CHAPTER II

LITERATURE REVIEW

This chapter presents general review of literature on the following subjects: Literature review on wood, chemical composition of wood, lignin removal methods, enzymatic system, xylanase and laccase.

2.1 Literature Review on Wood

Seed baring plants or Spermatophytae are divided into two groups: gymnosperms, which are known as softwoods or conifers, and angiosperms which are subdivided into monocotyledons and dicotyledons, the latter of which are known as hardwoods or deciduous trees (ณรงค์ โทณานนท์, 2544; Baker et al., 1974; Gullichsen et al., 2000; Sjostrom et al., 1999). The terms hardwood and softwood do not indicate wood actual hardness or softness (Baker et al., 1974; Gullichsen et al., 2000). Teak (*Tectona grandis*) is a hardwood (ณรงค์ โทณานนท์, 2544). Hardwoods and softwoods are different in their structures, chemical composition, and types of lignin.

2.1.1 Structure of Wood

The diagram of cross – sectional view of a tree trunk, as shown in Figure 1, reveals, from the inner center to the outer trunk respectively, the pith, heartwood, sapwood, cambium, inner bark and outer bark (Baker et al.,1974; Gullichsen et al., 2000).

The pith is located at the center of the tree trunk. It is the remain of the soft tissues that was produced during the plant's first year of life (Gullichsen et al., 2000). The bark, the outermost part of the tree trunk, consists of 2 layers which are the inner bark or phloem, and the outer bark or cork (Gullichsen et al., 2000). The inner bark or phloem is used to transport photosynthesis products to different part of wood (ณรงค์ โทณานนท์, 2544). It lives only for one year and then it dies and becomes part of the outer

bark (ฉรงค์ โทณานนท์, 2544). The outer bark's function is to protect the tree against the environment (Gullichsen et al., 2000).

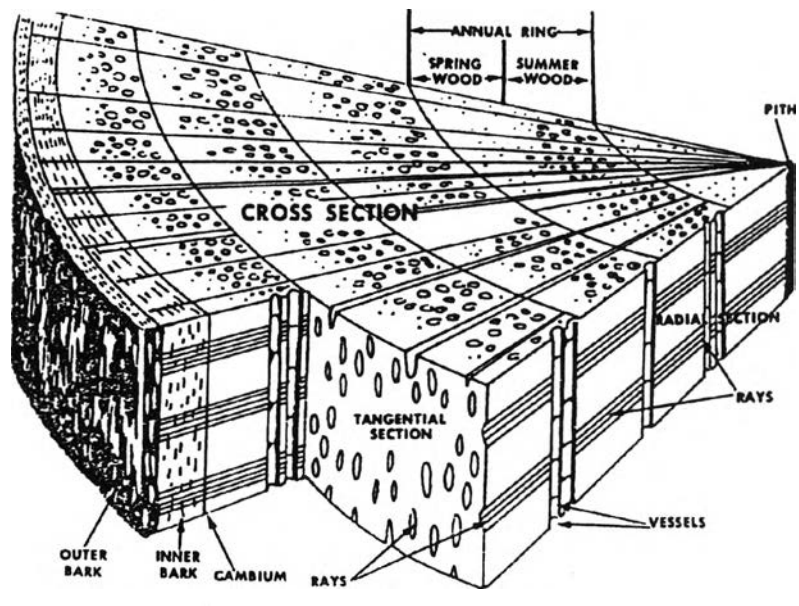


Figure 2.1 Structure of wood (Baker et al., 1974)

The cambium, a very thin layer of living cells between inner bark and sapwood, is a place where cells production occurs (Gullichsen et al., 2000). The cells produced from the cambium on the outer side of the tree trunk are called phloem or inner bark and those produced from the inner side are called xylems or wood cells (ฉรงค์ โทณานนท์, 2544; Wenzl et al., 1970). The new xylem becomes part of sapwood (ฉรงค์ โทณานนท์, 2544). The cambium is continuously producing new cells. The characteristic of the wood cells produced varies with seasons. In the first part of growing season which is in spring in the temperate zones, the tree requires an efficient water and nutrient transportation system for tree growth; therefore, the wood cells have a large cross – sectional area (Gullichsen et al., 2000). These rapidly growing cells are called earlywood or springwood cells (Harlow, 1975). They have a large cross – sectional area, a large and open center (lumen), and thin cell walls (Gullichsen et al., 2000). The slow growing wood cells formed during the later part of the growing season, which are called latewood or summerwood cells, have thicker cell walls and

narrower lumen (Gullichsen et al., 2000; Harlow, 1975). Together the earlywood and latewood layers form an annual ring or growth ring (Wenzl et al., 1970). The outer latewood layer is darker and denser than the inner earlywood layer which is porous (Sjostrom et al., 1999; Harlow, 1975). The annual rings of most of the hardwood in the tropical zone are not detectable; however, in the case of teak, the annual ring is detectable (ณรงค์ โทณานนท์, 2544).

The function of the sapwood is to give support, to store food and to transport water and nutrient from the roots to the leaves (ณรงค์ โทณานนท์, 2544; Gullichsen et al., 2000). It lives only for a few years and then it gradually transforms into heartwood (ณรงค์ โทณานนท์, 2544). As sapwood transforms into heartwood, the moisture content decreases and as a result the sapwood is softer than the heartwood (Baker et al., 1974). As transformation progresses, the starch decomposes into glucose which in turn converts to aromatic compounds (Wenzl et al., 1970). The transformation from sapwood to heartwood occurs with both physical and chemical changes (Wenzl et al., 1970).

The heartwood – the inner, usually darker, denser, drier, lifeless, rigid portion of the xylem – provides strength and support for the tree (Baker et al., 1974; Gullichsen et al., 2000). Heartwood has lower permeability to water and oxygen than sapwood (Eaton et al., 1993; Stamm, 1964). This is one of the reasons that make heartwood more durable than sapwood (Eaton et al., 1993). Unlike permeability, diffusion through sapwood is only slightly greater than diffusion through heartwood (Stamm, 1964).

Juvenile wood or corewood is formed by the young cambium around the pith during the first 5 – 20 years, 10 – 20 years for teak, of the tree life (ณรงค์ โทณานนท์, 2544; Gullichsen et al., 2000; Sjostrom et al., 1999). Juvenile wood differs from mature or adult wood. Juvenile wood has lower density and higher earlywood to latewood ratio (Gullichsen et al., 2000; Westermark et al., 1999).

The properties of the wood as a whole depend on the ratio of juvenile wood to mature wood, earlywood to latewood and heartwood to sapwood (Westermark et al., 1999).

2.1.2 Cell wall

In a fully mature cell, the cell contents die and only the cell wall is left (Lodish et al., 1995). The cell wall is composed of 2 layers: the primary wall and the secondary wall (Meylan et al., 1972). The outer and thin primary wall is developed first. When the cell reach its full size, no longer need to expand, the secondary wall is then developed on the inside of the primary wall and it grows toward the center of the cell (Eaton et al., 1993). The secondary wall is thick and it is subdivided into three layers: the outer layer (S1), the middle layer (S2) which is the thickest, and the inner layer (S3) (Meylan et al., 1972). Sometimes, the inside of S3 layer next to cell lumen is covered with a thin membrane called the warty layer, named after its small protrusions appearance (Meylan et al., 1972). After the cells are full grown and reach their full size, the primary walls of these cells are then bound together by the intercellular substance (ณรงค์ โทณานนท์, 2544). This layer between the primary wall is called middle lamella (ML) which is rich in lignin (Meylan et al., 1972). The primary wall, S1, S2, S3 and middle lamella all have different percentage of chemical component and different types of lignin (Sjostrom et al., 1999; Eaton et al., 1993). The schematic diagram of the cell wall is shown in Figure 2.2.

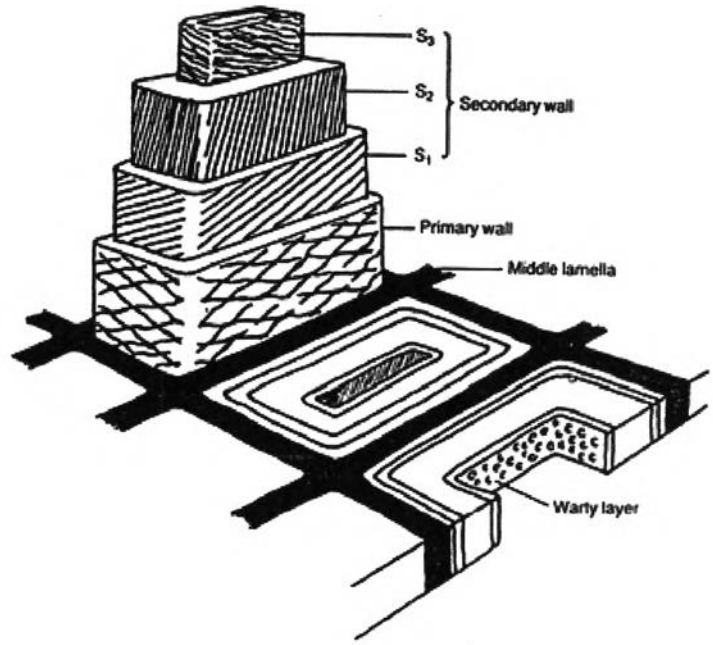


Figure 2.2 Schematic diagram of cell wall (Eaton et al., 1993).

2.1.3 Pits and perforations (Meylan et al., 1972)

Solution can pass from one cell to another by mean of perforations and pits in the cell wall. Perforations are simply the openings in the cell wall while pits still have membranes to separate the cells. The pit membrane is made up of both the primary walls of the two adjacent cells and the middle lamella that bind the two cells (P – ML – P). There are two types of pits: the bordered pits (which include its reduced form called reduced bordered pits) and simple pits.

2.2 Literature Review on Chemical Composition of Wood

Chemical composition of wood varies from softwood to hardwood, from species to species and even from the different parts of the same tree. The average values for hardwood, acquired from common pulpwood species, are shown below, and unless otherwise state are taken from Sjostrom and Westermark (Sjostrom et al., 1999) (See Table 2.1 and Figure 2.3).

Table 2.1 Composition of wood

constituent	% of dry wood weight
Cellulose	39-45
Hemicelluloses	30-35*
- Glucuronoxylan	15-30
- Glucomannan	2-5
Lignin	20-25
Extractives	2-4

- Gullichsen, J.; and Paulapuro, H., 2000.

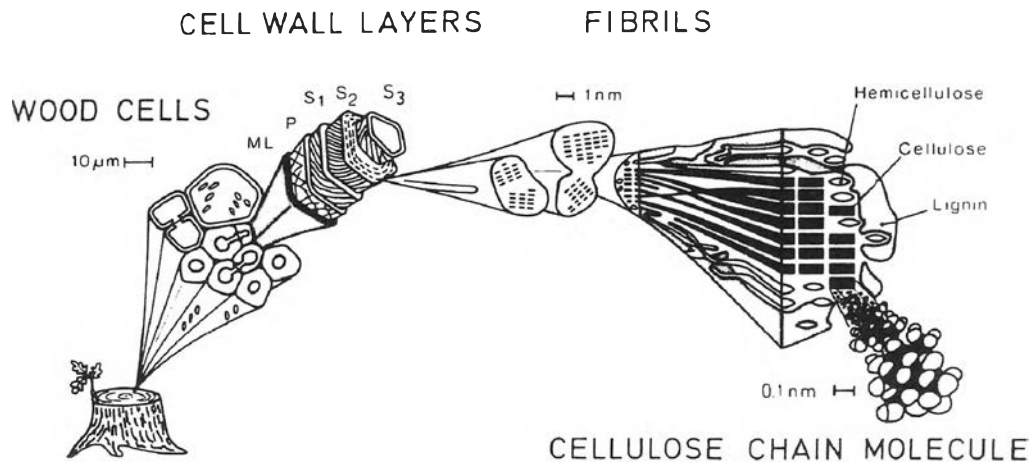


Figure 2.3 Constituent of wood (Hon et al., 2001)

2.2.1 Cellulose

A very high molecular weight relatively hydrophilic, water – insoluble, linear homopolysaccharide of D – glucose units linked together by β(1→4) linkages (as shown in Figure 2.4), cellulose is the major polysaccharide in wood cells and the world's most abundant biopolymer (Sjostrom et al.,1999; Esau, 1953; Mathews et al., 1996; Ritter, 1996). There can be up to roughly 15,000 of D – glucose units in a cellulose molecule (Ritter, 1996). The structure of a cellulose molecule is shown in Figure 2.4.

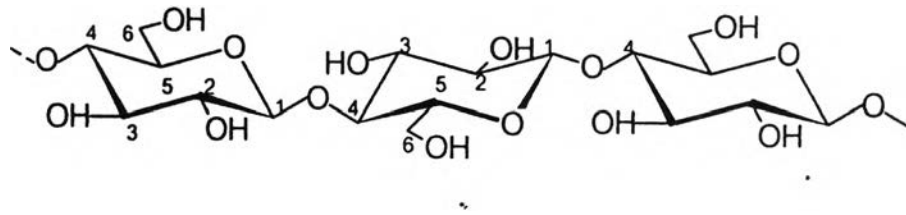


Figure 2.4 Structure of cellulose molecule (Lodish et al., 1995)

The cellulose molecules are connected by hydrogen bonds to form microfibrils (Sjostrom et al., 1999). The set of microfibrils are arrange in layers about 20 – 40 nm apart and are connected to each other with long hemicelluloses molecules by hydrogen bonds (Alberts et al., 2002).

2.2.2 Hemicelluloses

Hemicelluloses, the branched heteropolysaccharides, are one of the major components of cell wall (Sjostrom et al., 1999). The branches help bind hemicelluloses to each other and to other cell wall components (Lodish et al., 1995). The major hemicelluloses in hardwood are glucuronoxylan (xylan), shown in Figure 2.5, and glucomannan which constituent to 15 – 30% and 2 – 5% of dry wood weight respectively (Gullichsen et al., 2000; Sjostrom et al., 1999).

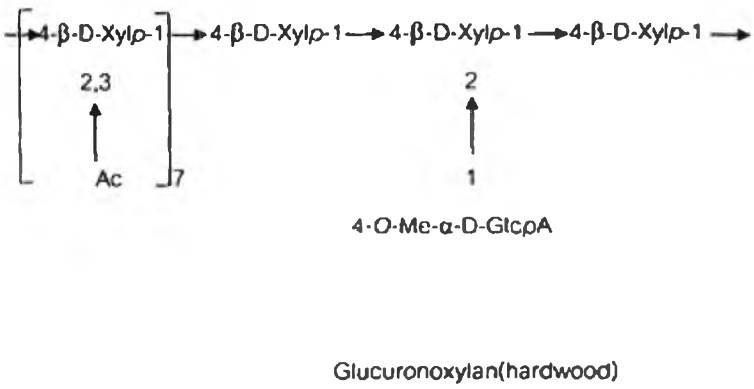
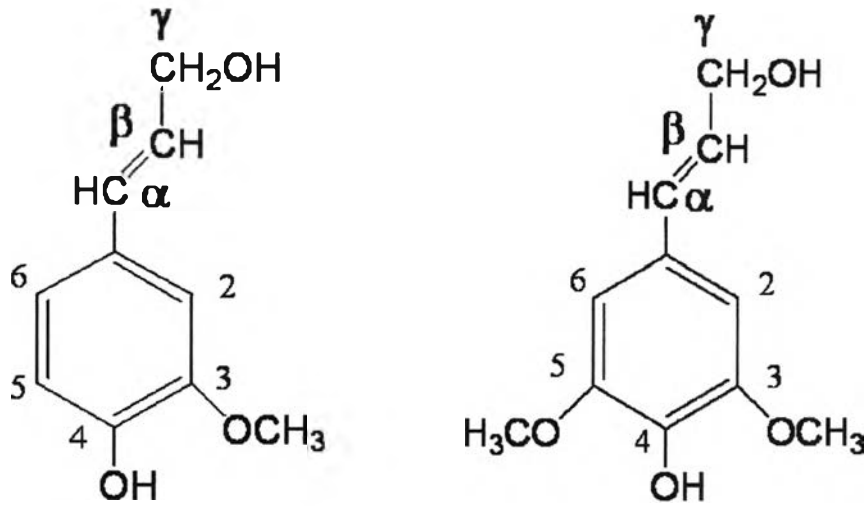


Figure 2.5 Structure of hard wood xylan (Sjostrom et al., 1999)

The hardwood xylan, O-acetyl-4-O-methyglucuronoxylan, consists of β-xylopyranose residues link together by β-1, 4-glycosidic bonds (Beg et al., 2001). Every of the tenth xylose residue are linked to 4-O-methylglucuronic acid (Beg et al., 2001). Hardwood xylan contains a lot of acetyl groups which make xylan partially soluble in water (Beg et al., 2001). Xylans are link to cellulose with hydrogen bonds and are linked to lignin with covalent bond (Beg et al., 2001).

2.2.3 Lignin

Lignin, a major nonpolysaccharide component in wood, is a complex polymer composed of aromatic hydrocarbon (Tsai et al., 1998; Campell, 1991). A complex phenolic polymer, lignin is composed of coniferyl, sinapyl and *p*-coumaryl alcohols, all of which are *p*-hydroxycinnamyl alcohol, in various combinations and proportions (Mackay et al., 1997; Christensen et al., 1998). The chemical structure and atoms designation of coniferyl and sinapyl alcohols is shown in Figure 2.6. The ratio of these alcohols in wood varies from species to species (Brunow et al., 1999). The three alcohols are derived from phenylalanine and tyrosine (Mathews et al., 1996). One of the published biosynthetic pathways for the three alcohols, taken from MacKay et al. (Mackay et al., 1997) is shown in Figure 2.7. Coniferyl alcohol is called guaiacyl lignin or G lignin and sinapyl alcohol is called syringyl lignin or S lignin (Tsai et al., 1998; Li et al., 2000). In hardwood, which teak is, lignin is composed of a mixture of G and S lignin monomers (Tsai et al., 1998; Li et al., 2000; Osakabe et al., 1999). In gymnosperm lignin consists of almost entirely of G lignin monomer (Osakabe et al., 1999).



G lignin S lignin

Figure 2.6 Lignin unit structure and atoms designation (Brunow et al., 1999; Mackay et al., 1997)

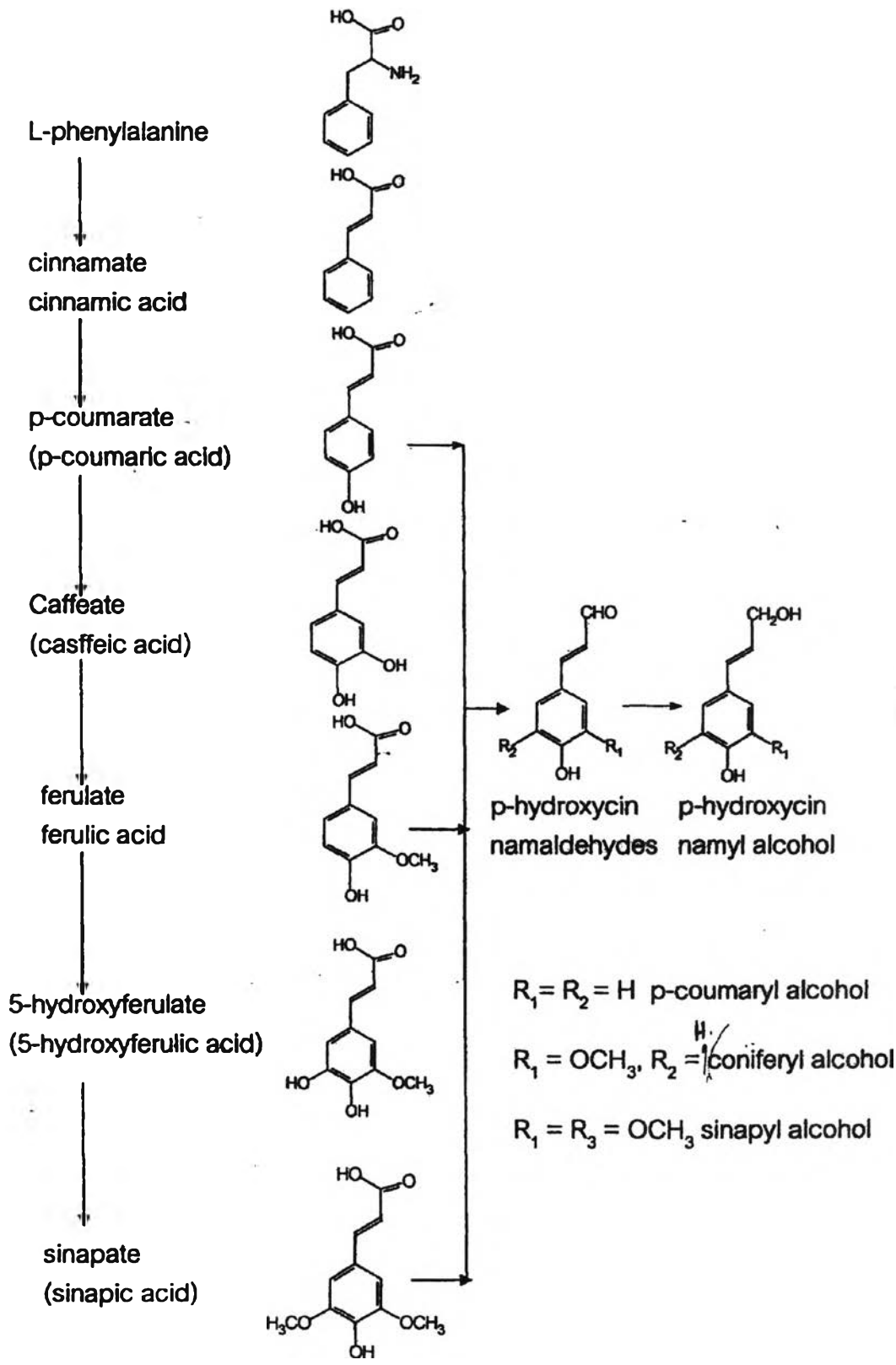


Figure 2.7 Mackay et al.'s published biosynthesis pathway for coniferyl, sinapyl and *p*-coumaryl alcohols (Mackay et al., 1997).

Lignin monomers are linked together by various numbers of linkages of carbon – carbon and ether linkages (Pérez et al., 2002). The examples of these linkages are shown in Figure 2.8. The chemical structure of lignin is irregular because the lignin monomers are not linked to each other in any particular order (Brunow et al., 1999). The example of chemical structure of lignin is presented in figure 2.9. The other linkages, such as β-O-4, are frequent and not as difficult to break as the much less frequent C-C linkages especially the biphenyl 5-5 bonds (Gullichsen et al., 2000; Lapierre et al., 1999). The 5-5 bond can occur only with G-G interunit linking not with G-S or S-S linking because the C5 position in S unit is not available for C-C bonding (Lapierre et al., 1999). Therefore, delignification of the softwood which consists entirely of G units is more difficult than that of the hardwood which consists of both S and G units (Lapierre et al., 1999).

According to Crawford (Crawford, 1981), there are 3 kinds of lignins:

1. Guaiacyl lignin (found in most softwood).
2. Guaiacyl – syringyl lignin (found in hardwoods).
3. Guaiacyl – syringyl – *p*-hydroxyphenyl lignin (found in highly evolved glasses).

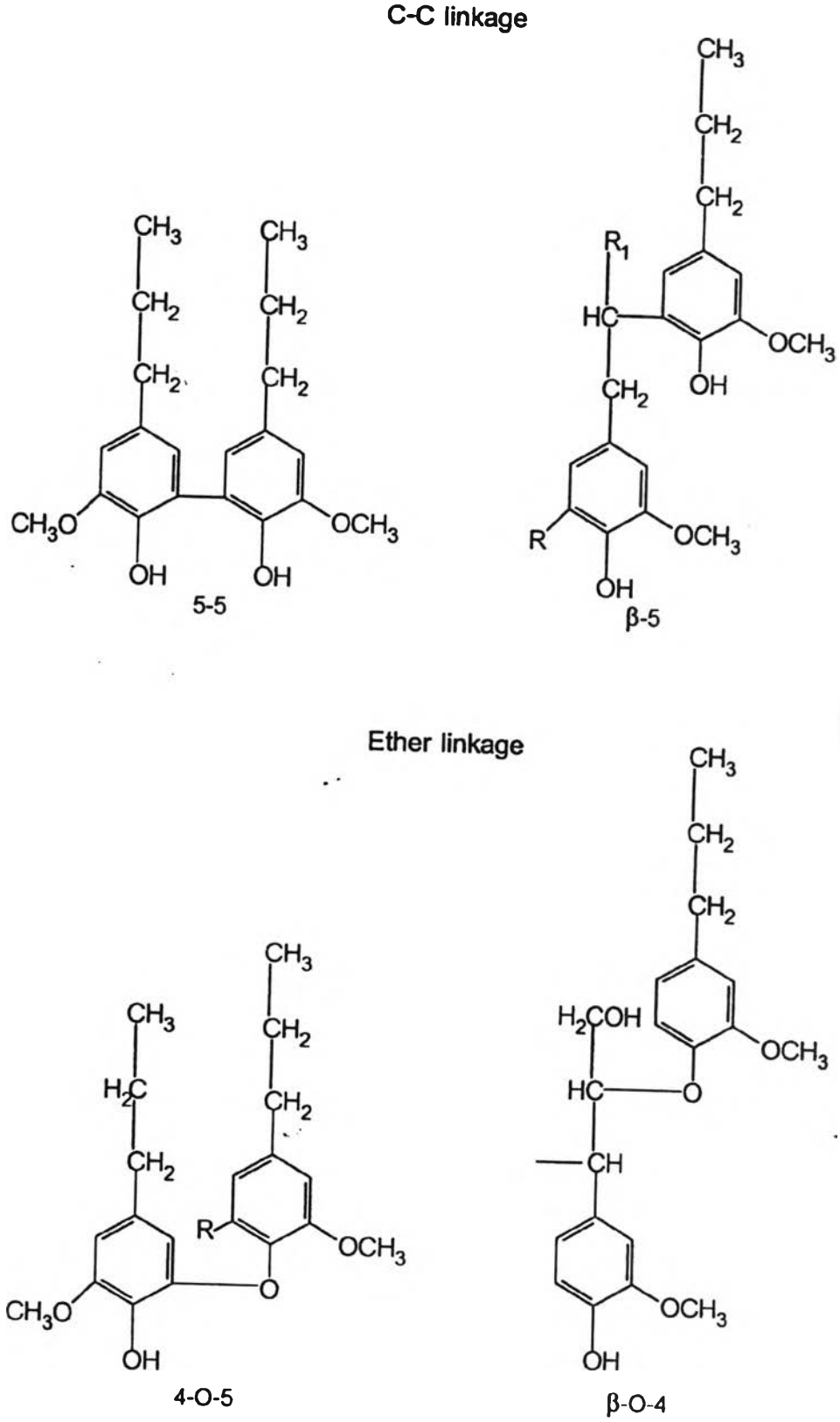


Figure 2.8 Lignin linkages (Gullichsen et al., 2000; Lapierre et al., 1999)

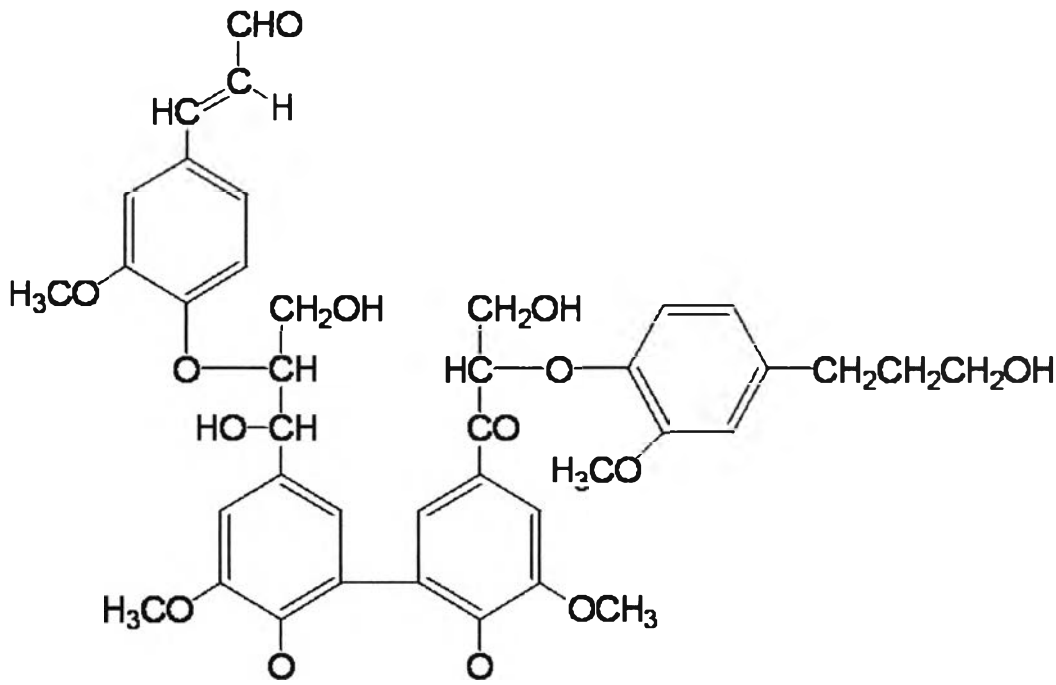


Figure 2.9 Example of lignin chemical structure (Brunow et al., 1999)

2.3 Literature Review on Lignin Removal Methods

There are three major methods in extraction of lignin. They are chlorine bleaching; ECF or elemental chlorine free bleaching and TCF or total chlorine free bleaching (Hampp, 2001). In chlorine bleaching, the most effective, selective and inexpensive method, lignin is extracted using elemental chlorine. This process, however efficient, releases organochlorines, one of which is dioxin which is one of the most acutely toxic compounds (Hampp, 2001; Tolan et al., 1997). In ECF process, chlorine dioxide is used instead of elemental chlorine (Hampp, 2001). This process is less efficient in lignin removal and still release organochlorine but the amount released is much lower than that of chlorine bleaching (Hampp, 2001). When used in combination with oxygen delignification, it reduces the amount of chlorine dioxide used; therefore, reduces the amount of pollutant (Hampp, 2001). In the case of TCF bleaching, which used no chlorine – containing compounds, lignin is extracted using chemicals such as oxygen, ozone or hydrogen peroxide (Hampp, 2001 and Del Rio et al., 2000). These chemicals are less effective than chlorine or chlorine – containing compound (Hampp, 2001). Example of TCF process is from the work of Vincent et al. (Vincent et al., 1997). However, these TCF processes are still releasing some toxic waste (Del Rio et al., 2000) and they are expensive since they require large amount of expensive chemical such as hydrogen peroxide (Hampp, 2001).

There are other methods in lignin removal. Some researchers (Tsai et al., 1998; Mackay, 1997; Christensen et al., 1998; Li et al., 2000; Osaka be et al., 1999; Lapierre et al., 1999; Meyermans et al., 2000) work on tailoring a plant to have lower lignin concentration and/or to have more S lignin than G lignin since S lignin is easier to extract than G lignin (Lapierre et al., 1999). Hampp (Hampp, 2001) invented an electrochemical process suitable for pulp delignification.

Another method is to use enzymes. The use of enzymes will reduce the toxic waste producing. Enzymes are special in that they are highly selective by nature in choosing their targets which is suitable in industry that need to extract lignin without damaging other compounds. Enzymes can attack lignin directly or may do so indirectly by attacking hemicelluloses (Hampp, 2001). Since lignin, cellulose and xylan are interconnected with each other, removal of xylan will help free the entrapping lignin allow it to be extracted by the use of chemicals (Hampp, 2001;

Suurnakki et al., 1997; Kirk, 1995). Examples of enzymes that attack lignin directly are lignin peroxidase, manganese peroxidase, and laccase (Hampp, 2001; Tolan et al., 1997). Xylanase is believed to remove some of xylan from the lignin – xylan – cellulose matrix (Suurnakki et al., 1997). Removal of some of xylan is known to increase the bleach ability of lignin in pulping industry (Suurnakki et al., 1997; Kirk, 1995).

2.4 Literature Review on Enzymatic System, (Beg et al., 2001; Perez et al., 2002; Tolan et al., 1997)

Enzymes are target specific catalyst. They often have catalytic power greater than the synthetic or inorganic catalysts. They are also not consumed in the process. Two types of extracellular enzymatic systems are produced by microorganisms. They are:

1. The hydrolytic enzymatic system.
   It produces hydrolases and is responsible for cellulose and hemicelluloses degradation
2. The oxidative enzymatic system.
   It depolymerizes lignin.

The hydrolytic enzymes are subdivided into cellulases which attack celluloses and hemicellulases which attack hemicelluloses. The hemicellulases are often classified according to their action on the hemicelluloses. The hemicellulases for xylan can be furthered divided into 3 groups:

1. Endo-1,4-β-xylanase (endoxylanase)
   This enzyme cleave xylan main linkages, the β-1,4-D-xylopyranose linkage, and yields xylan oligosaccharides.
2. Xylan-1,4-b-xylosidase
   This enzyme degrades xylan oligosaccharide to xylose.
3. Accessory enzymes
   These enzymes cleave size chains, for example:
   - Acetyl esterase removes acetyl group
   - α-glucuronidase removes 4-O-Me-glucuronic group

The oxidative enzymes for lignin or lignolytic enzymes can be subdivided into 2 major groups:

1. Peroxidases: These enzymes use low molecular weight mediators to perform lignin degradation. The examples of these enzymes are Lignin Peroxidase (LIP) and Manganese – dependent Peroxidase (MnP).
2. Laccase: This enzyme also uses low molecular weight mediators to perform lignin degradation.

2.5 Literature Review on Xylanase and Laccase

Due to the lack of research done in veneer delignification, the works cited here came from research in pulp and paper field. Delignification method using enzymes is growing fast in the past several decades and chief among the enzymes employed are xylanase and laccase. Both xylanase and laccase will be used in this research.

Xylanase is the enzyme that reduces xylan which is the major hemicelluloses in hard wood; it will not degrade lignin directly. However, since cellulose, xylan and lignin are interconnected and bonded by covalent and non covalent bond (Perez et al., 2002), removal of xylan will help free the lignin allow it to be extracted by the use of chemicals (Sigoillot et al., 2002). Lately it has been used in the field of pulp and paper to aid in removing lignin (Whitmore, 1997).

Laccases (E.c.1.10.3.2) are copper- containing oxidase that reduce oxygen to water and simultaneously catalyze the one-electron oxidation of many aromatic substrates such as phenols (Srebotnik and Hammel, 2000; Bournonnais et al., 1997). Laccase is an oxidative enzyme that degrades lignin directly (Perez et al., 2002). Laccase alone can only degrade phenolic lignin but cannot degrade non-phenolic lignin in lignin structure (Sigoillot, 2002). With the use of a mediator, laccase can then degrade non phenolic lignin (Bourbonnais et al., 1995). However, mediator can be both expensive and toxic. Non toxic mediator including transition metal complexes are being investigated (Kenealy and Jeffries, 2003).

In this research only partial removal of lignin is required; therefore, it was decided that laccase is to be used without mediators.