BIOLOGICAL CONVERSION OF LIGNIN FOR CHEMICAL PRODUCTION

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Abstract

Lignin, a derivative of wood is one of the abundant aromatic polymers, and a cell wall component which provides mechanical support for wood fibres, regulates the transport of water through the cell wall xylem and protects plant against destructive attacks from insects and enzymes by building an impenetrable membrane surrounding the inner polysaccharides components. Lignin has high energy content due to its complex polymer structure and thus makes it a potential renewable resource for chemicals and fuels. The biological conversion of lignin is achieved chemically, physically or biologically. The biological conversion is of utmost importance and this article takes a critical look at the methods used for its conversion and the products obtainable from lignin.

Keyword: Lignin, biochemical, biological conversion, renewable resources

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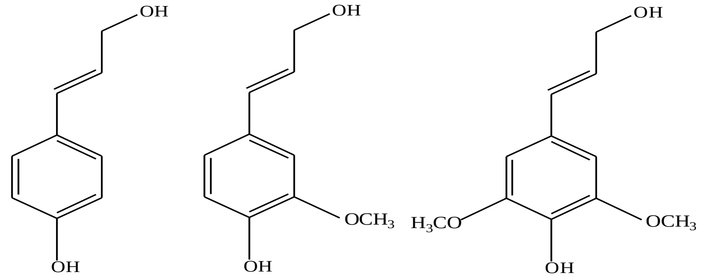
Introduction

Lignin is compound name given to a vast group of aromatic polymers. The word lignin is derived from the Latin term lignum, which means wood (Sarkanen et al., 1971). Lignin is a derivative of wood and one of the most abundant aromatic polymers on earth (Lora and Glasser 2002; Kudanga and Rose-Hill 2014). It is one of the three major components of plant materials: lignin, cellulose and hemicellulose. In plants, it is predominantly a cell wall component and makes up between 15-25 % of woody plant materials representing a large amount of the world non-fossil carbon reserve (Kudanga and Rose-Hill 2014). Its presence in plant play lots of roles such as provision of mechanical support for wood fibres, regulation of water transport through the cell wall xylem and protection of plant against destructive attacks from insects and enzymes by building an impenetrable surrounding the inner polysaccharides components (Sjostrom, 1993; Sammond et al 2014). Although it is abundant, it is underutilized as less than 5% lignin have been put into productive use (Kudanga and Rose-Hill, 2014; Kleinert and Barth, 2008). Lignin contains high energy content due to its complex polymer structure and this makes it a potential renewable resource for chemicals and fuels (Liu et al., 2014). The use of lignin as a renewable source of energy is highly encouraging due to the current instability in the demand and prices of petroleum and products (Liu et al., 2014). It is also a means of sustainable energy source as it has less hazardous effect on environment compared to first generation fossil fuels (Clark and Deswarte 2015). Lignin is majorly generated as effluent of paper and pulp industries, while its intended production runs to about 50 million tons/year (Suhas et al., 2007). Due to the economic importance of this polymer, International Lignin Institute, Switzerland was established to promote technologies for conversion of lignin to environmentally friendly products (www.ili-lignin.com).

Biosynthesis of lignin

Lignin structure is extensively complex, heterogeneous, dimensional, irregular, branched and an optically inactive organic polymer. The three main components (monolignols) of lignin structural units are p-coumaryl alcohol [4-hydroxycinnamyl alcohol or its 3- and/or 3, 5-methoxylated derivatives], coniferyl and sinapyl alcohol (Roberts, 2006) (Fig. 1). Upon their final incorporation into the lignin moiety, these monolignols become p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S).



The overall biosynthesis of lignin is a complex process which can be categorized into three discreet but concerted cellular processes. These processes involves the synthesis of monolignols within the cytosol, the transport of monomeric precursors across plasma membrane and the oxidative polymerization of monolignols to form macromolecules within the cell wall (Liu, 2012).

Synthesis of the monolignols

The first stage of the synthesis proceeds in the cytosol by the synthesis of the monolignols from a single precursor, L-phenylanine (Fig. 2). The synthesis begins with the series of reactions leading to the glycosylation of the amino acid. This process renders the amino acids water soluble and less toxic due to the glucose molecules attached to them (Boerjan et al., 2003).

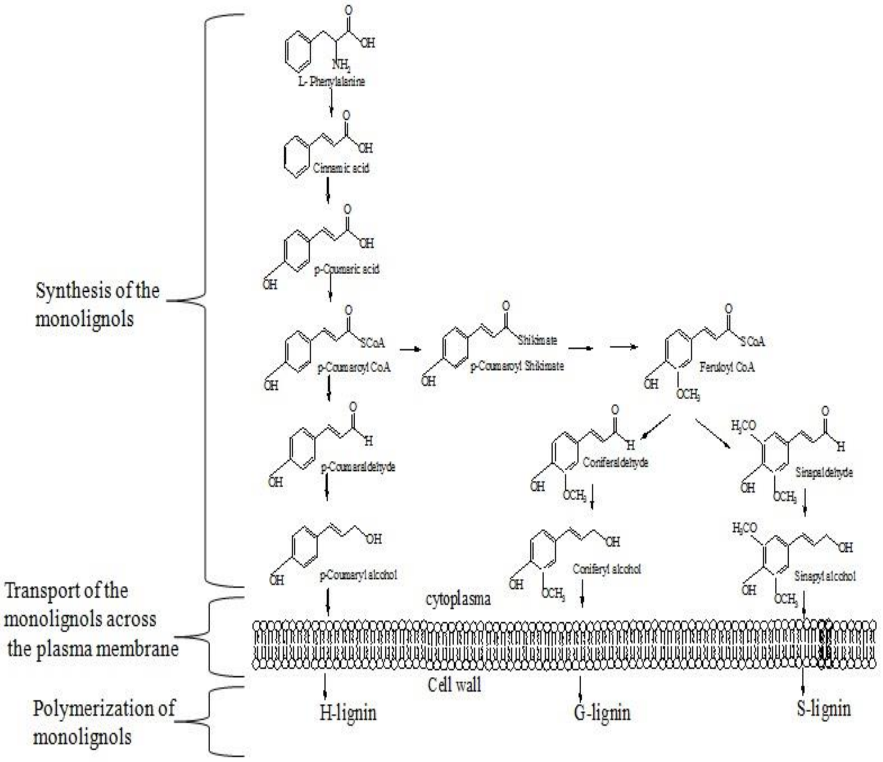


Figure 2. The phenylpropanoid-lignin biosynthesis pathway (Liu et al. 2014).

The linear pathway of this phase of the synthesis ends at a critical branching step where an intermediate precoursor, *p*-coumaric CoA, is subject to two fates. This intermediate could either form *p*-coumaryl shikimate by the action of enzyme: hydroxycinnamoyl-CoA:shikimate/ quinate hydroxycinnamoyl transferase (HCT) or continued in the linear pathway to produce *p*-coumaric aldehyde by the action of CCR, cinnamoyl-CoA reductase (Humphreys and Chapple, 2002). This step is critical in that it coordinates the subsequent direction of the carbon flux precursor to the synthesis of guaiacyl and syringyl lignin precursors in the phenylpropanoid pathway (Niggeweg et al., 2004).

Transport of monomeric precursors across plasma membrane

Upon the complete synthesis of the monolignols in the cytosol, they are exported across the plasma membrane and deposited into the cell wall (Fig. 2). The entrance of the monolignols into the accompanied by the dissociation of the attached glucose unit and polymerization of the units follows (Samuels et al., 2002).

Although, the intrinsic mechanism of the translocation of the monolignols is yet unclear, transcriptomics, proteomics and modern auto radiographic studies have suggested the involvement of ATP-binding cassette (ABC) transporters in plant lignification (Nilsson, et al., 2010; Smith et al.,2013).

Oxidative Polymerization of Monolignols

The polymerization is initiated by oxidation/dehydrogenation of the phenolic hydroxyl groups to form radicals. This step is supposed to be catalysed by oxidative enzymes: laccase and peroxidise present on the plant cell wall (Ralph et al., 2004). The radicals formed are stabilized in a series of radical-radical coupling to one another in positions of the unpaired electron.

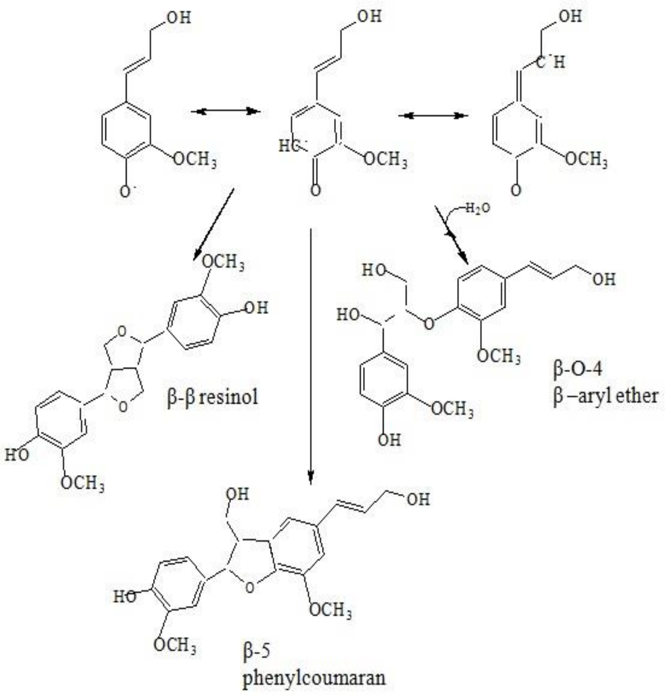


Figure 3. Dimerization of two dehydrogenated coniferyl alcohol monomers (Adler, 1977).

The combination of monomeric radicals through only β-O-4, α-O-4 and β-5 coupling would lead to the formation of a dilignol. The dilignols could subsequently undergo further endwise polymerization with one another or a new oxidized monolignol.

Structure of lignin

Due to the complex nature of lignin, its chemical structure remains unresolved after many years of study. However, advances in crystallography and structural elucidation studies have improved the identification of lignin molecules (Liu, 2012). Lignin structure is heterogeneous, dimensional and irregular. Its structure is branched and optically inactive organic polymer.

The lignin molecule is a product of high number of polymerization reactions and this yield a highly-branched, interlocking networked polymer. Figure 4 is a schematic representation of a softwood lignin proposed by Adler (1977).

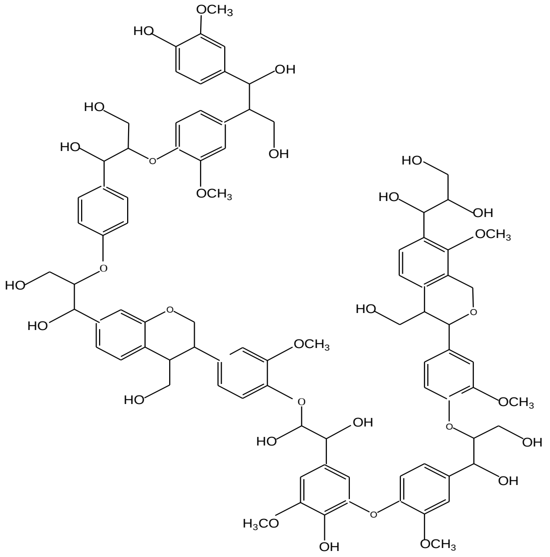


Figure 4. Chemical structure of lignin (Adler, 1977)

Conversion of Lignin for Production Biofuel and Chemicals

Efficient utilization of lignin for production biofuel and chemicals is greatly hampered by its recalcitrance (Roberts, 2006). Depolymerisation of lignin can generally be categorized into three broad spectrums: physical, chemical and biological depolymerisation for chemical production (Figure 5). The physical deploymerisation process involves: pyrolysis, hydrolysis, hydrogenolysis and gasification (Clark and Deswarte, 2015). Generally, chemical depolymerization of lignin can be carried out according to different chemicals applied in the depolymerization process, which includes: acid/base-catalyzed, metallic catalyzed, ionic liquids-assisted and supercritical fluids-assisted lignin depolymerizations (Ralph and Baucher, 2003)

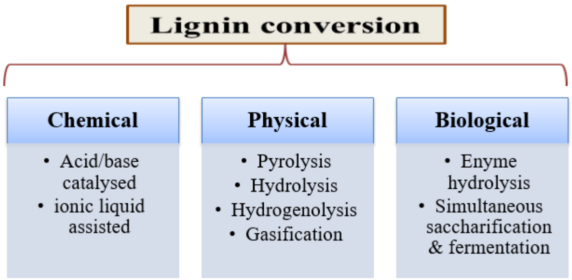


Figure 5. Methods for lignin conversion

According to The International Lignin Institute, Switzerland, there are four broad categories in which the applications and products derivable from lignin can be classified: multi-polarity products, Agriculture applications, high-purity value products and materials (www.ili-lignin.com). Table 1 shows a summary of products and the applications of lignin under the above mentioned categories.

Table 1: Applications and Products derivable form lignin (www.ili-lignin.com)

Multi-polarity	**Agriculture**	**High-purity value**	**Materials**
Ceramics	Soil rehabilitation	Antioxidants	Polyurethanes
Tiles	Granulation	Antibiotics	Particle boards
Dyes	Fertilizer	HIV Inhibitors	Epoxides
Electrolytes	Soil stabilization	Growth stimulators	Polyesters
Wax	Pelletizing	Biofuels	Carbon fibres
Grinding aids	Insecticide	Foam stabilizer	Biodegradables
Cements	Manure treatment	Binder	Activated carbons
Water softening	Soil stabilization	Tanning agents	Paper bounding
Dust control	Artificial fertilizer	Enzymes	Phenolic resins
Metal cleaners	Controlled fertilizer release	Absorbent	Carbon sieves

Bioconversion of Lignin

The biological conversion of lignin involves the utilization of living organisms or enzymes to catalyze the conversion of biomass into specialty and commodity chemicals (Fig. 6).

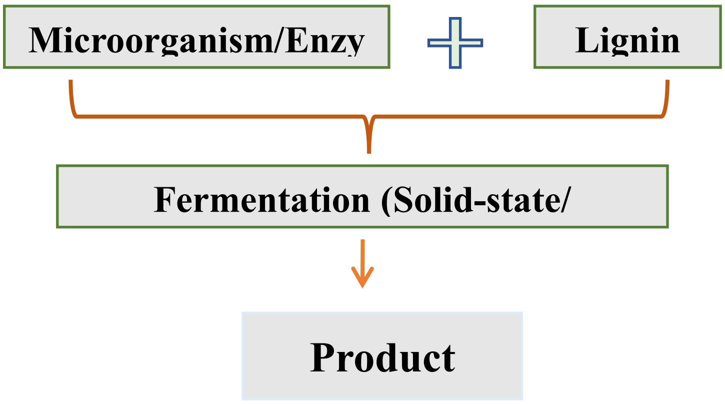


Figure 6. Schematic representation of lignin biological conversion

Bioconversion has a unique advantage due to its flexibility when compared to other methods of conversion. It also provides the pathway to obtaining numerous industrial products. (Dale, 2003). The utilization of physical and chemical processes for lignin conversion involves high temperatures and pressures, whereas, biological conversion operates under mild conditions. The advantages of this conversion over other techniques include: low energy input, mild operation conditions, high yield of products, cheap starting materials and cost-effective production.

Fermentation is one of the primordial methods of food transformation and chemical production. This technique has advanced with biotechnology for biological transformation of bio-materials. Fermentation has been in use for centuries in preserving and processing food and beverages. It is only in the last several decades ago due to current advancements in biotechnology, that it has been used to bring to market a wide variety of fermentation-based obtainable from agricultural waste and materials include industrial chemicals, organic acids and enzymes, biofuels and biogas, and pharmaceuticals.

Some commercial bulk chemicals, such as ethanol, lactic acid, citric acid, acetone, and butanol, have been produced via yeast, fungal and bacterial fermentation processes (Dhillon et al., 2011; Chen, 2013 and Mitchell et al., 2000). Advances in utilization of biocatalysts has enabled the transformation of renewable bioresources into biochemicals. However, not much of desired chemicals could be produced via fermentation as a result of specificity in microorganism metabolic pathways. (Danner and Braun, 1999). With advances in genetic engineering, modification of microorganisms to perform specified metabolic functions is now possible (Zha et al 2004). Recombinant DNA technology, also provides for genetic manipulations of organisms to be cloned for specialized biochemical production (Danner and Braun, 1999). Therefore, it is important to explore these technological advances to produce recombinant strains that can efficiently utilize all components of lignocellulosic biomass (Chen and He, 2012). Currently, research efforts are ongoing to isolate, identify, characterise, and even tailor microorganisms and enzymes in order to better utilize renewable resources to produce structurally diverse and complex chemicals. However, problems such as capital cost, pretreatment, low yield of products in innate organism and downstream recovery of the products still bedevils the biotransformation process (Danner and Braun, 1999). Further research and considerable investment is required to make biotransformation processes highly efficient and rewarding (Dodds and Gross, 2007). Pretreatments can be carried out by various means to breakdown the recalcitrant lignocellulosic structures to obtain maximum efficiency (Chen 2013; Chen and He, 2012; Brodeur et al, 2011). The well-arranged three-dimensional structure of lignocellulosic wastes serves as an impediment to its efficient hydrolysis and subsequent conversion of sugar derived to various important products. This also depends upon various other factors, such as crystalline structure of cellulose, amount and nature of lignin present, and production of various inhibitory compounds during hydrolysis (Chen and He, 2012; Brodeur et al., 2011; Dodds and Gross, 2007; Chen and Liying, 2007). Lignin residues obtained from such hydrolyisis often serve as fuel in some combustion engines thereby reducing the effects of greenhouse gas emissions from fossil fuels (Kaur et al., 2013).

High-Value Chemical Derivatives from Lignin Bioconversion

Currently, research efforts are ongoing to isolate, identify, characterise, and even tailor microorganisms and enzymes in order to better utilize renewable resources to produce structurally diverse and complex chemicals. Although bioconversion provides avenue for production of biochemical, the biotransformation technologies still suffers some impediment in upstream and downstream processes. The capital costs related to energy requirements, such as pretreatment, sterilisation, production, agitation, aeration, temperature control, and finally recovery of target products from aqueous systems with low product concentration, result in high-cost processes (Dodds and Gross.2007). Further, considerable investment is required to make processes highly efficient and continuous (Danner Braun, 1999). The drive to develop bio-based economy from the conversion of lignin provides lots of research opportunities whose results could lead to production of important biochemicals. Biological conversion also known as biotransformation is well defined process with fermentation and anaerobic digestion as the key methods utilized for converting lingo-cellulosic materials. The lignin residues could be used as fuel for the energy required and even providing surplus energy, resulting in significantly improved energy balances and resulting potential reductions in greenhouse gas emissions. Generally, the production of high-value biochemicals from lignin derivatives are regarded as safe products and even their uses in food and beverages (Xu et al., 2007). Converting lignocellulosic materials provides ample opportunity to generate lots of raw materials and make them cheaply available for production of various industrial chemicals and products.

Other few examples of chemicals obtainable from lignin conversion include, Biofuels (Liu and Cheng 2010; Wang and Liu, 2009)' Industrial chemicals and enzymes such as; cellulose (Vu et al.,2011), Succinic acid (Lee et al., 2022), Lactic acid (Huang et al., 2005), Lignolytic enzymes (Szabo et al., 2015), Antibiotics, (Asagbra et al., 2005; Barrios-Gonzalez and Mejia, 1996) and Antioxidants (Knoblich et al., 2005; Ajila et al., 2011).

Conclusion

The current problem faced by production of most high-value chemicals from fossil fuels poses a major challenge due to climate change and fossil-resource depletion. Thus, there is an increasing demand for sustainable production of bio-based platform chemicals using lignin contained biomass as substrates for fermentation by microorganisms. From time immemorial, microbes have shown great potentials for a broad range of chemical products with high market value. Improved molecular biology and genetics have likewise contributed immensely into the development of microbial strains with higher ability to produce desired chemicals. The ability of researchers to manipulate the genetic systems and re-engineering metabolic pathways provide an ever-growing design space for the production of chemicals of interest. Likewise, the development of numerous pretreatment technologies has greatly encouraged the efficiency of the downstream process of microbial production of high-value chemical and improved output. All these put together could act as impetus to the use of renewable, inexpensive, and most readily lignin and lignocellulosic wastes thus reducing the production cost of biopolymer production process and will attract more the industries.

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