and D (nm)	Increase in α-C (%)
			L	H	α-C		
1:50	10:1	**20**	**1±0.75**	**3±0.75**	**93±1.24**	147-238 bundle 2.4-4.83μm	1
		25	1±0.75	3±0.99	93±0.24		
		30	1±0.24	3±0.75	92±1.24		
	15:1	**20**	**1±0.24**	**4±0.49**	**90±0.99**	219-549 bundle - 6.18μm	0
		25	1±0.49	4±0.24	90±0.75		
		30	1±0.99	4±1.24	90±0.49		
	20:1	**20**	**1±0.49**	**4±0.75**	**90±0.24**	286-690 bundle - 4.38μm	0
		25	1±0.99	4±0.24	90±0.75		
		30	1±0.24	4±0.49	90±1.24		

For the optimum condition under 1:30 fiber to liquor ratio, the cellulose microfiber was observed as bundle of size 4.82μm (Figure 4.7c) and when these cellulose microfibers were subjected to ultrasonication (method IOU) the bundle size was reduced to half the size 2.33μm (Table 4.18) of the cellulose bundle isolated by method IO. Similarly, the cellulose microfibers isolated by method IO under optimum conditions for 1:50 fiber to liquor ratio as presented in Figure 4.7d, are aggregated to form a bundle of diameter 4.3μm indicating that cellulose microfibers bond tightly among themselves. The range of diameters of the cellulose microfiber isolated by method IO under these conditions was 223nm-317nm. However, when these fibers were further subjected to ultrasonication (Method IOU), the size of the fibers varied in the range of 194nm - 477nm. But as observed in Table 4.18, under the optimum condition in method IOU, the isolated cellulose microfibers are defibrillated from the bundle and are dominant compared to cellulose microfibers isolated by method IO under corresponding conditions (Figure 4.7d).

The SEM images of cellulose microfiber isolated from Pongamia seed hull by treatment method IOU presented in Table 4.19, show that the diameter of the smallest cellulose microfiber has reduced with an increase in sodium chlorite concentration. At fiber to sodium chlorite liquor ratio of 1:30 and at optimum acetic acid to nitric acid ratio and sonication time, the smallest cellulose microfiber obtained was of diameter 277nm whereas for the optimum condition corresponding to fiber to sodium chlorite liquor ratio of 1:50, the smallest cellulose microfiber diameter has reduced to 145nm. The diameter of smallest cellulose microfiber has reduced from 159nm to 145nm in case of treatment method IOU as compared to treatment method IO (Table 4.21). However, from SEM images presented in Figure 4.8 (method IO) and Table 4.19 (method IOU), it can be observed that the cellulose microfibers run through the surface of cellulose fiber bundle dominantly in case of cellulose microfibers isolated by method IOU as compared to that obtained by method IO. The range of diameters of cellulose microfiber isolated by method IO is 159nm-509nm and by method IOU is 145nm-404nm. The smallest cellulose microfiber of dimeter 145 nm is isolated from Pongamia seed hull for the

optimum fiber to liquor ratio of 1:50, acetic acid to nitic acid ratio of 15:1 and ultrasonication time of 25min.

The maximum observed increase in cellulose concentration from method IO to Method IOU is 6%, in case of cellulose microfibers isolated by Finger millet straw and appreciable defibrillation of cellulose microfibers is also observed after ultrasonication treatment (Method IOU). It is also observed that, higher the cellulose concentration, smaller is the size of the isolated fibers. The action of ultrasonication in defibrillation is more effective when the cellulose concentration is higher. It is due to the effect of cavitation energy mainly oriented on the surface of cellulose fibers (Hu et al. 2015) as the concentration of matrix components, lignin and hemicellulose is minimal. The larger bundles seen in the cellulose microfiber isolated by treatment method IO in Figure 4.9 have been broken down to smaller bundles by the action of sonication energy incorporated in method IOU (Table 4.20). From Table 4.21 it is observed that the range of diameter of the cellulose microfiber isolated from method IOU is 147nm-238nm which is lesser than the fiber diameter range of 153nm-458nm obtained for cellulose microfiber isolated by method IO. For optimum fiber to liquor ratio of 1:50, acetic acid to nitic acid ratio of 10:1 and ultrasonication time of 20min, the smallest of the cellulose microfibers isolated is of diameter 147nm.

Table 4.21 illustrate the reduction of fiber size by IOU method compared to IO method which proves the effectiveness of ultra-sonication treatment on defibrillation of cellulose microfibers. The cellulose concentration has also varied by incorporation of ultrasonication treatment after IO treatment, which is due to release of loosely bound hemicellulose and lignin from the cellulose structure by ultrasonication. From Table 4.21, it can be observed that the cellulose microfibers isolated from Jatropha seed shell by treatment method IOU had highest cellulose concentration of 90% for optimum fiber to sodium chlorite ratio of 50:1, acetic acid to nitric acid ratio of 10:1 and sonication time of 30min. Similarly, from Table 4.19 it can be observed that cellulose microfiber isolated from Pongamia seed hull fibers by treatment method IOU have the maximum cellulose

concentration of 85% for optimum fiber to sodium chlorite liquor ratio of 1:50, acetic acid to nitric acid ratio of 20:1 and sonication time of 25 min.

Table 4.21. The cellulose concentration and range of diameter (D) of cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet hull fibers after treatment by Method IOU, at optimized fiber to liquor (sodium chlorite) ratio, acetic acid to nitric acid ratio and ultrasonication time (t_u).

Lignocellulosic source	Fiber to liquor ratio per cycle	Acetic acid to nitric acid ratio	α-C (method IO) (dry wt %)	t_u (min)	α-C (method IOU) (dry wt %)	D (nm)	
						method IO	method IOU
Jatropha seed shell	1:50	10:1	89±0.24	20	90 ±0.99	225-317	194-477
Pongamia seed hull		1:15	84±0.49	25	85±0.75	159-509	145-404
Finger millet straw		1:10	92±0.75	20	93±1.24	153-458	147-238

As per Table 4.20, the cellulose microfiber isolated from Finger millet straw by treatment method IOU for the optimum fiber to liquor ratio of 1:50, acetic acid to nitric acid ratio of 10:1 and sonication time of 20 min has the maximum cellulose concentration of 93%.

From Table 4.21, it is also clear that the treatment method IOU has resulted in marginal increase in cellulose concentration in the cellulose microfiber by only 1% as compared to method IO. But the defibrillation of cellulose microfibers is prominent as observed from the SEM images and also there is a considerable reduction in size of the cellulose microfibers. Thus, in case of cellulose microfibers isolated from Jatropha seed shell,

Pongamia seed hull and Finger millet straw fibers by treatment method IOU under optimum condition, it is observed that the maximum of sonication energy was applied on to the surface of cellulose fibers for defibrillation.

4.2.6 Isolation of cellulose microfiber by method IOE

The cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw fibers by treatment method IO under the optimum conditions which yielded highest cellulose content were further subjected to enzymatic hydrolysis in order to check the effect of hydrolysis on isolation of cellulose and defibrillation of cellulose microfibers. For the enzymatic treatment, the cellulose microfibers isolated by IO method under the optimum conditions involved the treatment with the enzyme endoglucanase. The effect of enzyme concentration (0.02% and 0.5%) on the cellulose isolation and defibrillation was studied. Table 4.22–4.24 present the chemical composition and SEM images along with the range of diameter of cellulose microfibers isolated by treatment method IOE from Jatropha seed shell, Pongamia seed hull and Finger millet straw fibers respectively.

4.2.6.1 Effect of enzymatic hydrolysis on isolation of cellulose fiber

From Tables 4.22-4.24 it is observed that the cellulose concentration of isolated cellulose microfibers has increased only marginally (≤1%) in case of cellulose microfibers isolated from Jatropha seed shell and Pongamia seed hull fibers and by ≤2% in cellulose microfibers isolated by Finger millet straw fibers after treatment with enzymes (method IOE) compared to cellulose concentration obtained after treatment method IO. As discussed in case of OE treatment method in section 4.2.3, the enzymes act on the cellulose fibers by increasing the cell wall swelling (Henriksson et al. 2007), breaking glucosidic bonds (Manley et al. 1964, White et al. 1981) and degrade amorphous region of cellulose microfibers (Tang et al. 2015). Treatment with 0.02% (by wt.) enzyme concentration is below the saturation limit of enzymes to bind into cellulose fibers and has proven to be marginally better in isolation of cellulose microfibers as compared to

that with 0.5% of enzyme concentration which may be above the saturation level. Enzyme concentration above the saturation level may also lead to degradation of the isolated cellulose, thus leading to lower increase from the IO method as compared to that with 0.02% (by wt.) enzyme concentration.

4.2.6.2 Effect of enzymatic hydrolysis on morphology of cellulose microfiber

In case of cellulose microfiber isolated from Jatropha seed shell, it is observed that the enzymatic hydrolysis has contributed marginally in increasing the cellulose concentration with maximum of 1% increase in cellulose concentration in the cellulose microfibers as compared to those isolated by method IO. However, the comparison of the SEM images of the cellulose fibers isolated by IO method (Figure 4.7) with that of the cellulose fibers isolated by IOE method (Table 4.22), it is observed that the enzymatic treatment has resulted in defibrillation of the isolated fibers owing to breakage of glucosidic bonds by the enzymes in the isolated cellulose. Table 4.22 shows that, under the optimum condition with 1:30 fiber to liquor ratio, though there is no significant reduction in bundles size of cellulose microfibers isolated by IOE method, the defibrillation is clearly indicated as observed from the exposed microfibers on the surface of fiber bundles when compared with that of cellulose microfibers isolated by method IO (Figure 4.7c), However, under the optimum condition with 1:50 fiber to liquor ratio the cellulose fibers appear to be more defibrillated after IOE treatment, with separated cellulose microfibers predominantly visible on the surface. The range of diameter of the cellulose microfibers isolated by IOE treatment is 222 nm-474 nm and the maximum cellulose content of 90% is obtained under optimum fiber to liquor ratio of 1:50, acetic acid and nitric acid ratio of 10:1 and with enzyme concentration of 0.02%.

Table 4.22. The chemical composition of the cellulose microfibers isolated from Jatropha seed shell fibers after treatment by Method IOE, at different fiber to liquor (sodium chlorite) ratio, acetic acid to nitric acid ratio and enzyme concentration (E) with SEM images of cellulose microfibers and range of diameter (D).

fiber to Liquor ratio	Acetic acid to nitric acid ratio	Composition of cellulose fiber dry wt %) by IO method			SEM images and D (nm)	E (wt%)	Composition of cellulose fiber in (dry wt %) by IOE method			SEM images and D (nm)
		L	H	α-C			L	H	α-C	
1:30	20:1	12±0.99	10±1.24	74±1.24	319-718	0.02	11±0.49	10±0.75	75±0.24	212-382 bundle – 4.64µm
						0.5	11±0.99	10±0.24	75±0.75	
1:50	10:1	2±0.75	6±0.49	89±0.24	225-317	0.02	2±0.49	5±0.49	90±1.24	222-474
						0.5	2±0.24	5±0.75	89±0.49	

Table 4.23. The chemical composition of the cellulose microfibers isolated from Pongamia seed hull fibers after treatment by Method IOE, at different fiber to liquor (sodium chlorite) ratio, acetic acid to nitric acid ratio and enzyme concentration (E) with SEM images of cellulose microfibers and diameter range (D).

fiber to liquor ratio	Acetic acid to nitric acid ratio	Composition of cellulose fiber (dry wt %) by IO method			SEM images and D (nm)	E (wt%)	Composition of cellulose fiber (dry wt %) by IOE method			SEM images and D (nm)
		L	H	α-C			L	H	α-C	
1:30	20:1	6±0.49	11±0.75	79±1.24	230-1.12 µm	0.02	6±1.24	10±0.24	80±0.75	211-397
						0.5	6±0.49	11±0.99	79±0.24	
1:50	15:1	3±0.99	10±1.24	84±0.49	159-509	0.02	3±0.99	8±0.49	85±0.75	227-466
						0.5	3±0.24	9±0.75	85±0.99	

Table 4.24. The chemical composition of the cellulose microfibers isolated from finger millet straw fibers after treatment by Method IOE, at different fiber to liquor (sodium chlorite) ratio, acetic acid to nitric acid ratio and enzyme concentration (E) with SEM images of cellulose microfibers and diameter range (D).

fiber to liquor ratio	Acetic acid to nitric acid ratio	Composition of cellulose fiber (dry wt %) by IO method			SEM images and D (nm)	E (wt%)	Composition of cellulose fiber (dry wt %) by IOE method			SEM images and D (nm)
		L	**H**	**α-C**			**L**	**H**	**α-C**	
1:30	20:1	3±0.75	9±1.24	81±0.99	195-834	0.02	3±0.49	8±0.24	**83**±0.75	200-1097
						0.5	3±0.99	8±0.49	82±0.24	
1:50	10:1	1±0.49	3±0.24	**92**±0.75	153-458	0.02	1±0.49	3±0.24	**93**±0.75	324-1208
						0.5	1±0.75	3±0.99	92±0.24	

The lignin content in cellulose microfibers isolated from Jatropha seed shell by method IO (Table 4.22) is 12% under optimum conditions with 1:30 fiber to liquor ratio where as it is 2% under optimum condition with 1:50 fiber to liquor ratio. When the lignin concentration is high, due to the shielding effect of the lignin the accessibility of enzymes to the cellulose is lower. This results in lower defibrillation effect in the case of fibers isolated under optimum condition with 1:30 fiber to liquor ratio as compared to that isolated under optimum condition with 1:50 fiber to liquor ratio as it is clear in Table 4.22.

Similar observations are made in case of cellulose fibers isolated by method IOE from Pongamia seed hull. As observed from Table 4.23 the maximum increase in cellulose content after treatment method IOE is only 1% under optimum conditions with both 1:30 and 1:50 fiber to liquor ratio. The cellulose microfiber isolated under the optimum conditions with 1:30 fiber to liquor ratio by method IO as presented in the SEM image, reveal presence of isolated cellulose microfibers running through the length of the fiber bundle with smallest cellulose microfiber of diameter 230nm whereas in case of cellulose microfibers isolated by method IOE (Table 4.23), shortened cellulose fibers have appeared with reduction in diameter of the smallest cellulose microfiber by 19nm. Under optimum conditions with 1:50 fiber to liquor ratio the cellulose fibers isolated by method IO, have microfiber bundles with the exposed cellulose microfibers of the diameter range of 159nm -509nm, whereas prominently protruding microfibers with diameter range of 227nm-466nm (Table 4.23) are visible in the cellulose fibers isolated by method IOE. The cellulose fibers isolated by method IOE are distinctly separated and do not show the presence of bundles as in the cellulose fibers isolated by method IO, thus indicating that defibrillation of cellulose microfibers is favoured by the enzymatic treatment. The maximum cellulose content of 85% and the smallest cellulose microfiber of diameter 227nm is obtained under optimum fiber to liquor ratio of 1:50, acetic acid and nitric acid ratio of 15:1 and enzyme concentration of 0.02%.

In case of cellulose microfibers isolated from Finger millet straw, maximum of 2% increase in cellulose concentration for 1:30 fiber to liquor ratio and 1% for 1:50 fiber to

liquor ratio was reported after treatment method IOE. The images in Table 4.24 show the defibrillated cellulose microfibers when compared to the cellulose microfibers isolated by method IO. From Table 4.25 it is observed that the cellulose microfibers isolated by method IOE for optimum fiber to liquor ratio of 1:50, acetic acid to nitric acid ratio of 10:1 and enzyme concentration of 0.02%, have fiber diameters in the range of 324nm-1.2μm. Whereas, cellulose microfibers isolated by method IO have smaller fiber diameters in the range of 153nm-458nm. The cellulose microfibers isolated by method IO are observed to be bundles with no significant appearance of cellulose microfibers on the bundle surface however, the cellulose microfibers isolated by method IOE have cellulose microfibers dominant on the surface ensuring defibrillation of cellulose microfibers by enzymatic action. The number of defibrillated fibers are lesser in the fibers isolated by IO method, whereas the fibers isolated by IOE method are clearly seemed to be defibrillated. The reduction in fiber size after enzymatic treatment is not observed, rather the cellulose fibers are found to bond among themselves to form aggregates of larger diameter compared to that of fibers isolated by method IO. For optimum treatment condition of 1:50 fiber to liquor ratio, 10:1 acetic acid to nitric acid ratio and enzyme concentration of 0.02%, the maximum cellulose content obtained was of 93% and the smallest cellulose microfiber isolated is of diameter 324nm.

As discussed in section 4.2.3, and on comparison of Tables 4.13 and 4.25, it is observed that the defibrillation by enzymatic treatment is favoured when the cellulose content is higher as observed both in method OE and method IOE. The accessibility of the enzyme onto cellulose surface for defibrillation by the action of enzyme hydrolysis increases when the cellulose concentration is higher and thus the cellulose microfiber has been defibrillated and/or reduced in diameter. The enzyme concentration of 0.02% has been found to be favourable for all the lignocellulosic sources; Jatropha seed shell, Pongamia seed hull and Finger millet straw fibers owing to higher increase in cellulose content, defibrillation of cellulose microfibers and also by being economical owing to less requirement of enzymes.

Table 4.25. The cellulose concentration and range of diameter (D) of the cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw fibers by Method IO, IOU and IOE, at optimized fiber to sodium chlorite liquor ratio, acetic acid to nitric acid ratio, ultrasonication time (t_u) and enzyme concentration (E).

Lignocellulosic source	Fiber to liquor ratio per cycle	Acetic acid to nitric acid ratio	t_u (min)	E (%)	α-C (dry wt %)			D (nm)		
					IO	IOU	IOE	IO	IOU	IOE
Jatropha seed shell	1:50	10:1	20	0.02	89±0.24	90 ±0.99	90±1.24	225-317	194-477	222-474
Pongamia seed hull		15:1	25		84±0.49	85±0.75	85±0.75	159-509	145-404	227-446
Finger millet straw		10:1	20		92±0.75	93±1.24	**93**±0.75	153-458	147-238	324-1208

4.3 Comparison of cellulose microfibers isolated by the combination of different methods

Cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by Method O (Organosolv), Method OU (Organosolv-Ultrasonication), Method OE (Organosolv-Enzymatic), Method IO (Inorganic-Organosolv), Method IOU (Inorganic-Organosolv-Ultrasonication) and Method IOE (Inorganic-Organosolv-Enzymatic) under optimized process parameters were compared in order to choose a favourable method for isolation of cellulose microfibers rich in cellulose and of smaller diameter. The effect of combination of methods under optimum conditions in terms of cellulose concentration, the size of the cellulose microfiber bundles and cellulose microfiber diameter are presented in Table 4.26.

From Table 4.26 it is observed that the cellulose microfibers isolated by treatment method O and IO show maximum isolation of cellulose microfibers when compared to

untreated fibers whereas treatment methods OU, OE, IOU and IOE have facilitated in defibrillation of cellulose microfibers with marginal change in cellulose composition. In case of cellulose microfibers isolated from Jatropha seed shell and Finger millet straw it can also be observed that the isolation of cellulose microfibers with higher cellulose composition is observed by method IO compared to cellulose composition in cellulose fibers isolated by treatment method O. However, the cellulose composition of cellulose microfibers isolated from Pongamia seed hull remains same for both the treatment method O and IO, owing to difficulty in breaking the matrix structure as the lignin and hemicellulose is high in untreated fiber of Pongamia seed hull compared to matrix composition of untreated Jatropha seed shell and Finger millet straw fibers. However, when the diameter of smallest cellulose microfibers isolated from Pongamia seed hull by these two methods are compared, it is observed that there is significant reduction in diameter of cellulose microfibers isolated by method IO. Thus, the Method IO is favourable in isolation of cellulose microfibers from all the three lignocellulosic sources.
The ultrasonication treatment (IOU) incorporated after method IO has further enhanced the cellulose microfiber isolation, defibrillation of cellulose microfibers and also accounted significant reduction in size of smallest cellulose microfiber. Thus, method IOU is found to be favourable compared to other methods as high cellulose content and finer cellulose microfibers are isolated from lignocellulosic sources. Further the cellulose microfiber isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by method IOU were compared with cellulose microfibers isolated from different sources and by different methods as reported in literature.

Table 4.26. Comparison of cellulose concentration (α-C) (wt %) of cellulose microfibers, surface morphology (S) of cellulose microfibers and the diameter (D) range of cellulose microfiber (nm) isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by methods O, OU, OE, IO, IOU and IOE under optimized condition.

Diameter of cellulose microfibers (nm) (D), cellulose content (wt %) (α-C) and surface morphology (S)												
Sourc e	**Untreated**			**O**			**OU**			**OE**		
	D	**α-C**	**S**	**D**	**α-C**	**S**	**D**	**α-C**	**S**	**D**	**α-C**	**S**
Jatrop ha seed shell	NA	41	Plane surface without cellulose fibers on the surface	277-747	82	Cellulose fiber bundles of size 4.05μm with microfibers barely seen on the surface	231-468	84	No Bundles, smaller microfiber is distinct on the surface	248 -452	83	No Bundles, smaller microfibers are defibrillated
Ponga mia seed hull	NA	42	Plane surface without cellulose fibers on the surface	319-768	85	Cellulose microfiber of size 9.24 μm are emerged out from the surface of cellulose fiber bundle	251-614	86	No Bundles, microfiber s are defibrillate d	274-757	86	No Bundles, Defibrillate d microfibers with distinct surface
Finger millet straw	NA	50	Plane surface without cellulose fibers on the surface	329 -522	84	Bundle NA, distinct microfibers on the surface of fiber bundle	153 -359	86	No Bundles, smaller microfiber defibrillate d e	178-389	86	No Bundles, smaller microfibers predominan t on the surface

Table 4.26. Continued…..

Source	Diameter of cellulose microfibers (nm) (D), cellulose content (wt %) (α-C) and surface morphology (S)								
	IO			IOE			IOU		
	D	α-C	S	D	α-C	S	D	α-C	S
Jatropha seed shell	225-317	89	Cellulose fibers bundle of size 4.3 µm with few distinct microfibers	222-474	90	No Bundles, microfibers defibrillated	**194-477**	**90**	No Bundles, microfibers defibrillated to smaller diameter with fibers emerging out from the surface
Pongamia seed hull	159-509	84	Cellulose fibers bundle of size 4.1 µm with microfibers bond to each other	227-466	85	No Bundles, microfibers defibrillated but appear to be bond with one another	**145-404**	**85**	No Bundles, microfibers are reduced in size and are bond within themselves
Finger millet straw	153-458	92	Cellulose fibers bundle size 7.8 µm with very few microfibers barely seen	324-1208	93	No Bundles, distinct microfibers on the surface	**147-238**	**93**	Bundle size reduced to 4.8 µm with smaller microfibers dominant on the surface

The comparisons of diameter of the cellulose fiber isolated and method of isolation are presented in Table 4.27. It is observed that the cellulose microfibers isolated from other different sources have shown reduction in diameter as the number of treatment methods are increased. The diameter range of cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw are lesser when compared to diameter of cellulose microfibers isolated from Helicteres isora plant barks (10µm) (Chirayil et al 2014), Agave tequilana bagasse and barley husk (6.5-44.8µm and 27.7µm) (Espino et al 2014), by multiple chemical and mechanical treatments which consists of many stages of treatments than the one incorporated in the present work and cellulose microfiber isolated from Energycane bagasse (12±5µm) (Yue et al. 2015) by solely chemical treatments. However, comparatively smaller diameter cellulose microfibers are isolated from other sources by combination of several treatment steps which are either complete chemical treatments involving acid hydrolysis using high concentration Sulphuric acid or combination of high energy consuming mechanical treatments.

The observation made from the chemical composition and morphology of the cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw fibers by treatment method IO and IOU have shown removal of matrix components, lignin and hemicellulose and isolation of cellulose microfibers.

From Table 4.26, the cellulose microfiber isolated by method IOU from Finger millet straw fibers have the highest cellulose composition of 93%, which is followed by Jatropha seed shell fibers having of 90% and the least of 85% cellulose concentration in Pongamia seed hull. The cellulose microfibers isolated from coconut sheath by method IO by Maheswari et al. (2012), had final cellulose concentration of 89.6% and lignin concentration of 4.1%. Xu et al. (2016) have reported that the cellulose fibers isolated from bamboo by bleaching and alkaline treatment had final cellulose composition of 83.67% and 0.13% lignin. Similarly, Reddy and Yang, (2009) have also reported that the cellulose and lignin composition of the cellulose fibers isolated from milk weed stem fibers by strong alkali and maceration (nitric acid and citric acid) treatment was about 74.5% and 4.1% respectively. The cellulose composition of the cellulose microfibers

isolated from all the three lignocellulosic sources in the present work are on the higher side compared to the ones reported in the literature. Thus, in the present work the method IOU has not led to any compromise in cellulose composition and size of the isolated cellulose microfibers.

Table 4.27. Comparison of different sources treated by different methods for isolation of cellulose microfibers and diameter of the isolated cellulose fibers.

Sl.no	Source	Type	Diameter	Isolation method	Reference
1	Wood powder	powder	5-20 nm	Bleached, alkaline, ultra-sonication	Yu et al. (2011)
2	Wheat	straw	10–50 nm	Alkali, steam explosion coupled with high shear homogenization	Kaushik and Singh (2011)
3	Semi-chemical kraft bleached eucalyptus	pulp	580 nm	Sonication	Urruzola et al. (2012).
4	Dry softwood pulp	Bleached softwood pulp	30 nm	high shear homogenization	Zhao et al. (2013)
5	Switchgrass and cotton	-	35- 200 nm 50 -140 nm	Bleached, sulfuric acid hydrolysis, dialysis	Wu et al. (2013)
6	Agave tequilana and barley	Bagasse husk	6.5 -44.8 µm 27.7 µm	Acid hydrolysis, dialysis, homogenisation, ultrasonication	Espino et al (2014)
7	Banana peel bran	bran	10.9-7.6 nm	alkaline, bleaching, and acid hydrolysis and enzymatic	Tibolla et al. (2014)
8	Poplar wood	-	5- 20 nm	Bleached, alkaline, ultrasonication	Chen et al. (2011)
9	Coconut fibers	husk	5 nm	Bleached, acid hydrolysis	Rosa et al (2010)
10	Oil palm	biomass	10 nm-20nm	Acid hydrolysis, sonication	Haafiz et al. (2014)
11	Kapok fiber	-	450-850 nm	Bleached, alkaline,	Draman et al. (2014)
12	Alfa fibers	-	5 -10 nm	Alkaline, bleaching, acid hydrolysis	Trache et al. (2014)
13	Bagasse	-	200 nm	Acid Hydrolysis, alkali and bleached	Bhattacharya et al. (2008)
14	Rice	husk	6 - 14 nm	Alkali, ultrasonication, H_2O_2TAED and acid hydrolyse	Rosa et al. (2012)
15	De-pectinated sugar beet pulp	pulp	10–70 nm	Alkali, bleaching, high pressure homogenization	Li et al. (2014)
16	Norway spruce	bark	2.8 nm	Bleached, acid hydrolysis and dialyzed	Normand et al. (2014)

Table 4.27. Continued….

Sl.no	Source	Type	Diameter	Isolation method	Reference
17	Bamboo fibers	pulp	10-50 µm	Dialysis and ultrasonication	Zhang et al. (2014)
18	Posidonia oceanica	leaves and balls	7-8 nm	Alkali, bleaching, acid hydrolysis ultrasonication	Bettaieb et al. (2014)
19	Helicteres isora plant	barks	10 µm	Alkaline, bleaching, acidic steam and homogenization	Chirayil et al. (2014)
20	Natural Pine	needle	30 - 70 nm	Ultrasonication	Xiao et al. (2015)
21	Tomato	peels	42 nm	Bleaching, chlorine-free alkaline peroxide and dialyzed	Jiang and Hsieh (2015)
22	Posidonia oceanica	balls and leaves	7nm	Acid hydrolysis and ultrasonication	Bettaieb et al. (2015)
23	Oil palm	trunk	7.67 nm - 7.97 nm	Bleached, alkaline, sonicated, homogenised, acid hydrolysis	Lamaming et al. (2015)
24	Banana	pseudo-stem	-	Bleached, liquefaction, alkali	Li et al. (2015)
25	Energycane	bagasse	12±5 µm	Alkali, Bleached	Yue et al. (2015)
26	Cotton	stalks	3–15 nm	Acid hydrolysis, TEMPO mediated oxidation , alkaline, Bleached, Ultrasonication	Soni et al. (2015)
27	Pomelo	fruit	10–20 nm	Alkali and acid hydrolysis	Yongvanich (2015)
28	Garlic	straw	6 nm	Alkali extraction, bleached and acid hydrolysis	Kallel et al. (2016)
29	Culinary banana	peel	43.8 - 10.3 nm	Alkali-acid hydrolysis, high-intensity ultrasonication	Khawas et al. (2016)
30	Ushar (calotropis procera)	seed	14–24 nm 10–20 nm	Acid hydrolysis and TEMPO-mediated oxidation	Oun and Rhim (2016)
31	Jatropha	seed shell	194-477nm	Bleaching, NaOH, organosolv, and ultrasonication	Present study
32	Pongamia	seed hull	145-404nm		
33	Finger millet	straw	147-238nm		

4.3.1 Visual observation

The variation in appearance of cellulose microfibers isolated from the lignocellulosic source after each treatment step by method IOU are presented in Figure 4.10.

As observed in Figure 4.10, the dewaxed fibers have retained the brownish colour similar to that of untreated fibers shown in Figure 4.1. Further with sodium chlorite treatment, the fiber colour changes to white by bleaching. Later the cellulose microfibers were subjected to treatment with NaOH and then organosolv treatment. The fibers dispersed in the organosolv after treatment retain the white colour and appear finer compared to the dewaxed fibers. The dispersion of finer fibers indicates that the fibers are rich in cellulose. These isolated cellulose microfibers after separation from the organosolv, when dispersed in distilled water settle down at the bottom of the container. However, after ultrasonication treatment it is observed that the cellulose microfibers are well dispersed. The dispersion of cellulose microfibers in distilled water after ultrasonication treatment is due to the effect of sonication energy (Urruzola et al. 2012) which breaks the bonds between the cellulose and resulted in defibrillation and reduction in the size of the cellulose microfibers.

4.3.2 SEM analysis

The untreated and the isolated cellulose microfibers obtained from the three lignocellulosic sources after different stages of treatment by IOU method when observed under scanning electron microscope showed morphological changes which are presented in Figure 4.11. As observed from Figure 4.11, it is clear that the untreated fibers (Figure 1a, 2a, 3a) have smooth plain surface when compared to dewaxed fibers (Figures 1b, 2b, 3b) due to the presence of waxes on the surface. Further on subjecting the dewaxed fibers to method IO (Figures 1c, 2c, 3c), the large fiber bundles of cellulose fibers appear. These fiber bundles show the presence of microfibers which are being exposed on the surface. However, they are agglomerated to form bundles. After ultrasonication treatment (method IOU), the cellulose microfibers have defibrillated from the cellulose bundle and are dominant at the surface of the bundle (Figures 1d, 2d, 3d).

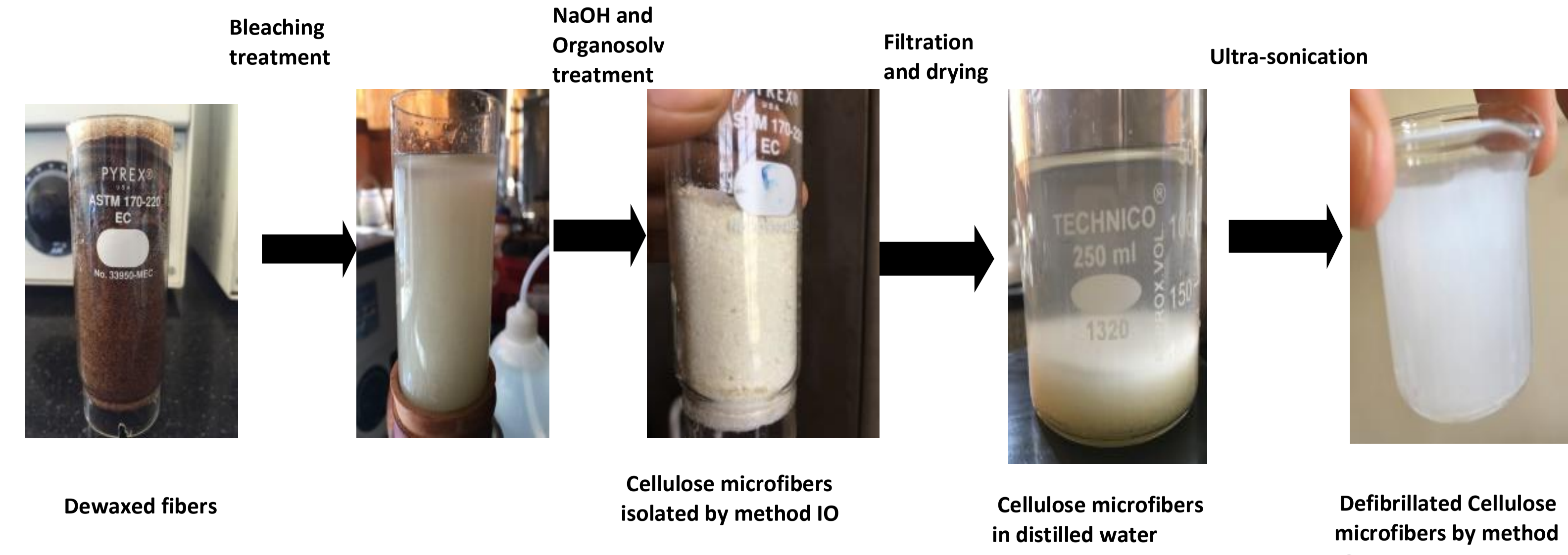


Figure 4.10. Comparison of variation in appearance of fibers isolated by method IOU

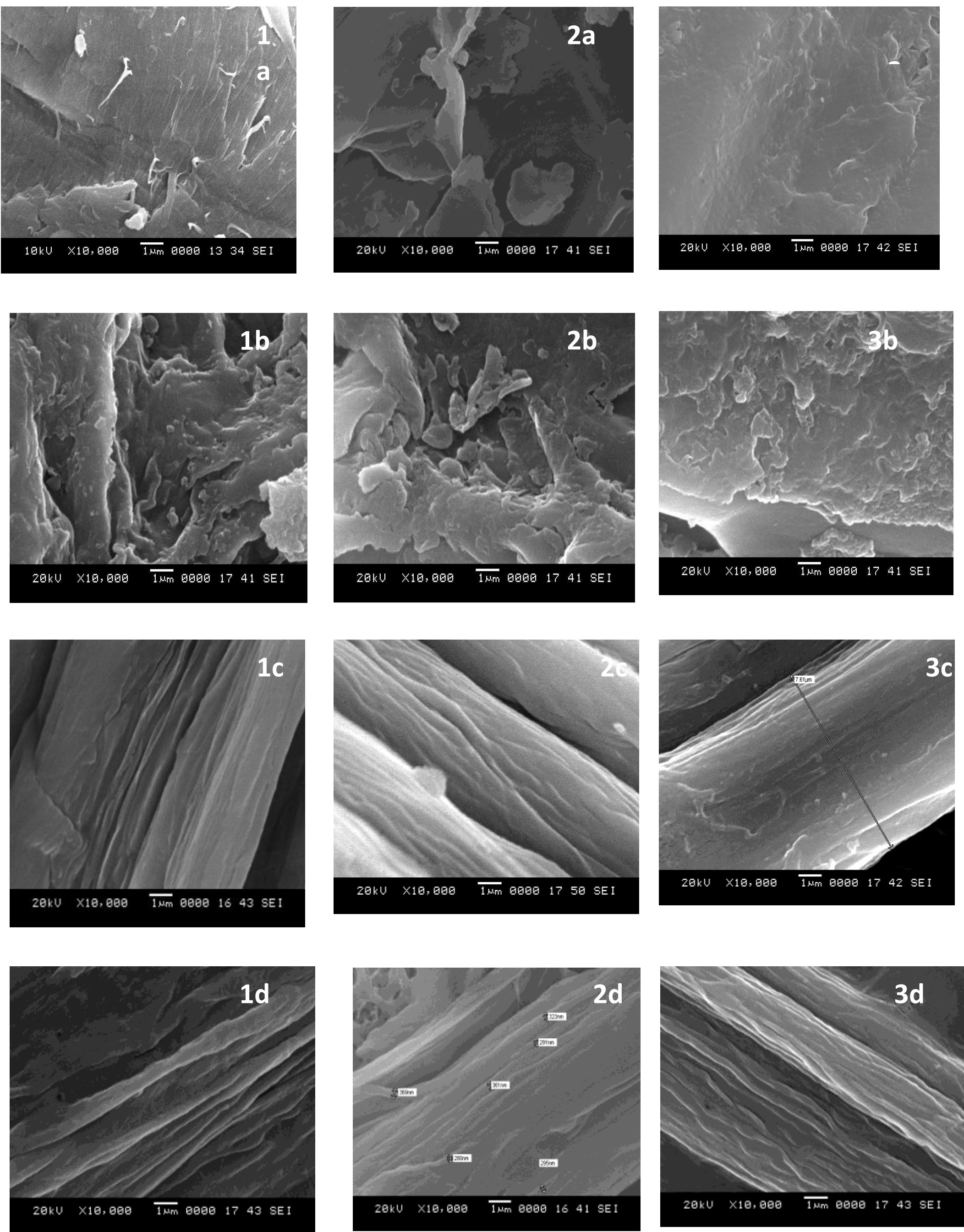


Figure 4.11. Scanning electron microscope images of a) untreated fibers, b) dewaxed fibers and cellulose microfibers isolated c) by method IO and d) method IOU respectively from 1. Jatropha seed shell, 2. Pongamia seed hull and 3. Finger millet straw respectively.

The cellulose microfibers obtained from Jatropha seed shell and Finger millet straw appear to be distinctly separated after IOU treatment. However, though the cellulose microfibers obtained from Pongamia seed hull appear to be defibrillated, they are not distinctly separated due to agglomeration of the fibers. However, for better understanding of the cellulose microfiber properties and to further confirm the removal of matrix components, the untreated and isolated cellulose microfiber from Jatropha seed shell, Pongamia seed hull and Finger millet straw fibers by treatment method IO and IOU were characterized by various techniques such as Nuclear magnetic resonance (NMR) analysis, X- Ray diffractometer analysis, Fourier transform infrared radiation (FTIR) analysis, Thermogravimetric analysis (TGA) and Particle size analyser (DLS -dynamic light scattering).

4.4 Characterization of untreated lignocellulosic sources and the cellulose microfibers isolated by method IO and method IOU

The morphological and chemical composition of untreated lignocellulosic sources and the isolated cellulose microfibers from Jatropha seed shell, Pongamia seed hull and Finger millet straw fibers, by treatment method IO and IOU are discussed in detail in the previous sections. The untreated and the cellulose microfibers were subjected to further characterization by NMR, TG, XRD, FTIR and DLS (fiber dimension) analyser. The untreated fibers of Jatropha seed shell, Pongamia seed hull and Finger millet straw are herein after represented as JR, PR and FR respectively, whereas cellulose microfibers isolated by method IO are represented as J-IO, P-IO and F-IO respectively and cellulose microfibers isolated by treatment method IOU are referred as J-IOU, H-IOU and R-IOU respectively.

4.4.1 DLS analysis

The fiber size distribution of cellulose microfibers isolated by method IO and IOU from Jatropha seed shell, Pongamia seed hull, and Finger millet straw are presented in Figure 4.12 and the mean fiber dimensions of these cellulose microfibers are presented in Table 4.28.

Table 4.28. The mean Diameter and length of cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull fibers and Finger millet straw by method IO and method IOU as observed in DLS analyser respectively.

Dimensions	Fiber dimension (nm)					
	J-IO	P-IO	F-IO	J-IOU	P-IOU	F-IOU
Mean Diameter	199.9	353.0	218.5	173.8	178.3	151.9
Mean Length	3670.3	2527	905.8	1053.0	770.6	741.8

The two peaks observed in the size distribution plots in Figure 4.12 are corresponding to the longer and shorter dimensions owing to the fibrous structure of cellulose fibers representing the length and the diameter. de Carvalho Mendes et al. (2015) has also reported such two peaks in DLS histogram and it is generally attained in aqueous dispersion of cellulose fibers. The mean hydrodynamic dimensions of the isolated cellulose fibers are summarized in Table 4.28. The ultrasonication treatment has resulted in defibrillating the cellulose microfibers obtained by treatment method IO, leading to decrease in fiber diameters by 26 nm, 175 nm and 66.6 nm, and the fiber length by 2617.3 nm, 1757 nm and 164 nm, from Jatropha seed shell, Pongamia seed hull and Finger millet straw respectively. The diameters of the cellulose microfibers obtained by particle size analyses is within the range of diameters obtained for cellulose microfibers observed under SEM after IOU treatment. Thus, the visual observation, SEM and particle size analysis show the reduction in cellulose microfiber size thus confirming the removal of matrix components and defibrillation to a large extent.

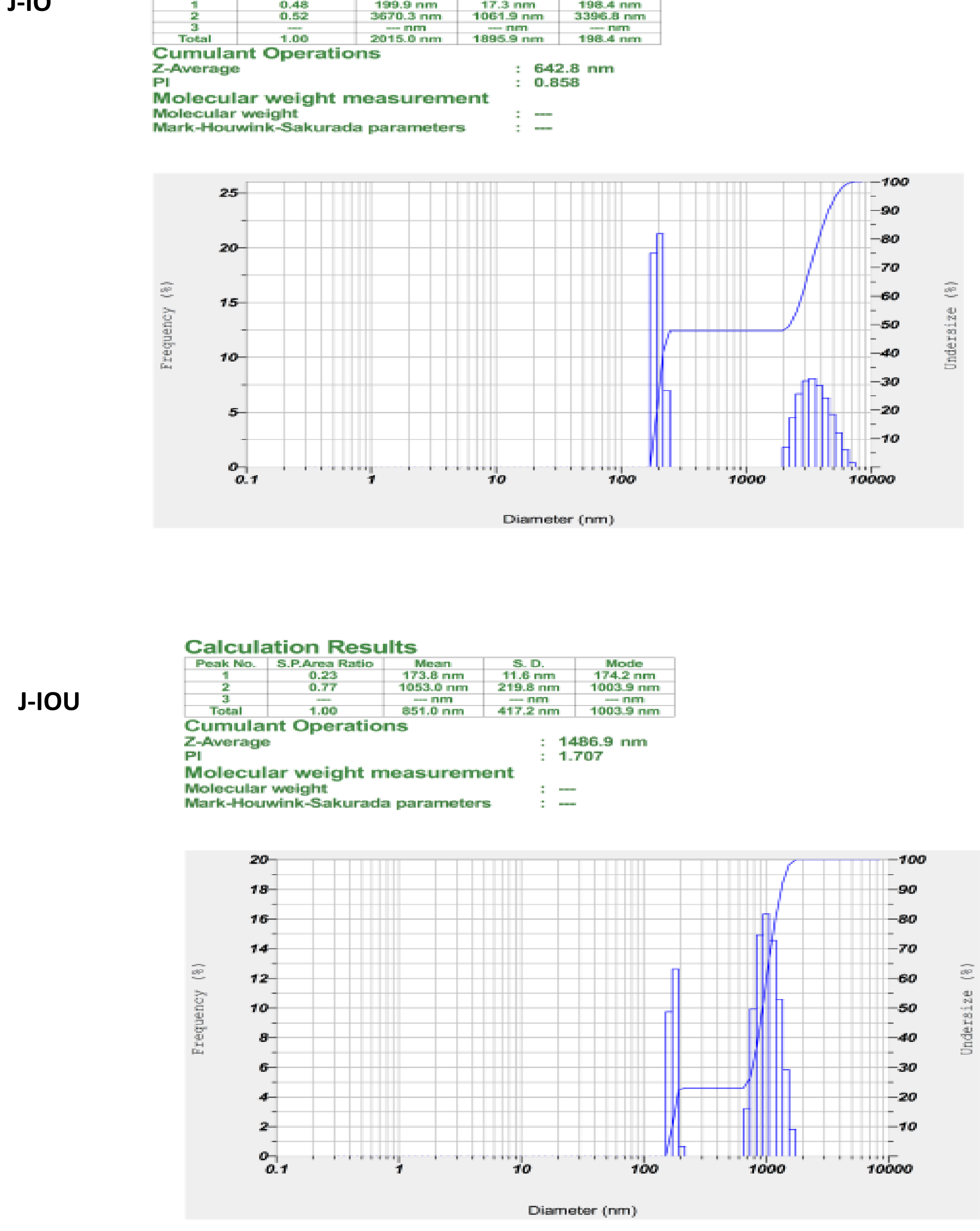
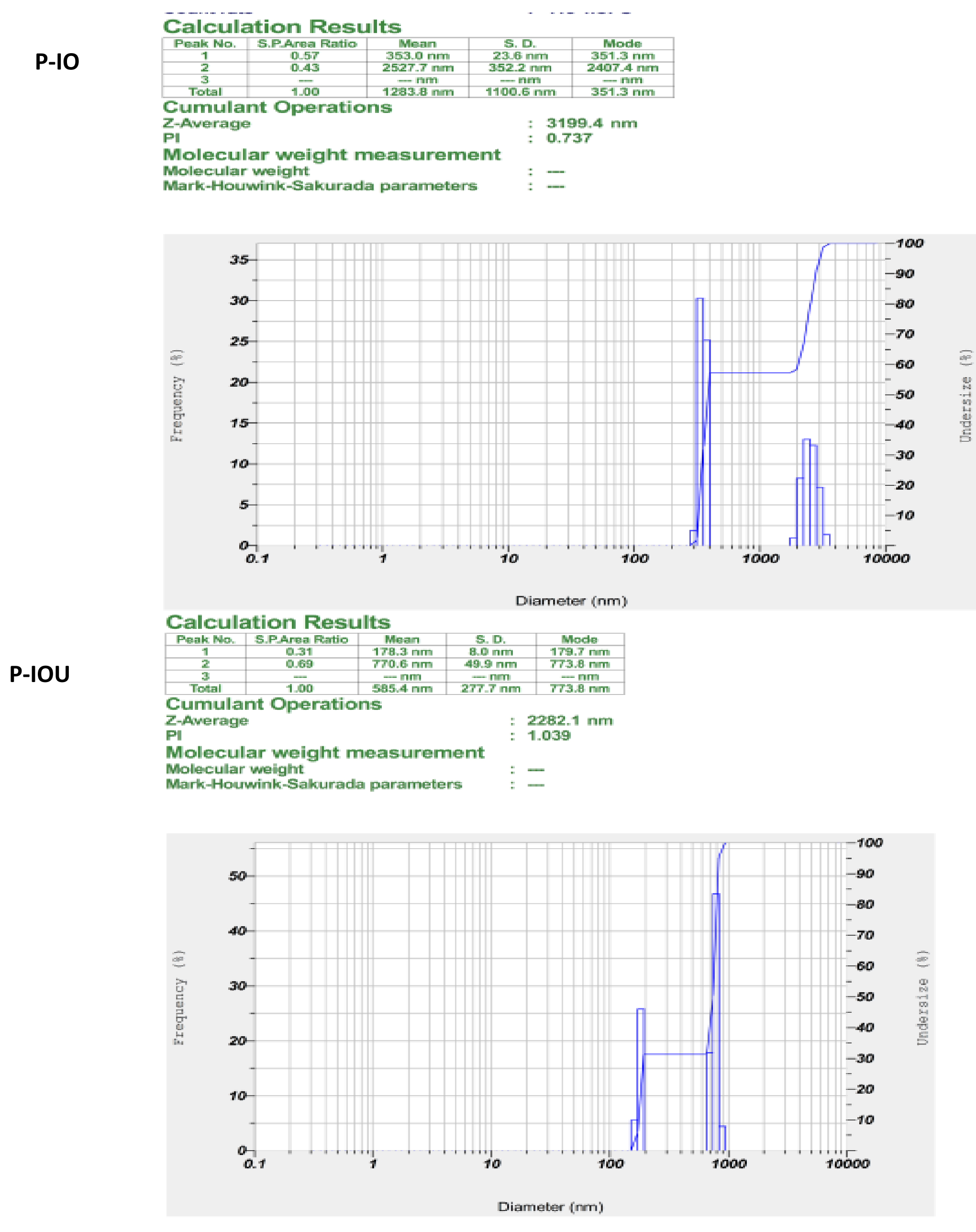


Figure 4.12a. Fiber size distribution curve obtained for cellulose microfiber isolated from Jatropha seed shell by method IO and method IOU



P-IO

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	0.57	353.0 nm	23.6 nm	351.3 nm
2	0.43	2527.7 nm	352.2 nm	2407.4 nm
3	---	--- nm	--- nm	--- nm
Total	1.00	1283.8 nm	1100.6 nm	351.3 nm

Cumulant Operations
Z-Average : 3199.4 nm
PI : 0.737
Molecular weight measurement
Molecular weight : ---
Mark-Houwink-Sakurada parameters : ---

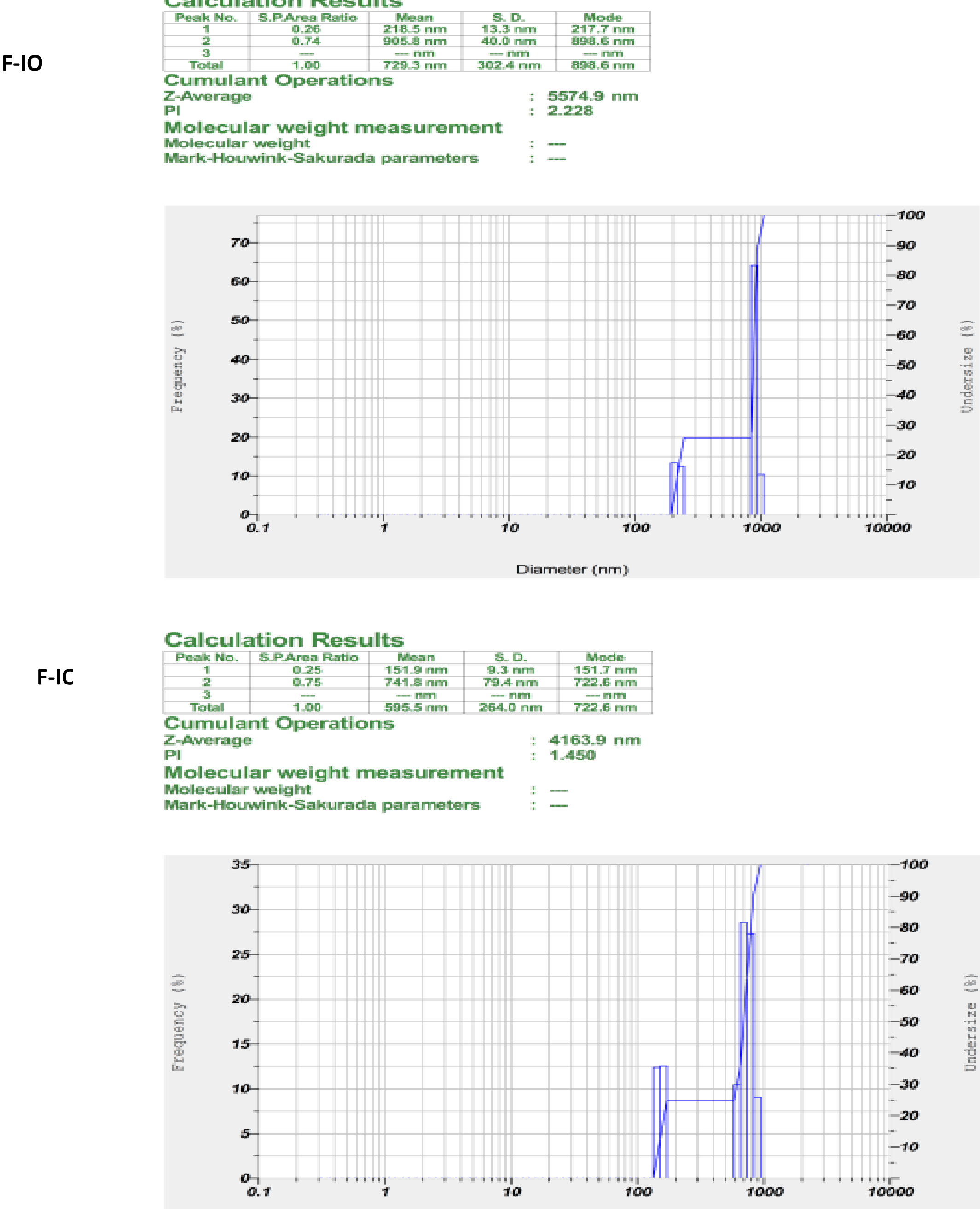
P-IOU

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	0.31	178.3 nm	8.0 nm	179.7 nm
2	0.69	770.6 nm	49.9 nm	773.8 nm
3	---	--- nm	--- nm	--- nm
Total	1.00	585.4 nm	277.7 nm	773.8 nm

Cumulant Operations
Z-Average : 2282.1 nm
PI : 1.039
Molecular weight measurement
Molecular weight : ---
Mark-Houwink-Sakurada parameters : ---

Figure 4.12b. Fiber size distribution curve obtained for cellulose microfiber isolated from Pongamia seed hull fibers by method IO and method.



F-IO

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	0.26	218.5 nm	13.3 nm	217.7 nm
2	0.74	905.8 nm	40.0 nm	898.6 nm
3	---	--- nm	--- nm	--- nm
Total	1.00	729.3 nm	302.4 nm	898.6 nm

Cumulant Operations
Z-Average : 5574.9 nm
PI : 2.228
Molecular weight measurement
Molecular weight : ---
Mark-Houwink-Sakurada parameters : ---

F-IC

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	0.25	151.9 nm	9.3 nm	151.7 nm
2	0.75	741.8 nm	79.4 nm	722.6 nm
3	---	--- nm	--- nm	--- nm
Total	1.00	595.5 nm	264.0 nm	722.6 nm

Cumulant Operations
Z-Average : 4163.9 nm
PI : 1.450
Molecular weight measurement
Molecular weight : ---
Mark-Houwink-Sakurada parameters : ---

Figure 4.12c. Fiber size distribution curve obtained for cellulose microfiber isolated from Finger millet straw by method IO and method IOU.

4.4.2 Solid state ^{13}C NMR spectra

The removal of matrix components such as lignin and hemicellulose from Jatropha seed shell, Pongamia seed hull and Finger millet straw fibers are further confirmed by NMR spectra presented in Figure 4.13a, b, c for the untreated fibers and for the cellulose microfibers isolated by treatment method IO and IOU. The peaks obtained in the NMR spectra for untreated fibers and cellulose microfibers are assigned to their respective functional groups in Table 4.29.

Table 4.29. Assignment of Peaks (ppm) for spectra of untreated fibers and isolated cellulose fiber from Jatropha seed shell, Pongamia seed hull and Finger millet straw by treatment method IO and IOU.

Peaks of isolated cellulose microfibers (ppm)			Assignment
Jatropha seed shell	Pongamia seed hull	Finger millet straw	
106	106	106	C-1- Cellulose I lattice
90	90	90	C-4- Cellulose I lattice of crystalline cellulose
83	84	85.2	C-4- Cellulose I lattice Amorphous cellulose
76	76	76	C-2, C-3, C-5 of cellulose
73	73	73	
66	66	66, 63	C-6 of Cellulose II lattice Crystalline cellulose
21,33	21,35	-	Methylene's in lignin
58	58	58	$-OCH_3$ groups in lignin and hemicellulose

The spectrum represented in Figure 4.13 a, b and c, illustrates the presence of corresponding signals for cellulose, hemicellulose and lignin in case of untreated fibers: JR, PR and FR, whereas spectra of isolated cellulose microfibers J-IO, P-IO, F-IO and J-IOU, P-IOU and F-IOU illustrates prominent peaks of only cellulose carbon atoms. Peaks between 107 to 60 ppm corresponding to six carbon atoms assigned to cellulose molecules are observed in all the spectra. In case of cellulose microfiber spectrum (J-IO,

P-IO, F-IO and J-IOU, P-IOU and F-IOU), the absence of peaks at 20-33 ppm and 110-140 ppm associated with methylenes in lignin and 58.896 ppm of $-OCH_3$ groups in lignin and hemicellulose, ensures removal of the matrix components, hemicellulose and lignin (Sun et al. 2004; Bhattacharya et al. 2008).

Further the absence of peaks at 168 ppm associated with lignin and 172.21 ppm of hemicellulose (Sherif and Keshk, 2015) in cellulose microfiber spectra (J-IO, P-IO, F-IO and J-IOU, P-IOU and F-IOU), signifies effectiveness of chlorination method in isolations of cellulose microfibers by removal of matrix components.

The carbon peaks are observed to be same throughout the 64 -105 ppm region of the spectra for both untreated and the cellulose microfibers of Jatropha seed shell, Pongamia seed hull and Finger millet straw, which ensures un-alteration of cellulose structure by chlorination treatment. The decrease in peaks at 70-80 ppm and 100-110 ppm in the spectra of cellulose microfibers isolated from Pongamia seed hull (Figure 4.13b), can be accounted to loss of non-cellulosic polysaccharides. Bleaching and alkaline treatments dissolve non-cellulosic polysaccharides which are associated with cellulose at the microfibrils surface (Heux et al. 1999) by strong interaction and due to strain/compression of the cellulose fibers (Motaung and Mtibe, 2015).

However, all the spectra show the cellulose carbon atom peak at 107.6 ppm associated with C1 (Halonen et al. 2013), peaks at 77-67 ppm are assigned to C2, C3 and C5 carbon atoms (Sun et al. 2004), peaks at 91.45-84.44 of C4 (Bhattacharya et al. 2008) and attributed to crystalline cellulose, and finally 65.305 -58 associated with C6 amorphous region of cellulose carbon atom (Sun et al. 2004; Sun X. F. et al. 2004).

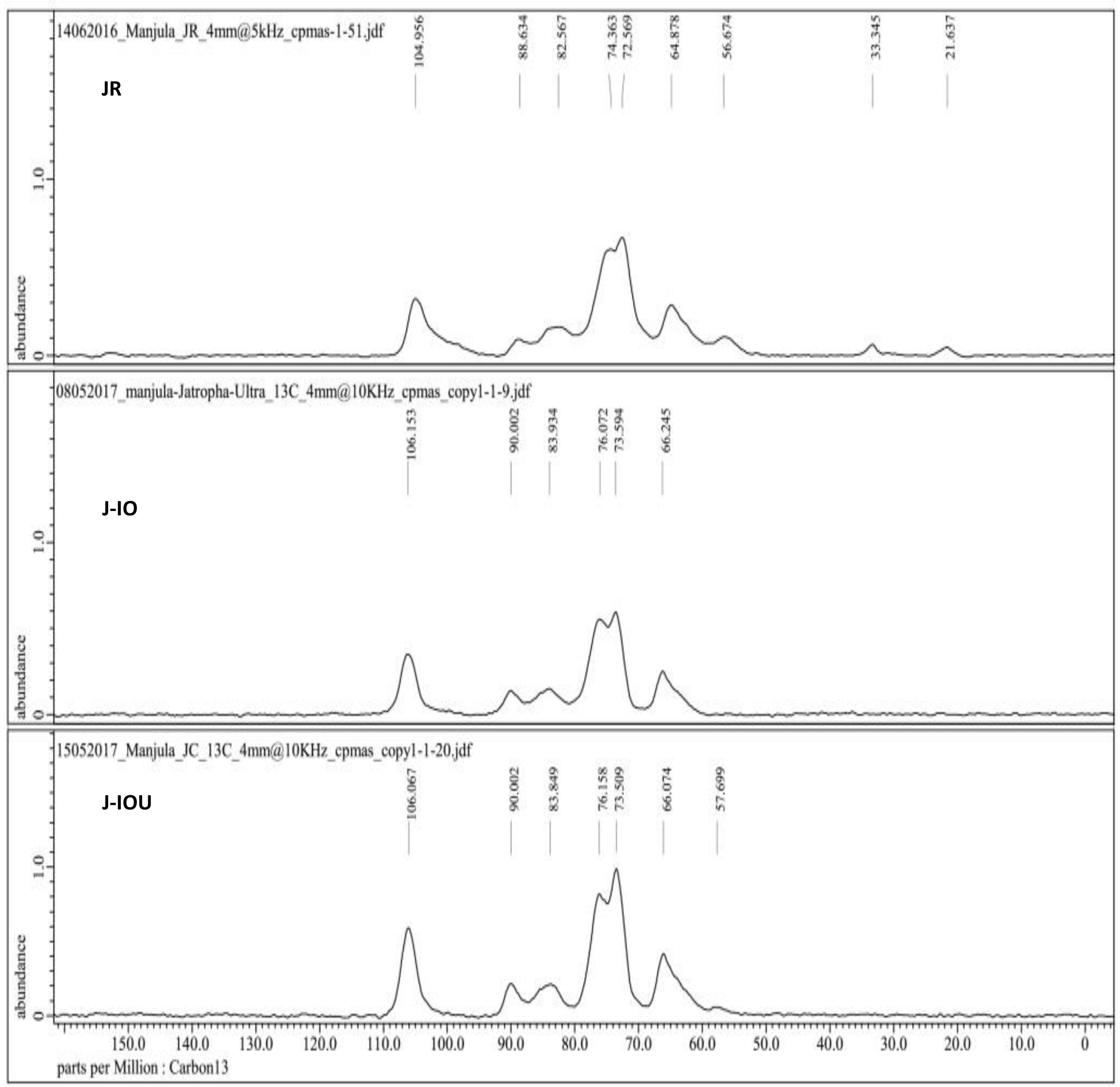


Figure 4.13a. ^{13}C NMR spectra of Jatropha seed shell raw untreated fibers (JR), cellulose microfiber isolated by treatment method IO (J-IO) and by treatment method IOU (J-IOU).

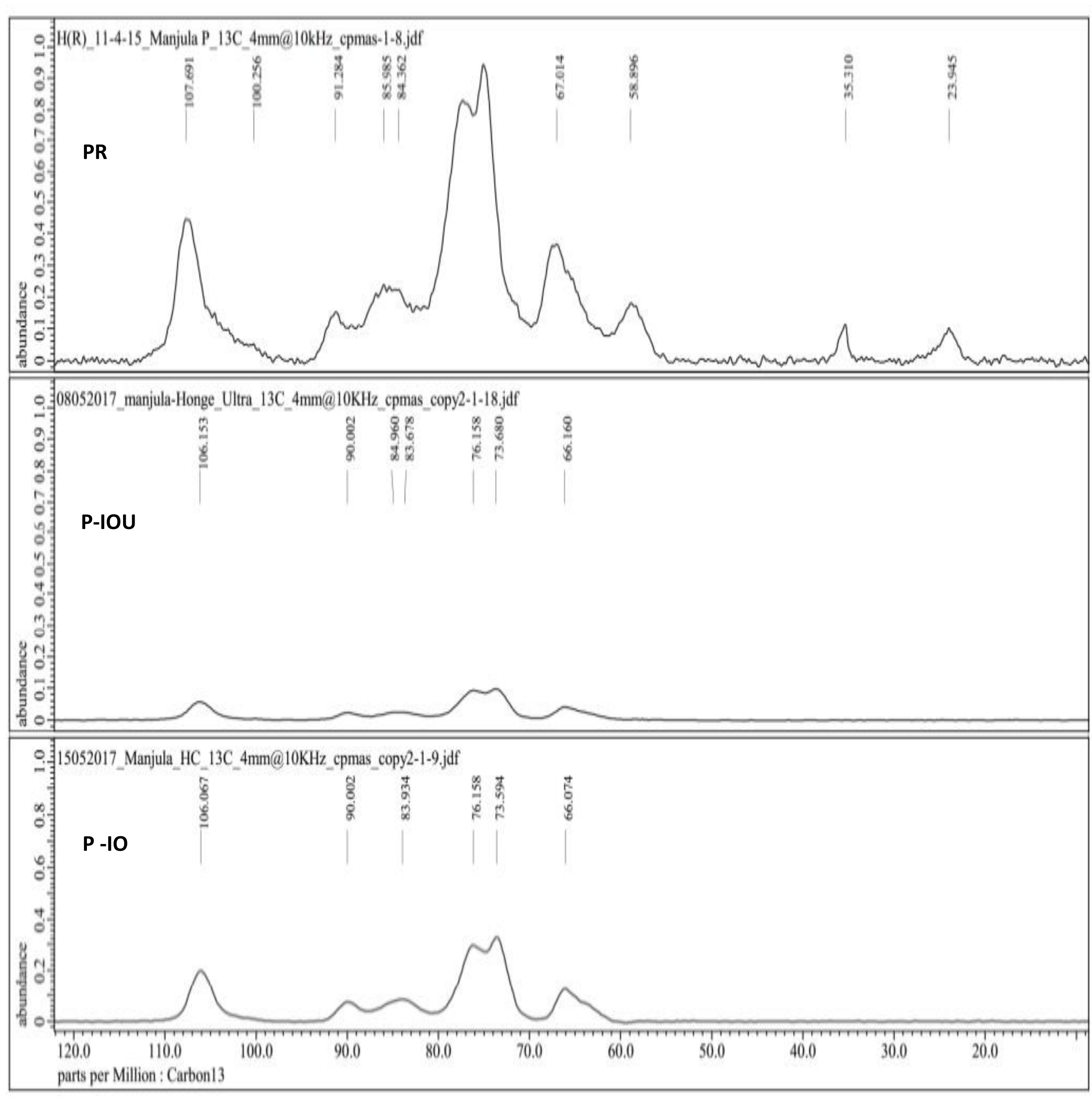


Figure 4.13b. ^{13}C NMR spectra of Pongamia seed hull, raw untreated fibers (PR), cellulose microfiber isolated by treatment method IO (P-IO) and by treatment method IOU (P-IOU).

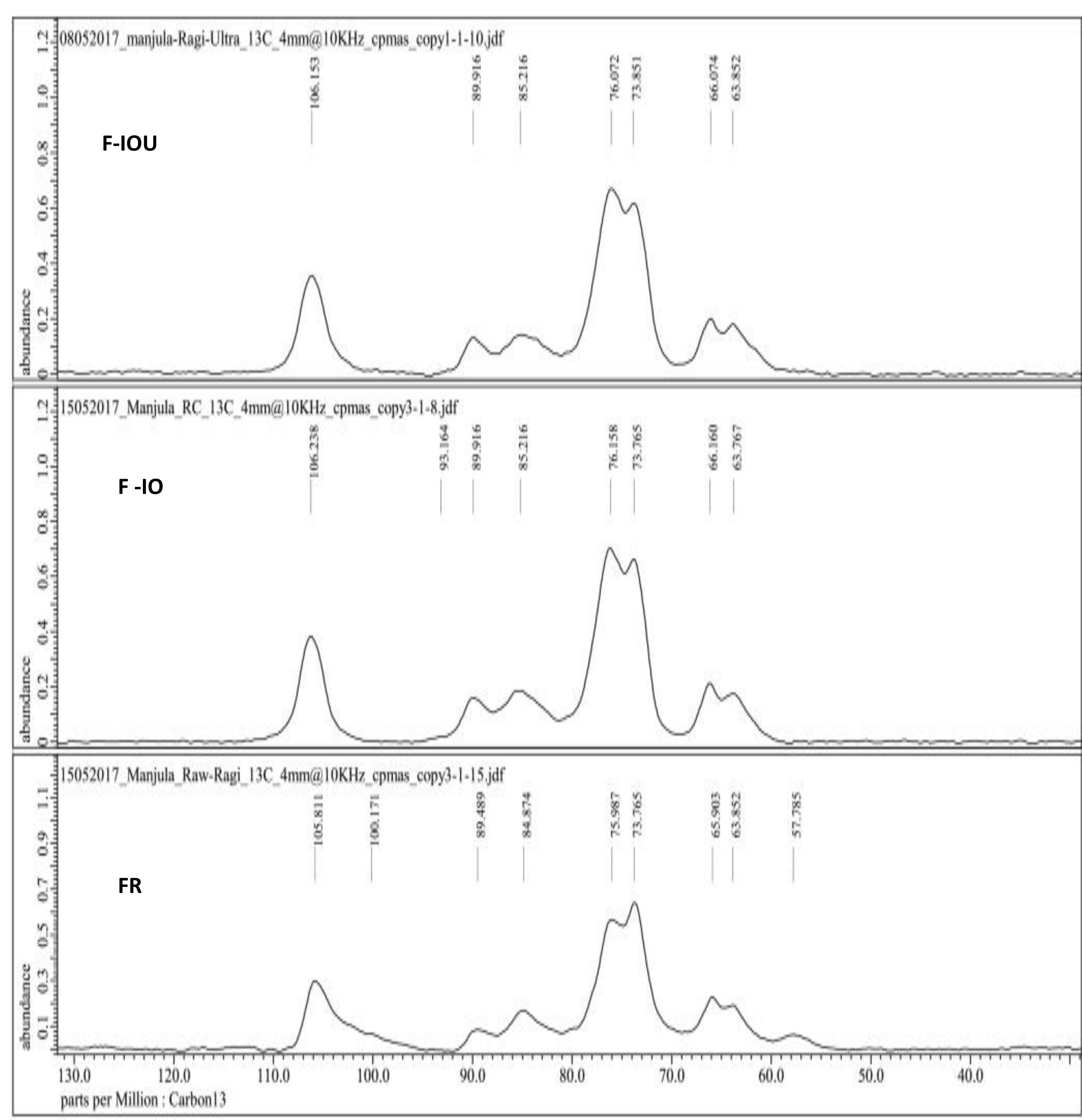


Figure 4.13c. ^{13}C NMR spectra of Finger millet straw, raw untreated fibers (FR), cellulose microfiber isolated by treatment method IO (F-IO) and by treatment method IOU (F-IOU)

4.4.3 X-ray diffractometer analysis

The XRD diffractograms of untreated and cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by method IO and IOU are presented in Figure 4.14 and the crystallinity index is presented in Table 4.30 respectively. The crystallinity index, CI% was calculated by Segal method using Equation (1) presented in section 3.7.4.

XRD patterns of untreated Jatropha seed shell and isolated cellulose microfibers are shown in Figure 4.14a. Diffractograms obtained by analysing the untreated fibers and cellulose microfibers of Jatropha seed shell show peaks at ~16°, and ~22°. Peaks at 2θ = 22° and 14°– 17° represent cellulose I (Bondeson et al. 2006). The crystallinity index (CI) obtained using Equation (1) for untreated Jatropha seed shell fibers (JR), cellulose microfibers isolated by method IO (J-IO) and method IOU (J-IOU) was 39.34%, 58.92 and 55.9% respectively as presented in Table 4.30. Increase in crystallinity of cellulose fiber by 33%, indicates the reduction of amorphous components by removal of amorphous lignin and hemicellulose in the cellulose fiber (Sonia and Dasan, 2013). Decrease in crystallinity of ultra-sonicated cellulose microfibers can be accounted to the effect of sonic energy on the crystalline surface of cellulose which leads to destruction of the crystalline part of the macromolecular area resulting in decrease in crystallinity (Sumari et al. 2013; Barbash et al. 2017).

Table 4.30. Crystallinity index of cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by method IO and method IOU.

Sample	Crystallinity index CI %		
	Jatropha seed shell	Pongamia seed hull	Finger millet straw
Untreated fibers	39.34	45.87	40.51
Cellulose microfibers isolated by method IO	58.92	48.64	47.59
Cellulose microfibers isolated by method IOU	55.9	60.47	48.11

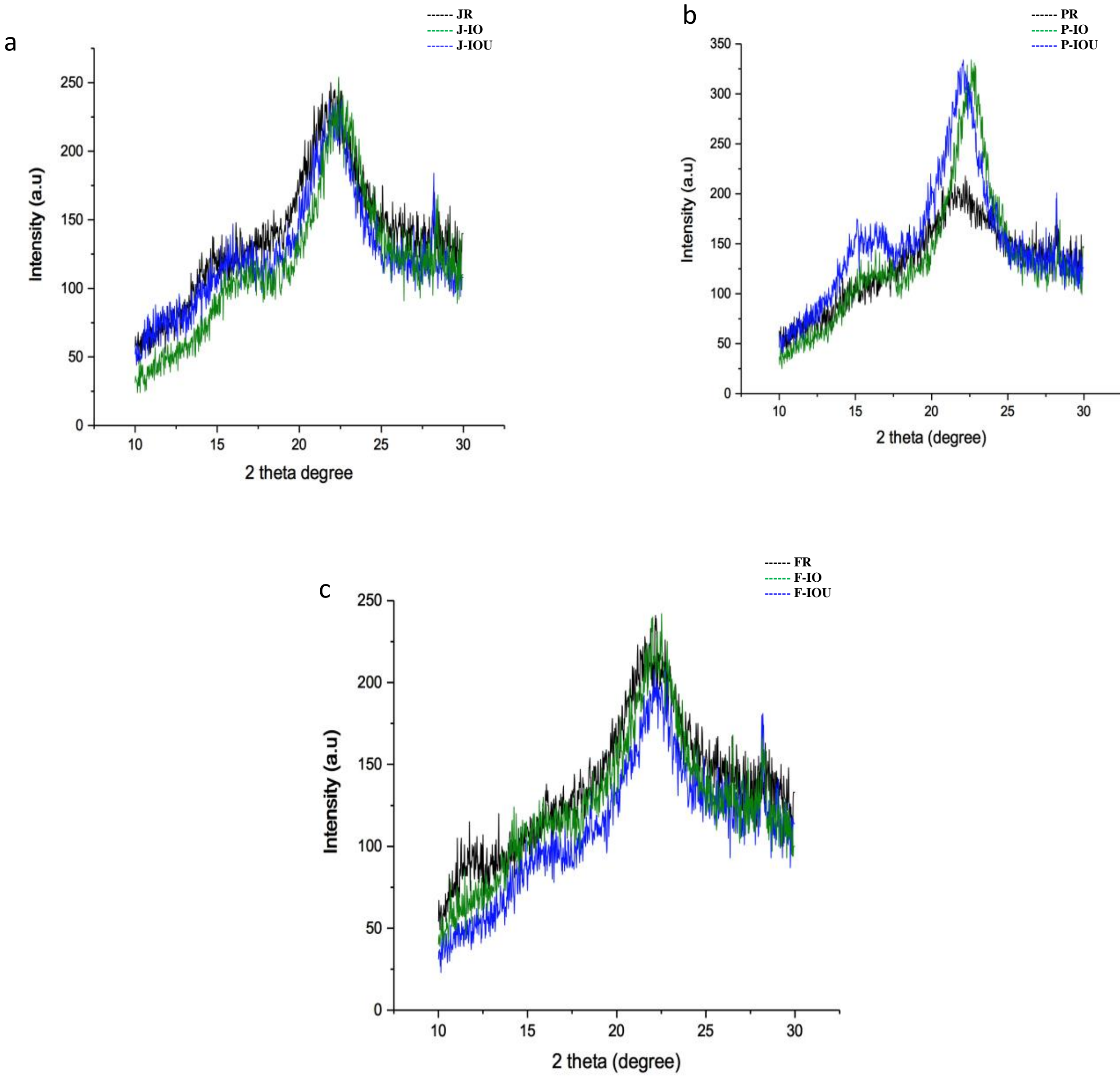


Figure 4.14. Powder X-ray diffraction patterns of a. Jatropha seed shell, b.

Pongamia seed hull fibers c. Finger millet straw, untreated and isolated cellulose microfibers by method IO and method IOU.

The diffractograms of untreated fibers and cellulose microfibers isolated by method IO and method IOU from Pongamia seed hull and Finger millet straw are presented in Figure 4.14b and 4.14c respectively. Two peaks are observed at $2\theta = 16^{\circ}$ and 22.6° for all the samples which are the characteristic of crystal polymorphs of cellulose I and cellulose II respectively (Bondeson et al. 2006; Novo et al. 2015). The peak at $2\theta = 16^{\circ}$ corresponds to the $(1\ 1\ 0)$ and $2\theta = 22.6^{\circ}$ correspond to the $(2\ 0\ 0)$. The crystallinity index (CI) calculated using equation (1) for untreated Pongamia seed hull fibers and isolated cellulose microfibers by method IO and IOU were 45.87 %, 48.64 and 60.47 % respectively. For Finger millet straw, the crystallinity increased from 40.51% (untreated fibers) to 47.59% (by IO treatment) and 48.11% (by IOU treatment) as presented in Table 4.30. The crystallinity of the cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by method IOU, increased by 29.62%, 24.4 % and 15.8 % respectively compared to that of untreated fibers. This increase in crystallinity can be accounted to the presence of crystalline cellulose and also removal of amorphous hemicellulose and lignin (Rosa et al. 2010) from isolated cellulose fibers by method IOU.

4.4.4 Fourier transform infrared spectroscopic (FTIR) analysis

FT-IR spectroscopy monitors the functional groups present in the fibers. The FTIR spectra of untreated lignocellulosic sources and cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by method IO and IOU are presented in Figure 4.15 a, b, c and the prominent spectral peaks are assigned to their groups in Table 4.31. The FTIR spectra presented in Figure 4.15 a, b, c, indicates the presence of band around 1040-1060 cm^{-1} (C-O-C stretching) in the spectra of fibers accounting to the presence of xylans associated with hemicelluloses which strongly bond with the cellulose fibers (Kaushik and Singh, 2011). However, the intensity of the peak at 1040-1060 cm^{-1} reduces for cellulose microfibers isolated by method IO and method IOU

which indicates the removal of lignin to a large extent.

Table 4.31. Peaks obtained in FTIR spectra of Jatropha seed shell, Pongamia seed hull and Finger millet straw, assigned to their functional groups.

Absorption peaks cm^{-1}									Functional Groups
Jatropha seed shell			Pongamia seed hull			Finger millet straw			
JR	J-IO	J-IOU	PR	P-IO	P-IOU	FR	F-IO	F-IOU	
3397.47	3437.38	3429.87	3425.71	3425.70	3428.93	3429.25	3424.46	3423.02	Amorphous cellulose
2922.08	2895.85	2894.37	2925.58	2895.79	2896.69	2925.07	2892.89	2893.39	CH stretching
1622.58	1629.97	1628.35	1628.54	1632.20	1628.27	1631.11	1631.16	1629.1	C=C aromatic ring, C-H deformation of hemicellulose and lignin. Skeletal vibration of lignin
1420.21	1437.91	1436.23	1509.99	1435.22	1438.60	1429.74	1435.42	1436.96	CH_2 scissoring of cellulose
1384.79	1368.44	1321.53	1376.82	1374.50	1376.62	1375.65	1372.53	1368.44	O-H bending of cellulose
1155.96	1164.13	1151.1	1102.84	1169.10	1176.39	1164.13	1160.87	1164.14	C-O antisymmetric bridge stretching
1061.71	1065	1063.61	1065.24	1063.82	1065.83	1040.90	1064.24	1068.72	–C-O-C- pyranose ring skeletal vibration

Reduction in peaks, 1630cm^{-1} (aromatic ring vibrations) (Bono et al. 2009; Draman et al. 2013), 1430 cm^{-1}(–C=C– stretch of the aromatic rings of lignin) (Juby et al. 2012; Sun et al. 2004; Kaushik et al. 2011; Elanthikkal et al. 2010; Haafiz et al. 2013) and disappearance of peaks at 775cm^{-1} (C-H deformations) (Rosa et al. 2010) in the spectra for cellulose microfibers as compared to those for untreated fibers assures the removal of lignin from cellulose microfibers. The strong band at around 1060 cm^{-1} is observed in the spectra of cellulose microfibers isolated from all the three sources, which is attributed to –C-O-C- pyranose ring skeletal vibration, indicating an increase in cellulose content (Sun et al. 2004; Elanthikkal et al. 2010). Broadening of vibration at 3400 cm^{-1} in cellulose fiber spectra ensures the presence of amorphous fraction of cellulose (Tibolla et al. 2014, Qiao et al. 2016; Shin et al. 2012; Kalita et al. 2015; Sun et al. 2004; Kaushik et al. 2011).

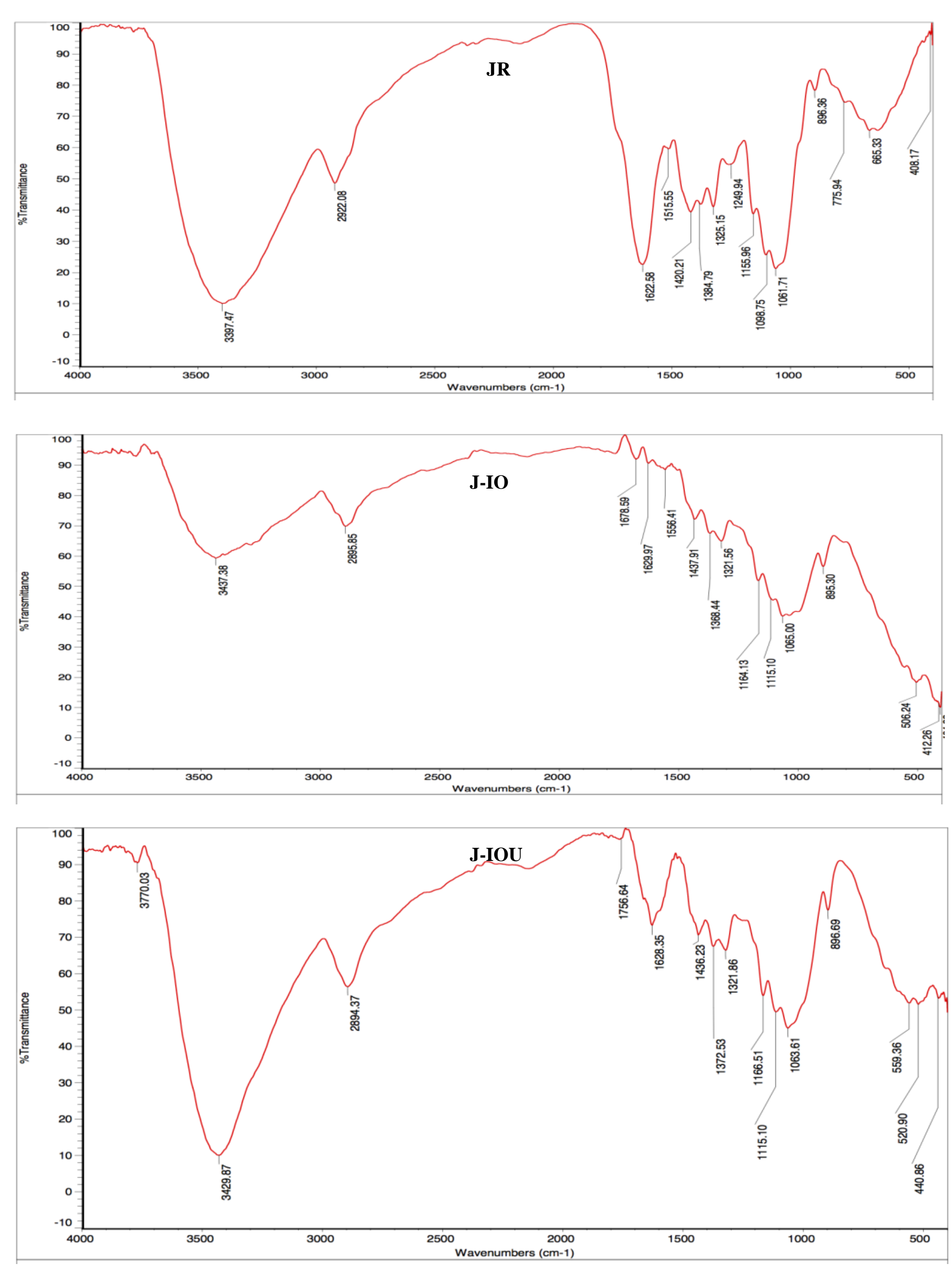


Figure 4.15a. FTIR spectra of untreated (JR) and cellulose microfibers isolated by method IO and IOU (J-IO and J-IOU) from Jatropha seed shell.

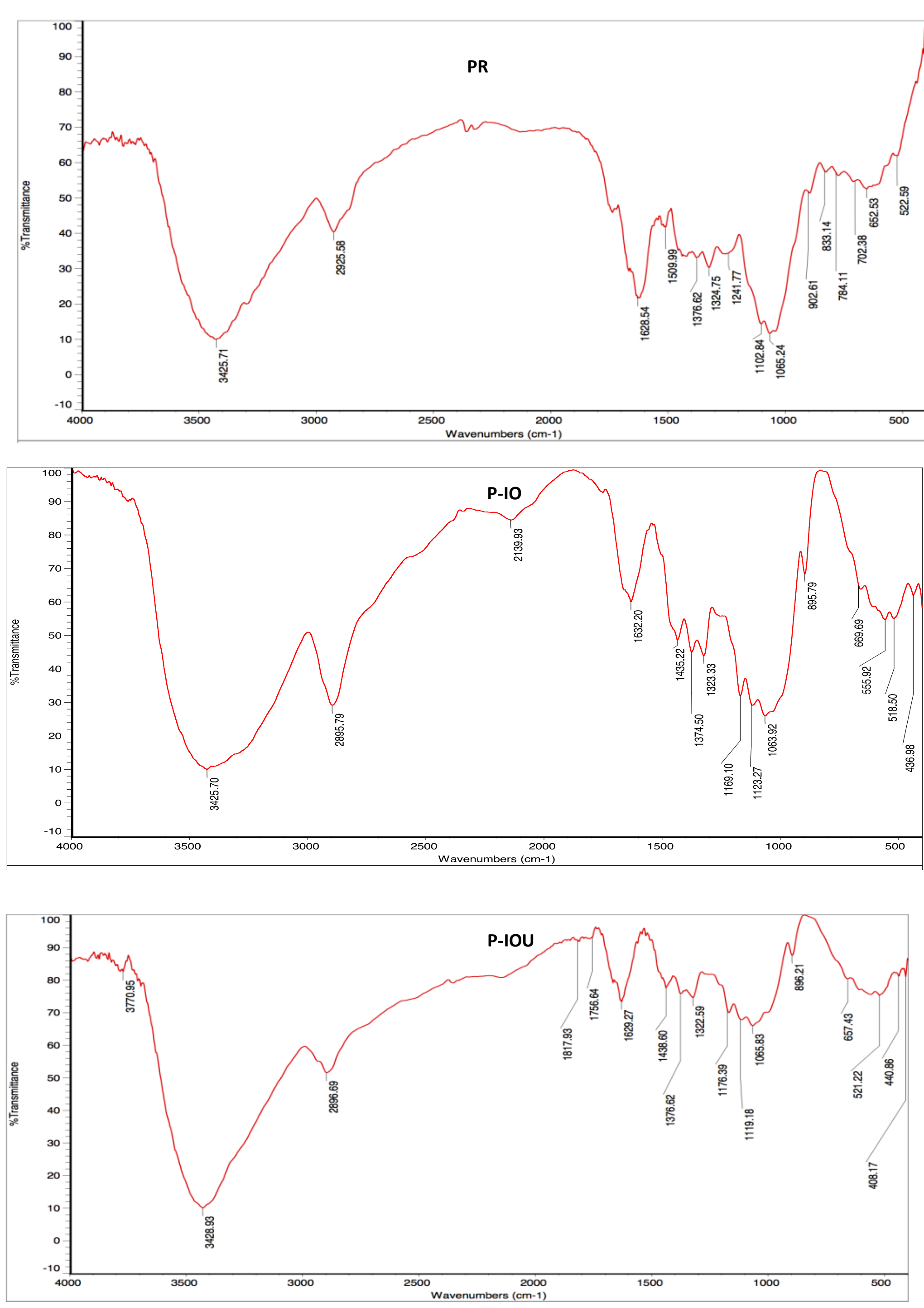


Figure 4.15b. FTIR spectra of untreated (PR) and cellulose microfibers isolated by method IO and IOU (P-IO and P-IOU) from Pongamia seed hull.

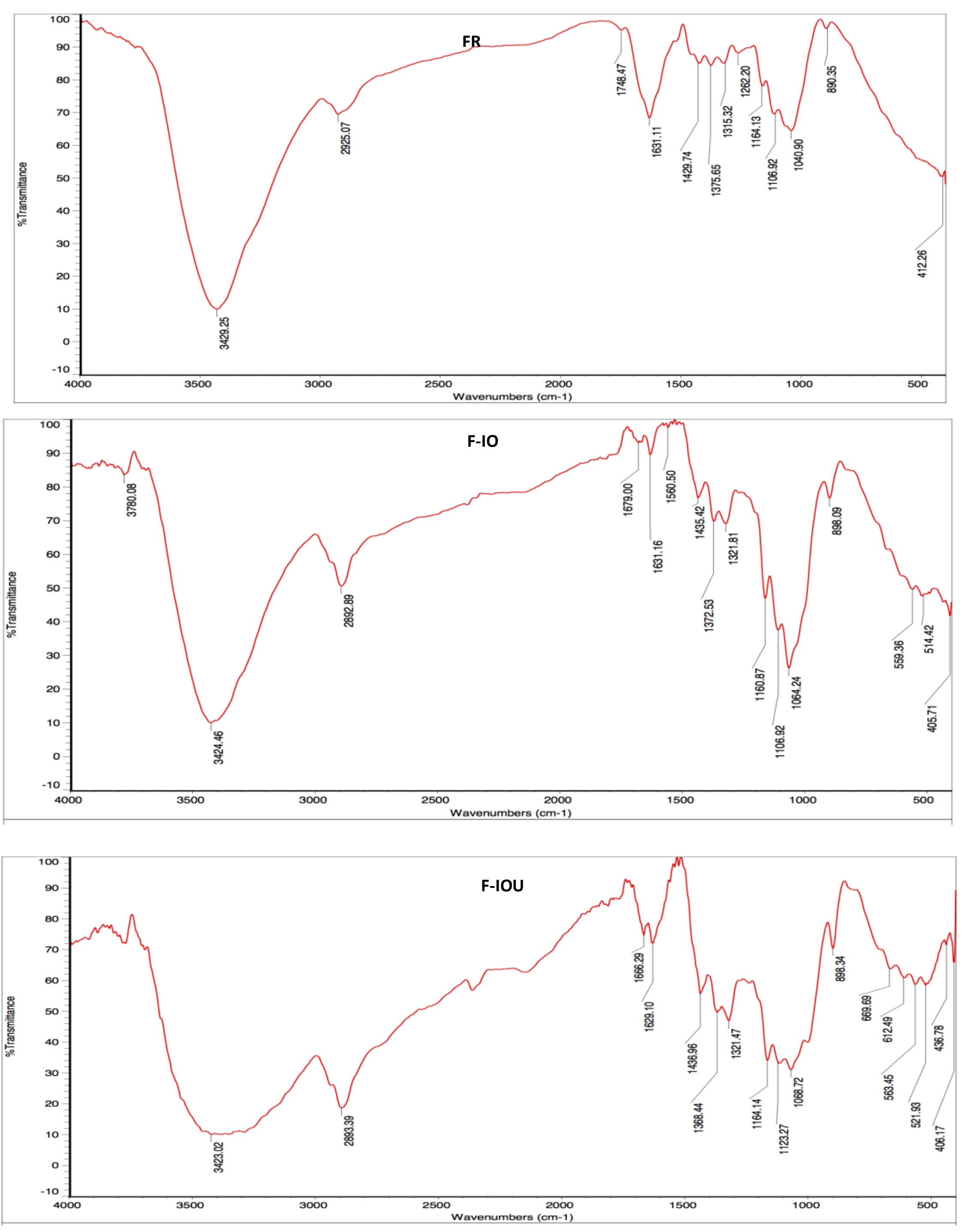


Figure 4.15c. FTIR spectra of untreated (FR) and cellulose microfibers isolated by method IO and IOU (F-IO and F-IOU) from Finger millet straw.

The slight absorption at 2900cm^{-1} band region correspond to the alkyl (C-H) stretching (asym and sym) vibration in lignin polysaccharide (hemicellulose and cellulose) and indicates the presence of trace amount of lignin (Reddy et al. 2012; Shin et al. 2012; Sun et al. 2004; Kaushik et al. 2011; Zhong et al. 2013; Haafiz et al. 2014). The peak at 1670 cm^{-1} is associated with adsorbed water (Hassan et al. 2010) which could be due to hydrophilicity of cellulose fibers. The increase in peak at around 1370 cm^{-1}, assigned to O-H bending of cellulose in the spectra of cellulose microfibers indicates an increase in cellulose in the cellulose microfibers after IO and IOU treatments (Maheshwari et al 2012). Broadening of the band at 1106cm^{-1} in cellulose spectra associated with cellulose I to cellulose II transition is due to the isolation of cellulose by chlorite and alkaline treatment (Korte et al. 2008). Peak at 1735.62 cm^{-1} is assigned to C=O stretching vibration of carbonyl, acetyl and uronic ester group of the ferulic and p-coumeric acids of lignin and /or xylan component of hemicellulose. The disappearance of these peaks in cellulose fiber spectra, confirms the removal of lignin and hemicellulose (Kalita et al. 2015; Kaushik et al. 2011; Sun et al. 2004; Elanthikkal et al. 2010; Rosa et al. 2012; Oun et al. 2016).

Thus, the observations made in NMR and FTIR spectra of untreated fibers and cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw have supported the removal of matrix components such as lignin and hemicellulose and thereby leading to isolation of cellulose by method IOU.

4.4.5 Thermogravimetric analysis

The change in thermal stability of the isolated cellulose microfibers after IO and IOU treatments are witnessed from thermogravimetric curves. Figure 4.16a, b, c presents the thermograms obtained for untreated fibers and cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw fibers by method IO and IOU.

The onset degradation temperature of untreated fibers and cellulose microfibers isolated from Jatropha seed shell by method IO and method IOU were recorded as 235°C, 267°C and 280°C respectively. The thermograms clearly indicate the increase in thermal stability by 45°C for cellulose microfibers isolated by method IOU compared to that of untreated Jatropha seed shell fibers, which can be accounted for removal of hemicellulose and lignin through chemical treatments (He et al. 2013; Abe et al. 2009). The maximum rate of degradation was observed between 235°C-347°C, 267°C-332°C and 280°C-342°C for untreated fibers and cellulose microfibers isolated from Jatropha seed shell by method IO and IOU respectively, with 50% reduction in weight which is mainly due to pyrolysis of cellulose and thermal depolymerisation of hemicellulose (Abraham et al. 2011; Li et al. 2015; Chen et al. 2011; Ludueña et al. 2011). Presence of residue was observed even at temperature of 700°C, which is due to the carbonaceous materials remaining after pyrolysis and the residual inorganic materials in the samples. The residue present after pyrolysis upto a temperature of 700°C was 26% for untreated fibers and reduced to 21% for cellulose microfibers isolated by method IOU. As the hemicellulose and lignin content in the untreated fibers is higher, more of the carbonaceous material remain as residue after pyrolysis even upto 700°C. Removal of hemicellulose and lignin by IOU method result in lower hemicellulose and lignin content thus resulting in lower residual carbonaceous material after pyrolysis (Marimuthu and Atmakuru, 2015). Some of the inorganic material in the untreated fibers may also get removed during the dewaxing process, thus reducing the inorganic residual material after pyrolysis of the isolated fibers during TGA.

The untreated fibers and isolated cellulose fibers after IO and IOU treatment of Pongamia seed hull fibers have the onset degradation temperatures of 219°C, 283°C and 291°C respectively. The thermal stability of cellulose microfibers has increased by 72°C after IOU treatment. The maximum rate of degradation with increase in temperature occurs at around 230°C-370°C for isolated cellulose fibers with percentage weight loss of 46%. Residuals present at 700°C in untreated fibers and isolated cellulose fibers from Pongamia seed hull by method IOU were observed to be 26% and 19% respectively.

Similarly, the untreated and isolated cellulose fibers from Finger millet straw have the onset degradation temperatures of 200°C, 272°C and 293°C respectively. The thermal stability of cellulose microfibers has increased by 93 °C after IOU treatment. The maximum degradation is observed at around 250°C-350°C, showing 41% degradation of cellulose. Presence of around 23% and 17% residue was observed at 730°C with untreated fibers and cellulose fibers isolated by method IOU owing to the residual carbonaceous material and inorganic materials of the fibers after pyrolysis.

The onset degradation temperatures for the isolated cellulose fibers have been observed to be higher than those for untreated fibers. The onset degradation temperature has increased with the increase in cellulose content of the fibers. The isolation process decreases the lignin and hemicellulose content but increases the crystallinity index of the fibers, thus showing the presence of larger quantity of crystalline cellulose. Higher crystallinity also contributes to better thermal stability (Rosa et al. 2012; Jawid et al. 2017). Thermoplastic processing is generally carried out at temperatures above 200°C (Sain and Panthapulakka 2006). The cellulose fibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by method IO and IOU have the onset degradation temperatures of above 200°C. Thus, these fibers may find application in thermoplastic processing.

Espino et al. (2014) have reported the onset degradation temperature of commercially available microcrystalline cellulose (MCC), cellulose nanocrystals (CNC) derived from MCC, CNC isolated from A. tequilana and Barley have shown decrease from 256, 227, 224 to 217 °C. This shows that the cellulose microfibers isolated from the three lignocellulosic sources have the onset degradation temperature are in line with that of microcrystalline cellulose reported in the literature.

The characterization of cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by method IOU, by FTIR, NMR, TG, XRD and DLS analysis support the removal of matrix components and the improved properties of the isolated cellulose microfibers such as crystallinity and thermal properties to facilitate

their application. Thus, the method IOU is found to be favourable in isolation and defibrillation of cellulose microfibers from Jatropha seed shell, Pongamia seed hull and Finger millet straw.

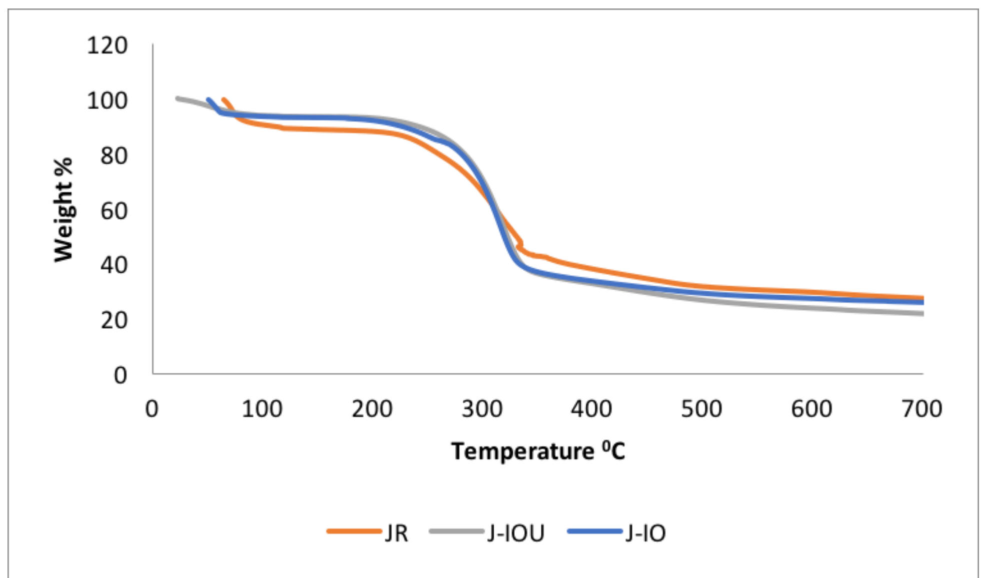


Figure 4.16a. Thermograms of untreated (JR), isolated cellulose microfibers by method IO (J-IO) and method IOU (J-IOU) from Jatropha seed shell.

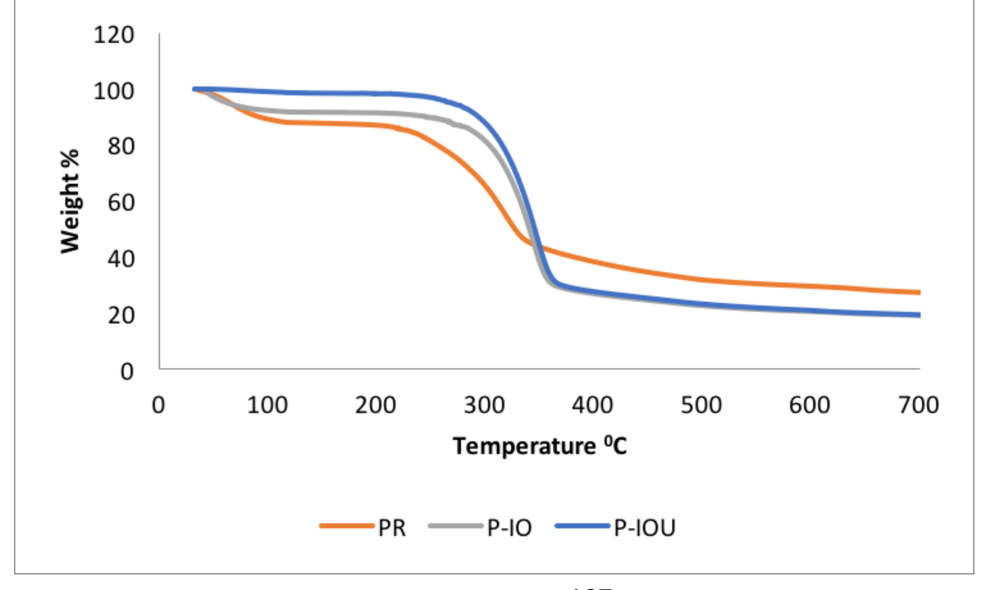


Figure 4.16b. Thermograms of untreated (PR), isolated cellulose microfibers by method IO (P-IO) and method IOU (P-IOU) from Pongamia seed hull.

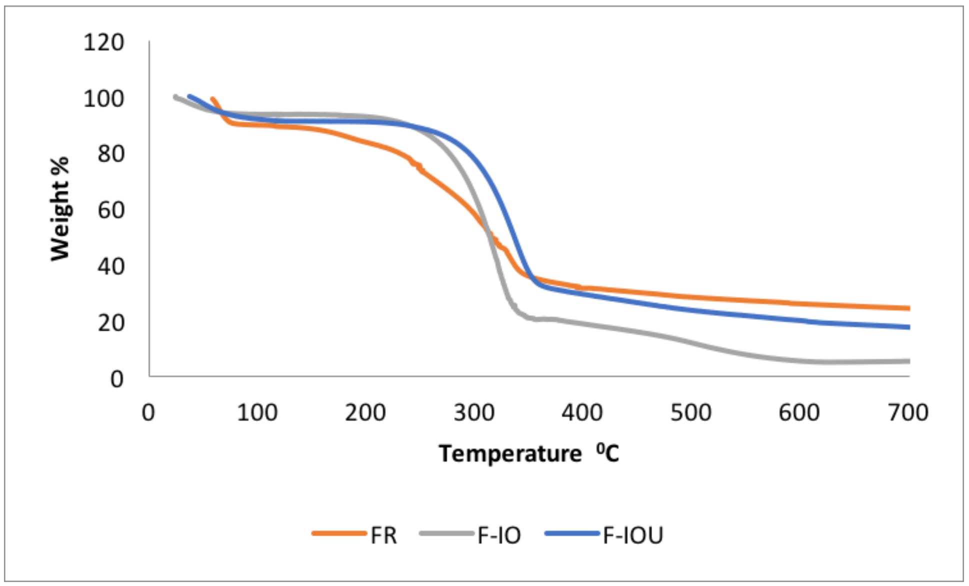


Figure 4.16c. Thermograms of untreated (FR), isolated cellulose microfibers by method IO (F-IO) and method IOU (F-IOU) from Finger millet straw.

4.5 Preparation and characterization of cellulose fiber reinforced PVA biocomposites

The objective of isolating cellulose microfibers from the lignocellulosic source was to exploit the potential of cellulose microfibers as reinforcement in the biocomposites. To achieve the said objective, the cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by IOU method were used for reinforcement at 5, 10, and 20 wt% fiber loading and the biocomposites were prepared by solution casting as described in section 3.2. These biocomposites were further characterized by SEM, TG, Universal testing machine, Oxygen Transfer rate test for their morphological, thermal, tensile and oxygen transfer properties followed by the test for biodegradability to investigate the potentiality of these composites in the field of packaging.

4.5.1 Cellulose fiber reinforced PVA biocomposites appearance and transmittance

The biocomposites of PVA reinforced with cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by IOU method were prepared using solution casting method with different fiber loading. The photographs of the biocomposites with different cellulose microfiber loading and that of neat PVA are presented in Figure 4.17. The neat PVA film was with a smooth surface and was more transparent as compared to cellulose microfiber reinforced composites as seen in Figure 4.17. For 5wt% cellulose microfiber loading, the surface of the composites was comparatively rougher than that of neat PVA film, which is due to dispersion of cellulose microfibers in the PVA matrix. The transparency of the composites reduced slightly as the fiber loading increased. This indicates that the lower loading of cellulose microfibers resulted in homogeneous dispersion (Liu et al. 2010) and as the loading increased, the higher concentration of opaque cellulose microfibers may decrease the transparency of the composite films. The reduction in transparency of the films can also be attributed to the increase in agglomeration of the fibers at higher concentration of cellulose microfibers (Liu et al. 2010; Littunen et al. 2013; Kiziltas et al. 2015). The agglomeration of cellulose microfibers at higher loading has also contributed to the roughness of the composite surface.

The neat PVA and cellulose microfiber reinforced PVA biocomposites films where analysed for transmittance by measuring the percent transmittance at 800 nm using a UV/ VIS spectrophotometer. The Figure 4.18 represents the percentage transmittance of the neat PVA and biocomposites films. The UV light transmittance through the films were measured between the wavelength range 200-800 nm. It is observed that the percentage transmittance of neat PVA at wavelength of 800 nm is very high compared to that of all the cellulose microfiber reinforced PVA biocomposites. The percentage transmittance of neat PVA is 77% whereas for the cellulose microfiber reinforced PVA biocomposites it is reduced as the fiber loading is increased.

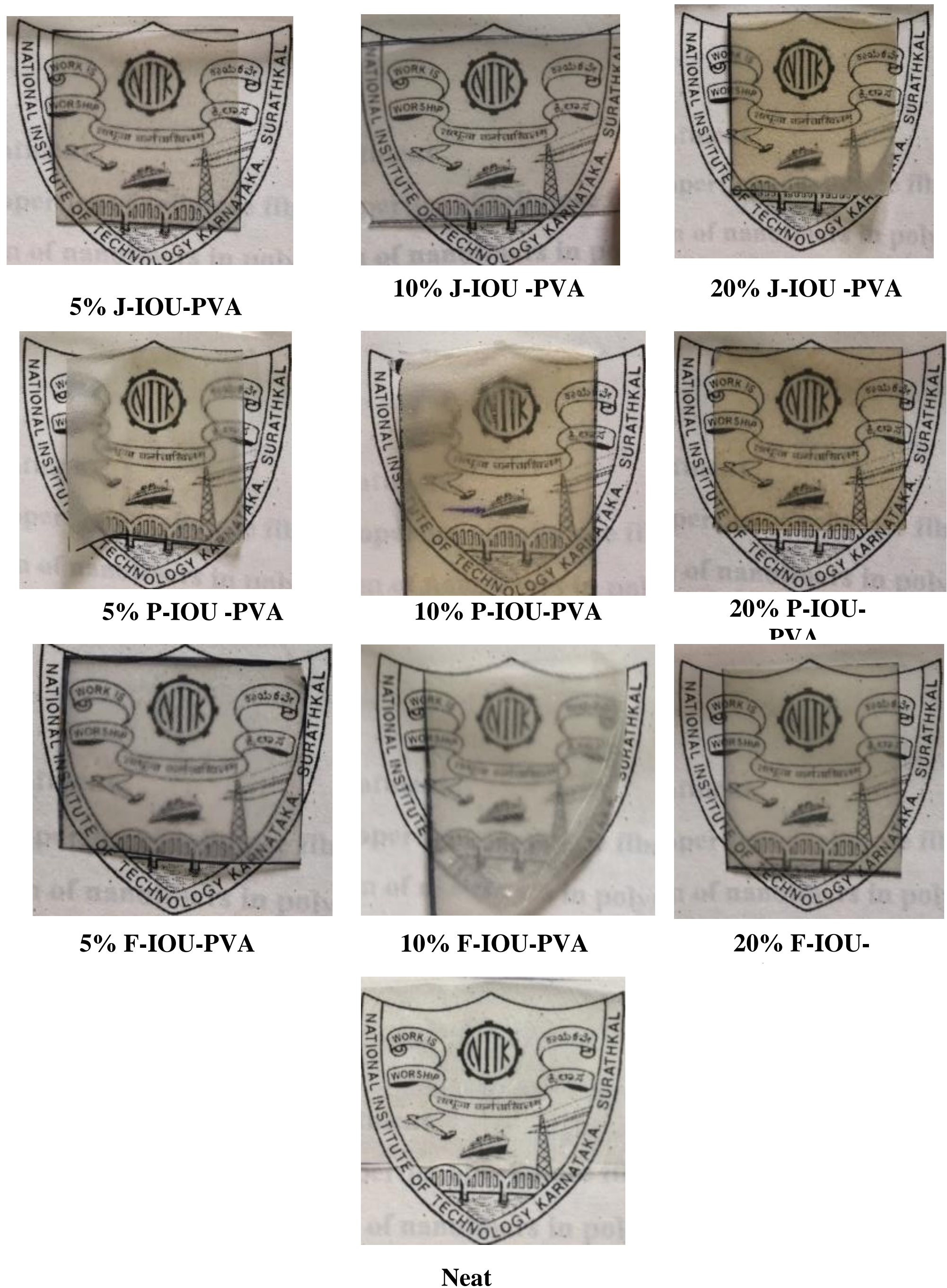


Figure4.17. Photographs of composites prepared by different loading of cellulose microfiber isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by method IOU and neat PVA.

With respect to 5 wt% of cellulose microfiber reinforcement in PVA, it is observed that the percentage transmittance of the biocomposites films is 27%, 22.8% and 30.8% for microfibers isolated from Jatropha seed shell, Pongamia seed hull and finger millet straw respectively, whereas for cellulose microfiber loading of 20 wt% the percentage transmittances has reduced to a lower value of 18.1%, 5.4% and 8.3% for microfibers isolated from Jatropha seed shell, Pongamia seed hull and finger millet straw respectively. This indicates that the presence of opaque particles i.e. cellulose fibers has obstructed the passage of light through the films and also the non-uniform distribution of cellulose microfibers resulting in agglomeration also would be one of the reason for decrease in transmittance of the biocomposites films.

In the literature, it is observed that the transmittance of the biocomposites depends on the thickness of the biocomposites films as well as on the amount of cellulose fiber reinforcement (Kumar Thakur and Kumari Thakur, 2015). Andrade-Pizarro et al. (2010) have also reported similar kind of lower transmittance in PVA/NFC nanocomposite films and have related it to the presence of light blocking particles, cellulose nano fibers in the PVA matrix. Similarly, Ching et al. (2015) have observed the transmittance decrease in nanocellulose reinforced PVA films as the loading of nano cellulose increased which was attributed to the presence of agglomeration of cellulose in the composites. Fortunati et al. (2016) have stated that the light barrier properties of the PVA composite films between 250-600 nm would be extremely beneficial in packaging for certain food products. Light transmittance may lead to degradation of amino acids, loss of certain vitamins, formation of aldehydes and methional kind of volatile compound which induce unpleasant smell in packaged dairy products (Bosset et al. 1994). UV light is known to play a major role in photochemical oxidation reactions (Coltro and Borghetti 2007), thus the cellulose fiber reinforced PVA biocomposites used for packaging can provide protection to larger extent from UV light and sunlight induced photo degradation.

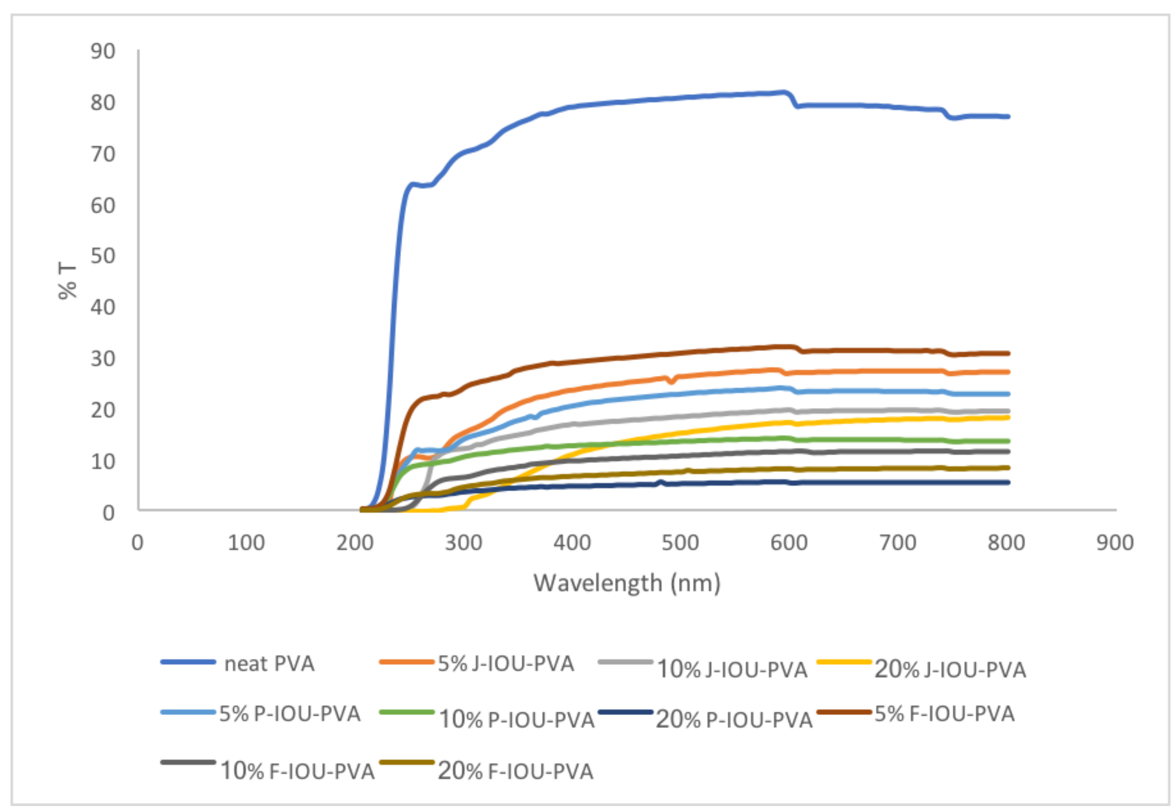


Figure 4.18. Percentage transmittance vs wavelength of biocomposites reinforced with cellulose fibers isolated from Jatropha seed shell, Pongamia seed hull fibers and Finger millet straw by method IOU treatment at different fiber loading and Neat PVA.

4.5.2 Scanning electron microscope analysis

The surface morphology of cellulose microfibers reinforced PVA biocomposites at different fiber loadings gives a picture of cellulose microfiber distribution in the PVA matrix and the same is presented through SEM images in Figure 4.19. The distribution of cellulose microfibers in PVA matrix is found to be different for biocomposites reinforced with cellulose microfiber isolated from different sources, which is due to difference in composition of cellulose microfibers. In case of reinforcement with cellulose microfibers isolated from Jatropha seed shell in PVA (Figure 4.18a, b, c), the cellulose microfibers are observed to be embedded in the PVA matrix. Interfacial adhesion between cellulose microfibers and PVA matrix has led to the bonding of cellulose microfibers to the matrix.

The composite prepared with cellulose microfibers isolated from Pongamia seed hull show the presence of cellulose microfibers at the surface of the composites (Figure 4.19 d, e and f), which can be attributed to week interfacial bonding of few cellulose fibers seen on the surface of composites. However, most of the cellulose microfibers are seen to be embedded within the PVA matrix. Similarly, in case of biocomposites with cellulose microfibers isolated from Finger millet straw, few cellulose microfibers seem to be pulled out from the matrix indicating week interfacial bonding between the reinforcement and the matrix (Figure 4.19 g, h and i) with majority of cellulose microfibers being bound with the matrix (Pöllänen et al. 2013). With higher concentration of cellulose in cellulose microfibers, tendency of cellulose fibers bonding with each other increases due to their hydrophilic nature. This bonding hinders the dispersion of cellulose microfibers in the PVA matrix. Higher cellulose fiber loading in the matrix leads to increase in the number of cellulose fibers on the surface of the matrix as observed from the SEM images in Fig 4.19. However, higher fiber loading may also lead to agglomeration of fibers within the matrix as observed with biocomposites prepared using cellulose microfibers isolated from Jatropha seed shell with 20% fiber loading. Agglomeration may reduce the uniform dispersion of the fibers in the PVA matrix.

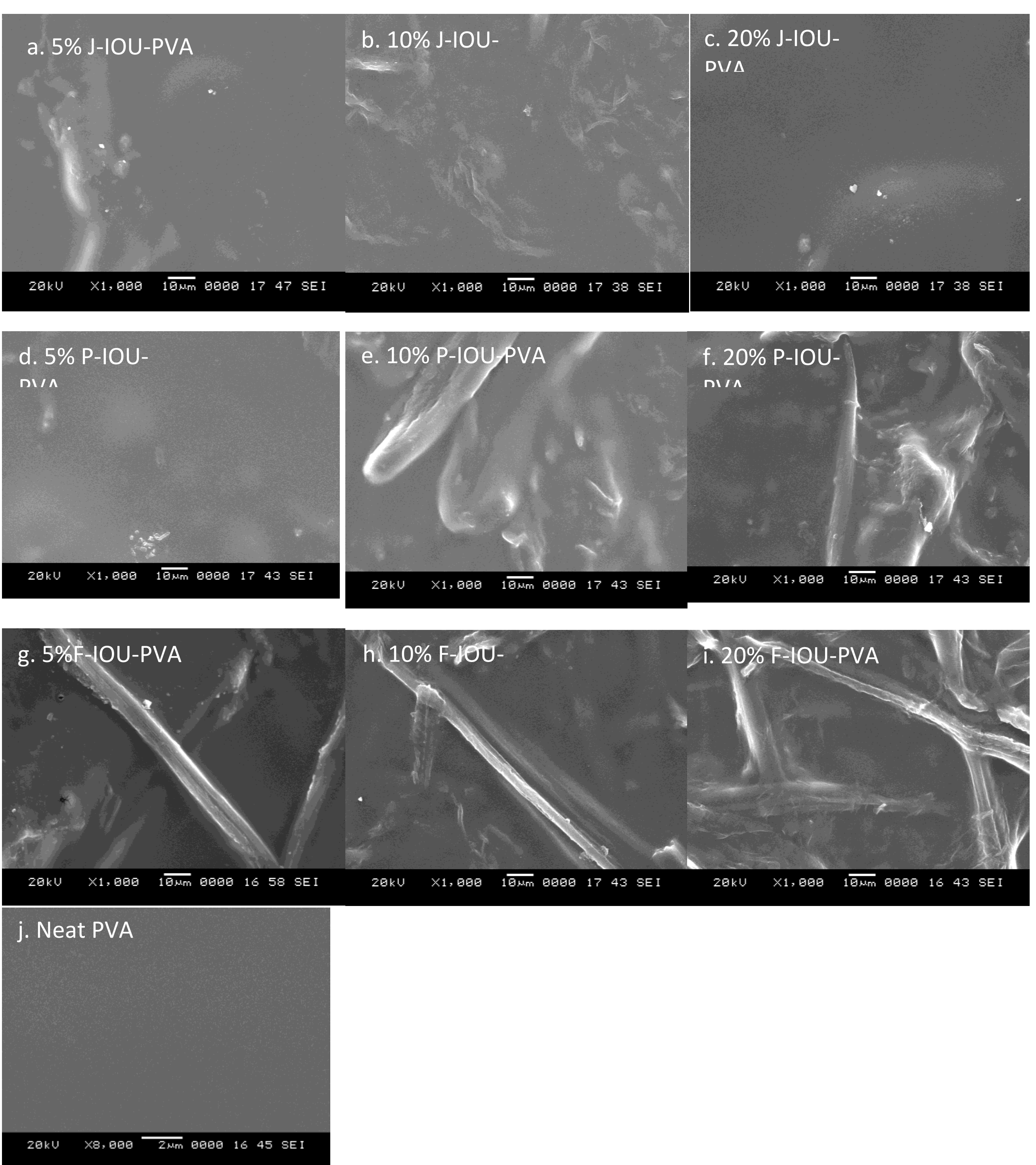


Figure 4.19. SEM images of biocomposites reinforced with cellulose fibers isolated from Jatropha seed shell, Pongamia seed hull fibers and Finger millet straw by method IOU treatment at different fiber loading and neat PVA.

4.5.3 Thermogravimetric analysis

The composites were further characterized by TG analysis in order to obtain the thermal properties which would assist in finding the potentiality of cellulose microfiber reinforced PVA composites for application in packaging industries. The thermograms obtained for cellulose reinforced PVA composites with different fiber loading and neat PVA are presented in Figure 4.20.

The onset degradation temperature of neat PVA is 270°C, whereas, the onset degradation temperature of PVA biocomposites comprising of cellulose microfibers isolated from Jatropha seed shell at 5%, 10%, and 20% fiber loading were recorded as 276°C, 279°C and 278°C respectively. The thermograms clearly indicate a maximum increase in onset degradation temperature by 9°C for cellulose microfibers loading of 10% compared to neat PVA. The composites consisting of cellulose microfibers isolated from Pongamia seed hull with loading of 5%, 10%, and 20%, have the onset degradation temperatures of 274°C, 281°C and 285°C respectively. Maximum thermal stability was exhibited by the PVA composite reinforced with 20% cellulose microfiber loading with the increase in onset degradation temperatures by 15°C as compared to that of neat PVA. Similarly, the thermograms obtained for PVA biocomposites reinforced with cellulose microfibers isolated from Finger millet straw at fiber loading of 5%, 10%, and 20%, have shown onset degradation temperature of 275°C, 281°C and 285°C respectively with a maximum increase of 15°C from that of neat PVA being observed with the 20% loading of cellulose in the composite. It has to be noted that the composites have intermediate degradation temperature between cellulose microfibers and neat PVA. The onset degradation temperature of cellulose microfibers isolated by method IOU from Jatropha seed shell, Pongamia seed hull and Finger millet straw are 280°C, 291°C and 293 °C respectively, which are higher than the values obtained for the composites. Modest adhesion of cellulose microfiber in PVA matrix has influenced the degradation temperature of the composites (de Medeiors et al. 2009). Thus, the reinforcement of cellulose microfibers as fillers in PVA matrix enhances the thermal property of biocomposites as a whole.

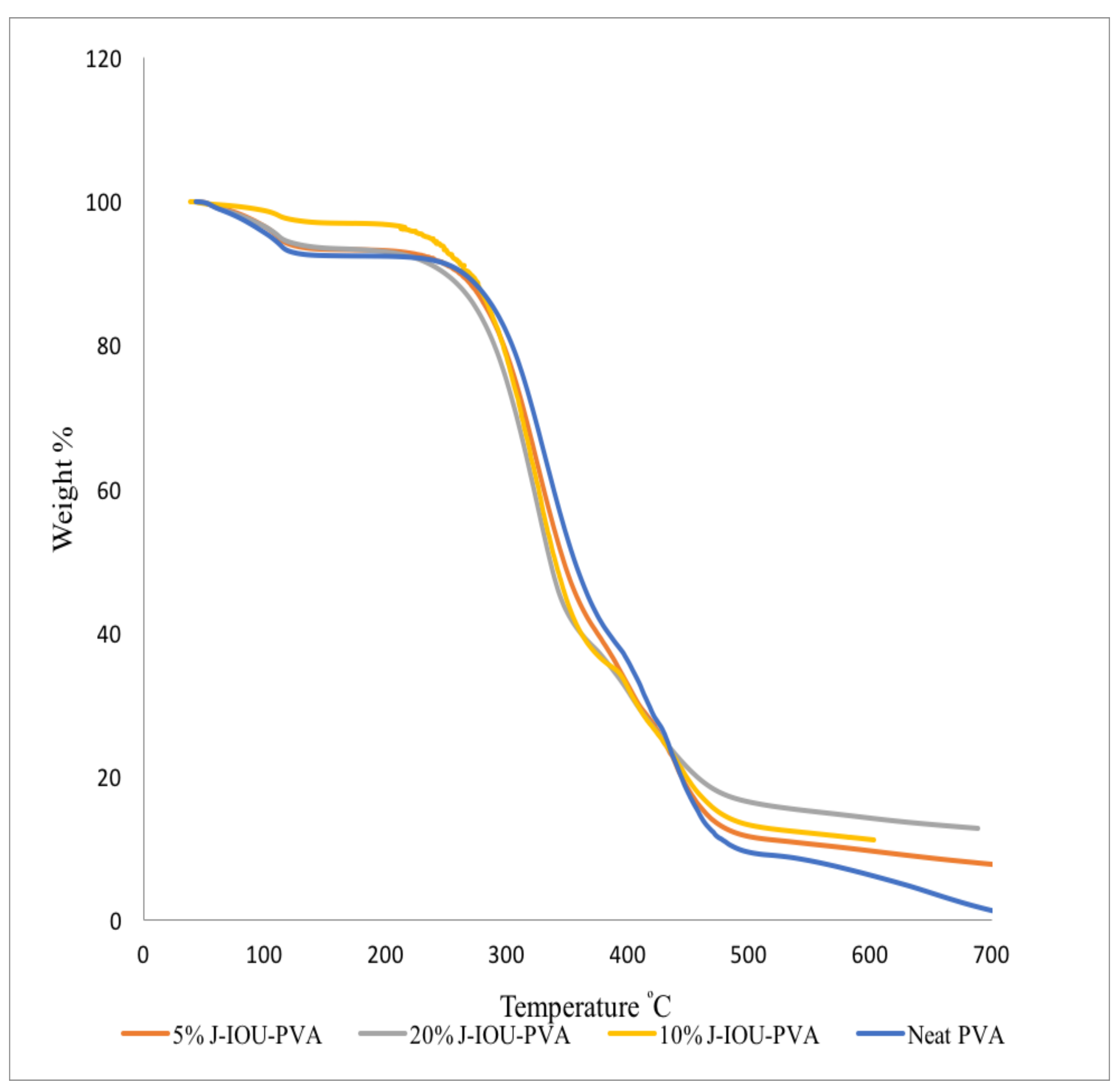


Figure 4.20a. Thermograms of neat PVA and biocomposites with different (wt%) of cellulose microfiber loading isolated from Jatropha seed shell in PVA matrix.

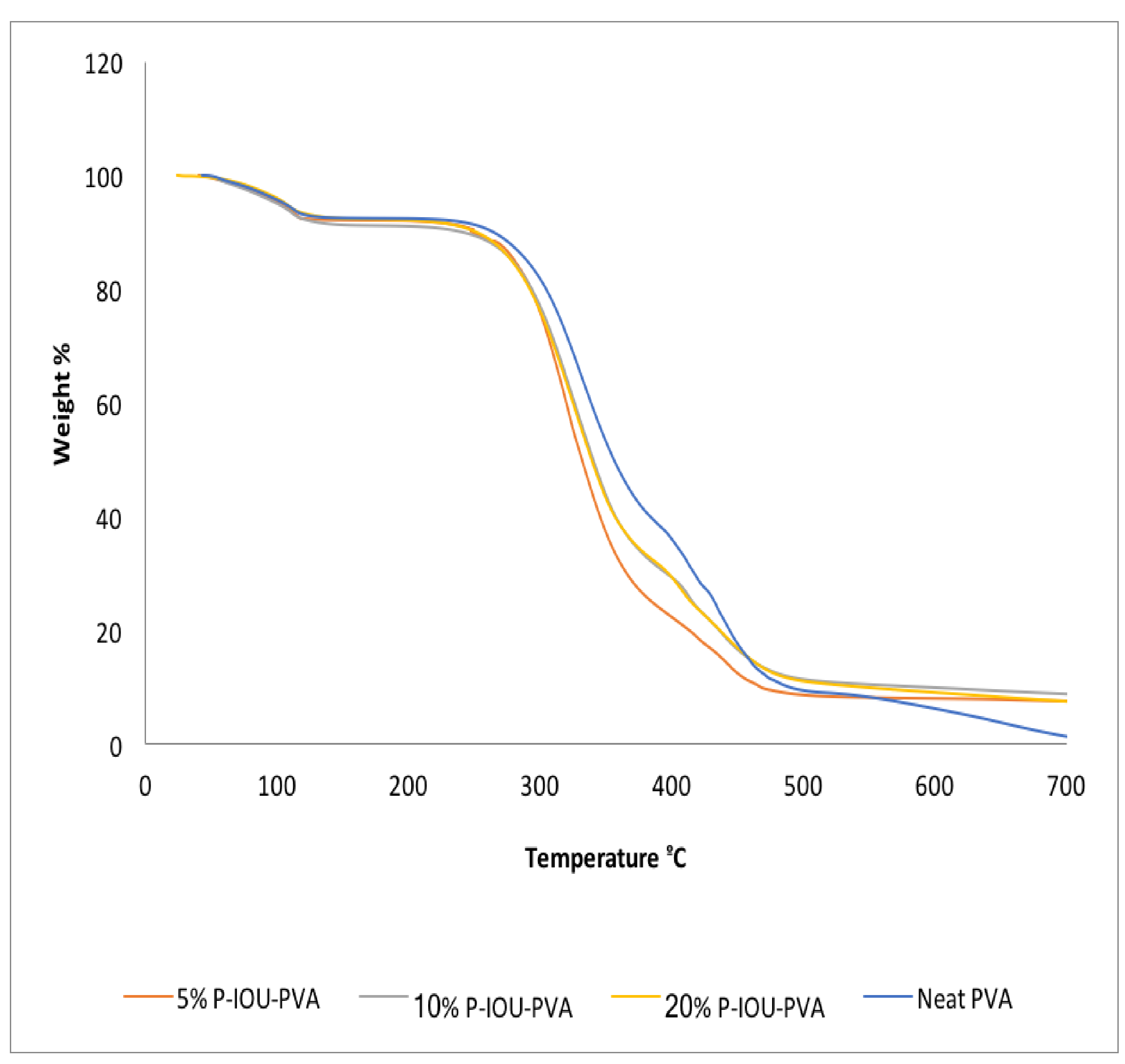


Figure 4.20b. Thermograms of neat PVA and biocomposites with different (wt%) of cellulose microfiber loading isolated from Pongamia seed hull (P) in PVA matrix.

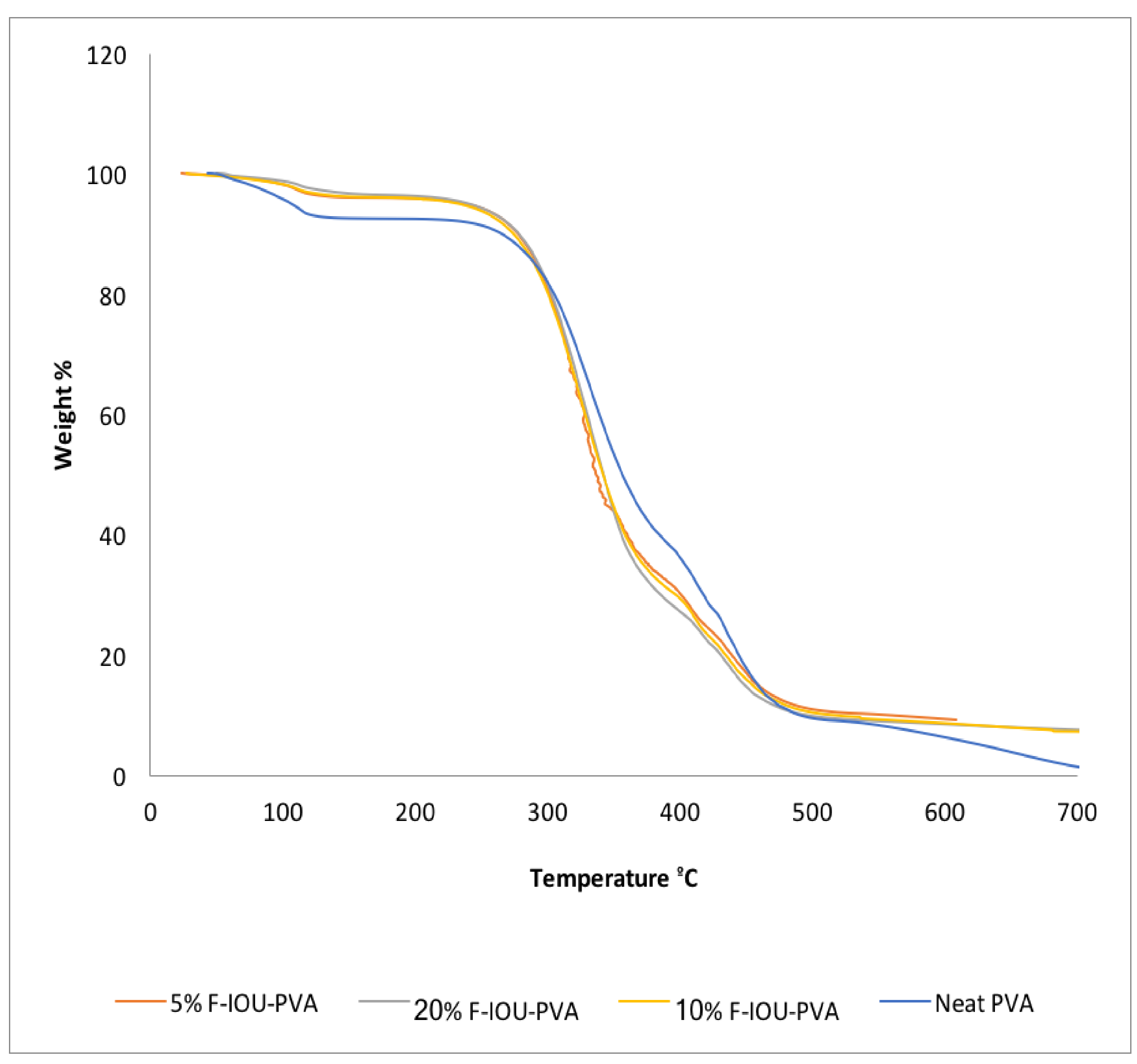


Figure 4.20c. Thermograms of neat PVA and biocomposites with different (wt%) of cellulose microfiber loading isolated from Finger millet straw (F) in PVA matrix.

The thermal stability of the cellulose microfibers reinforced composites depends upon the drying conditions followed while preparation of the films which directly influence different level of degradation stages (Lavoine et al.2012). Padal et al. (2014) have reported that the jute nanofiber reinforced epoxy composites showed increase in thermal stability by 26 $^{\circ}$C compared to that of pure epoxy film. However, the degradation temperature of the composites recorded in the present work is higher than that of the other active ingredients reinforced PVA biocomposites reported in literature for food packaging applications (Fortunati et al 2016) and is in agreement with apple pomace reinforced PVA composites, reported in literature for food packaging applications (Gaikwad et al. 2016). High thermal stability of the biocomposites reinforced with cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw is favourable for food packaging applications involving high temperature.

4.5.4 Biodegradability of cellulose reinforced PVA biocomposites

Recently there is a considerable interest on biodegradable materials for the protection of the environment from ever increasing plastic waste (Franco et al. 2004; Kim et al.2000; Okada et al.2002). Biodegradable polymers are degraded by the actions of enzymes secreted by microorganisms in appropriate environmental conditions. These enzymes break down the high molecular weight polymeric material into smaller segments, thus reducing its molecular weight and increasing the ease of its degradation in the environment into simpler compounds. Biodegradable materials can be completely degraded into natural ecosystems such as soil, river or ocean (Roy et al. 2015). The biocomposites used in packaging field after usage would end up in landfills and municipal waste dump yard (MWDY). Thus, any polymer composite material newly developed for packaging application need to be assessed for its biodegradability. Thus, the cellulose reinforced biocomposites developed in the present study were also assessed for their biodegradability. The extent of biodegradability of the isolated cellulose microfiber reinforced PVA composites assists in analysing the safe disposal potential of these composites. The cellulose microfiber reinforced PVA biocomposites with 5%, 10% and 20% fiber loading were subjected to biodegradation by soil burial test in two types of

soil: Garden soil and MWDY soil as discussed in section 3.5. The composites were buried in both garden soil and municipal dump yard soil for 4 weeks in duplicates and were tested for the biodegradability in terms of percentage weight loss of the biocomposites. The biodegradability of the biocomposites was evaluated in terms of the percentage loss in weight of the composites with time and is presented in Figure 4.21. The extent of biodegradability of these biocomposites are further compared with that of neat PVA. Around 70% and 80% of neat PVA was biodegraded in the first week in MWDY soil and in garden soil respectively. The percentage degradation of cellulose fiber reinforced PVA biocomposites in the first week is higher than that of the neat PVA both in garden soil and MWDY soil. It shows that, the biodegradation of the composites proceeds at a higher rate than that of neat PVA.

Complete degradation of cellulose microfiber reinforced PVA biocomposites was observed in the second week in garden soil, whereas >95% degradation was observed in the second week in MWDY soil, indicating that the rate of biodegradation of cellulose reinforced PVA biocomposites is higher in garden soil than that in MWDY soil. The garden soil is nourished with vermicompost which contain highly active microorganisms and the required nutrients due the action of which degradation of the composites would have resulted (Kawai 2010, Campos et al 2011) at a higher rate. MWDY soil may contain certain organic/toxic compounds which may have inhibited the growth and biodegradation rate. However, within third week complete degradation of the composites could be achieved in the MWDY soil. With increase in cellulose fiber loading in the composite the weight loss by biodegradation has increased both in MWDY and garden soil. PVA degrades in soil as it is susceptible to the microorganisms present in the soil (Pająk et al. 2010). Reinforcement with cellulose microfibers with PVA in the composite would decrease the weight percentage of polymer and also depolymerisation of cellulose and hemicellulose in soil leads to degradation of biocomposites (Tănase et al. 2016). Thus, the cellulose microfiber reinforced PVA biocomposites can be disposed by composting, where the microorganisms break the composite into carbon dioxide, water and biomass (Franchetti et al 2011).

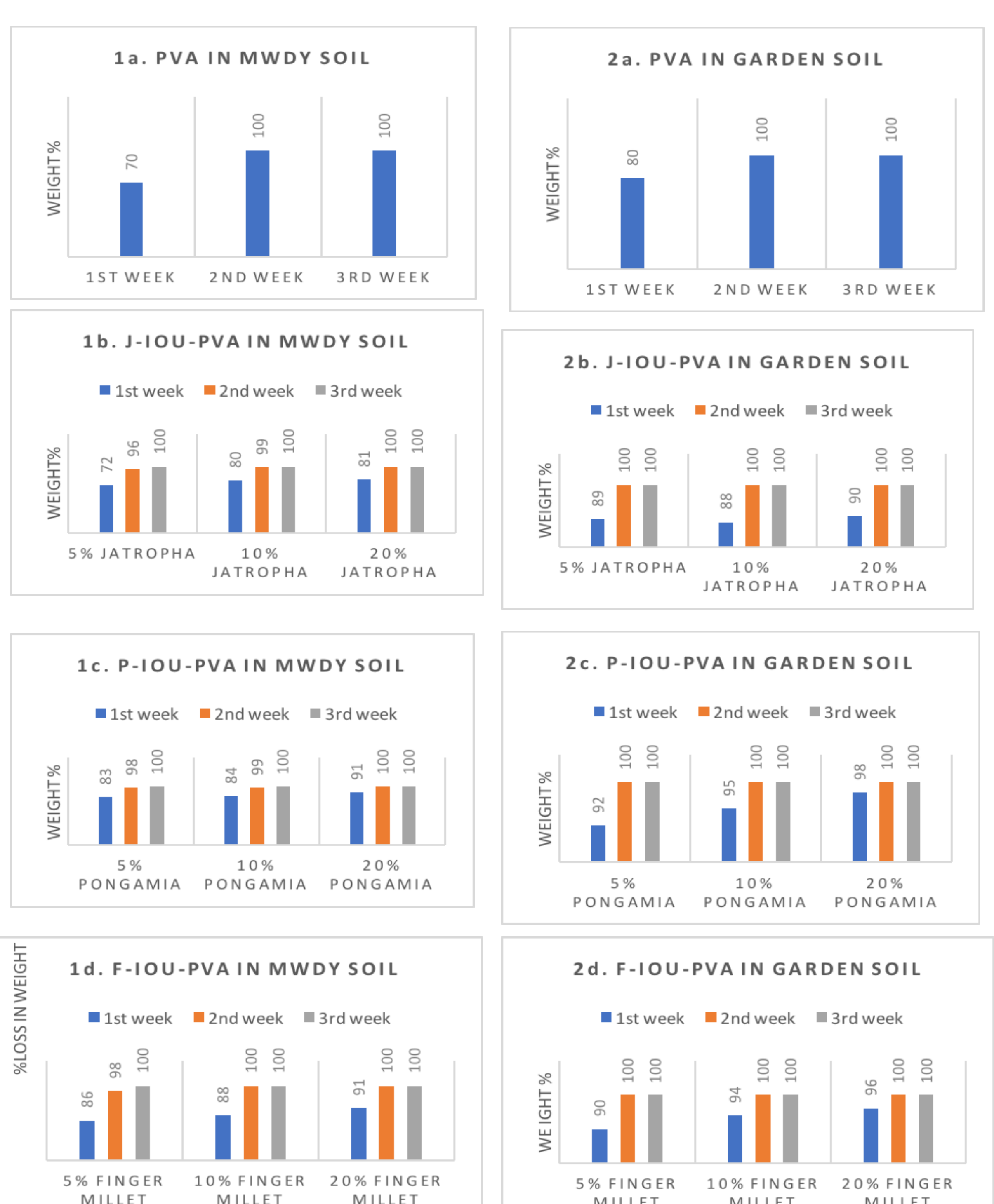


Figure 4.21. Percentage Weight loss due to biodegradation as a function of time (week) of neat PVA and cellulose reinforced PVA in municipal waste dump yard soil (MWDY soil) and garden soil.

4.5.5. Tensile properties of Composites

The tensile properties of cellulose microfiber reinforced biocomposites are analysed to determine the strength of the composites. Variation in tensile strength and Young's modulus of composites are shown in Table.4.32. The stress and strain behavior of PVA and the cellulose microfiber/PVA composites are represented in Figure 4.22.

Table 4.32. Tensile strength and modulus of biocomposites with cellulose microfiber isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw at different fiber loading in PVA matrix.

Reinforcement wt%	Tensile strength (N/mm^2)			Tensile modulus(N/mm^2)		
	P-IOU-PVA	J-IOU -PVA	F-IOU -PVA	P-IOU -PVA	J-IOU -PVA	F-IOU -PVA
0	11.64	11.64	11.64	$7.196x10^{-4}$	$7.196x10^{-4}$	$7.196x10^{-4}$
5	14.11	17.57	19.56	0.40	3.30	5.31
10	21.18	39.64	29.43	4.75	20.87	15.40
20	17.52	33.47	47.39	2.75	12.35	26.65

The tensile strength and the modulus values have increased on reinforcement with cellulose microfibers with PVA. Tensile properties have increased the increase in fiber loading of up to 10% and then decreased with further increase to 20 % for the composites prepared with isolated cellulose fibers from Jatropha seed shell and Pongamia seed hull. However, the values have increased with the increase in fiber loading up to 20% for the composites prepared with cellulose microfibers isolated from Finger millet straw. The tensile strength of the biocomposites has increased by three and two times with 10% reinforcement by cellulose microfibers isolated from Jatropha seed shell and Pongamia seed hull respectively, with reference to neat PVA. The tensile strength of the biocomposites reinforced with 20% cellulose microfibers isolated from Finger millet straw is four times that of neat PVA.

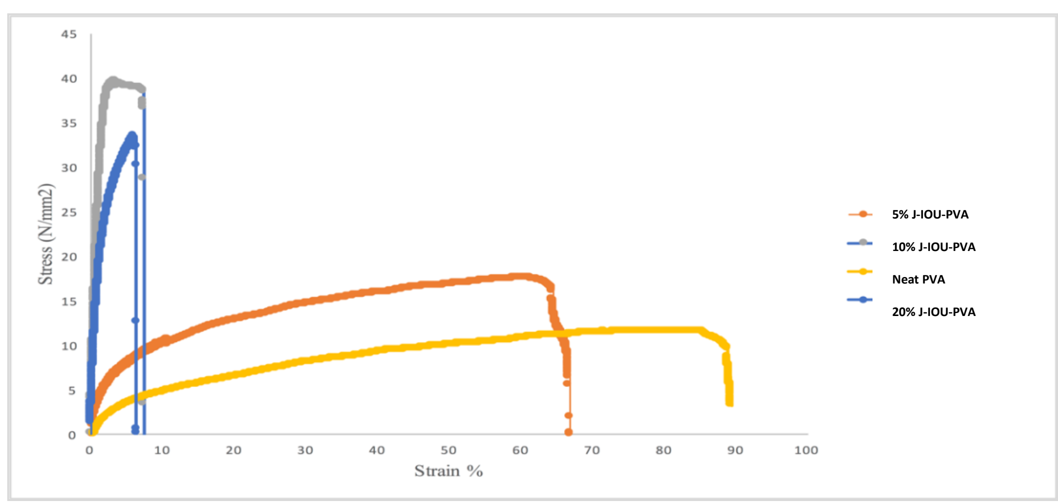


Figure 4.22a. Stress vs strain graph of neat PVA and biocomposites with cellulose microfiber isolated from Jatropha seed shell at different fiber loading in PVA matrix.

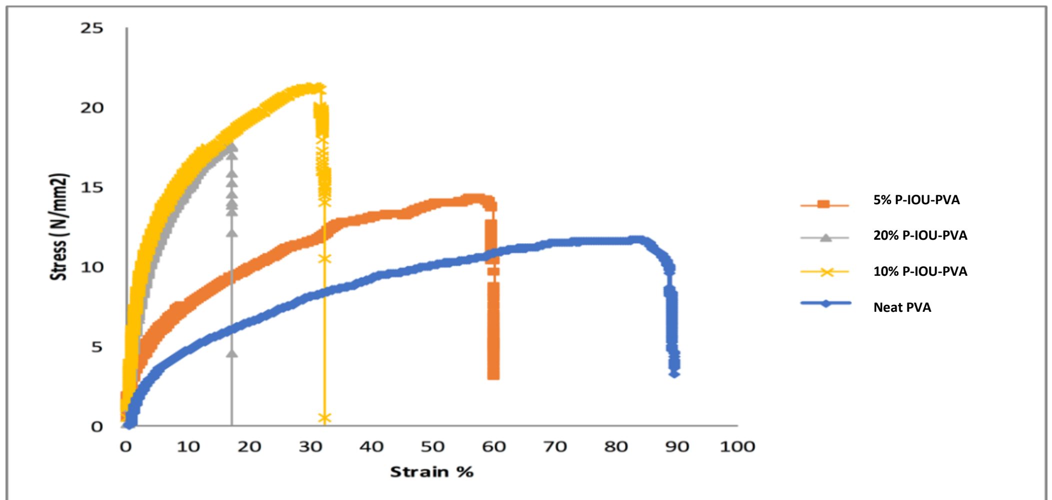


Figure 4.22b. Stress vs strain graph of neat PVA and biocomposites with cellulose microfiber isolated from Pongamia seed hull at different fiber loading in PVA matrix.

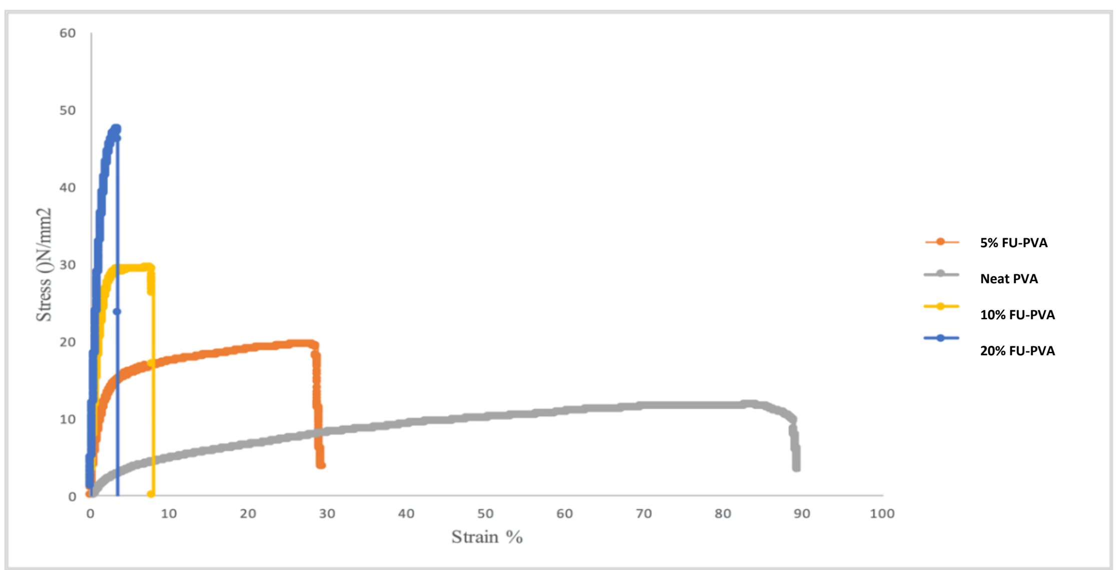


Figure 4.22c. Stress vs strain graph of neat PVA and biocomposites with cellulose microfiber isolated from Finger millet straw at different fiber loading in PVA matrix.

However, the Young's modulus has increased drastically with cellulose microfiber reinforcement. The increase in tensile strength and modulus with the increase in cellulose content indicates that stress successfully transfers from the matrix to the fiber (Augustine et al. 2014) due to effective distribution and adhesion of cellulose microfibers in the matrix (Sun et al. 2014) and availability of large interfacial area as a result of smaller diameter cellulose fibers used as fillers (Maheswari et al. 2014; Mitra 2014; dos Santos et al. 2016).

However, there is a slight reduction in tensile strength and modulus for the filler concentration of 20wt% in case of cellulose fibers isolated from Jatropha seed shell and Pongamia seed hulls.

This could be due the uneven distribution and agglomeration of cellulose microfiber at higher concentration which reduces effective aspect ratio in the PVA matrix (Enayati et al. 2016; Behzad et al. 2014). Cerpakovska & Kalnins et al. (2012) have also reported that cellulose reinforcement improves the mechanical strength of PVA.

4.4.6 Oxygen transfer rate (OTR) analysis

The biocomposites of highest tensile strength were further subjected to analysis of oxygen permeability in terms of oxygen transfer rate. The oxygen transfer rate values of the biocomposites are presented in Table 4.33. The composites prepared with cellulose microfibers isolated from Jatropha seed shell and Pongamia seed hull have shown marginal reduction in OTR values compared to that of neat PVA.

Table 4.33. Comparison of Oxygen transfer rate through composites reinforced by cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by method IOU at optimum loading in PVA.

Reinforcement wt %	OTR (cc/m^2.day)		
	Jatropha (J-IOU)	Pongamia (P-IOU)	Finger millet (F-IOU)
0	3.66	3.66	3.66
10/20	2.91	2.06	6.87

The OTR for neat PVA is 3.66 cc/m^2.day. The OTR for the optimized cellulose fiber loading of 10 wt% in J-IOU-PVA and P-IOU-PVA composites has reduced compared to neat PVA. Many factors govern the OTR value of a material. However, in the present study the reduction in OTR value would have resulted due to the presence of crystalline cellulose replacing the space that would otherwise be occupied by the permeable polymer (Sonia and Dasan, 2013), hindering the gas flow through the film owing to increase in diffusion path and thus reducing the permeability. However, the OTR value for the PVA composite reinforced with cellulose microfibers isolated from Finger millet straw, is higher than that of neat PVA. The increase in OTR value can be accounted to the influence of fiber size, orientation and agglomeration tendency of cellulose microfibers providing channels, pores, or micro crevices in the composites which may have decreased the tortuous path for the gas permeability (Petersson and Oksman 2006, Azizi et al. 2014; dos Santos et al. 2016).

Oxygen transfer rate plays a vital role in food packaging and lower values are preferred to protect the food material from oxidation. The OTR values reported in the present study are lesser than 10–20 cc/m^2day, which is the maximum prescribed for food packaging applications (Lavoine et al. 2012; Grumezescu, 2016). Laxmeshwar et al. (2012), have reported decrease in OTR value with increase in the cellulose fiber loading in composites reinforced by cellulose in PVA matrix. The lowest OTR value reported by them was 843 cc/m^2day and have suggested that these composites are potential in food packaging. However, for thicker composites (20-33 µm) reinforced with cellulose microfiber from bleached spruce sulphite pulp OTR values have been reported in the range of 17-18 cc/m^2day (Syverud and Stenius 2009). Even though the thickness of the composites (0.1mm) in the present study is smaller, much lower OTR values have been observed indicating lower oxygen permeability through the composite film.

PVA in general is used in packaging industry for packaging of detergents, dyes and industrial polarizers due to resistance to greases, oils and solvents and also in food packaging industries (Russo et al. 2009; Cerpakovska et al 2012; Abdullah et al. 2016). Since the reinforcement of PVA with cellulose microfiber isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by method IOU has improved the thermal stability and mechanical strength of the biocomposites and has resulted in OTR values which are within the limit specified for the packaging requirements. The cellulose fibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw can find potential utilization in packaging field, specifically in food packaging as effective reinforcement in biocomposites.

CHAPTER 5

SUMMARY AND CONCLUSIONS

The main objective of the present work was focused on isolation of cellulose microfibers from non-edible and abundantly available, potential wastes (residues) of industrial and agricultural sectors. These residues are alike the ones reported in the literatures as they are being underutilized and most important factor being non-consumable by both human and animals. The cellulose composition and fiber dimensions of the cellulose microfibers play a major role in enhancing the overall properties of biocomposites. Thus, the cellulose fibers were aimed to be isolated by combination of well-established chemical treatments, low energy consuming mechanical treatments and eco-friendly enzymatic treatments in order to obtain cellulose fibers without compromising with their cellulose composition and lower fiber dimensions. These isolated cellulose fibers were reinforced in hydrophilic PVA matrix to avoid further modification treatments, in order to make the composite preparation economical and also for the reason PVA being extensively used in packaging industries. These biocomposites were further prepared by a economical method; solvent casting and were tested for their potentiality in food packaging by subjecting to several characterization techniques.

The major findings of the present research work are summarized below

- Jatropha seed shell, Pongamia seed hull and Finger millet straw, which are the biofuel industry and agricultural residues, are the potential sources of cellulose and cellulose microfibers are isolated from these sources by adopting suitable isolation methods.
- Organosolv treatment(O) involving hydrolysis by organic acid and combined inorganic and organosolv treatment (IO) which involved bleaching with sodium chlorite, alkali treatment and hydrolysis by organic acid have contributed majorly in removal of lignin and hemicellulose and in the cellulose isolation.

- After the treatment by Method O on the untreated fibers of Jatropha seed shell, Pongamia seed hull and Finger millet straw which contained 41%,42% and 50% cellulose respectively, the cellulose concentration in the respective isolated fibers have increased to 82%,85% and 84%.
- Treatment by Method IO on the untreated fibers has increased the cellulose concentration in the isolated cellulose fibers of Jatropha seed shell, Pongamia seed hull and Finger millet straw to 89%,84% and 92% respectively.
- Combining ultrasonication treatment(U) or enzymatic treatment (E) with Method O or Method IO on cellulose isolation in terms of increasing the cellulose concentration in the isolated fibers is not very significant. However, they aided in further defibrillation of the isolated cellulose fibers leading to decrease in fiber diameter.
- The isolation process conditions such as time and temperature of acid treatment in organosolv treatment, fiber to liquor ratio for the inorganic treatment; sonication time in ultrasonication treatment and enzyme concentration in enzymatic treatment were found to influence the extent of cellulose isolation and defibrillation.
- Method IOU was chosen as the favorable method for isolation of cellulose fibers from the three lignocellulosic sources, as this process under optimum conditions yielded cellulose microfibers with the maximum cellulose content and minimum fiber diameter.
- The optimum conditions chosen for IOU treatment were
  - (i) Fiber to liquor (sodium chlorite) ratio of 1:50
  - (ii) Acetic acid to nitric acid ratio of 10:1 for Jatropha seed shell and finger millet straw and 15:1 for Pongamia seed hull.
  - (iii) Ultrasonication time of 20 min for defibrillating cellulose microfibers isolated from Jatropha seed shell and finger millet straw and sonication time of 25 min for Pongamia seed hull.

- The cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and Finger millet straw by Method IOU under optimum conditions contained 90%, 85% and 93% of cellulose with smallest fiber diameters of 194, 145 and 147nm respectively.
- Characterization of cellulose microfibers by SEM, DLS, FTIR, NMR and XRD analysis have ensured the removal of matrix components and isolation of cellulose microfibers.
- The isolated cellulose microfibers have shown the degradation temperature of >280°C and they have shown superior thermal properties as compared to the untreated fibers. The enhanced thermal properties of the isolated cellulose fibers have widened the scope of their application in the field of composites.
- These cellulose microfibers were further reinforced in PVA matrix with different fiber loading (5, 10, and 20 wt%) by solution casting to form biocomposites. These biocomposites were transparent and also showed enhanced thermal properties compared to that of neat PVA.
- The biocomposites have shown better tensile properties as compared to neat PVA and the tensile properties have improved with the increase in fiber loading. The biocomposites reinforced with 10 wt% loading of cellulose microfibers isolated from Jatropha seed shell, Pongamia seed hull and 20 wt% loading of cellulose microfibers isolated from Finger millet straw have shown maximum tensile strength and Young modulus compared to the bio composites with other fiber loading.
- The cellulose reinforced biocomposites have also shown the OTR values lower than the maximum applicable for food packaging applications.
- The biodegradability of these biocomposites proceeds at a higher rate than that of neat PVA. The percentage degradation of cellulose fiber reinforced PVA biocomposites in the first week is higher than that of the neat PVA both in garden soil and MWDY soil.

Thus, the biocomposites prepared using cellulose microfibers isolated by method IOU from Jatropha seed shell, Pongamia seed hull and Finger millet straw reinforced in PVA matrix with better mechanical and remarkable thermal properties compared to that of neat PVA, can find several applications. Their lower affinity for transfer of oxygen, good tensile and thermal properties makes them as ideal choice in the field of food packaging. These biodegradable composites prepared using the cellulose isolated from industry and agricultural residues as fillers, can serve as economical and eco-friendly replacements for the composites reinforced with synthetic fillers.

SCOPE FOR FUTURE STUDIES

- Study of the parameters affecting fiber distribution in PVA matrix such that the properties of the biocomposites are further enhanced.
- To assess the suitability of the developed biocomposites for real time food packaging applications.

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APPENDICES

Appendix I

ASTM D1105-96 Standard test method for extractive- free wood.

Designation: D 1105 – 96 (Reapproved 2007)

Technical Association of Pulp and Paper Industry
Standard Method T 12 os-75

Standard Test Method for Preparation of Extractive-Free Wood[1]

This standard is issued under the fixed designation D 1105; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers the preparation of extractive-free wood and is applicable to all North American woods. Extractives in wood consist of materials that are soluble in neutral solvents and that are not a part of the wood.

1.2 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* Specific precautionary statements are given in 4.2.

2. Significance and Use

2.1 Extractives are materials soluble in neutral solvents. They are not generally considered part of the wood polymer structure. These materials should be removed before any chemical analysis of the wood. Ethanol-benzene extracts waxes, fats, some resins, and portions of wood gums. Hot water extracts tannins, gums, sugars, starches, and coloring matter.

3. Apparatus

3.1 *Soxhlet Extraction Apparatus*—A glass Soxhlet extraction apparatus of suitable size for containing the sample, and fritted-glass or Alundum extraction thimbles of medium to coarse porosity, will be required. Bags of cotton cloth of fine weave and thoroughly washed, of a suitable size to fit within the body of the extractor, are also satisfactory in place of the thimbles. Alternatively, a small wad of cotton or a wire screen may be placed in the discharge tube of the extractor and the entire body of the extractor filled with the wood sample. A thin wire screen disk placed over the top of the material will prevent channeling by the dripping condensate.

4. Reagents

4.1 *Ethyl Alcohol* (95 %).

4.2 *Ethanol-Toluene Mixture*—Mix 1.0 absolute ethanol and 427 mL toluene. (**Warning**—Avoid inhalation of vapors and contact with skin.)

5. Sample

5.1 The sample shall consist of air-dry sawdust that has been reduced by means of a Wiley mill so as to pass through a 250-μm sieve and be retained on a 180-μm sieve.

6. Procedure

6.1 Place a suitable quantity of the sample in the extraction thimble, being certain that the wood does not extend above the level of the top of the siphon tube. Extract for 4 h with ethanol-toluene mixture in the Soxhlet extraction apparatus. Transfer the wood to a Büchner funnel, remove the excess solvent with suction, and wash the thimble and wood with alcohol to remove the toluene. Return the wood to the extractor and continue the extraction with ethanol for 4 h, or longer if necessary, until the ethanol siphons over colorless.

6.2 If the thimble is nearly full, a Gooch crucible of suitable size may be placed in the rim of the thimble to keep the sample together. Extraction with each solvent should be carried out at a rate of not less than four siphonings per hour. Remove the wood from the thimble, spread it out in a thin layer, and allow it to dry in the air until free of alcohol. Transfer the material to a 7.5-L Erlenmeyer or Florence flask and extract successively with three 1-L portions of distilled water, heating the flask with each change of water for 1 h in a hot-water bath at 100°C. The water should be at boiling temperature before the addition of the wood and the flask in the bath should be entirely surrounded by the boiling water. After the third extraction with water is complete, filter on a Büchner funnel, wash with 500 mL of boiling distilled water, and allow the extracted material to become thoroughly dry in the air.

7. Precision and Bias

7.1 Statements of precision and bias are not applicable to this method.

8. Keywords

8.1 extractive-free wood; wood

[1] This test method is under the jurisdiction of ASTM Committee D07 on Wood and is the direct responsibility of Subcommittee D07.01 on Fundamental Test Methods and Properties.

Current edition approved April 1, 2007. Published April 2007. Originally approved in 1950. Last previous edition approved in 2001 as D1105 – 96 (2001).

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Appendix II

ASTM D1102-84 Standard test method for ash wood

Designation: D1102 – 84 (Reapproved 2007)

Modification of
Technical Association of Pulp and Paper Industry
Standard Method T 211 OM-80

Standard Test Method for Ash in Wood[1]

This standard is issued under the fixed designation D1102; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers the determination of ash, expressed as the percentage of residue remaining after dry oxidation (oxidation at 580 to 600°C), of wood or wood products.

1.2 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Significance and Use

2.1 The ash content is an approximate measure of the mineral content and other inorganic matter in wood.

3. Apparatus

3.1 *Crucibles,* with tightly fitting lids, having a capacity of 30 mL or more, shall be used. Platinum crucibles are preferred, but silica or porcelain crucibles may be used.

3.2 *Muffle Furnace*—An electric furnace is recommended for igniting the wood sample. A furnace fitted with an indicating pyrometer, so that the desired temperature can be maintained, is preferable.

3.3 *Analytical Balance,* sensitive to 0.1 mg.

3.4 *Drying Oven,* with temperature controlled between 100 and 105°C.

4. Test Specimen

4.1 The test specimen shall consist of approximately 2 g of wood that has been ground to pass a No. 40 (425-μm) sieve. Care shall be taken to ensure that it is representative of the entire lot of material being tested.

[1] This test method is currently under the jurisdiction of ASTM Committee D07 on Wood and is the direct responsibility of Subcommittee D07.01 on Fundamental Test Methods and Properties.

Current edition approved April 1, 2007. Published April 2007. Originally approved in 1950. Last previous edition approved in 2001 as D1102 – 84 (2001). DOI: 10.1520/D1102-84R07.

5. Procedure

5.1 Ignite the empty crucible and cover over a burner or in the muffle at 600°C, cool in a desiccator, and weigh to the nearest 0.1 mg. Place the 2-g test specimen in the crucible, determine the weight of crucible plus specimen, and place in the drying oven at 100 to 105°C with the crucible cover removed. After 1 h, replace the cover on the crucible, cool in a desiccator, and weigh. Repeat the drying and weighing until the weight is constant to within 0.1 mg. During the cooling and weighing periods, keep the crucible covered to prevent absorption of moisture from the air. Record the weight (crucible plus specimen minus weight of crucible) as the weight of the oven-dry test specimen.

5.2 Place the crucible and contents, with the cover removed, in the muffle furnace and ignite until all the carbon is eliminated. Heat slowly at the start to avoid flaming and protect the crucible from strong drafts at all times to avoid mechanical loss of test specimen. The recommended temperature of final ignition is 580 to 600°C. Avoid heating above this maximum.

5.3 Remove the crucible with its contents to a desiccator, replace the cover loosely, cool, and weigh accurately. Repeat the heating for 30-min periods until the weight after cooling is constant to within 0.2 mg.

6. Calculations and Report

6.1 Calculate the percentage of ash, based on the weight of the moisture-free wood, as follows:

$$\text{Ash, } \% = (W_1/W_2) \times 100 \quad (1)$$

where:

W_1 = weight of ash, and
W_2 = weight of oven-dry sample.

6.2 Report the results to two decimal places.

7. Precision and Bias[2]

7.1 Data obtained by testing 60 wood samples in one laboratory gives a repeatability as ash content of 0.03 % and as

[2] Data in this section was obtained from the Technical Association of the Pulp and Paper Industry, P.O. Box 105113, Atlanta, GA 30348.

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Appendix III

ASTM D1106-96 Standard test method for acid-insoluble lignin in wood

Designation: D1106 – 96 (Reapproved 2007)

Technical Association of Pulp and Paper Industry
Standard Method T 222-om-83

Standard Test Method for Acid-Insoluble Lignin in Wood[1]

This standard is issued under the fixed designation D1106; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method[2] covers the determination of the acid-insoluble lignin content of wood.

1.2 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* Specific precautionary statements are given in 6.1.

2. Referenced Documents

2.1 *ASTM Standards:*[3]
D1107 Test Method for Ethanol-Toluene Solubility of Wood

3. Principle of Method

3.1 When wood is treated with strong acids the carbohydrates are hydrolyzed, leaving an insoluble residue which is determined as lignin. Since some of the wood extractives (oils, resins, fats, waxes, tannins, gums, and starch) would remain insoluble with the lignin, these are first removed by proper solvents. The 72 % sulfuric acid method for lignin contains two and sometimes three preliminary extractive treatments, namely: (*1*) with alcohol, to remove the catechol tannins; (*2*) with alcohol-benzene solution, to remove the resins, oils, fats and waxes; and (*3*) with hot water, to remove the remaining water-soluble materials.

3.2 The alcohol extraction is necessary in analysis of woods high in tannin; that is, oak, chestnut, redwood, etc. It has not been shown necessary in the more common pulpwoods, such as the various species of spruce, pine, fir, hemlock, poplar, birch, beech, and maple. It is recommended that for these woods the alcohol extraction be omitted unless it is desirable for a special purpose. In analysis of woods not listed, the desirability of the alcohol extraction depends upon the purpose of the analysis and the report should state whether or not alcohol extraction was used.

4. Significance and Use

4.1 Wood contains approximately 20 to 30 % lignin. Removal of the lignin is the primary objective of pulping and bleaching procedures. Determination of the lignin content provides information for the evaluation and application of these processes.

5. Apparatus

5.1 *Extraction Apparatus*—A compact form of Soxhlet extraction apparatus, with ground-glass joints, is preferable. The apparatus shall consist of the following items:

5.1.1 *Soxhlet Extraction Flask,* having a capacity of 250 mL.

5.1.2 *Soxhlet Extraction Tube,* 45 to 50 mm in inside diameter, having a capacity to the top of the siphon of approximately 100 mL and a siphon tube approximately 55 mm in height. Extraction tubes of these dimensions siphon more rapidly than extractors with higher siphon tubes.

5.1.3 *Condenser,* of the Hopkins inner-cooled type.

5.1.4 *Extraction Crucibles,* of Alundum or fritted glass and of medium or fine porosity.

5.2 *Filtering Crucibles*—Alundum, porous porcelain, or fritted-glass crucibles (all of fine porosity), or Gooch crucibles with a glass-fiber mat, are recommended for filtering the separated lignin. Glass crucibles cannot be used if the lignin is to be ashed.

6. Reagents

6.1 *Ethylene-Toluene Solution*—Mix 1.0 L absolute ethanol and 427 mL toluene. (**Warning—**Avoid inhalation of vapors and contact with skin.)

[1] This test method is under the jurisdiction of ASTM Committee D07 on Wood and is the direct responsibility of Subcommittee D07.01 on Fundamental Test Methods and Properties.

Current edition approved April 1, 2007. Published April 2007. Originally approved in 1950. Last previous edition approved in 2001 as D1106 – 96 (2001). DOI: 10.1520/D1106-96R07.

[2] For further information on this test method the following references may be consulted:

Bray, M. W., "Methods Used at the Forest Products Laboratory for the Chemical Analysis of Pulps and Pulpwoods," *Paper Trade Journal*, Vol 87, No. 25, December 20, 1928, p. 29.

Ritter, G. J., Seborg, R. M., Mitchell, R. L., *Industrial and Engineering Chemistry*, Analytical Edition, Vol 4, 1932, p. 202.

Ritter, G. J., and Barbour, J. H., *Industrial and Engineering Chemistry*, Analytical Edition, Vol 7, 1935, p. 238.

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

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6.2 *Sulfuric Acid* (72 %)—Carefully pour 665 mL of H_2SO_4 (sp gr 1.84) into about 300 mL of water, with vigorous stirring, and after cooling, dilute to 1 L. Standardize against standard NaOH solution, using methyl orange indicator. Adjust the H_2SO_4 to a strength of 72 ± 0.1 % by addition of water or H_2SO_4 (sp gr 1.84) as may be found necessary. If desired, the solution may be standardized by an accurate determination of its specific gravity. For 72 % H_2SO_4 the specific gravity at 20/4°C is 1.6338; for use of this specific gravity method appropriate tables should be consulted.

7. Test Specimen

7.1 The test specimen shall consist of 1 g of wood that has been ground to pass a 425-µm (40 mesh) sieve and thoroughly air-dried.

8. Procedure

8.1 Weigh two 1-g test specimens in tared glass-stoppered weighing bottles. Dry in an oven for 2 h at 100 to 105°C, replace and stopper, and cool in a desiccator. Loosen the stopper to equalize the pressure and weigh. Continue the drying for 1-h periods until the weight is constant. Calculate the percentage of moisture-free wood.

8.2 Weigh in the extraction crucibles two additional 1-g test specimens for the lignin determination in duplicate. Place the extraction crucible containing the specimen in a Soxhlet extraction apparatus. Extract with 95 % alcohol for 4 h, unless the wood is known not to contain catechol tannins, in which case this extraction with alcohol will not be required. Then extract the test specimen with ethanol-toluene solution as described in Test Method D1107. Remove as much of the solvent by suction as possible and wash by suction with 50 mL of ethanol to remove the toluene. Remove the excess ethanol, transfer to a beaker, and digest with 400 mL of hot water in a steam or hot-water bath at approximately 100°C for 3 h. Filter, wash with 100 mL of hot water, and finally with 50 mL of ethanol to facilitate the removal of the test specimen from the crucible. After these preliminary extractions, let the specimen dry in the air.

8.3 Transfer all of the air-dried test specimen to a glass-stoppered weighing bottle or a small beaker with a glass cover and add slowly, while stirring, 15 mL of cold (12 to 15°C) H_2SO_4 (72 %). Mix the specimen well with the acid by stirring constantly for at least 1 min. Allow to stand for 2 h, with frequent stirring, at a temperature of 18 to 20°C. A water bath may be necessary to keep the temperature within these limits. Wash the material into a 1-L beaker or Erlenmeyer flask, dilute to a 3 % concentration of H_2SO_4 by adding 560 mL of distilled water, and boil for 4 h, either under a reflux condenser or in the nearly constant volume condition maintained by the occasional addition of hot water to the flask.

8.4 After allowing the insoluble material to settle, filter into a filtering crucible that has been dried at 100 to 105°C and weighed in a glass-stoppered weighing bottle. Wash the residue free of acid with 500 mL of hot water and dry the crucible and contents in an oven for 2 h at 100 to 105°C. Place in the weighing bottle, cool in a desiccator, loosen the stopper of the bottle, and weigh the contents of the crucible as lignin. Repeat the drying and weighing until the weight is constant.

8.5 If a correction for ash is desired, transfer the lignin to a tared platinum crucible and determine the ash by igniting at 900°C. If the lignin cannot be quantitatively transferred, it may be ashed in the filtering crucible, provided the latter has been ignited to constant weight before filtration of the lignin residue. Ignition cannot be performed in fritted-glass crucibles.

9. Report

9.1 Report the results as percentage by weight of lignin in moisture-free unextracted wood. If the wood was extracted with alcohol, or if the lignin was corrected for ash, state this in the report.

10. Precision and Bias[4]

10.1 An interlaboratory study conducted by nine laboratories on six woods indicates that the precision both within and between laboratories is approximately constant throughout the range of lignin content. A range of content from 19 to 30 % gave a repeatability of 0.34 and a reproducibility of 0.79.

11. Keywords

11.1 acid-insoluble lignin; wood

[4] Data in this section obtained form the Technical Association of the Pulp and Paper Industry, P.O. Box 105113, Atlanta, GA 30348.

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RESEARCH PUBLICATIONS

Articles in Journals

- Manjula, P., Srinikethan, G., and Shetty, K. V. (2017). "Biofibres from biofuel industrial byproduct-Pongamia pinnata seed hull." *Bioresources and Bioprocessing*, 4:14, 1-10, DOI: 10.1186/s40643-017-0144-x.
- Manjula, P., Srinikethan, G., and Shetty, K. V. (2017). "Biocomposite composed of PVA reinforced with cellulose microfibers isolated from biofuel industrial dissipate: *Jatropha Curcus L.* seed shell." Journal of Environmental Chemical Engineering, 5(2), 1990-1997, doi.org/10.1016/j.jece.2017.04.004.

Articles in Conference Proceedings

- Manjula, P., Srinikethan, G., and Shetty, K. V. (2014). "Isolation and characterization of cellulose microfibrils from agricultural residue- Jatropha Carcus L seed shell." International Conference on Polymer Composites, NITK, Surathkal, 19th-20th December, OP-114, pp-53.
- Manjula, P., Srinikethan, G., and Shetty, K. V. (2015). "Extraction and characterisation of cellulose microfibrils from Pongamia pinnata seed shell." 20th International Conference on Composite Materials, Copenhagen, Denmark, 19th - 24th July, OP- 5210-3, pp-100.
- Manjula, P., Srinikethan, G., and Shetty, K. V. (2015). "Isolation of cellulose fibers from biofuel waste; Jatropha Curcus L seed shell and reinforcement with PVA to form Biocomposites". 10th Asian-Australasian conference on composite materials, Bexco, Korea, 16th -19th, October, OP-M112-2, pp-28.

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- Doctor of philosophy in Chemical Engineering from National Institute of technology Karnataka, Surathkal, Karnataka with CGPA of 7.33.
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Research articles published

- Manjula, P., Srinikethan, G., and Shetty, K. V. (2017). "Biofibres from biofuel industrial byproduct-Pongamia pinnata seed hull." *Bioresources and Bioprocessing*, 4:14, 1-10, DOI: 10.1186/s40643-017-0144-x.
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- Manjula, P., Srinikethan, G., and Shetty, K. V. (2015). "Isolation of cellulose fibers from biofuel waste; Jatropha Curcus L seed shell and reinforcement with PVA to form Biocomposites". 10th Asian-Australasian conference on composite materials, Bexco, Korea, 16th -19th, October, OP-M112-2, pp-28.

Academic projects

- Postgraduation dissertation entitled " Biological removal of hexavalent chromium using chromium vi resistant microorganisms" conducted at Department of Chemical Engineering, National Institute of technology Karnataka, Surathkal, Karnataka (2007-2009).
- Graduate research project entitled " Extraction from tinospora cordifolia and isolation of glycosides" conducted at Department of Chemical Engineering, Siddaganga Institute of Technology, Tumkur (2007).

Conferences / workshops attended

- 10th Asian-Australasian conference on composite materials (ACCM-10), Bexco, Korea, October 16-19, 2016. "Isolation of cellulose fibers from biofuel waste; Jatropha Curcus L seed shell and reinforcement with PVA to form Biocomposites".

- 20th International Conference on Composite Materials in Copenhagen (ICCM20), COPENHAGEN, DENMARK, July 19-24, 2015, Participant number: ICCM150701-I/1460. "Extraction and characterisation of cellulose microfibrils from pongamia pinnata seed shell".

- International Conference on Polymer Composites (ICPC 2014), National Institute of Technology Karnataka, December 19-20, 2014. "Isolation and characterization of cellulose microfibrils from agricultural residue- Jatropha Carcus L seed shell".
- International conference on "Convergence of science & Engineering in Education & Research- A global perspective in new millennium- ICSE-2010" organised by DSCE, Bengaluru, April 21-23, 2010. "Biological Removal of Hexavalent Chromium using Cr VI resistant microorganisms".

- National Conference on "Information Technology trends in Engineering Applications, NEC-2009" organised by DSCE, Bengaluru, March 19-21 2009.

"Biological Removal of Hexavalent Chromium using Cr VI resistant microorganisms" and won the best paper award.

- International conference-ICACE-2015 "INTERNATIONAL CONFERENCE ON ADVANCES IN CHEMICAL ENGINEERING" on DECEMBER 20-22, 2015, organized by Department of Chemical Engineering, National Institute of Technology Karnataka, Surathkal, India.
- Indo-German Conference – IGC-2011 on "Cities in the era of Climate Change" on 17-18 January 2011, conducted jointly by IIT Bombay and Goethe-Institut class, Max-Mueller Bhavan, Mumbai.
- 4–Day workshop on "RECENT CHALLENGES IN ATMOSPHERIC AND EARTH SCIENCES (RCAES – 2014), held at Department of Chemical Engineering, NITK Surathkal, Karnataka, India from 27th – 30th December 2014.
- Two days TEQIP Workshop on "Fundamentals and Applications of Nanofibers", held at Department of Chemical Engineering, Indian Institute of Technology, Hyderabad (IITH), India from 4th – 5th July 2014.
- Workshop on "Sustainable Polymers" conducted at the Department of Chemical Engineering, IITG, India from 6th - 11th January 2014.
- Chemcon-2011, MSRIT, Bengaluru, Karnataka, India, from 27th - 29th December 2011.
- Conducted Workshop on "Food Technology" in DSCE Bengaluru, Karnataka, India on 29th September 2011.
- 3-day Workshop on "Advances in material research" from 25th - 27th August 2010, organized by Poorna Pragna Institute of scientific Research in association with Indian National Science Academy, National Academy of Science India.
- Workshop attended on "Emerging Trends in National Symposium on Emerging Trends in Novel Separation Science & Technology" on 13th February 2010, organized by Indian Institute of Chemical Engineers, IIChE, Bangalore Regional Centre (BRC), Department of Chemical Engineering, MSRIT, Bengaluru, Karnataka, India

- In plant training in Mangalore Refinery and Petrochemicals Limited, Surathkal, Mangalore, Karnataka, India for a period of 60 days from May – June 2008.
- In plant training in Foundry & Forge Division, HAL, Bengaluru, Karnataka, India for a period of 15 days in February 2006.
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Declaration

I hereby declare that all the above details furnished by me are true and correct to the best of my Knowledge and belief. I understand that the organization can take action against me if I am found to be guilty of furnishing any wrong information.

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