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METHODS FOR IDENTIFYING LIGNIN DEGRADING MICROORGANISMS

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Abstract

Lignocellulosic biomass has a tremendous potential for obtaining value-added products useful in applications in medicine, food and feed, textile, biofuels, carbon fibres etc. Amongst the three main components, lignin is the most abundant natural aromatic polymer that is the least valorised. The main enzymes involved in lignin degradation are mainly oxidases (laccases and peroxidases), but several esterases (hydroxycinnamic acid esterases) are also important for their activity on releasing lignin from hemicellulose and cellulose. This study is focused on describing the most used compounds that can mimic the structure of lignin components and presents the available methods used for identifying microorganisms capable of degrading lignin. Also, it provides an array of industrial applications for depolymerised lignin.

Key words*: lignin, lignocellulose, oxidase, peroxidase.*

INTRODUCTION

With the increase of demand for agricultural production due to rapid growth of world population, there is also an increase in agro-waste, mainly called lignocellulose biomass. This biomass is comprised of three main components: cellulose, hemicellulose and lignin, which are interconnected and interspersed, making it difficult to depolymerise. Although, the biomass derives mainly from agricultural waste, it can also result from forest residues, first generation feedstock, paper/pulp waste, or recycling stations (Usmani et al., 2021). Recent reports suggest that the annual production of lignocellulosic biomass is approximately 181.5 billion tons (Mujtaba et al., 2023), out of which 173.7 billion tons are not used properly and usually are discarded or burned to produce energy or steam. Advancements in this area are made for producing biofuels, environmentally friendly bioproducts, bioplastics, bio-composites (substrates for medical applications, environment remediation or inks for additive manufacturing) etc. (Coulibaly et al., 2023; Rusu et al., 2022; Mujtabai et al., 2023; Usmani et al., 2021).

Lignin is the most abundant natural aromatic polymer with a heterogenous structure and a recalcitrance to depolymerisation. The importance of degrading lignin derives from the increased degradation yield of lignocellulose materials and the possibility to obtain chemical compounds that could replace petroleum products (Ohta et al., 2012).

The valorisation of lignin from lignocellulose is one of the primary barriers to its recovery and utilization. The following issues should be considered: reducing the environmental impact of burning; preventing carbon loss and completely utilizing the lignin from waste sources (Sharma et al., 2023).

Lignin is comprised of phenylpropanoid units: coniferyl, sinapyl and p-coumaryl alcohol groups (Figure 1) which form an integral part of the cell walls of softwood (25-35%), hardwood (18-25%) or grass (15-25%) (Sharma et al., 2023). The most common linkages found in lignin are: β-O-4, α-O-4, 4-O-5, β-5, β-1, 5-5 and β- β (Figure 1).

Currently, the microorganisms used for degrading lignin are white and brown-rot fungi from *Basidiomycetes* division. Some bacteria can also degrade lignin, but their pathways are not fully elucidated. The microorganisms are producing oxidases such as laccase, manganese peroxidase, lignin peroxidase, or versatile peroxidase. Other enzymes are also known as helpful with lignin degradation and are known as auxiliary enzymes: ferulic acid esterase, p-coumaric acid esterase or acetyl xylan esterase.

These enzymes are used in the cleavage of linkages that connect lignin to hemicellulose. Although they are considered secondary enzymes, they are important in releasing lignin from the lignocellulosic materials and enabling the access to lignin structures of oxidases.

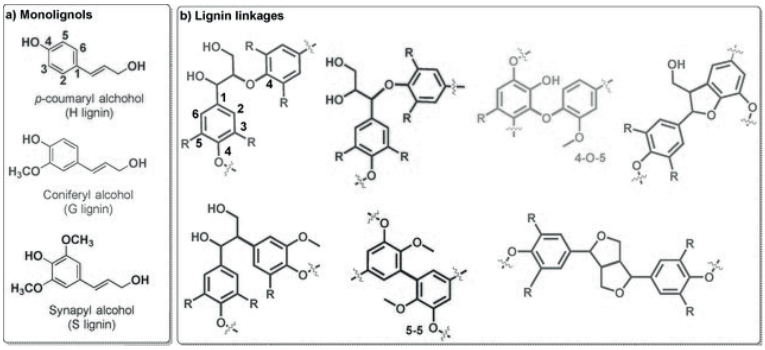

Figure 1. Structural units (a) and linkages (b) found in lignin (Garedew et al., 2020)

This study is focused on describing the most used compounds that have similar structure to lignin components and presents the available methods used for identifying microorganisms able to degrade lignin.

MOLECULAR STRUCTURES OF AROMATIC COMPOUNDS USED AS LIGNIN MODELS

In order to identify microbial strains with the ability to degrade lignin from lignocellulosic materials, the first step would be selecting substrates that can mimic structural components of lignin. According to Gonçalves (2020), the substrates used more often are either chromogenic ones, precursors of lignin or aromatic dyes. With chromogenic substrates, it is possible to quantify the oxidases enzymatic activity and therefore to develop high-throughput screening methods.

The most used chromogenic substrates suitable for oxidases (laccases or peroxidases) involved in lignin degradation are: ABTS (2,2′-Azinobis-(3-Ethylbenzthiazolin-6-Sulfonic Acid)), 2,6-DMP (2,6-dymethylphenol), guaiacol, o-dianisidine, L-DOPA (l-3,4-dihydroxyphenylalanine), veratryl alcohol, 2,4-DCP (2,4-dichlorophenol), phenol red, TMPD (N,N,N',N'-Tetramethyl-p-phenylenediamine dihydrochloride), vanillylacetone (Figure 2).

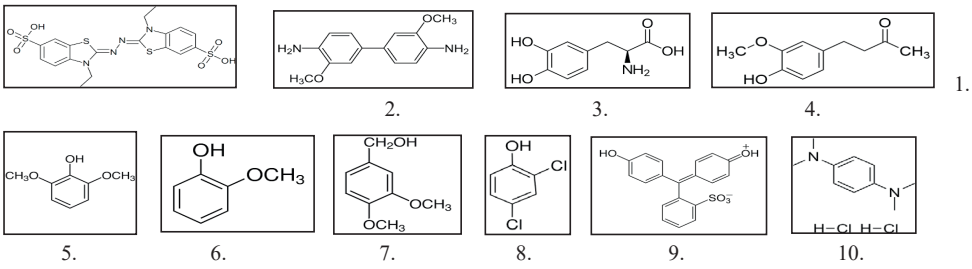

Figure 2. Substrates used for investigating lignin degradation: ABTS (1), o-dianisidine (2), L-DOPA (3), vanillylacetone (4), 2,6-DMP (5), guaiacol (6), veratryl alcohol (7), 2,4-DCP (8), phenol red (9), TMPD (10)

The aromatic dyes used are: Azure B, Remazol Brilliant Blue R, toluidine blue, Remazol Marine Blue, malachite green, induline, methylene blue (Figure 3). The use of dyes in liquid media (malachite green, methylene blue, toluidine blue) allow the evaluation of lignin degradation by measuring the optical density at 620 nm (Bandounas et al., 2011; Melo-Nascimento et al, 2018).

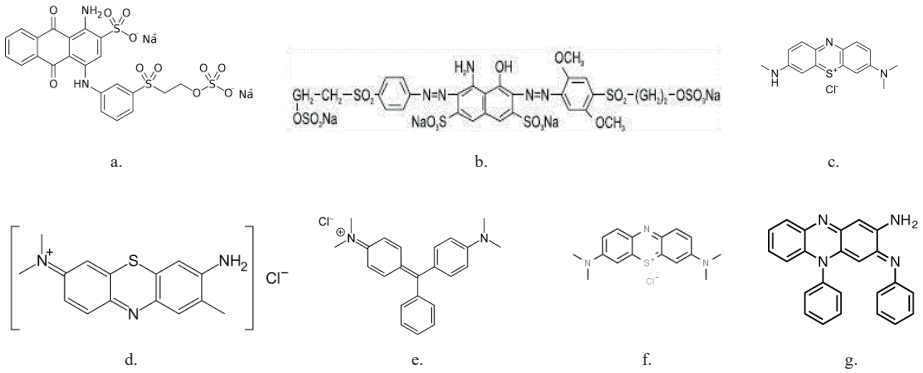

Figure 3. Aromatic dyes used for identifying microorganisms capable of degrading lignin
a. Remazol Brilliant Blue R, b. Remazol Marine Blue, c. Azure B, d. toluidine blue, e. malachite green, f. methylene blue, g. induline

Other studies (Ohta et al., 2012) suggest to use compounds with similar structures as lignin precursors: p-coumaryl alcohol (p-coumaric acid, 4-vinyl phenol, protocatechuic acid, p-hydroxybenzoic acid), coniferyl alcohol (ferulic acid, 4-vinyl guaiacol, vanillin, veratryl alcohol) or sinapyl alcohol (sinapic acid, 4-vinyl syringol, syringaldehyde) (Figure 4).

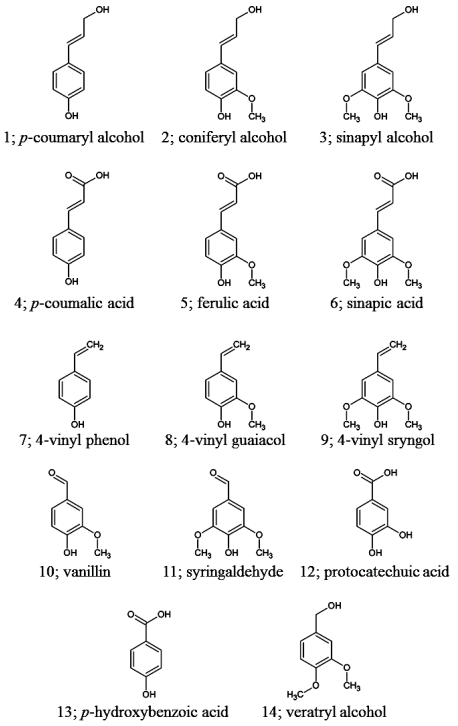

Figure 4. Aromatic compounds with similar structure to lignin precursors (Ohta et al., 2012)

For auxiliary enzymes mentioned before, the most used substrates for identifying microbial producers are: α- or β-naphtyl acetate, p-methylumbelliferyl acetate, p-nitrophenyl acetate or even chemically acetylated xylan (for acetyl xylan esterase); methyl ferulate, ethyl ferulate, vinyl ferulate, prenyl ferulate, L-arabinose ferulate, p-nitrophenyl ferulate, feruloyl glucose (for ferulic acid esterase); methyl p-coumarate, ethyl p-coumarate, p-coumaroyl glucose (for p-coumaric acid esterase) (Popa et al., 2020a; Popa et al., 2020b; Antonopoulou et al., 2019; Li et al., 2019; Ramírez-Velasco et al., 2016; Collombel et al., 2019).

METHODS FOR DETECTING LIGNIN DEGRADING ACTIVITIES

Lignocellulose biorefineries could convert lignocellulose biomass to high value-added bioproducts. Due to lack of high producers of multiple enzymes that could degrade lignin, there is a need for screening novel microorganisms for their ability to depolymerise lignin. Therefore, screening methods for lignin degradation have to be optimised or developed in order to detect lignin degrading activities rapidly and at lower costs.

The most widely used methods for isolating microorganisms that produce ligninases are cultivation-based methods, some of them being well established.

Usually, the qualitative screening of lignin degrading fungi or bacteria is performed using

natural substrates that resembles the structure of lignin.

There are many methods employed for evaluating lignin degradation that vary in detection limit and sensitivity, but usually the most frequently used are colorimetric and enzymatic assays.

Growth-based assays

An easy and efficient method for evaluating lignin-degrading activities is described by Ravi et al. (2017). In this study, an enriched vegetal compost is used to isolate three *Pseudomonas* strains with high ligninases activities and are compared to the activity of *Pseudomonas putida* KT2440. Several lignin model compounds are used in order to characterise the growth of the isolates: vanillin, vanillate, ferulate, 4-hydroxybenzoate, p-coumarate and benzoate. The results indicate that specific growth rates were considerably higher on p-coumarate, benzoate and 4-hydroxybenzoate than those on ferulate and vanillate. There is no growth on vanillin, mainly due to the fact that the substrate is rapidly converted to vanillate. The results also highlight the fact that vanillin, benzoate and 4-hydroxybenzoate are preferentially consumed first and ferulate last. With this research, the scientists are able to understand better the metabolic pathways of microorganisms and how they include aromatic compounds. However, the method can only be used for identifying microbial strains that are easy to work with (Ravi et al., 2017).

Colorimetric assays

Bandounas et al. (2011) isolates soil bacteria (*Pandoraea norimbergensis* LD001, *Pseudomonas* sp. LD002, *Bacillus* sp. LD003) by enrichment of Kraft lignin and use them for comparing their lignin degrading activities. The capacity to degrade lignin is determined based on three assessments: ability to grow on high and low-molecular weight lignin fractions, ability to use lignin aromatic monomers and ability to decolourise several dyes (Azure B, toluidine blue O, Congo red, methylene blue). The results indicate that the strains have a slow and small growth on the lignin fractions, the ability to use lignin monomers is also limited for the isolates and the best dye-decolourizing activity is noted for *Bacillus* sp. LD003. The advantage of this method was that it resulted in an accurate evaluation of degradation of lignin monomers. The disadvantage was that the activities of ligninases can vary with different types of lignin.

Another screening method (Jeong et al., 2012) is based on identifying enzymes capable of degrading synthetic organophosphates (based on fluorescence). The advantage of this method is that it is possible to improve the enzymatic activity by using phenolics responsive transcription activator. The disadvantage is that the enzymatic activities registered with synthetic molecules may not be similar to those in natural substrates with high lignin content.

In the method proposed by Melo-Nascimento et al. (2018), 21 ligninolytic strains (*Klebsiella* spp.) are screened based on their ability to grow on culture media containing lignin as the main carbon source. In media supplemented with dye (methylene blue, toluidine blue, Congo red, malachite green), the best decolourization (98% after 48 hours) is observed with *Klebsiella* P3TM1 in the presence of methylene blue. The advantage of this method is that it provides a better understanding of the mechanism of depolymerisation of lignin, since the study concluded that the breakdown of lignin is based mostly on the extracellular activities of peroxidases produced by *Klebsiella* spp. The disadvantage of this method is that results may vary based on using different lignin models.

A method for isolating lignin degrading microorganisms is presented by Taylor et al. (2012). In this research, microbial isolates (*Microbacterium* sp., *Ochrobactrum* sp., *Micrococcus* sp., *Thermobifida fusca*, *Rhodococcus erythropolis*, *Sphingobacterium* sp., *Rhizobiales* sp.,) are cultivated on high and low molecular Kraft lignin. Lignin degradation is measured with the products obtained (protocatechuic acid and oxalic acid), the best activity being registered with *Sphingobacterium* isolate. Although the method is successful in detecting new microbial strains able to produce ligninases, it is only applicable to samples with nitrated lignin.

A method proposed by Zhou et al. (2017) uses Azure B as a target molecule for identifying microorganisms capable of degrading lignin. The microorganisms tested are isolated from termite gut (*Enterobacter hormaechei*, *Bacillus licheniformis*). The method is also used to

identify fungi capable of producing lignin peroxidase. The advantage of this technique was that it is low cost, but it is however time consuming and requires high amounts of enzymes.

Enzymatic assays

An easy and accessible method for evaluating degradation of ABTS with *Trametes versicolor* laccase can be performed spectrophotometrically, as suggested by Alcade et al. (2005). The presence of enzymatic activity is observed when a green colour is developed suggesting the formation of ABTS radical. Although the substrate used is soluble, stable and available, this screening method is less sensitive, therefore not accurate enough and time consuming (Gonçalves et al., 2020).

In a research lead by Huang et al. (2013), 140 bacterial strains are isolated from soil and screened based on their ability to oxidise ABTS (known as a substrate for laccase). The two selected strains (*Bacillus pumilus* and *Bacillus atropheus*) are able to degrade guaiacylglycerol-b-guaiacylether (the most abundant linkage in lignin) and also fragments of kraft lignin. Although the method allows the identification of ligninolytic strains, it is also time consuming and only limited to laccases activities (Gonçalves et al., 2020).

Laccase activity can also be identified using guaiacol supplemented PDA plates, the enzymatic activity being observed as reddish-brown zones around microbial colonies (Abd El Monssef et al., 2016).

Spectrophotometric assays with nitrated lignin

Ahmad et al. (2011) describes a scalable and semiquantitative method for identifying microorganisms able to degrade lignin using nitrated milled wood lignin. The breakdown of chemically nitrated lignin produces nitrated phenol products which lead to changes in absorbance at 430 nm. With this assay, the researchers identified several bacterial strains (*Rhodococcus jostii* and *Pseudomonas putida*) that produce peroxidases that can degrade lignin to low molecular weight phenolic byproducts. Unfortunately, the method is growth dependant and time consuming (Gonçalves et al., 2020).

The research proposed by Taylor et al. (2012) involves spraying a nitrated lignin solution on inoculated agar plates. The bacterial strains are identified based on a signal (fluorescent yellow) produced which is measured spectrophotometrically at 430nm.

Chromatographic methods

Reverse phase HPLC can be an useful method to detect the products of lignin depolymerisation. In a study (Taylor et al., 2012), *Microbacterium* sp., *Rhodococcus erythropolis* and *Sphingobacterium* sp. are cultivated on Luria-Bertani broth supplemented with wheat straw lignocellulose in order to establish the amount of oxalic acid produced (formed by oxidation of glycolaldehyde generated). Additionaly, *Microbacterium* sp. produces protocatechuic acid, an intermediate molecule in aromatic degradation pathways. The disadvantage of this analytical method is that same mass to charge ratio (m/z) in various compounds may give deceiving results (Gonçalves et al., 2020).

Other studies (Ohta et al., 2012) use LC/MS or GC/MS to identify and quantify products obtained from lignin degradation such as: ferulic acid and p-coumaric acid. The substrates related to lignin structure are: vanillin, p-hydroxybenzoic acid, protocatechuic acid, sinapic acid, p-hydroxybenzaldehyde, ferulic acid, p-coumaric acid and veratryl alcohol. Supplementation with ferulic acid and p-coumaric acid is justified, since research shows that they can boost enzymatic activity of ligninases (especially laccases) (Kuhar and Papinutti, 2014). Although this method is able to highlight the biotransformation pathways of lignin depolymerisation, compounds with similar m/z ratio can lead to deceiving results (Gonçalves et al., 2020).

FTIR

Fourier-transform infrared spectroscopy is used as a technique for evaluating the changes in the chemical composition of lignin (from lignocellulosic materials) during degradation with enzymes produced by *Penicillium simplicissimum* (Liu et al., 2014). The analysis shows cleavage of ether linkages, demethylation and oxidation, lignin being transformed to pseudo-lignin.

Native gel electrophoresis

Native gel electrophoresis was employed for detection and quantification of lignin degradation since 1989 (Adhi et al., 1989), and

since then several researches optimised this method (Kumar et al., 2017; Sun et al., 2004). The polyacrylamide gels contain different substrates in order to detect enzymatic activities: O-tolidine (laccase activity) or L-DOPA (lignin peroxidase activity). The microorganisms tested with this technique are: *Streptomyces badius*, *Trametes gallica* and *Ganoderma lucidum*. Although these methods are accessible, rapid and can work with crude enzymes, they require a substantial amount of enzyme since they are less sensitive (Gonçalves et al., 2020).

Capillary electrophoresis

Kudo et al. (2017) describes a capillary electrophoresis method used for evaluating the activities of manganese peroxidase and lignin peroxidase (produced by *Phanerochaete chrysosporium*). The substrates required are veratryl alcohol and Mn(II) malonate. Since lignin peroxidase activity is overestimated in the presence of manganese peroxidase and vice versa, with this method the researchers are able to distinguish between the activity of both enzymes in a mixture without separation. However, with this method there are several disadvantages such as protein adsorption to capillary walls and small injectable sample size.

Chemiluminescent method

The base for several diagnostic immunoassays is peroxide-dependent oxidation of luminol by horseradish peroxidase, but peroxidases from other sources can also catalyse luminol oxidation.

Mercer et al. (1996) presents a chemiluminescent assay with an Amerlite analyser used to examine the peroxidase activity of a taxonomic range of actinomycetes (*Saccharomonospora* sp., *Thermomonospora* sp., *Streptomyces* sp., *Actinomadura* sp.). With this method, the researchers are able to identify producers with high extracellular peroxidase activity. Although the chemiluminescent method is sensitive to peroxidase and permits the use of the crude enzyme, it has short luminescence, weak signals, is sensitive to external factors and the apparatus is not widely available.

To address the issues with this method, Zhang et al. (2018) suggests the importance of high chemiluminescent intensity in providing low detection limit for analytes and high sensitivity. This is obtained with the use of enhancers such as derivatives of: lophine, p-phenol, substituted boronic acid or combination of N-alkyl phenothiazines and nucleophilic acylation catalysts as co-enhancer. Also, the use of amplification techniques can lead to higher sensitivity of chemiluminescence detection.

$^{14}CO_2$-autoradiography

Temp et al. (1998) develops a method for screening lignin degrading microorganisms using synthetic lignin (DHP-^{14}C ring labelled dehydrogenation polymerizate). Although the use of this method can easily, rapidly and accurately detect microbial strains with the ability to depolymerise lignin, is sensitive to culture conditions, it requires specific expertise and is time consuming and laborious.

Sequence-based methods

DNA-based methods can allow the ability to evaluate lignin degrading activity by searching and selecting genes encoding for key enzymes involved in lignin depolymerisation. Several databases (NCBI, PATRIC, KEGG, UniProt, Pfam, ExPASy) are useful for this approach, however eLignin is considered to contain the most published research regarding aspects such as: microbial strains, enzymatic systems, metabolic pathways, metabolites etc. (Gonçalves et al., 2020). Also, several annotation tools offer the possibility to study the molecular functional diversity at different levels: metabolic context subsystems, protein families and individual.

Biosensor-based methods

Biosensor-based methods are expected to overcome the shortcomings of conventional screening methods, including identifying novel enzymes and/or aromatic molecules involved in lignin degradation (Gonçalves et al., 2020).

Several studies (Eggeling et al., 2015; Rinaldi et al., 2016; Fritzsch et al., 2012; Ho et al., 2018; Siedler et al., 2017) are focused on whole-cell biosensors used to measure aromatic lignin derivatives such as p-coumaric acid, vanillin and syringaldehyde. These methods have limited sensitivity and accuracy but they are able to detect extracellular products and can have a broad range of applications (Gonçalves et al., 2020).

Other biosensors are employed to detect genes encoding for enzymes involved in lignin

depolymerisation (Uchiyama and Miyazaki, 2013; Alvarez-Gonzalez and Dixon, 2019; Mannan et al., 2017). Genetic Enzyme Screening System (GESS) is a biosensor that targets the phenol and cresol molecules. It is a quantitative enzyme biosensor and has a high sensitivity to targeted molecules. However, it only measures intracellular concentrations of compounds (Choi et al., 2014).

Substrate Induced Gene Expression (SIGEX) is a biosensor that measures aromatic compounds such as: chlorohydroquinone, 4-chlorocatechol, 3-methyl catechol and salicylate (Uchiyama and Miyazaki, 2013). This biosensor can be used to identify targeted genes, but it only measures intracellular concentrations. Also, most of the transcription regulator functions are still unknown (Gonçalves et al., 2020).

APPLICATIONS OF DEPOLYMERISED LIGNIN

Lignin degradation can generate various value-added products that can be tough, water and heat resistant, durable, friendly to the environment (Sharma et al., 2023). The bio-products obtained with lignin depolymerisation are: acids (ferulic acid, muconic acid, p-coumaric acid, acetic acid, acylic acid, lactic acid hydroxybenzoic acid, 3-hydroxypropinoic acid, adipic acid), benzene, pyrogallol, xylene, guaiacol, toluene, polyhydroxyalkanoates etc. These compounds are useful to various biotechnological applications: biofuels (biogas, bio-oils), bioremediation, pharmaceutics, cosmetics, bioplastics, textiles, biosensors, agrochemicals (biofertilisers, regulators for plant growth), nanocomposites, carbon fibres, dyes, flavoring compounds, sustainable construction materials etc. (Burlacu et al., 2018; Sharma et al., 2023; Ahmad et al., 2021; Xia et al., 2021; Chauhan et al., 2021; Sivagurunathan et al., 2021; Jędrzejczak et al., 2021).

Biofuels

Although bioethanol production from cellulose is environmentally friendly, the cost of the process is more expensive than that of fossil fuels. Therefore, lignin valorisation to obtain biogas could enhance the economics of lignocellulose biomass refineries. Biogas can be obtained through anaerobic digestion from hydrolysed lignin produced through steam explosion pretreated biomass. With this process, a high yield of volatile compounds is produced (even higher with microbial fermentation) facilitating the complete valorisation of lignocellulosic biomass (Sharma et al., 2023).

Lignin derived compounds can be converted by microorganisms into lipids through aromatic catabolism and later the lipids can be used for biodiesel production (Saini et al., 2022).

Pharmaceutics and biomedicine

Lignin has low cytotoxicity, biocompatibility, UV absorption capacity, and antioxidant and antibacterial qualities. Furthermore, many processed biomaterials can have their mechanical strength increased by lignin. As a result, lignin is a potential aromatic raw resource for the biomedical and pharmaceutical industries. The recent developments in the valorization of lignin involves creation of wound dressings, tissue engineering, medication and gene delivery systems, and sunscreen actives (Domínguez-Robles et al., 2020).

Bioplastics

By creating biodegradable and environmentally friendly bioplastics from renewable lignocellulosic biomass, the petrochemical industries may become less dependent on them. Using lignocellulosic wastes as substrates, a range of microorganisms can create polyhydroxyalkanoates (PHAs), or biopolyesters. PHAs have the potential to replace non-renewable petrochemical plastics because of their superior thermomechanical properties, biodegradability, UV stability, and biocompatibility. The development of synthetic biology and metabolic engineering methods creates new avenues for the affordable synthesis of PHAs from bacteria utilizing lignin as the substrate (Xia et al., 2021). Brown et al. (2022) research involves the overproduction of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) from the breakdown product of lignin, p-coumarate.

Biosensors

Due to the tremendous rise of portable point-of-care testing devices based on disposable electrodes, it is necessary the production of disposable electrodes in large quantities in an economical and environmentally friendly way.

Meng et al. (2022) provides a method for converting lignin biomass, patterning, reducing the lignin derived graphene electrodes, water lift-off and sequential laser lithography to obtain electrochemical lactate biosensors. These disposable electrodes made from lignin may help ensure the sustainable production of biosensors for point-of-care and point-of-use applications by combining low-energy manufacture and patterning with renewable resource utilization.

Agrochemicals

In the recent years, there is a growing trend in producing biodegradable agrochemicals that can replace toxic chemicals and fertilizers. Lignin degradation of lignocellulosic waste can be a solution for this task. With a growing interest in research and development on lignin-derived agrochemicals, several value-added products could be used for biofertilizers, plant growth regulators, insecticides and soil improvers (Sharma et al., 2023).

Nanocomposites

Two types of uniform lignin nanoparticles (LNPs) are prepared by self-assembling of deep eutectic solvent (DES) and ethanol-organosolv extracted technical lignins derived from a two-stage fractionation pretreatment approach, respectively. In contrast to the DES-LNPs, which displays a more homogeneous particle size distribution, both LPNs display sphere shape and a distinct core-shell nanostructure. These LPN products show great potential to form a transparent nanocomposite film with additional UV-shielding efficacy and antioxidant properties when incorporated into the traditional polymeric matrix such as poly(vinyl alcohol) (Tian et al., 2017).

Carbon fibres

Out of all the possible applications of lignin depolymerisation, obtaining carbon fibres has attracted a great interest during these last years. Lignin-based carbon fibres can lower the production cost of this fiber by more than 35% (Souto et al., 2018). Since final properties of lignin based carbon fibers are not satisfactory (mechanical properties), several attempts are made in this direction including optimization of processing parameters (Wang et al., 2022).

Flavoring compounds

Aromatics like guaiacol, syringol, catechol and vanillin could be obtained through enzymatic conversion of lignin. These aromatic compounds are in demand due to their usefulness as flavouring components.

CONCLUSIONS

Since lignin's chemical composition is complicated and varies with the source, the substrates are limited in the full structural resemblance with native lignin. Therefore, the next step would be to screen the selected microorganisms with natural substrates that have a high lignin content.

The methods presented in this paper are important for providing several ways to identify microbial strains with lignin degrading capabilities, while broadening the understanding of metabolic pathways of lignin depolymerisation. With these methods it is also possible to identify new biocatalysts, enzymes or even genes involved in lignin degradation, which can be useful for future biotechnological approaches for lignin valorisation.

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