Chapter 1

Introduction

Plant fibers and fiber-reinforced polymer composites have received much attention because of low density, nonabrasive, combustible, nontoxic, low cost, and biodegradable properties of plant fibers. However, the lack of good interfacial adhesion between fibers and matrix, low melting point, and water sensitivity make the use of plant fiber-reinforced composites less attractive. By modifying the surface of the plant fibers, surface roughness is increased and moisture absorption is decreased (Kalia et al., 2009). Various methods have been explored in order to improve the compatibility between hydrophilic plant fibers and hydrophobic polymer matrices (John and Anandjiwala, 2008; Li et al., 2007). Most of the chemical surface treatments of plant fibers involve silylation (Valadez-Gonzalez et al., 1999; Mehta et al., 2006; Ganan et al., 2005; Pothen et al., 2006), acetylation (Tserki et al., 2005), benzoylation (Nair et al., 2001), maleated coupling agents (Mishra et al., 2000), isocyanate treatment (George et al., 1996) and grafting of synthetic polymers (Kaith and Kalia, 2008). Although these hydrophobizing treatments can alter the wettability of plant fibers, the appropriate handling and disposal of the large amounts of hazardous chemicals that is often involved is unattractive and an additional cost to the production. Moreover, chemical treatments of plant fibers do not always result in improvement in composite properties because of the anisotropic nature of plant fibers. Therefore, surface modification of plant fibers using chemical treatments can be avoided by alternative methods. Instead, efforts should be focused on environmentally friendly methods to increase the hydrophobicity of plant fibers and to avoid the shrinkage problem of plant fibers during thermal processing (Lee et al., 2011).

1.1. Plant fibers

Plant fibers can be classified on the basis of their origin and they are grouped according to their type of leaf: abaca, cantala, curaua, date palm, henequen, pineapple, sisal, banana; seed: cotton; bast: flax, hemp, jute, ramie; fruit: coir, kapok, oil palm; grass: alfa, bagasse, bamboo and stalk: straw (cereal). The most commonly used types in the composite applications are the bast and leaf, i.e. the hard fibers (Kalia et al., 2009; Williams and Wool, 2000; Torres and Diaz, 2004). Jute, cotton, hemp, flax, sisal, ramie, kapok, henequen and coir are the examples of commonly used plant fibers. Plant fibers, which have been used by man for many generations, have
remained an attractive material for a variety of potential applications (McDougall et al., 1993). These fibers possess a distinctive characteristic, which makes them excellent materials for soil conservation (Thomas et al., 2011), textile applications (Lewin and Pearce, 1998), as alternate materials especially wood substitutes in the construction market (Singh and Gupta, 2005) and as reinforcement of composite materials to produce automotive structural components (Suddell and Evans, 2005). Plant fibers possess sufficient strength and stiffness but are difficult to use in load bearing applications by themselves because of their fibrous structure. Most plastics themselves are not suitable for load bearing applications due to their lack of sufficient strength, stiffness and dimensional stability (Mohanty et al., 2005). One of the important applications of plant fibers is ‘fiber-reinforced composites’. In fiber-reinforced composites, fibers give strength and stiffness to the structure while the plastic matrix serves as the binder to hold the fibers in place (Thomas et al., 2011). In plant fibers, an amorphous lignin matrix helps in the combination of helically arranged cellulose microfibrils, which results in the formation of composite fiber. Lignin plays very important role in the plant fiber such as water holding capacity, provide protection against biological attacks and strengthened the stem against wind and gravity forces. Hemicellulose found in the plant fibers is believed to be a compatibilizer between cellulose and lignin (Hansen and Bjorkman, 1998).

Lignocellulosic biomass is a starting raw material for many industrial processes. It is renewable, less expensive, non-toxic, biodegradable material and mainly used for ethanol production. Because of structural characteristics of the lignocellulosic biomass, it has been gaining continuous interest in many other important fields including developing antibacterial functional biopolymers. Lignocellulosics biomass refers to the organic matter produced by trees, shrubs, and agricultural crops and is major feedstock for the pulp & paper industry, composite industry and packaging material. Carbohydrate polymers such as cellulose, hemicellulose and lignin as an aromatic polymer are the major macromolecular constituents of ligneous cell walls which are distinguished by a hierarchical fibrilar composite micro structure (Greil, 2001). Lignocellulosic biomass results from plant photosynthesis which converts solar energy to organic material with the benefits of biodegradable and renewability (Dey and Brinson, 1984). Lignin component of lignocellulosic biomass is a polymer of monolignols. The monolignols are synthesized from phenylalanine through the
general phenylpropanoid and monolignol-specific pathways. Phenylalanine is derived from the shikimate biosynthetic pathway in the plastid (Rippert et al., 2009; Vanholme et al., 2010). Renewable sources of lignocellulosic biomass are natural fibers (flax, hemp, sisal, bamboo, ramie etc.), agricultural residues (wheat straw, corn stover, sugarcane bagasse, switchgrass, salix) (U.S. Department of Energy Biomass Program, 2009) and forest products (hardwood and softwood) (Hoadley, 2000). These agricultural wastes and forest feedstocks are sufficiently abundant and generate very low net greenhouse emissions. Forest wood products contain more lignin and less ash content, which makes them attractive to cost-effective transportation in comparison to agricultural residues (Zhu and Pan, 2010).

1.2. Structure of lignocellulosic biomass

Lignocellulosic biomass (plant fiber) consists of cellulose, hemicellulose, lignin, organic extractives (mixture of different organic com-pounds) and some inorganic components, which turn into ash following combustion (Wiselogel et al., 1996). Cellulose, hemicellulose and lignin, constitute more than 75% of the lignocellulosic material, and are composed of organic polymers of high molecular weight (Abril et al., 2009). The composition of cellulose, hemicellulose and lignin can vary from one plant species to another. Lignocellulose is the primary building block of the plant cell walls and cellulose is the main constituent in plant cell walls (Kumar et al., 2009). Plant cell walls are subdivided as primary and secondary walls. The distribution of cellulose, hemicellulose and lignin varies considerably among these layers. The secondary wall (SW) is composed of SW1, SW2 and SW3 where SW2 is usually thicker than the others and contains the major portion of cellulose. The middle lamella, which binds the adjacent cells, is almost entirely composed of lignin (Pandey, 2009). The cellulose chains are packed into microfibrils through hydrogen bonds which are further attached to each other by hemicelluloses and amorphous polymers of different sugars as well as other polymers such as pectin and covered by lignin. The cellulose microfibrils which are present in the hemicellulose–lignin matrix are often associated in the form of bundles or macrofibrils (Figure 1.1) (Menon and Rao, 2012). Some parts of the microfibrils have a less ordered, noncrystalline structure referred to as amorphous region (Arantes and Saddler, 2010). Cellulose is a linear syndiotactic polymer of glucose linked together by β-(1/4)-glycosidic bonds whereas hemicellulose
is a branched heteropolymer of d-xylose, l-arabinose, d-mannose, d-glucose, d-galactose and d-glucuronic acid. In biomass, cellulose is present in both crystalline and amorphous forms. Crystalline form of cellulose comprises the major proportion of cellulose, whereas a small percentage of unorganized cellulose chains form amorphous cellulose. Amorphous form of cellulose is more susceptible to enzymatic degradation (Beguin and Aubert, 1994).

**Figure 1.1. Lignocellulosic framework.** Reprinted from (Menon and Rao, 2012), with permission from Elsevier.

Variable amorphous structure of hemicellulose comprised of heteropolymers including hexoses (d-glucose, d-galactose and d-mannose) as well as pentose (d-xylose and l-arabinose) and may contain sugar acids (uronic acids) namely, d-glucuronic, d-galacturonic and methylgalacturonic acids (McMillan, 1993; Saha, 2003). Lignin is a complex, large molecular structure containing cross-linked polymers of phenolic monomers. Lignin imparts structural support, impermeability, and resistance against microbial attack to primary cell wall (Perez *et al.*, 2002). Softwoods have the highest lignin contents, whereas herbaceous plants such as grasses have the lowest contents of lignin. Lignin is composed of three phenolic monomers of phenyl propionic alcohol namely, coumaryl, coniferyl and sinapyl alcohol. Forest woody biomass is primarily composed of cellulose and lignin polymers. Softwood barks have the highest level of lignin (30–60%) followed by the hardwood barks (30–55%). Grasses and agricultural residues contain the lowest level of lignin i.e. 10–30% and 3–15%, respectively (Demirbaz, 2005; Peettersen, 1984; Limayem and Ricke, 2012).
Table 1.1. Percentage composition of cellulose, hemicellulose and lignin in some agricultural residues and fibers. Reprinted from (Bledzki et al., 1996), with permission from John Wiley & Sons and from (Juntaro et al., 2008).

<table>
<thead>
<tr>
<th>Lignocellulosic material</th>
<th>Cellulose (%)</th>
<th>Hemicellulose (%)</th>
<th>Lignin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardwood stems</td>
<td>40-55</td>
<td>24-40</td>
<td>18-25</td>
</tr>
<tr>
<td>Softwood stems</td>
<td>45-50</td>
<td>25-35</td>
<td>25-35</td>
</tr>
<tr>
<td>Nut shells</td>
<td>25-30</td>
<td>25-30</td>
<td>30-40</td>
</tr>
<tr>
<td>Corn cobs</td>
<td>45</td>
<td>35</td>
<td>15</td>
</tr>
<tr>
<td>Grases</td>
<td>25-49</td>
<td>35-50</td>
<td>10-30</td>
</tr>
<tr>
<td>Paper</td>
<td>85-99</td>
<td>0</td>
<td>0-15</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>30</td>
<td>50</td>
<td>15</td>
</tr>
<tr>
<td>Sorted refuse</td>
<td>60</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>leaves</td>
<td>15-20</td>
<td>80-85</td>
<td>0</td>
</tr>
<tr>
<td>Cotton seed hair</td>
<td>80-95</td>
<td>5-20</td>
<td>0</td>
</tr>
<tr>
<td>News paper</td>
<td>40-55</td>
<td>25-40</td>
<td>18-30</td>
</tr>
<tr>
<td>Waste papers from chemical pulps</td>
<td>60-70</td>
<td>10-20</td>
<td>5-10</td>
</tr>
<tr>
<td>Primary waste water solids</td>
<td>8-15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solid cattle manure</td>
<td>1.6-4.7</td>
<td>1.4-3.3</td>
<td>2.7-5.7</td>
</tr>
<tr>
<td>Coastal Bermuda grass</td>
<td>25</td>
<td>35.7</td>
<td>6.4</td>
</tr>
<tr>
<td>Switch grass</td>
<td>45</td>
<td>31.4</td>
<td>12</td>
</tr>
<tr>
<td>Swine waste</td>
<td>6.0</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Hemp</td>
<td>74.4</td>
<td>17.9</td>
<td>3.7</td>
</tr>
<tr>
<td>Jute</td>
<td>61.0</td>
<td>20.4</td>
<td>13</td>
</tr>
<tr>
<td>Flax</td>
<td>71</td>
<td>18.6</td>
<td>2.2</td>
</tr>
<tr>
<td>Ramie</td>
<td>68.6</td>
<td>13.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Sisal</td>
<td>78</td>
<td>10</td>
<td>8</td>
</tr>
</tbody>
</table>
Lignin provides mechanical strength to lignocellulosic biomass by binding fibers together and involved in water transport in plants and forms a barrier against microbial destruction by protecting the readily hydrolysable polysaccharides (Hofrichter, 2002). Percentage composition of cellulose, hemicellulose and lignin in common agricultural residues, wastes and fibers is depicted in Table 1.1 (Jorgensen et al., 2007; Bledzki et al., 1996), respectively. There are various factors which affect the properties of plant fibers such as variety, maturity, climate, harvest, decortications, disintegration (mechanical, steam explosion treatment), fiber modification, retting degree, textile and technical processes, i.e. spinning and carding (Van de Valde and Kiekens, 2001). Factors such as size, maturity and the way by which the extraction of fiber was carried out, affect the quality of fibers. Internal structure and chemical composition of fibers are responsible for the density, electrical resistivity, tensile strength and initial modulus (Gassan and Bledzki, 1996). Excellent tensile strength and modulus, high durability, low bulk density, good moldability and recyclability are the most required properties for fibers (Kalia et al., 2009; Li et al., 2007).

1.3. Green modifying agents for surface modification of natural fibers

Green modifying agents play important role in the surface modification of natural fibers by green methods such as plasma, bacterial cellulose, bacterial cellulase enzyme, fungi and laccase enzyme.

1.3.1. Plasma

In 1879, Sir William Crooks suggested the concept of plasma as the ‘fourth state of matter’. Irving Langmuir, American chemist first used the term ‘plasma’ in 1928. Plasma contains the mixture of reactive species like free radicals, electrons and heavy particles, which makes it a unique and diverse media for surface modification of plant fibers. Plasma is defined as a gaseous environment composed of charged and neutral species with an overall zero charge density. It offers numerous advantages over the conventional chemical processes, as it is a clean and dry process (Morent et al., 2008).
Surface chemistry and topography can be changed by plasma treatment and this treatment does not affect the bulk properties of the polymers (Poll et al., 2001). Change in the surface chemistry occurs when the excited and energetic ions, radicals, electrons and metastables are bombarded onto the textile or polymer surface. Plasma can bring out two types of interactions with surface: (1) chain scission on the surface which results in surface etching, cleaning or activation, which is obtained by using non-polymerizing gases like helium, oxygen, air and nitrogen and; (2) plasma induced polymerization or grafting, which is carried out by using various polymerizing gases and precursors like fluorocarbons, hydrocarbons and silicon containing monomers (Figure 1.2). Due to the various potentials and unique properties of plasma, it has been successfully used in different areas of electronics, tool making industries, automotive, general plastics and films industries (Kale and Desai, 2011). Low pressure, low temperature, atmospheric glow discharge (AGD) (Kim et al., 2008) and atmospheric pressure plasma jet (APPJ) (Wolter et al., 2009) treatments are mostly used to modify the surface of plant fibers rather than using the chemical treatments. High voltage radio frequency (RF) excitation at KHz frequency ranges is the main plasma sources to develop the AGD. The AGD plasma polymerization can be used to modify the surface properties of wood powder as well as plant fiber (Kim et al., 2008). The industrial uses of low pressure and atmospheric pressure plasmas have increased for industries for the pretreatment purposes. Atmospheric pressure plasma jets (APPJ) are widely used because they are easy to integrate into existing production lines and they can selectively treat specific parts of a substrate (Tendero et al., 2006). In comparison to the corona treatments and dielectric barrier discharges, APPJs are not only limited to the two-dimensional structures but can also be used for the three-dimensional structures (Wagner et al., 2003). Applications of APPJs extend towards the treatment of the temperature sensitive surfaces such as biological materials, for example, plasma-living cell, tissues and bacterial interactions and these interactions play important role in cultivation and treatment of diseases (Wolter et al., 2009).
Figure 1.2. (a) Etching/cleaning/ablation with plasma and (b) grafting/polymerization with plasma. Reprinted from (Kale and Desai, 2011), with permission from NISCAIR.

1.3.2. Bacterial cellulose

Cellulose is the fundamental unit of the most plant substances. Some bacterial genera like Acetobacter, Sarcina ventriculi and Agrobacterium can also produce protective covering of cellulose around the cell. Bacterial or microbial cellulose is different from the plant cellulose in various respective ways such as their high degree of purity, better water holding capacity, mold ability and greater strength (Jonas and Farah, 1998). The basic formula of bacterial cellulose is \((C_6H_{10}O_5)_n\) (Jonas and Farah, 1998) and its basic structure is shown in Figure 1.3 (Lee et al., 2011).

Figure 1.3. Structure of cellulose. Reprinted from (Lee et al., 2011), with permission from Springer.

In 1886, A.J. Brown had discovered the bacterial cellulose as extracellular gelatinous material from the Acetobacter xylinum (Brown, 1886). Cellulose synthesis has two main steps, i.e. synthesis of uridine diphosphoglucose (UDPGlc) and polymerization
of the glucose. Production starts with the carbon compounds (hexoses, glycerol, pyruvate, etc.), which enter the kreb cycle, gluconeogenesis or pentosphosphate cycle depending upon what carbon source is available. After this phosphorylation takes place, it is followed by the isomerization of intermediates known as UDPGIc pyropho-sphorylation to convert compounds into UDPGIc, which is a precursor to the production of cellulose. Afterwards, polymeriza-tion of glucose starts, resulting in the formation of cellulose (Delmer and Amor, 1995). Majority of bacteria synthesize extra cellular polysaccharides such as the homopolymeric cellulose. Cellulose production is well known by Gram-negative bacteria species such as Acetobacter, Azotobacter, Rhizobium, Pseudomonas, Salmonella, Alcaligenes and Gram-positive species such as S. ventriculi (Shoda and Sugano, 2005). At the industrial level, the use of microbial cellulose has increased owing to products with high tensile strength, higher degree of polymerization and high crystallinity index (Panesar et al., 2009). Of the previously mentioned species, much attention has been given to A. xylinum due to the unique mechanical properties of cellulose that it produces which may have potential applications in biotechnology, microbiology and materials science. A. xylinum can produce high amounts of cellulose using different carbon and nitrogen sources. This ability makes the A. xylinum a good substrate for basic and applied research for cellulose (Bielecki et al., 2005).

1.3.3. Bacterial cellulase

Cellulase refers to a class of enzymes that catalyze cellulolysis, i.e. the hydrolysis of cellulose. Cellulase generally hydrolyzes the β-(1,4)-linkage in cellulose. Cellulase consists of three different enzymes that act synergistically in hydrolyses of cellulose. Endoglucanase (EG) randomly hydrolyzes the β-(1,4)-linkages within the water-insoluble cellulose chain, cellulbiohydrolase (CBH) hydrolyzes the linkages at the reducing ends of cellulose chain to form cellobiose and cellobiase and β-glucosidase converts the water soluble cellobiose into two glucose residues (Almeida and Cavaco-Paulo, 1993). Srisodsuk et al. (1998) has explained the mode of action of cellulase onto cellulose. Extracellular cellulases can degrade both crystalline cellulose and soluble cellulose derivatives; others can degrade only the latter. Properties of fabric and fiber were improved without any damage due to the slow enzymatic degradation of crystalline cellulose. As a result of the enzymatic hydrolysis, the surface and pore
structure of cellulose fibers are expected to change. Cellulase is being used in many applications such as domestic fabric to clean fiber surface to improve appearance and color brightness, bioconversion of cellulose to glucose and in bioprocessing of plant fibers (Cavaco-Paulo, 1998). Removal of the ink from the papers and enhancement in the properties of the recycled fibers are some of the effects of cellulase. Use of the enzyme for deinking is completely an environment friendly method in comparison to chemical methods (Esteghlalian et al., 2002).

1.3.4. Fungi

Fungus belongs to the eukaryotic family; a large group of this family involves the microorganisms such as yeast, mold and mushrooms. Fungi can be classified into four categories: basidiomycetes, ascomycetes, zygomycetes and deuteromycetes. Baldrian and Valaskova (2008) have reported the degradation of cellulose by basidiomycetous fungi. Basidiomycetes utilize a set of hydrolytic enzymes typically composed of endoglucanase, cellobiohydrolase and β-glucosidase for the degradation of cellulose. The white rot fungi from the basidiomycetes are the only fungi known to degrade lignin by producing extracellular oxidases to expose the cellulose and hemicelluloses for metabolizing (Pickering et al., 2007). Apart from degrading lignin, the white rot fungus can also degrade the hydrophobic constituents of plant fibers such as triglycerides and fatty acids (Gutierrez et al., 2001) and other microbial degradation-resistant molecules such as sitosterol, sitosterol esters and resin acid (Leone and Breuil, 1998).

1.3.5. Enzymes

One of the environmentally friendly alternatives to chemical methods is the modification of polymers with enzymes. There are some enzymes which are most commonly used for polymer modification such as from the class hydrolases: glycosidases, proteases and lipases and from the class oxidoreductases: tyrosinase, laccase and peroxidase (Gulitz and Paulo, 2003). The use of oxidative enzymes for cross-linking and functionalization of lignaceous compounds has been reported in 2003 (Gronqvist et al., 2003). Modification of the polymers by the enzyme is safer and more advantageous as compared to the chemical methods because of high
reaction specificity of enzymes, milder reaction conditions and non-destructive transformations on the surface of polymer (Pallesen, 1996). Enzyme retting is a promising technique for improving the quality of fibers but so far this has not replaced commercial retting methods. There are some factors which have limited the use of enzymes on large scale such as high cost associated with enzymes and equipment, wastewater treatment systems and the lack of industry support (Lee et al., 2011; Akin, 2013; Akin et al., 2002). Chelator enhances efficiency of enzymatic-retting, by withdrawing the calcium from the solution. Enzymes and chelators were used to modify the surface of flax fiber by removing pectin and calcium, resulting to enhanced interfacial adhesion between the fiber and matrix (Adamsen et al., 2002). The action of the enzyme on the fiber cell wall has been shown in Figure 1.4 (Wheeler, 2009). Enzyme modification results in the degradation of cellulose in the fiber wall structure which then initiates wall stripping, causes the generation of fine fibrils and leaves the fibers less hydrophilic.

![Figure 1.4. Action of enzyme on plant cell. Reprinted from (Wheeler, 2009), Open access.](image)

1.3.5.1. Laccase enzyme

Enzyme-assisted modification of lignocellulosic fibers, is one of the green method used by researchers all over the world in order to develop improved raw material for composite materials and other industries (Kalia and Vashistha, 2012; Kalia and Sheoran, 2011; Kalia and Vashistha, 2011; Kalia and Sheoran, 2013; Kalia et al., 2013). Enzymatic processes are the best environment friendly tools for the development of lignocellulosics-based functional polymers with wide range of applications such as textile fibers, composite boards, and packaging material with
novel properties (antimicrobials properties, hydrophobic properties, attractive shed colors, etc.) (Nyanhongo et al., 2011; Chandra and Ragauskas, 2002; Chandra and Ragauskas, 2002a; Chandra et al., 2004; Schroder et al., 2007). Enzymes such as laccases and peroxidases can oxidize a wide variety of natural and synthetic molecules and thus generate reactive species such as phenoxy radicals. These versatile biocatalysts can enhance the reactivity of molecules/polymers, which forms the basis of their application in polymer chemistry (Nyanhongo et al., 2010). Biografting of polymers and other molecules makes lignocellulosics more hydrophobic and hence more compatible with polymer matrices for use in composite materials (Fackler et al., 2008). Laccase, peroxidase and lipases are the most important enzymes used in biografting to copolymerize lignocellulosics with other organic molecules and polymers. Laccase activation of lignin-containing cellulosic fibers and biografting of antibacterial and hydrophobic molecules has received much scientific attention to improve antibacterial, hydrophobic, mechanical and other properties (Kalia et al., 2013; Gronqvist et al., 2003; Wong et al., 2000; Chandra et al., 2004; Buchert et al., 2005; Elegir et al., 2007).

Laccase is oxidoreductase, an “eco-friendly” enzyme as it works in the presence of air and produce water as by-product. Laccase is a member of blue copper proteins or blue copper oxidases, which catalyze the oxidation of various aromatic compounds, especially phenols by concomitant reduction of oxygen to water. The molecular size of laccase, i.e. 60–100 kDa corresponding to 70 Å × 50 Å × 45 Å, limits the extent of oxidation in pulp applications to the surface of pulp material (Gianfreda et al., 1999; Xu, 1999; Ducros et al., 1998; Paice et al., 1995). Three major classes of blue copper-containing and related proteins are there; small blue proteins (plastocyanin, azurin, pseudoazurin, amicyanin, and phytocyanin), the blue oxidases (ascorbate oxidase, laccase, ceruloplasmin, and cytochrome c oxidase), and the coagulation factors (factor V and factor VIII). The blue oxidases have the ability to transfer four electrons from a reducing substrate to a molecule of oxygen (which is thereby reduced to water) and are the only enzymes known to catalyze this four-electron transfer reaction. Laccase and ascorbate oxidase contain peptide chains of 540–570 amino acid residues, while ceruloplasmin has a single peptide chain that is 1046 amino acid residues long (Messerschmidt and Huber, 2005). Laccase generally requires four copper atoms to carry out the catalytic activity. There are three types of four copper atoms. Type 1 (T1) is a paramagnetic blue copper with a typical maximum
absorbance at 610 nm (ox.) and which is responsible for providing blue color. Type 2 (T2) is a paramagnetic nonblue copper, while Type 3 (T3) coppers display a maximum absorbance at 330 nm and form a diamagnetic spin-coupled copper–copper pair (ox.) (Strong and Claus, 2011). Substrates release electrons which are transferred sequentially to the T1 copper and T2/T3 center present in the active center of laccase and then to molecular oxygen, resulting in its reduction to water (Solomon et al., 1996; Xu, 1999; Solomon et al., 2008).

Laccase activity has been found in plants, some insects (Kalia and Vashistha, 2011; Kramer et al., 2001), and few bacteria (Claus, 2003). Biotechnologically, laccases of fungi origin are the most useful (i.e. those with high redox potentials). Over 60 fungal strains belonging to Ascomycetes, Deuteromycetes and especially Basidiomycetes show laccase activities. Among bacidiomycetes, white-rot fungi are the highest producers of laccases but also litter-decomposing and ectomycorrhizal fungi secret lac-cases (Baldrian, 2006). The highest redox potential of a laccase reported so far does not exceed 800 mV, which is believed not to be high enough to oxidize a non-phenolic lignin structure. Laccase can oxidize only phenolic groups of lignin to phenoxy radicals due to the random polymer nature of lignin and to the laccase lower redox potential (Li et al., 1999; d’Acunzo et al., 2002). These phenoxy radicals can result in lignin depolymerization or polymerization (Figure 1.5) (Kalia et al., 2014).

Figure 1.5. Laccase-assisted grafting of functional molecule on lignocellulosics. Reprinted from (Kalia et al., 2014), with permission from Elsevier.
Laccase is more readily available and easier to manipulate in comparison to other enzymes, and its substrate specificity is low, as long as a good match of oxidation potentials is provided (Xu, 1996; Itoh et al., 2000; Guillen et al., 2000; Kersten et al., 1990). Laccase-assisted oxidation of non-phenolic lignin units can follow an electron transfer, a radical hydrogen atom transfer or an anionic mechanism, depending upon the particular mediator (Barreca et al., 2004). The characteristics of an alkali-stable enzyme with fungal laccase activity were studied and the reactive capability of fungal laccase on alkali lignin was also investigated. Fungal laccase was isolated from Mycelia Sterilia YY-5, an entophytic fungus. The modified reaction of alkali lignin results indicated that fungal laccase could oxidize the free radical graft at a free position of the aromatic ring and phenolic hydroxyl was the most important site for lignin copolymerization (Weihua and Hongzhang, 2008). Applications of laccases within different industrial fields include the detoxification of industrial effluents, mostly from the paper and pulp, textile and petrochemical industries, use as a tool for medical diagnostics and as a bioremediation agent to clean up herbicides, pesticides and certain explosives in soil. Laccases are also used as cleaning agents for certain water purification systems, as catalysts for the manufacture of anti-cancer drugs and even as ingredients in cosmetics (Couto and Herrera, 2006). Applications of laccases in pulp and paper industry include pulp delignification, pitch removal and deinking etc. Lignin acts as a barrier to the penetration of enzymes into the lignocellulosic structure and is responsible for the color of pulp and it is a principal component of wastewater. Laccase treatment not only removes lignin, but also increases the brightness and significantly affects the color remediation and toxicity of chlorinated lignin degradation products (Virk et al., 2012).

Research work is being carried out on degradation of lignin by laccase, laccase-assisted biografting, coupling and effect of these processes on the properties of the lignocellulosic materials (Riva, 2006). Enzymatic activation of lignocellulosic surfaces is of great interest due to its specificity. As a result of activation, radicals are generated in the lignocellulosic surfaces and these radicals can possibly be exploited in functionalization of lignocellulosics by various means (Gronqvist et al., 2003). Laccases and peroxidases are main enzymes studied for the surface functionalization of lignocellulosic biomass. However, laccases are usually preferred as peroxidases.
require hydrogen per-oxide as cofactor which makes the process more expensive (Kudanga et al., 2009). In laccase-catalyzed oxidation of wood fibers, phenoxy radicals are formed in the lignin matrix (Widsten et al., 2002; Felby et al., 1997). The oxidation is thought to be due to direct oxidation of surface lignin or alternatively mediated by dissolved and colloidal material (Felby et al., 1997; Hassingboe et al., 1998). It has been suggested that presence of water-soluble extractives (i.e. low molecular weight compounds adsorbed to the dry fibers before a treatment) is necessary for radical formation in lignin. Some phenolic extractives may play the role of mediators and extend the laccase catalytic range to the fiber surfaces where phenoxy radicals may be created (Hassingboe et al., 1998; Barsberg and Thygesen, 1999). In 2006, Hernandez et al. (2006) indicated that new functional groups were introduced into lignin when incubated with partially purified laccase. The effects of laccase-natural mediator systems (LMS) on sisal pulp and their potential for either for biobleaching or functionalizing its fibers were investigated. Four different plant phenols (sinapic acid, ferulic acid, coniferyl aldehyde and sinapyl aldehyde) were used as laccase redox mediators. It was resulted that selected natural mediators proved ineffective for bleaching of sisal pulp; in fact, they exhibited a tendency to couple onto fibers, which can be useful with a view to functionalizing lignocellulosic fibers by laccase-aided biografting (Aracri et al., 2009). According to international norms and standards, food packaging requires lot of attention in terms of safety and quality. Biografting of antibacterial molecules onto lignocellulosic biomass can provide new food packaging materials with improved mechanical and antimicrobial properties. The role of laccase in lignin modification is being explored and many processes based on laccase and its mediators have been developed.

1.4. Surface modification of plant fibers

The surfaces of plant fibers can be modified by conventional/ chemical treatments and environment friendly methods for better binding between fibers and matrix for a wide range of applications. Chemical treatments include mercerization, acetylation, peroxide, benzylation, coupling agents and polymer grafting. Environment friendly methods include treating fibers with plasma, enzyme, fungi and coating with nanocellulose.
1.4.1. Conventional methods

Mercerization, i.e. alkali treatment is one of the common methods used to get the fibers with better qualities (Ray et al., 2001). Mercerization results in the breaking of bundles of composites fibers into the microfibrils. Mercerization results in the decreased fiber diameter and roughness on the fiber surface, which further results in better fiber–matrix interfacial adhesion with improved mechanical properties (Joseph et al., 2000). As mercerization causes the removal of the lignin and hemicellulose from the plant fibers, it affects the chemical composition of plant fibers, molecular orientation of the cellulose crystallites and degree of polymerization (Kim and Netravali, 2010). Acetylation involves the reaction of cell wall hydroxyl groups of lignocellulosic materials with acetic or propionic anhydride at elevated temperatures, which increase the hydrophobicity of the plant fibers (Hill et al., 1998). Hydroxyl groups of the lignin and hemicelluloses (amorphous material) react with the reagents whereas the hydrogen bonding on the closely packed hydroxyl groups of crystalline cellulose prevent the diffusion of reagent and thus, result in very low rates of reaction (Rowell, 1998). Peroxide treatment is a very simple method and results in the increased mechanical properties. Peroxide free radical (RO) reacts with the hydrogen groups of matrix and fiber cellulose (Sreekala et al., 2000). Benzoyl chloride is most commonly used in benzylation of the plant fibers. The inclusion of benzoyl (C₆H₅COO) group is responsible for the decreased hydrophilic nature of the treated plant fibers (Kalia et al., 2009; Joseph et al., 2000). Improvement in the degree of crosslinking in the interface region and strong bonding has been achieved by the use of coupling agents. Silanes were reported as effective coupling agents for modifying the fiber-matrix interface. Silane treatment showed best results for mercerized fibers as alkaline treated fibers having more reaction sites in comparison to untreated fibers. Therefore, fibers were pretreated with the NaOH for half an hour before the silane treatment. Fibers were then thoroughly washed with distilled water and dried afterwards. The number of cellulose hydroxyl groups in fiber-matrix interface has been reduced by the silane treatment. Silanols formation takes place in the presence of moisture by hydrolizable alkoxy groups. Hydroxyl group of fibers then reacts with the silanols to form stable covalent bond and get chemisorbed on to the fiber surface. Therefore, silane treatment restrains the swelling of fiber by providing the hydrocarbon chains to form cross-linking network between the fiber and matrix
Among the various chemical treatments, graft copolymerization of synthetic polymers onto plant fibers is the best method for surface modification (Kalia et al., 2009). Grafting of synthetic polymers onto plant fibers incorporate targeted properties in backbones for specialized applications without affecting their biodegradability (Bhattacharya and Misra, 2004). Graft copolymers can be prepared by various chemical methods. The most common method is to generate an active site on the previously existing polymeric backbone. In an ionic polymerization or condensation process, the active site may be either a free radical or a chemical group. Polymerization of an appropriate monomer onto this activated backbone polymer leads to the formation of a graft copolymer. C2, C3, and C6 hydroxyls and C–H groups are the active cites for grafting in cellulosics (Kalia et al., 2009; 2011).

### 1.4.2. Environment friendly methods

Green methods such as coating with bacterial nanocellulose, fungal, enzymatic and plasma treatments are better methods for the surface modification of plant fibers. Coating of bacterial nanocellulose onto plant fibers increases the hydrophobicity as bacterial cellulose forms the hydrogen bonding with the hydroxyl groups present on the surface of the plant fibers. Fungal treatment causes the removal of noncellulosic material from the plant fibers and exposes the cellulose and hemicellulosic material. Cellulose present in the plant fibers can be hydrolysed by the bacterial cellulase. Plasma treatment results into the surface etching and cleaning or activation of the plant fibers. All these methods can bring about changes in the morphology, thermal behavior, crystallinity and mechanical properties of the plant fibers without using any harmful chemicals. Modified fibers can be used to prepare composites with green image, as materials for the textile industries as well as promising antimicrobial materials. Biografting of phenolic and other organic molecules on lignocellulosic biomass is an environmentally friendly and best approach to incorporate desired functionalities for successful industrial applications. Functionalized antibacterial and hydrophobic lignocellulosic biomass can be potentially used for packaging material, composite materials and textile & paper industries. Lack of availability of suitable antibacterial molecules in large amounts and costeffectiveness are the major problems for the commercialization of this method. In order to generate better raw material for
the industrial applications, it’s very necessary to understand this mechanism and we have to develop new functional molecules and new methods for biografting on other major constituents of ligno-cellulosic biomass.

In present work, our approach is towards the use of green or biological methods to modify the surface of natural fibers. We have studied the effects of biological methods on the thermal, crystalline, moisture retention and antibacterial behaviour of the modified natural fibers and then use of these modified fibers as reinforcing material to synthesize biocomposites.

### 1.5. Problem statement

Pretreatments of natural fibers with environment benevolent methods for the development of polymer composites for advance structural applications.

### 1.6. Objectives

This whole project consists of the following objectives:

1. To modify the surface of ramie fibers using bacterial cellulase.
2. To modify the surface of ramie fibers using fungal cellulase.
3. To modify the surface of coconut fibers by laccase-catalyzed biografting of phenolic compounds.
4. To characterize the original and modified natural fibers by FTIR, SEM, TGA, XRD techniques in order to know their functionality, surface morphology, thermal stability and crystallinity.
5. To study the thermal behavior and moisture absorption properties of modified fibers
6. To study the antibacterial behavior of biographed coconut fibers
7. Synthesis of biocomposites using original and modified natural fibers.
8. To study the mechanical properties of synthesized biocomposites such as tensile strength and flexural strength.
9. To study the morphology of fractured surface of synthesized biocomposites using SEM technique.