CHAPTER 2

Characterization of Mechanically Destructured and Non-Destructured Bamboo (*Dendrocalamus strictus*)

2.1 Introduction

Worldwide cellulose pulp production is from hardwood, softwood and agro residues. Hardwood and softwood pulping accounts to ~95% of the total worldwide pulp production. The rest 5% pulp comes from non-wood raw materials, mainly agro residues and grasses (Jimenez et al., 2005). India and China are the major non-wood pulp producers and meet out 45-50% of their pulp requirement from these raw materials.

In India pulp and paper industry uses a variety of raw materials from wood and non-wood sources (Wanrosli et al., 2007; Mendes et al., 2009). Hardwoods such as eucalyptus, popular and casuarina are the major woody raw materials and about 50% share of the pulp comes from these raw materials. Bamboo is also one of the major raw materials in India and about 2 lakhs tonnes of pulp is made by using 100% bamboo (Kamthai, 2007). A large quantity of bamboo is also used by wood based mills in quantities varying from 5-20% in their fiber furnish. Agricultural residues such as rice straw, wheat straw, bagasse and sabi grasses etc. are other non-wood raw materials used by Indian pulp and paper industry and they constitute about 50% of the virgin pulp produced in the country.

Bamboo is one of the forest based raw materials which was found suitable for pulping way back in early 1913 by Forest Research Institute, Dehradun as a substitute for imported pulp wood during the early days of Indian pulp and paper industry (Singh, 1991). It was one of the major raw materials till 1980’s, however it’s share gradually decreased 1990 onwards.

Though bamboo is one of the oldest raw materials used by the paper industry, a little work has been carried out on its biopulping to improve the yield as well as fiber characteristics. Bamboo has dense surface on which fungal hyphae penetration is difficult and it does not allow the growth of fungi under normal conditions. In the present chapter
mechanical destructuring of bamboo has been tried to open the compact chips structure thereby converting them to spongy material with increased surface area for effective fungal growth.

2.1.1 Bamboo - An Important Fiber Resource for Pulp and Paper Industry

Bamboo is a perennial, giant, woody grass belonging to the group angiosperms (Chapman, 1996) and the order monocotyledon (Latif et al., 1990). The grass family Poaceae (or Gramineae) can be divided into one small subfamily, Centothecoideae, and five large subfamilies, Arundinoideae, Pooideae, Chloridodeae, Panicoideae, and Bambusoideae. In distinction to its name, bamboos are classified under the subfamily Bambusoideae (Chapman, 1996 and 1997). There are about more than 70 genera and over all 1200 species (Gyansah and Kwofie, 2011) of bamboo worldwide. About half of these species grow in Asia, most of them within the Indo-Burmese region, which is also considered to be their area of origin (Grosser and Liese, 1971). Most of the bamboos need a warm climate, abundant moisture, and productive soil, though some do grow in reasonably cold weather (below –20°C) (Wang and Shen, 1987).

According to Grosser and Liese (1971) bamboo grows particularly well in the tropics and subtropics, but some taxa (taxonomic category or group) also thrive in the temperate climate of Japan, China, Chile and the USA. Lee et al. (1994) stated that the smaller bamboo species are mostly found in high elevations or temperate latitudes, and the larger ones are abundant in the tropic and sub-tropic areas. Bamboos are also adaptable to various types of habitat. They grow in plains, hilly and high altitude mountainous regions, and in most kinds of soils, except alkaline soils, desert, and marsh. Latif and Razak (1991) mentioned that bamboo could grow from sea level to as high as 3000 meter. Bamboo is suitable on well drained sandy to clay loom or from underlying rocks with pH of 5.0 to 6.5.

Wong (1995); McClure (1966); Dransfield (1992) represented the general structure of bamboo. Bamboo is divided into 2 major portions, the rhizomes and the culms. The rhizome is the underground part of the stem and is mostly sympodial or, to a much lesser degree, monopodial. This dissertation is concerned with the upper ground portion of the stem, called the culm. It is the portion of the bamboo tree that contains
most of the woody material. Most of bamboo culms are cylindrical and hollow, with diameters ranging from 0.25 inch to 12 inches, and height ranging from 1 feet to 120 feet \cite{Lee_1994}. It is without any bark and has a hard smooth outer skin due to the presence of silica \cite{Tewari_1992}. The culm is complimented by a branching system, sheath, foliage leaves, flowering, fruits and seedlings. Bamboo is distinguishable from one another by the differences of these basic features, along with the growth style of the culm, which is either strictly erect, erect with pendulous tips, ascending, arched or clambering.

Bamboo is a fast growing species and a high yield renewable resource. Bamboo growth depends on species, but generally all bamboo mature quickly. \textit{Aminuddin and Latif} \cite{Aminuddin_1991} stated that bamboo might have 40 to 50 stems in one clump, which adds 10 to 20 culms yearly. Bamboo can reach its maximum height in 4 to 6 months with a daily increment of 15 to 18 cm (5 to 7 inches). \textit{Wong} \cite{Wong_1995} stated that culms take 2 to 6 years to mature, which depends on the species. It is suggested that with a good management of the bamboo resource, the cutting cycle is normally 3 years. According to \textit{Lee et al.} \cite{Lee_1994} bamboo matures in about 3 to 5 years, which means its growth is more rapid than any other plant on the planet. Some bamboo species have been observed to surge skyward as fast as 48 inches in one-day \cite{Farrelly_1984}. The fast growth characteristic of bamboo is an important incentive for its utilization in pulp and paper industry. Due to the fact that it is abundant and cheap, bamboo should be used to its fullest extent.

The anatomical characteristics in relation to the mechanical properties of bamboo have been studied by \textit{Latif et al.} \cite{Latif_1990}. The three species, 1 to 3 year old \textit{Bambusa vulgaris}, \textit{Bambusa bluemeana} and \textit{Gigantochloa scortechinii} were used for their study. They concluded that vascular bundle size (radial/tangential ratio) and fiber length correlated positively with modulus of elasticity (MOE) and stress at proportional limit. The authors implied that the increase in the size (mature stage), and fiber length could be accompanied by an increase in strength properties. They mentioned that bamboo that possesses longer fiber might be stiffer, if it has a greater vascular bundle size. The correlation between fiber length and shear strength was negative. The fiber wall thickness correlates positively with compression strength and MOE, but negatively with modulus of rupture (MOR). There was also a correlation between lumen diameter and all of the
mechanical properties, except compression strength. The effects of anatomical characteristics on the physical and mechanical properties of *Bambusa bluemeana* were determined by Latif et al. (1991 and 1993). The studies were carried out by using nine culms of 1, 2 and 3-year-old bamboo from Malaysia. This study found that the frequency of vascular bundles does not significantly vary with age and height of the culm. They observed that the highest mean concentration of vascular bundles was at the top location of the 2-year-old culm, and the lowest mean concentration was in the middle location of the 1-year-old culm. The high density of vascular bundles at the top was due to the decrease in culm wall thickness (Grosser and Liese, 1971). The size of vascular bundles was not significantly different with height and age. There was no correlation of vascular bundles with age, but there was a significant decreased with height of the culm. They explained that the reason for the higher ratio of vascular bundle size near the basal location was due to the presence of mature tissues. The radial diameter decreases faster than the longitudinal diameter of the vascular bundles within the height of the culm. The fiber length of the species of bamboo studied did not significantly differ with age and culm height. Fiber wall thickness is not significant by age or height of the culm. They observed that there is a decrease of lumen diameter with the increase of age and height of the culm. Chew et al. (1992) analyzed the fiber of Buloh Minyak (*Bambusa Vulgaris*). The macerated fiber was stained with safranin-C and mounted on slides. They then measured 300 fibers for their length, width and lumen width using a visopan projection microscope. Their study shows that the fiber is long and slender, with a narrow lumen. The average fiber length and width was found to be 2.8 mm and 0.013mm, whilst the lumen width and cell-wall thickness was 0.003mm and 0.005mm respectively.

Latif and Tarmizi (1993) studied the anatomical properties of three Malaysian bamboo species, 1 to 3 year old *Bambusa vulgaris* (buluh minyak), *Bambusa bluemeana* (buluh duri) and *Gigantochloa scortechinii* (buluh semantan). The bamboo was cut at about 30 cm above the ground level. Each stem was marked and cut at about 4 m intervals into basal, middle and top segments. Disks were cut and used for the determination of vascular bundles distribution and fiber dimensions respectively. This study showed that the highest mean concentration of vascular bundles was observed in the top location of the 2 year old *B. bluemeana* (365 bundles/cm²), *B.vulgaris* (307
bundles/cm²) and *G. scortechini* (223 bundles/cm²). The lowest mean concentration of vascular bundles was in the middle location of the 1 year old *G. scortechini* (132 bundles/cm²), 2 year old *B. vulgaris* (215 bundles/cm²) and 1 year old *B. bluemeana* (200 bundles/cm²). The radial/tangential ratio, which was used earlier by Grosser and Liese, 1991 is the ratio of radial diameter (length of vascular bundle) to the tangential diameter (width of the vascular bundle). According to this study, age does not significantly affect the radial/tangential ratio, and the trend is to decrease with height except for *G. scortechini*. It was concluded by this study that vascular bundle size is larger at the basal and gradually decreases at the top. The fiber length between species to species was significantly different. Age does not significantly affect fiber length.

The main species used for papermaking in India is *Dendrocalamus strictus* (*Singh et al.*, 1991). This species occupies 53 percent of total bamboo area in India. This is one of the predominant species of bamboo in Uttar Pradesh, Madhya Pradesh, Orissa, and Western Ghats (*Limaye, 1952*). Widely distributed in India in semi dry and dry zone along plains and hilly tracts, usually up to an altitude of 1000 meters, bamboo are commonly cultivated throughout the plains and foot hills. *D. strictus* is widely adaptable to temperatures as low as -5°C and as high as 45°C. This species is mainly found in drier open deciduous forests in hill slopes, ravines and alluvial plains. It prefers well-drained, poor, coarse, grained and stony soils (*Farrelly, 1984*). It occurs naturally in tracts receiving as low as 750 mm of rainfall and also in extensive gregarious patches or as an understory in mixed forests and teak plantations. It generally grow in all types of soils, with good drainage characteristics, except water-logged soil such as pure clay or clay mixed with lime.

*Dendrocalamus strictus* is commonly recognized as Calcutta bamboo (*Farrelly, 1984*), but also known as male bamboo (*Tewari, 1992*), and solid bamboo (*Anon, 1992*). Local names for this species are bans, bans kaban, bans khurd, karail, mathan, mat, butu mat, salis bans, halpa, vadur, bhiru, kark, kal mungil, kiri bidaru, radhanapavedru, kauka, myinwa, Phai Zang, bambu batu and pring peting (*Anon, 1972; Farrelly, 1984; Anon, 1992*).

According to *Wong (1995)* and *Tewari (1992)* the color of standing *D. strictus* culm is dark green, lightly and ephemerally white-waxy, glabrous. They described *D.*
strictus culm to be 16 to 26 feet (5 to 8 meters) tall when small-culmed, and 30 to 50 feet (10 to 15 meters) when bigger. The authors described the diameter as 1 to 1.5 inches (2.5 to 3.5 cm) in small culm and 1.5 to 3.0 inches (3.5 to 7.5 cm) diameter in big culm. There is no specific dimension reported for the culm wall thickness. Tewari (1992) described D. strictus as being thick-walled and sometimes with solid culms. The average internode length is between 9 to 18 inches (25 to 45 cm). The average fiber length of D. strictus is 2.2 mm, diameter 0.015 mm and thus ideal for paper production, all kinds of paper are manufactured from it (Aminuddin and Latif, 1991).

The selection of bamboo species for pulp and paper industry is not only related to physical and mechanical properties but also to the chemical composition (Tomalang et al., 1980). The main constituents of bamboo culms are holocellulose (60-70%), pentosans (20-25%), hemicellulose and lignin (each amounted to about 20-30%) and minor constituents like resins, tannins, waxes and inorganic salts. The proximate chemical compositions of bamboo are similar to those of hardwoods, except for the higher alkaline extract, ash and silica contents (Ogunsile and Uwajeh, 2009). The chemical components are distributed throughout the cell wall which is composed of primary and secondary wall layers. Chemical composition varies from plant to plant, and within different parts of the same plant. Chemical composition also varies within plants from different geographic locations, ages, climate, and soil conditions (Jeffries, 1994).

Yusoff et al. (1992) studied the chemical composition of one, two, and three year old bamboo (Gigantochloa scortechinii). The results indicated that the holocellulose content did not vary much among different ages of bamboo. Alpha-cellulose, lignin, extractives, pentosan, ash and silica content increased with increasing age of bamboo. Fujji et al. (1993) investigated the chemistry of the immature culm of a moso bamboo (Phyllostachys pubescens Mazel). The results indicated that the contents of cellulose, hemicellulose and lignin in immature bamboo increased while proceeding downward of the culm. The increase of cellulose in the lower position was also accompanied by an increase in crystallinity.

2.1.2 Chemical Composition of Bamboo
The chemical composition of bamboo is similar to that of wood (Li, 2004; Li et al., 2007). The main constituents of bamboo culms are cellulose, hemi-cellulose and lignin, which amount to over 90% of the total mass. The minor constituents of bamboo are resins, tannins, waxes and inorganic salts. Compared with wood, however, bamboo has higher alkaline extractives, ash and silica contents (Malanit et al., 2009; Amu and Babajide, 2011). The chemical components are distributed throughout the cell wall which is composed of primary and secondary wall layers. Chemical composition varies from plant to plant, and within different parts of the same plant. Chemical composition also varies within plants from different geographic locations, ages, climate, and soil conditions (Jeffries, 1994). A more detailed description of these constituents is as under:

2.1.2.1 Carbohydrates

The carbohydrate portion of cell wall is composed of holocellulose and minor amounts of other sugar polymer such as pectin and starch. The carbohydrate fraction constitutes 70-75% of wood cell wall.

Holocellulose

The combination of cellulose and hemicelluloses are called holocellulose and usually accounts for 65–70% of the plant dry weight. These polymers are made up of simple sugars, mainly, D-glucose, D-mannose, D-galactose, D-xylose, L-arabinose, D-glucuronic acid and lesser amounts of other sugars such as L-hamnose and D-fucose. These polymers are rich in hydroxyl groups, which are responsible for moisture absorption through hydrogen bonding (Han and Rowell, 1996).

Cellulose

Cellulose is a linear homopolysaccharide that consists of glucose (D-glucopyranose) units linked together by β-(1-4) glycosidic bonds (β-D-glucan). Van der Waals forces and hydrogen bonding interactions between and within cellulose molecules, however, make natural cellulose structurally complex; the individual cellulose molecules are arrayed in bundles known as micro fibrils, each of which contains approximately 40 individual cellulose molecules. Within these bundles the cellulose is highly ordered and
thus appears crystalline in diffraction measurements. Several elementary fibrils with an average thickness of 3.5 nm can associate with one another to form cellulose crystallites whose dimensions depend on the origin and treatment of the sample.

In addition, there are a small percentage of non-organized cellulose chains, which form amorphous cellulose. The extent of amorphous and crystal regions alternate irregularly, as also impeding solubility of cellulose in organic solvents and degrading polymer structure by acidic hydrolysis or enzyme-mediated catalysis. This polysaccharide is widespread in nature, occurring in both primitive and highly evolved plants. Cellulose makes up about 45% of the dry weight of wood. The size of the cellulose molecule is normally given in terms of its degree of polymerization (DP), i.e., the number of anhydroglucose units present in a single chain. However, the conformational analysis of cellulose indicated that cellobiose rather than glucose is its basic structural unit (Ramos, 2003; Kirk and Cullen, 1998; Perez et al., 2002).

Cellulose consists of up to 10,000 D-glucose units. Its closest relative polysaccharide is starch, which differs from cellulose only with α-glycosidic linkages. Cellulose is thermo chemically more stable than hemicelluloses. It has a tendency to form tight hydrogen bonds with neighboring molecules, which makes fiber structure of wood even harder and thus provides mechanical support for wood cells (Harinen, 2004). Cellulose is insoluble in most solvents and has a low accessibility to aqueous acids and cell-free enzymes. Cellulose is soluble only in a few solvents, of which the most common are cupriethylenediamine (CED) and cadmiumethylenediamine (Cadoxen) (Zahedifar, 1996; Sjoström, 1993).

![Fig. 2.1: Structure of Cellulose.](image-url)
Hemicellulose

After cellulose, hemicelluloses are the second prevalent components of cell walls in bamboo fibers. Hemicelluloses are plant hetero polysaccharides whose chemical nature varies from tissue to tissue and from species to species. Hemicelluloses are generally found in association with cellulose in the secondary walls of plants, but they are also present in the primary walls. Hemicelluloses make up 25 to 30% of the weight of bamboo. These polysaccharides are formed by a wide variety of building blocks including pentoses (xylose, rhamnose and arabinose), hexoses (glucose, mannose and galactose) and uronic acids (4-O-methyl-glucuronic and galacturonic acids). Generally, they fall into four classes: (a) unbranched chains such as (1-4)-linked xylans or mannans; (b) helical chain such as (1-3)-linked xylans; (c) branched chains such as (1-4)-linked galactoglucomannans; and (d) pectic substances such as polyrhamnogalacturonans (Ramos, 2003).

The main difference with cellulose is that hemicellulose has branches with short lateral chains consisting of different sugars, sugar acids, and acetyl esters. These groups render hemicelluloses non crystalline or only poorly crystalline, so that they exist more as a gel than as oriented fibers (Kirk and Cullen, 1998). In contrast to cellulose, they are easily hydrolyzable polymers. They do not form aggregates, even when they are co-crystallized with cellulose chains (Perez et al., 2002). Hemicellulose is more soluble than cellulose and is frequently branched with DP (Degree of Polymerization) of 100 to 200. Xylan is the most common hemicellulose component of grass and wood (Kuhad et al., 1997). Xylan is composed of chains of 1-4 linked β-D-xylopyranose residues. Different plants may contain the same basic xylan structure but different arrangements with other sugar residues, especially L-arabinose. Xylans from cereals and grasses are generally characterized by the presence of L-arabinofuranose residues linked to the backbone as single-unit side-chains, usually to position 3 of xylose. Similarly, D-glucuronic acid and/or 4-O-methyl-D-glucoronic acid residues are also present in a similar proportion (Zahedifar, 1996). The hemicellulose from bamboo consist of a backbone polymer of D-xylopyranose, linked β-(1–4) with an average of every eight xylose units containing a side chain of d-glucuronic acid attached glycosidically to the 2-position of the xylose sugar (Han and Rowell, 1996).
Pentosans

Part of the hemicellulose fraction consists of pentose sugars, mainly D-xylose and L-arabinose. The polymers containing these five carbon sugars are referred to as pentosans. Identification of this fraction in a plant material has been important to indicate its potential utilization for furan-type chemicals. A rapid and simple methods for determining total pentosans is based on the conversion of pentosan into furfural by boiling them in 13.5% HCl. Furfural is collected in the distillate and determined by spectrophotometric methods. Detailed of the procedure is given in TAPPI standard T223-cm-84.

2.1.2.2 Lignin

Lignin is the most abundant natural non-carbohydrate organic compound in fibrous materials. Lignin in bamboo accounts for 25-30% of the plant dry weight. The
bulk of the lignin is in the thick secondary cell walls, but highest lignin concentrations are in the middle lamellae (intercellular regions), where the lignin cements the plant cells together, thereby providing rigidity and strength to the plant, conferring structural support, impermeability, and resistance against microbial attack and oxidative stress (Oluwdare and Asagbara, 2008). Structurally, lignin is an amorphous heteropolymer, non-water soluble and optically inactive; it consists of phenylpropane units joined together by different types of linkages. The polymer is synthesized by the generation of free radicals (Perez et al., 2002). During biosynthesis the precursors are transformed, through enzymatic dehydrogenation reactions, to phenoxy radicals, which then are polymerised by forming the final lignin structure. Plant peroxidises catalyze the one-electron oxidation of these precursors to generate phenoxy radical intermediates which diffuse away from the enzyme and couple with each other. Bonds connecting lignin precursors together comprise 60-80% of ether linkages, of which the most common type is β-O-4-bonding. Rest part of linkages has been identified as carbon-carbon (C-C) bonds and ester (C-O-C) bonds (Harinen, 2004; Cullen and Kersten, 1996).

Softwood lignin is composed mainly of guaiacyl units originating from the predominant precursor, trans-coniferyl alcohol. While hardwood lignin is composed of both guaiacyl and syringyl units derived from trans-coniferyl and trans-sinapyl alcohols, respectively. Grass lignin contains p-hydroxyphenyl units derived from trans-p-coumaryl alcohol, besides units originating from the foregoing two precursors. However, strictly speaking, almost all plants consist more or less of all three units, namely, guaiacyl, syringyl and p-hydroxyphenyl moieties (Sakakibara and Sano, 2001). Softwood lignins are made up of approximately 80% coniferyl, 14% p-coumaryl and 6% sinapyl alcohols. In contrast, hardwood lignins are composed of 56% coniferyl, 4% p-coumaryl and 40% sinapyl alcohols. Grass lignins are rich in p-coumaryl units (Zahedifar, 1996). Lignin has no optical activity, in contrast to other compounds, because the radicals formed by enzymatic dehydrogenation couple with one another at random to give the lignin polymer.

Lignin is poorly degraded under anaerobic conditions but extensively degraded by white-rot fungi in presence of oxygen. The presence of many complex carbon-to-carbon linkages between the units makes it difficult to degrade the polymer to low-molecular-
weight fragments. Determination of lignin has not been an easy task, as lignin is always associated with cell wall polysaccharides and also artifacts produced during cell wall preparation can interfere with the determination of lignin. There are several methods for lignin determination. In the Klason method hydrolysis of plant cell wall by sulphuric acid produces a residue containing lignin (Jeffries, 1994; Zahedifar, 1996; Sakakibara and Sano, 2001).

Fig. 2.4: Structure of Lignin Precursors.

2.1.2.3 Extractive

Along with cellulose, hemicelluloses and lignin solid material of bamboo includes less than 3%-5% various extractives which contribute to properties such as colour, smell, decay resistance, density and flammability. Extractives are considered as lipophilic or hydrophilic organic compounds, which can be extracted from the samples by using neutral (non-polar), organic solvents (methyl-tert-butyl ether, acetone, benzene, tetrahydrofuran) or water, respectively. Water soluble extractives are, among others, some carbohydrates and tannins. In contrast lipophilic extractives, containing carbon atoms from 10 to 30, are classified into aliphatic hydrocarbons, fatty alcohols, fatty acids, resin acids and sterols as well as fats and waxes (Han and Rowell, 1996; Harinen, 2004).
Extractives cause production and environmental problems in the pulp and paper industry. The accumulation of small amounts of extractives can result in blockages causing a shutdown of operations. These blockages are responsible for reduced levels of production, higher operating costs and an increased incidence of quality defects. The viscous lumps that accumulate on equipment and the specks in pulp and paper are referred to as pitch and they contain considerable amounts of lipophilic compounds. Lipophilic extractives are comprised mainly of fatty acids, resin acids, waxes, alcohols, terpenes, sterols, sterol esters and glycerides and the different classes of extractives have different chemical behavior during and after pulping. In neutral to acidic processing of the wood, the lipophilic extractives are difficult to remove, and resinous woods, are more of a problem in pitch control. However, in alkaline processing, such as kraft process, the total extractives content may not be as important as the composition of the extractives. During kraft pulping, the glycerol esters are completely saponified and the fatty and resin acids dissolved. However, sterols and some sterol esters and waxes, do not form soluble soaps under the alkaline conditions used in kraft pulping, and therefore, have a tendency to deposit and cause pitch problems (Gutierrez et al., 1999).

2.1.2.4 Inorganic

The inorganic content of a bamboo is usually referred to as ash content, which is an approximate measure of the mineral salts and other inorganic matter in the fiber after combustion at a temperature of 600±5°C. The inorganic content can be quite high in bamboo containing large amounts of silica (Han and Rowell, 1996). Some of the inorganic elements present in bamboo are essential for growth, whereas others are not necessarily required. Metallic elements are often absorbed into the bamboo clum through the root system and transported to all areas (Saka, 2001). The density and mechanical properties of bamboo plays an important role in its durability and service life from fungi, mold and borers attack.

The main biological challenge observed in biopulping, after reviewing the studies done by various researchers on different raw materials is that fungal hyphae could not penetrate the core of wood chips, only surface phenomenon occurred during treatment stage. In view of this there arose a need to develop some new methods for providing large
surface for more lignin removal. Thus in present work an approach has been put forward to reduce the density of wood sample thereby increasing the surface area for fungal hyphae. It is presumed that destructured samples with large surface area and low density would allow mycelia to penetrate into the core of the fiber on pre-treatment with fungal culture.

2.2 Materials and Methods

2.2.1 Raw Material Collection

For the present study bamboo (*Dendrocalamus strictus*) was taken as raw material collected from the plantation of Forest Research Institute, Dehradun.

Kingdom 
*Plantae* – Plants

Subkingdom
*Tracheobionta* – Vascular plants

Division
*Magnoliophyta* – Flowering plants

Class
*Liliopsida* – Monocotyledons

Order
*Cyperales*

Family
*Poaceae* – Grass family

Genus
*Dendrocalamus* Nees

Species
*Dendrocalamus strictus* (Roxb.) Nees – male bamboo

2.2.2 Sample Preparation

Bamboo samples were prepared in two different forms i.e. non destructured and destructured. Sampling was done using engineered sampling method T257 cm-02. Fig 2.5 shows the processes of non destructured and destructured samples preparation:

2.2.2.1 Non Destructured Sample (Chips)

The bamboo (*Dendrocalamus strictus*) was chipped in pilot plant chipper. The chips so obtained were dried in sunlight to the normal moisture content and packed in poly bags for further experiments (Fig 2.7a).
2.2.2.2 Destructured Sample

Destructured sample was prepared with the help of impressafiner. It is the device which is used for separating fibers from chips. This unit completely compresses the chips and squeezes out soluble material along with water. The unit was designed and locally fabricated for the capacity of handling 2-5 kg/hr at one time. For the experiments about 10 kg raw material was taken on oven dry basis. The soaking was carried out in water for overnight. The soaked chips (after draining) were dewatered in compression-cum dewatering unit at 8 r.p.m and 6000 psi so that desirable fiber efficiency could be achieved. The unit is therefore designed to make the material spongy without damaging the fiber. The spongy bamboo samples after subjecting the chips in the impressafiner were dried in sunlight to the normal moisture content and packed in poly bags for further experiments. Schematic layout of compression cum dewatering unit and the destructured samples produced are shown in Fig 2.6 and 2.7(b) respectively.

2.2.3 Chemical Analysis of the Samples

Chemical composition of the plant gives an idea of how feasible the plant is as raw material for papermaking. The fibrous constituent is the main important part of the plant. Since plant fibers consist of cell walls, the composition and amount of fibers is reflected in the properties of cell walls (Hartley, 1987; McDougall et al., 1993). Cellulose is the principal component in cell walls and fibers (Taiz and Zeiger, 1991; Philip, 1992; Cassab, 1998). The amount and composition of the cell wall compounds differ among plant species and even among plant parts and they affect the pulping properties of plant material (McDougall et al., 1993). Some of non woody fiber plants contain more pentosans (over 20%), holocellulose (over 70%) and less lignin (about 15%) as compared with hardwoods (Hunsigi, 1989). They have also higher hot water solubility, which is apparent from the easy accessibility of cooking liquors. The low lignin content in non woody plants lowers the requirement of chemicals for cooking and bleaching (Hunsigi, 1989). The exact standards that were followed for chemical analysis are presented in Table 2.1.
Table 2.1: Standards Followed for Chemical Analysis.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Standard Methods</th>
<th>TAPPI No.</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Alcohol-Benzene Solubility</td>
<td>T 204 cm– 97</td>
</tr>
<tr>
<td>2</td>
<td>Hot-water Solubility</td>
<td>T 207 cm – 99</td>
</tr>
<tr>
<td>3</td>
<td>Cold-water Solubility</td>
<td>T 207 cm – 99</td>
</tr>
<tr>
<td>4</td>
<td>N/10- NaOH Solubility</td>
<td>T 212 cm – 02</td>
</tr>
<tr>
<td>5</td>
<td>Holocellulose</td>
<td>Useful method – 249</td>
</tr>
<tr>
<td>6</td>
<td>Alpha-cellulose</td>
<td>T 203 cm – 99</td>
</tr>
<tr>
<td>7</td>
<td>Klason Lignin</td>
<td>T 222 cm – 02</td>
</tr>
<tr>
<td>8</td>
<td>Pentosan</td>
<td>T 223 cm – 01</td>
</tr>
<tr>
<td>9</td>
<td>Ash Content</td>
<td>T 211 cm – 02</td>
</tr>
</tbody>
</table>

2.2.3.1 Water Content of Bamboo Dust

2 g air dried dust of both non destructured and destructured samples were placed in pre weighted weighing bottles. The weighing bottles with the dust particles were then kept in oven at 105°C for overnight. Then the weighing bottles were removed and placed in the dessicator for cooling it to room temperature before reweighing. The following formula was used to calculate the moisture content (dry-weight) of bamboo sample.

\[
\text{Dryness %} = \frac{(W_2 - W_1) \times 100}{\text{Weight of sample}}
\]

\(W_2\) - Stands for weight of weighing bottle + sample.
\(W_1\) - Stands for weight of empty weighing bottle.

2.2.3.2 Alcohol-Benzene Solubility of Bamboo

The extraction apparatus consisted of a soxhlet extraction tube which connected with a reflux condenser on the top and joined at the bottom to a boiling flask. 2 g (O.D.) samples from non destructured and destructured bamboo were placed into filter paper extraction thimbles. The thimbles were placed in a soxhlet extraction tubes. The boiling flasks contained a 2:1 solution of benzene and distilled alcohol respectively were placed on heating mantles. The extraction was conducted for four hours at the rate of approximately six siphoning per hour. After extraction, the thimbles were removed from soxhlet tubes and dried at 105±2°C for overnight. The materials were removed from
thimbles and weighed. The following formula was used to obtain the alcohol-benzene solubility content of bamboo:

\[
\text{Alcohol-Benzene Solubility} \% = \frac{(W_2 - W_1) \times 100}{\text{O.D. weight of sample}}
\]

- \(W_2\) - Stands for O.D. weight of the sample before extraction.
- \(W_1\) - Stands for O.D. weight of the sample after extraction.

### 2.2.3.3 Hot-Water Solubility of Bamboo

2 g (O.D.) samples from non destructured and destructured bamboo were placed into 500 ml flat bottom flasks with 300 ml of distilled water. Reflux condensers were attached to the flasks and the apparatus were placed on hot plate at 35-40°C for one hour. Samples were then removed from the hot plate and filtered by vacuum suction into G-2 glass crucibles of known weight. The residues were washed with distilled water. The crucibles were oven-dried at 105±2°C for over night. Crucibles were then cooled in a desiccator and weighed until a constant weight was obtained. The following formula was used to obtain the hot-water solubility of bamboo:

\[
\text{Hot Water Solubility} \% = \frac{(W_2 - W_1) \times 100}{\text{O.D. weight of sample}}
\]

- \(W_2\) - Stands for O.D. weight of sample.
- \(W_1\) - Stands for weight of crucible with sample – Weight of empty crucible.

### 2.2.3.4 Cold-Water Solubility of Bamboo

2 g (O.D.) samples from non destructured and destructured bamboo were placed into 500 ml flat bottom flasks with 300 ml of distilled water. The flasks were kept at room temperature for 48 hours. Samples were then filtered by vacuum suction into G-2 glass crucibles of known weight. The residues were washed with distilled water. The crucibles were oven-dried at 105±2°C for over night. Crucibles were then cooled in a desiccator and weighed until a constant weight was obtained. The following formula was used to obtain the cold-water solubility of bamboo:
Cold -Water Solubility % = \frac{(W_2 - W_1) \times 100}{\text{O.D. weight of sample}}

W_2 - Stands for O.D. weight of sample.
W_1 - Stands for weight of crucible with sample – Weight of empty crucible

2.2.3.5 N/10-NaOH Solubility of Bamboo

2 g (O.D.) samples from non destructured and destructured bamboo were placed into 500 ml flat bottom flasks with 300 ml of N/10 NaOH solution. Reflux condensers were attached to the flasks and the apparatus were placed on hot plate at 35-40°C for one hour. Samples were then removed from the hot plate and filtered by vacuum suction into G-2 glass crucibles of known weight. The residues were washed with distilled water. The crucibles were oven-dried at 105±2°C for over night. Crucibles were then cooled in a dessiccat and weighed until a constant weight was obtained. The following formula was used to obtain the N/10-NaOH solubility of bamboo:

\[ \text{N/10 - NaOH Solubility \%} = \frac{(W_2 - W_1) \times 100}{\text{O.D. weight of sample}} \]

W_2 - Stands for O.D. weight of sample.
W_1 - Stands for weight of crucible with sample – Weight of empty crucible

2.2.3.6 Holocellulose of Bamboo

2.5 g (O.D.) extractive-free samples from non destructured and destructured bamboo were placed into 250 ml flasks with small watch glass covers. The samples were then treated with 80 ml of distilled water, 0.5 ml of cold glacial acetic acid, and one gram of NaClO₂. The flasks were then placed into a water bath maintained between 70°C - 80°C. Every hour for three hours 0.5 ml of cold glacial acetic acid and 1 g of NaClO₂ were added and the contents of the flasks were stirred constantly. At the end of three hours, the flasks were cooled until the temperature of the flasks was reduced to 25°C. The contents of the flasks were filtered into G-2 glass crucibles of known weight followed by recycling. The residues were washed with acetone. The crucibles were then oven-dried at
105±2°C, then cooled in a dessicator, and weighed until a constant weight was reached. The following formula was used to determine the holocellulose content in bamboo

\[
\text{Holocellulose} \% = \frac{(W_2 - W_1) \times 100}{\text{O.D. weight of sample}}
\]

\(W_2\) - Stands for weight of crucible + sample. \\
\(W_1\) - Stands for weight of empty crucible.

### 2.2.3.7 Alpha-Cellulose of Bamboo

2 g oven-dried samples of holocellulose from non destructured and destructured bamboo were placed in 250 ml beakers with small watch glass covers. The samples were then treated with 10 ml of 17.5% NaOH and thoroughly mixed for 5 minutes. 15 ml of sodium hydroxide solution (17.5 %) was further added to the reaction mixture in three equal portions (3×5 ml) at an interval of 5 minutes with constant stirring. After the specimens were allowed to react with the solution for 30 minutes, 33 ml of distilled water was added in each flask and left for another one hour. The contents of the beakers were filtered by aid of vacuum suction into G-2 glass crucibles of known weight. The residues from each flask were washed first with 100 ml of 8.3% NaOH, then with 15 ml of 10% acetic acid and 1000 ml of hot tap water. The crucibles were oven-dried in an oven at 105±2°C, then cooled in a dessicator, and weighed until a constant weight was reached. The following formula was used to obtain α-cellulose in bamboo.

\[
\alpha - \text{Cellulose} \% \text{ (On the basis of Holocellulose)} = \frac{W_2}{W_1} \times 100
\]

\(W_2\) = Weight of the oven-dry α-cellulose residue  \\
\(W_1\) = Weight of the original oven-dry holocellulose sample.

\[
\text{Total alpha cellulose} \% = \frac{A \times B}{100}
\]

A- Alpha cellulose on the basis of holocellulose. \\
B- Percentage of holocellulose in the sample
2.2.3.8 Klason Lignin of Bamboo

1 g oven-dried extractive-free non destructured and destructured bamboo dusts were placed in 100 ml beakers. 15 ml of cold sulfuric acid (72%) was added slowly in each beaker while stirring and mixed well. The reaction proceeded for two hours with frequent stirring. When the two hours had expired, the specimens were transferred by washing it with 560 ml of distilled water into 2,000 ml flasks, diluting the concentration of the sulfuric acid to three percent. The flasks were placed on hot plates for four hours. The flasks were then removed from the hot plates and the insoluble materials were allowed to settle. The contents of the flasks were filtered by vacuum suction into G-3 glass crucibles of known weight. The residues were washed with distilled water and then oven-dried at 105±2°C. Crucibles were then cooled in a dessicator and weighed until a constant weight was obtained. The following formula was used to obtain the klason lignin content in bamboo:

\[
\text{Lignin \%} = \frac{(W_2 - W_1) \times 100}{\text{O.D. weight of sample}}
\]

\(W_2\) - Stands for weight of crucible + sample.
\(W_1\) - Stands for weight of empty crucible.

2.2.3.9 Pentosan of Bamboo

3 g (O.D.) samples from non destructured and non destructured bamboo were placed into 500 ml flat bottom flasks with 300 ml of 13.5% hydrochloric acid. Flasks were connected to pentosan apparatus and boiled the solution. Maintained the acid level in the round bottom flasks by adding 13.5% hydrochloric acid drop by drop continuously through separating funnels. 220 ml of distillates from both samples were collected and made it to 500 ml with distilled water in volumetric flasks. 1 ml from each mixture was diluted with 100 ml distilled water. The absorbances of distillates were noted at 280 nm using spectrophotometer. The following formula was used to obtain the pentosan percent in bamboo:

\[
\text{Pentosan \%} = \frac{\text{Absorbance at 280 nm} \times \text{dilution} \times 1.563 \times 0.5 \times 100}{151 \times \text{O.D. weight of sample}}
\]
2.2.3.10 Ash Content of Bamboo

Empty crucibles were ignited in the muffle at 600°C. After ignition crucibles were placed in a dessicator. When cooled to room temperature weighed the crucibles on the analytical balance. 2 g (O.D.) samples from non destructured and destructured bamboo were placed in the crucible. Crucibles with contents were placed in the muffle furnace and ignite for 2 hours. The temperature of final ignition was 600°C. Removed the crucibles with its contents to a dessicator, replaced the cover loosely, cooled and weighed accurately. The following formula was used to obtain the ash percent of bamboo:

\[
\text{Ash} \% = \frac{(W_2 - W_1) \times 100}{\text{O.D. weight of sample}}
\]

\(W_2\) - Stands for weight of crucible + sample.
\(W_1\) - Stands for weight of empty crucible

2.3 Results and Discussion

The results of proximate analysis of the non-destructured and destructured samples are presented in Table 2.2. Effect of mechanical operation on the chemical composition of bamboo samples were compared and are discussed below:

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Chemical Component (%)</th>
<th>Non Destructured</th>
<th>Destructured</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alcohol-Benzene Solubility</td>
<td>2.10±0.17</td>
<td>1.10±0.10</td>
</tr>
<tr>
<td>2</td>
<td>Hot-water Solubility</td>
<td>6.75±0.43</td>
<td>4.25±0.25</td>
</tr>
<tr>
<td>3</td>
<td>Cold-water Solubility</td>
<td>4.75±0.25</td>
<td>3.00±0.25</td>
</tr>
<tr>
<td>4</td>
<td>N/10- NaOH Solubility</td>
<td>18.00±0.50</td>
<td>16.50±0.43</td>
</tr>
<tr>
<td>5</td>
<td>Holocellulose</td>
<td>67.85±0.26</td>
<td>69.53±0.39</td>
</tr>
<tr>
<td>6</td>
<td>Alpha-cellulose</td>
<td>47.34±0.05</td>
<td>53.96±0.47</td>
</tr>
<tr>
<td>7</td>
<td>Klason Lignin</td>
<td>28.15±0.10</td>
<td>27.50±0.22</td>
</tr>
<tr>
<td>8</td>
<td>Pentosan</td>
<td>11.50±0.22</td>
<td>15.24±0.20</td>
</tr>
<tr>
<td>9</td>
<td>Ash Content</td>
<td>2.75±0.31</td>
<td>1.50±0.29</td>
</tr>
</tbody>
</table>

2.3.1 Hot and Cold Water Solubility in Bamboo
The hot-water procedure removes a part of extraneous components, such as inorganic compounds, tannins, gums, sugars, starches, and coloring matter present in bamboo. Fiber separation significantly affected solubility of these extractives in water. There was a noticeable difference between the hot and cold water solubility in both the destructured and non destructured bamboo samples. Hot water solubility was estimated to be 6.75% and 4.25% whereas cold water solubility was found 4.75% and 3.00% in non destructured and destructured samples respectively.

2.3.2 Alcohol–Benzene Extractives in Bamboo

The alcohol-benzene extractives of bamboo consist of the soluble materials, not generally considered part of bamboo substance. These are primarily the waxes, fats, resins and some gums as well as some water soluble substances. No single organic solvent is capable of removing all these substances, and different solvents remove different combinations. The mixture, ethanol-benzene, appears to provide the most complete removal of residual solvent-extractable substances in bamboo. Extraction with 1/3 ethanol and 2/3 benzene gives reproducible results.

Table 2.2 shows difference in extractive contents of non destructured and destructured samples. Mechanical operation had a significant effect on alcohol-benzene extractive contents. With the opening of compact chips extractive contents decreased from 2.10% to 1.10%. It showed a similar trend as that of hot and cold water solubilities. The epidermis of bamboo has an attractive green color due to the chlorophyll in its epidermis. After extraction with alcohol-benzene, the color of the extraction solution turned to a dark green color due to the extraction of chlorophyll (Chang et al., 1998 and 2001; Wu et al., 2002). Wax material attached to the inner layer also contributed to the higher alcohol-benzene extractive content.

2.3.3 N/10 Sodium Hydroxide Solubility in Bamboo

Hot alkali solution extracts low-molecular-weight carbohydrates consisting mainly of hemicellulose and degraded cellulose in wood. The sodium hydroxide solubility of raw materials could be due to degradation by heat, light, oxidation, etc. As the raw materials decays or degrades, the percentage of the alkali-soluble material
increases (Morgan, 1931; Procter and Chow, 1973). The solubility of sample indicates an extent of cellulose degradation during processes and has been related to strength and other properties of the further pulp of the sample (Anderson, 1937).

The results shown in Table 2.2 indicate that destructuring of the chips does not relate to cellulose degradation. According to the solubilities calculated for the two samples, there is more alkali soluble material in the compact chips than open spongy fibers on percentage basis. The value calculated was 18.00% in non destructured whereas 16.50% in destructured sample.

2.3.4 Holocellulose and Alpha-cellulose Contents in Bamboo

Holocellulose include alpha-cellulose and hemicellulose. Separation of the cellulose in pulp into alpha-, beta- and gamma-cellulose fractions is an empirical procedure, originally devised by Cross and Bevan around 1900, and has been widely used to evaluate samples for various purposes, such as aging characteristics and response to refining operations. In a modified form, the method was adopted first as a TAPPI tentative standard in 1931 by Willets. In general, the alpha-cellulose indicates undegraded, higher-molecular-weight cellulose content in pulp, the beta-cellulose indicates that of degraded cellulose, and the gamma-cellulose consists mainly of hemicellulose. Alpha-cellulose is the pulp fraction resistant to 17.5% and 8.3% sodium hydroxide solution under conditions of the test. Beta-cellulose is the soluble fraction which is reprecipitated on acidification of the solution; gamma-cellulose is that fraction remaining in the solution (Ranby, 1952; Wilson et al., 1955).

From Table 2.2 it is observed that non destructured and destructured samples had 67.85% and 69.53% content of holocellulose respectively. Thus the calculated holocellulose content is 1.68% higher in destructured sample, which seems to be a significant difference between the two sample forms. The apparent increase in percentage values of holocellulose in the destructured sample as compared with non destructured sample was due to the organic matter loss in the process of mechanical defiberization. Alpha-cellulose is the main constituent of bamboo. Analysis of alpha cellulose in the samples is presented in the Table 2.2 which shows the values as 53.96% and 47.34% for non destructured and destructured samples respectively.
2.3.5 Klason Lignin Content in Bamboo

Determination of lignin content in raw materials provides information for evaluation and application of the processes. Hardness, bleachability, and other pulp properties, such as color, are also associated with the lignin content.

The main objective of the study is based on the klason lignin content present in the raw material. The lignin content present in the compact chips before mechanical operation was found to be 28.15% which then decreased to 27.5% in destructured sample after compression through impressafiner.

2.3.6 Ash Content in Bamboo

The ash content was significantly different between the destructured and non destructured samples. Table 2.2 shows the ash content was 2.75% and 1.50% in non destructured and destructured samples respectively. It has been suggested that the higher ash content in chips is mainly due to the fact that almost all silica is located in the epidermis layers, with hardly any silica in rest of the cell walls (Satish et al., 1994). Mechanical operation results in the disruption of outer epidermal layers with a loss of silica content thereby decreasing the ash content in destructured sample.

2.4 Conclusion

Characterization of mechanically destructured and non destructured bamboo samples has been presented in order to understand the effect of sample form on delignification of bamboo by white rot fungi. The present work adds one more factor, sample form, to the growing list of variables described in the literature as having influence over the delignification process. Thus the bamboo chips when converted to open fibrous structures help in obtaining a large surface area for better delignification in the samples.

The compact chips before passing through impressafiner were submerged for 24 hrs in cold water to loosen the fibers and then it was passed under high pressure and stress. Since some of its chemical constituents were squeezed out during the conversion process, therefore the chemical compositions of two different forms of bamboo samples were determined separately. The results have shown that:
The overnight soaking of chips in water before destructuring had already resulted in removal of a part of extraneous components, such as inorganic compounds, tannins, gums, sugars, and coloring matter present in wood, therefore the cold water solubility was less in destructured sample (3.00%) than non destructured sample (4.75%). In addition hot water procedure resulted in loss of carbohydrate. Thus the observed hot water solubility of non destructured sample (6.75%) was more than destructured sample (4.25%) (James and Jeffrey, 1996; Harinen, 2004).

The mechanical operation removed most water-soluble and low molecular weight compounds that are also soluble in organic solvents (Alcohol and Benzene) in the destructured sample. Thus the alcohol- benzene solubility of destructured bamboo sample was found to be low, i.e., 1.10% in destructured samples compared to 2.10% in non-destructured sample respectively. The solvent extractable materials in saw dust of the sample were primarily resins and fatty acids, their esters, waxes, and unsaponifiable substances only.

It can be concluded from the results of 0.1N NaOH solubilities that 16.5% in destructured and 18.0% solubility in non destructured samples is due to compactness of chips, as non-destructured sample has more percentage of degraded cellulose and hemicellulose than open destructured samples. Thus the amount of undegraded cellulose and hemicellulose percent was expected to be more in destructured sample than compact chips.

The beta cellulose and hemicellulose are held together by some loosely bonded lignin polymers as lignin carbohydrate complexes (Morrison, 1974). These loosely bound lignin and carbohydrates can be removed easily by mechanical operation during destructed material preparation. Therefore the non destructured bamboo sample is found to have higher percentage of lignin than destructured sample.

The percentage of holocellulose content in the destructured sample (69.53%) was significantly more than non destructured (67.85%) sample. The apparent increase in percentage values of holocellulose in the destructured sample as compared to
non destructured sample was due to loss of organic matter during mechanical defiberization.

- The analysis showed that the ash content was 2.75% and 1.50% in non destructured and destructured samples respectively. Mechanical operation results in the disruption of outer epidermal layers with a loss of silica content thereby decreasing the ash content in destructured sample.
Fig. 2.5: Processes of Non Destructured and Destructured Samples Preparation.
Fig. 2.6: Schematic Layout of Compression Cum Dewatering Unit (Impressafiner).

Fig. 2.7: Bamboo Samples (a) Non Destructured, (b) Destructured.
2.5 References


