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NEW INSIGHTS ON LIGNIN DEGRADATION

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Abstract

Lignin is one of the primary components of lignocellulosic biomass, that hinders the depolymerization of the carbohydrate polymers. Lignin is an aromatic heteropolymer which has a complex chemical structure and a linkage heterogeneity which can lead to various aromatic compounds, that are converted into central carbon metabolism, through various microbial catabolic pathways and mechanisms. Lignin degradation can be obtained through different methods: chemical, enzymatic and microbial. Due to negative impact on the environment of the chemical approach, scientists suggest that the biotechnological pathways should be preferred. Multiple aromatic catabolic microorganisms actively secrete ligninolytic enzymes: peroxidases and laccases. The main lignin-degrading peroxidases include manganese peroxidase (MnP), lignin peroxidase (LiP) and versatile peroxidase (VP). Laccases, are usually found in plants, fungi, and bacteria and belong to the multicopper oxidase superfamily. In this review are described the most efficient approaches to depolymerize lignin in order to obtain value-added compounds.

Key words*: degradation; depolymerization; laccase; lignin; peroxidase.*

INTRODUCTION

Lignocellulosic materials consist of three major components: cellulose, hemicellulose and lignin. Lignin's matrix is closely linked to cellulose filaments and covalently connected with hemicellulose. Lignin can be found in plants, up to 30% of their cell walls. The heterogeneity of lignin determines in plants mechanical strength, defence against pathogens and water transportation to their tissues (Linger et al., 2014). Lignin is probably the most complex and least characterized molecular group among the wood components (Douglas, 1996). There is not one unique, well-defined lignin molecule with certain characteristic properties and functionalities. The structure of lignin depends on its origin, environmental factors during growth, the used extraction method and the applied pretreatment technology (Bruijnincx et al., 2016). Lignin is an amorphous biomacromolecule with a variable three-dimensional composition of p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) monomers (Figure 1). These three monomers differ in the methoxylation pattern of the aromatic ring (Douglas, 1996). The percentage of guayacil, syringyl and p-hydroxyphenyl units varies from species to species. For example: softwoods contain mainly G type lignins, so that means that are mainly G units; hardwoods contain mainly GS-type lignins, so we can find mixtures of G and S units and grass lignins contains a higher proportion of H units (Bugg et al., 2011).

Lignin biosynthesis occurs via oxidative coupling reactions starting from aromatic alcohols that differ in the degree of methoxylation: p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol (Beckham et al., 2016). The chemical composition of lignin consists of phenylpropanoid aryl-C_3 units linked through C-C and ether bonds.

ß-aryl ether is the most common connection, formed by an ether linkage to another aryl unit at C-2. The main sources of ß-aryl ether are softwoods, in which can be found up to 45-50% of units and 60% in hardwoods. Also, in softwoods a percentage of 20-25% units of biphenyl linkages can be found (Bugg et al., 2011). The diaryl propane in softwoods contains only a C–C bond to the second aryl ring, while the diaryl ether founded in hardwoods contains an ether linkage between both aryl rings (Bugg et al., 2011). These

bounds are formed through radical dimerization or polymerisation reactions from cinnamyl alcohol precursors (Freudenberg, 1965; Higuchi, 1971).

In Figure 1 are presented the major types of linkages found in lignin.

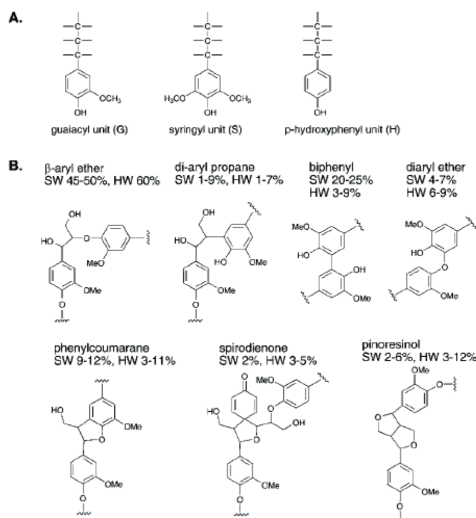

Figure 1. Structural units found in lignin,
A. Structures of G, S and H monomeric units.
B. Structures of chemical linkages found in lignin (shown as G units). SW-Softwood, HW-Hardwood (Bugg et al., 2011)

Linkages are generated by the hence reactions of the phenoxy radical which has stringent character on the "next to" aromatic carbon from the phenolic oxygen. The phenoxy radical is obtained through an oxidation by plant peroxidase and laccase enzymes, of the cinnamyl alcohol precursor (Bugg et al., 2011). Due to lignin heterogenic structure, most of the degradation processes lead to a mixture of aromatic compounds that usually are difficult to valorise (Vardon et al., 2015).

One of the main challenges in lignocellulose valorisation is considered to be lignin separation from lignocellulose components. Lignin removal is a necessary pretreatment, that will in return enhance hydrolysis of the complex polysaccharides left. This process is the most expensive step in the conversion of biomass into fermentable sugars (Karp et al., 2013). An advanced study on lignin structure and its degradation is imperative as it can lead to different value-added products, that can justify the importance of valorisation of all the components of lignocellulose.

Lignin degradation can be achieved through various types of methods: chemical, enzymatic and microbial.

CHEMICAL DEGRADATION OF LIGNIN

There are many methods used for lignin removal mainly being through thermo-chemical processes such as: organosolv process, alkaline hydrolysis, steam explosion, acid hydrolysis, wet oxidation, liquid hot water etc.

Acid hydrolysis is a method of lignin separation from biomass using dilute acid, most commonly sulfuric acid (Bruijnincx et al., 2016). In other experiments, several acids were used such as nitric, acetic, hydrochloric or phosphoric acid. Sulfuric acid process is considered to be the most efficient and low-cost method (Karp et al., 2013). An inconvenience in using these acid treatments is the formation of furfural or 5-hydroxy-methyl-furfural, that can act as inhibitors for microorganisms which are part of the degradation processes of cellulose and hemicellulose. Some organic acids such as fumaric and maleic acid were proposed as alternative treatments that will avoid the formation of 5-hydroxy-methyl-furfural (Karp et al., 2013), the efficiency being comparable with sulfuric acid process.

Alkaline hydrolysis is a pretreatment process often used to remove lignin from the biomass through saponification of ester bonds. It also removes acetyl groups from lignin and uronic acid units from hemicellulose (Karp et al., 2013).

This method uses either the Kraft process or the soda process. Lignin is dissolved in the pulping liquor (black liquor) and is present as lignin phenolate. This alkaline treatment usually performed with NaOH leads to a decrease of the degree of polymerization, crystallinity and a good degradation of the linkages between lignin and carbohydrates (Karp et al., 2013). This pre-treatment leads to extensive degradation of the native lignin structure. Kraft lignin, in contrast to soda lignin, has sulphur species incorporated in its structure.

The **Lignoboost technology** aims at extracting high quality lignin from a Kraft pulp mill. It works in conjunction with evaporation, and the

process begins by precipitating lignin from black liquor by lowering its pH with the help of CO_2. The precipitate is then dehydrated using a filter press, similar to those used in the mining and energy sectors. Conventional filtering and sodium separation problems are overcome by re-dissolving the lignin in spent wash water and acid. The resulting slurry is dehydrated again and washed with acidified water, to produce cakes of purified lignin. After acidification all phenols and carboxylic acids become protonated. The lignin obtained is highly pure with only a minor contamination of carbohydrates and ash, and 2-3% sulphur, half of which is being chemically linked to the lignin (Bruijnincx et al., 2016).

Acidic pulping of lignocellulose with excess aqueous (bi) sulphite together with either sodium, magnesium, calcium or ammonium hydroxide. The lignin ends up dissolved in the pulping liquor (black liquor) as lignosulfonate together with some degraded carbohydrates and some extractives. Lignosulfonates cannot be precipitated through by pH changing and are instead isolated through complete water evaporation (Bruijnincx et al., 2016).

Organosolv process is a treatment that uses an organic solvent mixture with or without an acid or alkaline catalyst (HCl, H_2SO_4, NaOH, NH_4OH etc.), that breaks the hemicellulose glycosidic bonds and degrade the internal structure of lignin (Sun & Cheng, 2002; Raita et al., 2017). Lignin is separated from the biomass through solubilization. The homogeneity of the organosolv processed lignin is higher than that of kraft lignins and lignosulfonates (Norgren & Edlund, 2014). Ethanol, methanol, acetone, cyclic ethers or organic acids, such as acetic acid and formic acid or combinations thereof, are often used for organosolv processing. The process is acid-catalysed, with the acid being formed in-situ from the hemicellulose fraction, or added deliberately (Bruijnincx et al., 2016).

The Bergius-Rheinau process, using concentrated hydrochloric acid at low temperature, produces a lignin fraction that is solid, water insoluble, high molecular weight and with less functional groups. Furthermore, the hydrolysed sugars and the lignin end up together with part of the hydrochloric acid, which must also be recovered (Bruijnincx et al., 2016).

Steam explosion is a most common used process for lignin degradation. The method can be performed with or without an acid or alkaline catalyst. The biomass is first grinded and then subjected to a high-pressure steam (0.69-4.83 Mpa) at 160°-260°C, followed by a rapid decompression. Besides lignin degradation, this process also helps with hemicellulose depolymerization (Karp et al., 2013). Also, fermentation to ethanol can be first performed followed by removal of the ethanol by distillation, leaving behind the lignin in the fermentation broth (Bruijnincx et al., 2016). This method is often used to depolymerize lignin after its isolation through some of the protocols described above.

Ammonia fibre expansion is another method used to degrade lignin, by suspending the lignocellulosic biomass in to a liquid ammonia at moderate pressure from 100 to 400 psi and temperature 70°-200°C. This process helps also with hemicellulose hydrolysis and decrystallizing of cellulose (Harmsen et al., 2010).

Other treatments such as **wet oxidation** performed at temperatures from 170°C to 200°C and at pressures from 10 to 12 bar O_2, for 10 to 15 minutes, are recognized as an efficient method for partial solubilization of lignin (McGinnis et al., 1983). Alkaline wet oxidation seemed to have a poor outcome of degradation compared with acid wet oxidation, where a significant part of the polysaccharides was lost (Karp et al., 2013).

Many new (catalytic) fractionation methods have been reported and might make use of **ionic liquids (Ils), liquid salts** such as zinc chloride hydrate or **deep eutectic solvents (DES)** to (selectively) dissolve lignin or cellulose from lignocellulosic biomass (Bruijnincx et al., 2016). Comparing all the main methods used for lignin degradation through chemical pathways, the major depolymerization of lignin was achieved with: alkaline treatments, organosolv process, wet oxidation and ammonia fibre expansion (Harmsen et al., 2010). Although these processes are considered effective in lignin depolymerization, they present several disadvantages, mainly high negative impact on

the environment due to the use of toxic compounds and harsh conditions or sometimes they are turning out to be expensive processes.

ENZYMATIC DEGRADATION OF LIGNIN

The most important enzymes known for their ability to oxidize lignin are: lignin peroxidase (LiP), manganese peroxidase (MnP), versatile peroxidase (VP) and laccase (Lac). Amongst them, laccase is considered to be the most suitable because it doesn't need a heme cofactor or a supply of hydrogen peroxide and the oxygen serves as the terminal electron acceptor for this enzyme (Woolridge, 2014). According to Thurston (1994) Laccase is a trinuclear blue copper enzyme involved in the catalysation of the oxidation of anilines, phenols and aromatic thiols, accompanied by four electron reduction of O_2 to H_2O, as seen in Table 2. Laccase's cluster is composed of four active site copper atoms; (Solomon et al., 1996). The copper atoms located at T2/T3 site conduct to oxygen reduction, while the copper atom located at T1 site determines the electrochemical potential (Morozova et al., 2007; Yaropolov et al., 1994). Laccases are known for their relative low redox potentials ($\leq$ 0.8 V) in comparison with ligninolytic peroxidases that have moderate redox potentials (>1 V) (Cañas & Camarero, 2010). According to Yanmis et al. (2016), it was observed that when laccase is used in combination with ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) leads to degradation of nonphenolic lignin model compounds and delignification of kraft pulp. When laccase is used in combination with a mediator (HBT – 1-hydroxybenzotriazole), veratryl alcohol is oxidized to veratraldehyde (Woolridge, 2014). Laccase coupled with a mediator is known as laccase mediator system (LMS) and usually the mediator has a low molecular weight and a low redox potential.

There are several mediators used in LMS such as NHA (N-hydroxyacetanilide), HPI (N-hydroxyphthalimide), ABTS, TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy), HBT and violuric acid. HBT and NHA combined with laccase seemed to lead to the best results (Woolridge, 2014). The mediators based on *N*-OH variety (NHA, HPI, HBT) and violuric acid were found to be the best for degradation of recalcitrant nonphenolic lignin. The nitroxyl radicals obtained oxidize their specific substrates by a mechanism that involves hydrogen atom transfer (Cañas & Camarero, 2010; Barreca et al., 2003; Cantarella et al., 2003). High redox potential laccases can oxidize the nitroxyl radical TEMPO to the oxoammonium ion through a non-radical, ionic mechanism (>N=O+) (Cañas & Camarero, 2010; Fabbrini et al., 2002). In laccase-HBT lignin was observed a better functionality of carboxylic acid than in laccase-NHA lignin (Chakar & Rgauskas, 2000). The LMS needs a low optimum oxygen pressure that can be 4tilized in used in lignin removal in the kraft pulp process. Depending on the treatments number of LMS using HBT as mediator and an alkaline extraction, the percentage of delignification can vary from 70% up to 80%. This is a strong proof that LMS with HBT as mediator is capable of degrading the most unreactive lignin (Bajpai et al., 2006). One thing observed during this degradation process was that the mediator has to be recycled constantly. However, the HBT mediator has proved to be less effective on converting to benzotriazole (Woolridge, 2014). Some concerns regarding the limitations of LMS using some mediators, and the toxicity and cost of HBT, were raised. One thing noticed was that the HBT oxidant derived from laccase is reactive, therefore supporting oxidation and inactivation of the latter, either due to adsorption to fibre either to adsorption to pulp, as seen in the case of bleaching flax pulp with *Pycnoporus cinnabarinus* laccase (Sigoillot et al., 2005).

A far better option as a mediator seems to be the synthetic NHA. This mediator is cheaper, biodegradable and sustains a better laccase retention of about 80% of LMS activity (Amann, 1997). Similar properties to NHA have been discovered in other two synthetic acids mediators: 7-cyano-4-hydroxy-2*H*-1,4-benzoxazine-3-one and *N*-(4-cyanophenyl)-acetohydroxamic acid, when utilised in the bleaching kraft process of softwood pulp (Geng et al., 2004). A 25% lignin degradation was observed when using natural phenolic mediators such as acetosyrignone and syringaldehyde (Camarero et al., 2007). A

higher rate of oxidation can be achieved with optimal pH activity (4-7) resulted from using laccases with higher redox potential together with N-OH compounds with lower redox potential.

Manganese Peroxidase (EC 1.11.1.13) catalyses the oxidation of Mn^{2+} to Mn^{3+} using H_2O_2 as an oxidizing substrate (Table 2). The reaction leads to a chelation of the manganese ion to an organic acid, such as oxalic or malonic acid. Being a strong oxidant, Mn^{3+} (1.54 V) can act on several phenolic structures found in lignin (Feijo et al., 2008). The MnP removes lignin and oxidizes residual lignin to a more accessible form to bleaching through alkaline process (Paice et al., 1997). High concentrations of Mn have been observed to lower the efficiency of the peroxide stage (Ni et al., 2000). These high concentrations, at high temperature and pH values, will generate darker pulps (Leary & Giampaolo, 1999). Manganese ion can be chelated by gluconic acid, formed when glucose oxidase and glucose produce H_2O_2 for MnP (Feijo et al., 2008). A significant impact on the fungal degradation of lignin could be represented by brightness amplification generated by MnP systems when unsaturated fatty acids (linoleic and linolenic acids) are added (Gruber et al., 1998). A reduction of a hydrogen atom from the benzyl position (Bao et al., 1994) or one electron oxidation of the aromatic ring (Srebotnik et al., 1997) are the result of the degradation of nonphenolic structures in lignin. Unsaturated lipids are oxidized to peroxyl radicals by Mn^{3+}-oxalate (Kapich et al., 1999). MnP-lipid system can oxidize compounds of nonphenolic β-*O*-4 lignin type (Jensen et al., 1996) compared to chelated Mn^{3+}, which cannot (Enoki et al., 1999).

Lignin peroxidase (EC 1.11.1.14) is a heme containing glycoprotein able to degrade non-specific lignin polymers, attacking non-phenolic compounds with β-O-4 linkages (Table 1) with redox potential up to 1.4 V (Dashtban et al., 2010). LiP can display its activity with the help of a redox mediators such as veratryl alcohol, oxidizing non-phenolic compounds that account for up to 90% of lignin, but its presence is not always need it. LiP can oxidize its substrates in multi-step electron transfers, forming unstable radical cations that will lead to non-enzymatic pathways such as rearrangements, removal of methyl groups, and cleavage of side chains (Falade et al., 2017).

Versatile peroxidase (EC 1.11.1.16) is a hybrid enzyme, because it combines the catalytic activities of LiP and MnP and therefore it is capable of degrading both phenolics and non-phenolics compounds in lignin (Table 2), including those with low and high redox potential (Dashtban et al., 2010). In comparison with MnP and LiP, VP is considered superior because it can display its activity without the help of veratryl alcohol (LiP) and Mn^{2+} (MnP).

According to Dashtban (2010), VP like other heme peroxidases (MnP, LiP) has the heme group deep inside the interior of the protein and has access to other medium through two channels, one that acts similarly to LiP (being hidden) and the other that acts like the one in MnP, where the oxidation of Mn^{2+} to Mn^{3+} happens. Lac does not need H_2O_2 to start the catalysis, compared to MnP, VP and LiP, but it does require the help of several mediators to be able to oxidize aromatic compounds in lignin.

Table 1. The main enzymes involved in lignin degradation

Enzyme	Oxidizing substrate	Cofactor	Lignin degradation
Lac	O_2	N/A	Phenolics, non-phenolics
LiP	H_2O_2	Heme	Non-phenolics
MnP	H_2O_2	Heme	Phenolics
VP	H_2O_2	Heme	Phenolics. Non-phenolics

In addition, new enzymes that act on specific linkages in lignin (ß-O-4 type), are described: aryl-alcohol oxidase (AAO), glyoxal oxidase (GOX), or aryl-alcohol dehydrogenase (AAD), as mentioned in Figure 2. One example is the ß-O-4 linkages (Beckham et al., 2016). Although enzymes can be sometimes expensive, compared with chemical approaches to lignin degradation, enzymatic depolymerization has a low cost for utilities, requires mild conditions and do not cause corrosion problems.

MICROBIAL DEGRADATION OF LIGNIN

In nature, it was observed that several microorganisms have developed some mechanisms to overpass lignin's heterogeneity. This mecha-

nism is using the so-called biological "funneling" pathways. The process is based on the microorganism's capacity to use the aromatic molecules formed as a carbon and energy source (Vardon et al., 2015). As a result of this abundance of aromatic carbon, microbes have evolved various catabolic pathways and mechanisms to utilize aromatic species. Aerobic bacteria employ 'upper pathways' for converting broad slates of aromatic compounds into a few central intermediates, such as catechol and protocatechuate (Fuchs et al., 2011). Dearomatization proceeds using reductive CoA thioesters that destabilize the aromatic ring structure and lead to common intermediates such as benzoyl-CoA (Fuchs et al., 2011; Boll et al., 2014). These intermediates can be further reduced by ATP-dependent (facultative anaerobic) or ATP-independent (obligate anaerobic) reductases to facilitate ring-opening and subsequent b-oxidation-like reactions to form central intermediates (Beckham et al., 2016). For biological funnelling to work, it will need to use a microbe being able to conduct efficient transport and catabolism of a wide spectrum of aromatic compounds simultaneously. Michalska et al. demonstrated that some bacterial ATP-binding cassette (ABC) transporters are able to transport a significant number of benzoate derivatives including vanillic acid and 4-hydroxybenzoic acid (Michalska et al., 2012). Scientists observed that microorganisms degrade lignin both through enzymatic pathways but also non-enzymatic ones. The enzymatic mechanisms involve either enzymes capable of oxidizing lignin, either enzymes that degrade cellulose and hemicellulose for a better access to lignin structure, as seen in Figure 2. The non-enzymatic mechanisms are usually assisted by oxidation through the formation of hydroxyl radicals. Therefore, many microorganisms were identified as lignin degraders (Dicu et al., 2020), mainly from fungal origin such as white and brown rot fungi. They produce H_2O_2 used either as a starter for MnP and LiP, either as a component of the Fenton reaction, in the end resulting in the formation of hydroxyl radicals, that will attack lignin and other lignocellulose components in a non-specific manner. It was also noted that these fungi produce low molecular weight compounds such as quinones, that are first converted into hidroquinones and then later subjected to Fenton reactions (Figure 2). Both white and brown rot fungi are associated with the quinone redox cycling (*Coniophora puteana, Phanerochaete. Chrysosporium).* Auxiliary enzymatic pathways for lignin degradation include: cellobiose dehydrogenase (CDH) and glycopeptides (Figure 2). CDH has been found to degrade cellulose, lignin and hemicellulose, providing hydroxyl radical through Fenton reaction. CDH is mainly synthesized by white rot fungi (*Ganoderma lucidum, Dichomitus squalens*) (Țețu, 2019). Brown and white rot fungi also produce glycopeptides (*P. chrysosporium, Gloeophyllum trabeum)*. These rot fungi are capable of catalysing redox reactions in the end also producing free hydroxyl radicals.

Several laccase isoenzymes with various optimum enzymatic activities and pH stability are synthesized by microorganisms such as *Trametes versicolor* or *Ceriporiopsis subvermispora*, that can oxidize with the lowest redox potential methoxybenzene congener (Woolridge, 2014). *C. subvermispora* could be utilized on both types of woods: hardwoods and softwoods, due to its selective properties towards lignin and extractives removal. One important aspect noted during several studies was that *C. subvermispora* can be cultivated on both *Eucalyptus grandis* and *Pinus taeda* wood chips, but only when *E. grandis* was used laccase production was detected, peroxidases being identified on both cultivation substrates (Ferraz et al., 2003). The results of different bleaching experimental studies with *C. subvermispora* and *T. versicolor* were that lignin degradation and MnP activity do not seem to be correlated (Addleman et al., 1995).

Several other microorganisms were considered as prominent solutions for microbial degradation of lignin: *Pseudomonas fluorescens, P. putida, Rhodococcus opacus, R. jostii*, *Acinetobacter baylyi*, *Amycolatopsis* sp.*, Sphingomonas* SYK-6*, Aspergillus nidulans* (Beckham et al., 2016; Albu et al., 2019). Studies showed that *P. putida* KT2440 offers significant potential, including reduced lag periods, increased biomass yield, higher growth rates, improved heterologous protein expression, higher tolerance to oxidative stress and improved cell survival in stationary phase.

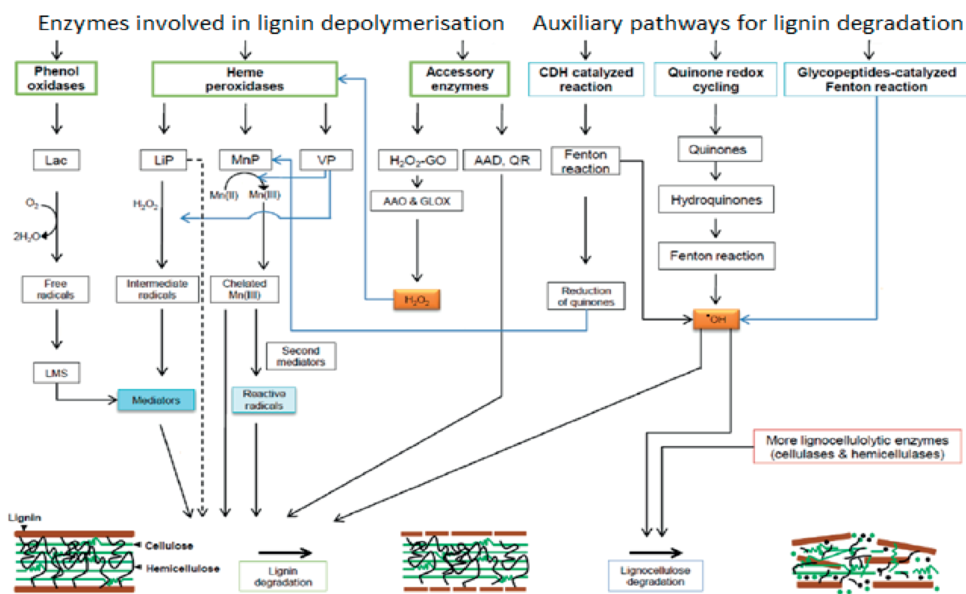

Figure 2. Microbial lignin degradation (adapted from Dashtban, 2010)

Several species have been described to accomplish aromatic catabolism via anaerobic mechanisms, including *Azoarcus* sp. CIB (Fernández et al., 2014), *Thauera aromatica* (Heider et al., 1998), *Aromatoleum aromaticum* (Trautwein et al., 2012), *Rhodopseudomonas palustris* (Fuchs et al., 2011), and others (Beckham et al., 2016). Microorganisms as *R. jostii* RHA1, *Amycolatopsis* sp., *Bacillus subtilis*, and *P. putida* MET94 have been useful in the discovery and description of lignin degrading enzymes. Salvachúa et al. (2015) recently demonstrated that multiple aromatic catabolic microorganisms actively synthesize ligninolytic enzymes, namely laccases and peroxidases, which leads to a significant extent of depolymerization of soluble, high molecular weight (HMW) lignin. According to Beckham, the resulting lower molecular weight compounds were taken up and converted to carbon storage products such as TAGs or PHAs, depending on the microbe. Subsequently, Yuan *et* al. demonstrated the addition of exogenous, commercial laccase from *Trametes versicolor* to *R. opacus* growth on commercial Kraft lignin, demonstrating a significant increase in TAG yield from 8 mg/L to 145 mg/L (Beckham et al., 2016). Delignification was realized over a wide range of acidic pH when *T. versicolor* laccase was utilized as part of a LMS (Bourbonnais & Paice, 1996). Biopulping process is fundamentally based on some fungi properties that makes them able to adsorb to wood chips and to synthesize extracellular enzymes, that will degrade lignin selectively, but will leave mostly intact the cellulose (Ferraz et al., 2008; de Souza-Cruz et al., 2004). Figure 3 presents the way guaiacol (2-methoxyphenol) is demethylated to catechol via a cytochrome P450 enzyme. Microorganisms capable of metabolizing aromatic monomers derived from depolymerized lignin, such as ferulate, phenol, 4-hydroxybenzoate, p-coumarate, vanillate or guaiacol, convert these molecules through ‘upper pathways’ to two central intermediates, protocatechuate and catechol. Protocatechuate can be decarboxylated by AroY to form catechol, which is then subjected to ring-opening to form cis–cis-muconate by CatA, a catechol 1,2-dioxygenase. Subsequently, muconic acid can be chemo-catalytically converted to (a) adipic acid via hydrogenation, or (b) terephthalic acid via isomerization, Diels–Alder reaction with ethylene, and dehydrogenation (Beckham et al., 2016). Vardon et al. demonstrated that cis, cis-muconate can be produced in an engineered

strain of P. putida KT2440 from aromatic components of lignin such as: ferulate and p-coumarate, and also the common model aromatic compound benzoate (Vardon D.R., 2015).

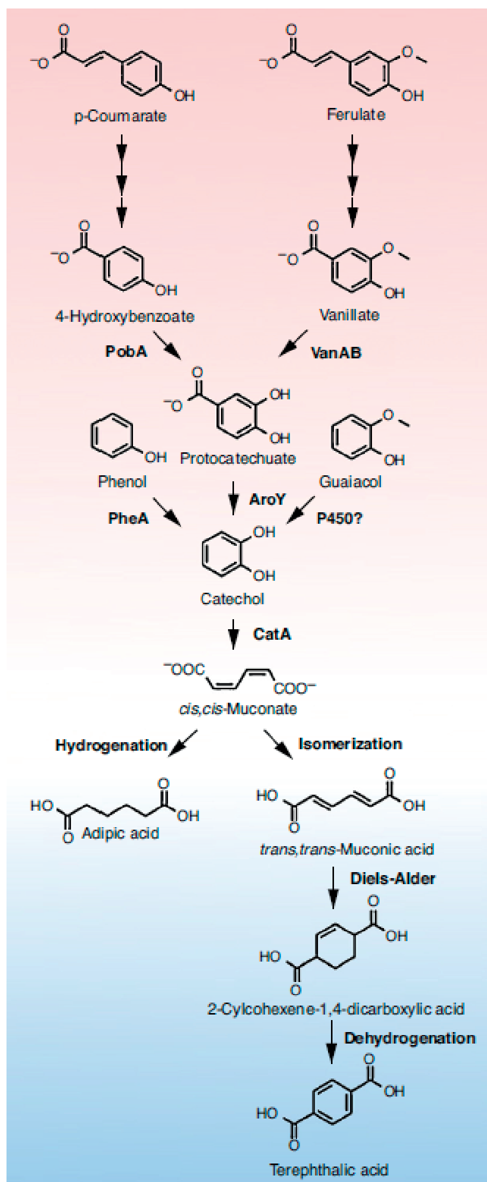

Figure 3. Metabolization of aromatic monomers derived from degraded lignin (Beckham et al., 2016)

T. versicolor and *Pycnoporus coccineus*, were proved to be some of the most prolific synthesizers of oxidative enzymes, especially laccase (Woolridge, 2014).

CONCLUSIONS

Lignin is one of the main components of lignocellulose, and has a distinctly different molecular structure and very different structural and chemical properties, that hinders the depolymerization of the carbohydrate polymers. The possibility of using native lignin for biotechnological applications is limited and therefore several steps are required in order to degrade lignin and provide value-added compounds.

In this paper, were presented the main pathways available for lignin degradation: chemical, enzymatic and microbial.

Although each one of them has both advantages and disadvantages, biotechnological approaches (enzymatic and/or microbial) are preferred due to low cost of utilities, mild conditions, lack of corrosive problems and overall less negative impact on the environment.

Enzymatic degradation of lignin involves a synergic action of four enzymes: laccase, lignin peroxidase, managanese peroxidase and versatile peroxidase, each of them with their own mechanism of action and very specific target components from lignin structure.

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