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Review Article

# Lignin from a disposable by-product to a repository of value-added compounds

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## ABSTRACT

Across the planet, lignin is widely distributed as one of the most pervasive polymers in existence. It naturally exists as an inherent component of the plant's structure. In the paper industry, kraft lignin (KL) disposal in large amounts as a waste by-product may potentially result in toxicity to aquatic ecosystems. We can get more value from this plentiful polymer and convert it to value-added products instead of its disposal. Several methods have been described to degrade lignin. These procedures are frequently severe and harmful to the environment. Out of them, biological methods are eco-friendly, ensure consistent production, and cause lower toxicity to the environment. Due to their extensive environmental adaptability and easy genetic engineering, numerous ligninolytic bacteria have been found and studied for lignin use. This includes biodegradation and valorization of lignin into commercial compounds such as fuels, phenolic compounds, ferulic acid, polyhydroxyalkanoates (PHAs), and vanillin. Besides, ligninolytic enzymes produced from several microorganisms have tremendous applications in other industrial fields including textile dye effluent decolorization and bioremediation. This review elucidates the current approaches to lignin degradation. We give an overview of the recent research on the discovery and application of bacterial ligninolytic enzymes and their various optimization strategies. This article also includes the new applications for the utilization of lignin as an economical and alternative-resource material either in the medical field or through biological conversion to value-added products and highlights future perspectives for the improvement of the lignin biodegradation process.

**Keywords:** *Kraft lignin; Ligninolytic enzyme; Lignin derived aromatics; Vanillin; Ferulic acid*.

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## 1. Introduction

Worldwide, lignin is considered one of the foremost widely distributed polymers. It exists naturally as an intrinsic part of lignocellulose, a structural component that makes up the plant biomass. In nature, the lignin polymer is not found alone; rather, cellulose and hemicellulose are firmly physically associated with it **[1]**. According to chemical structure, lignin is a complicated polymer made up of aromatic building blocks called phenylpropanoids, or monolignols. The main phenylpropanoid units are p-hydroxyphenyl, syringyl, and guaiacol, which are symbolized by the initials H, S, and G, respectively. These units vary mainly in the number of methoxy groups they have on the aromatic ring **[2, 3]**. The phenylpropanoid units are linked by each other through carbon-carbon and ether bonds to form lignin's structure **[4]**. The amount of lignin in the biosphere is more than 300 billion tons, and it grows by 20 billion tons annually **[5]**. We could consider lignin as an essential polymer. It is a tremendously significant

renewable supply of aromatic compounds. But even to this point, it has stayed to be the most insufficiently utilized substance **[6]**. The majority of the technical lignins that are currently on the market were retrieved from the pulping or delignification processes. There are many delignification techniques such as soda, sulfite, and kraft pulping **[6]**. Among them, the most common delignification procedure in the paper industry is kraft pulping. Almost about 130 million tons of kraft lignin (KL) are produced every year **[7]**. Disposal of KL in large quantities during the pulping process can cause serious pollution and toxicity in aquatic ecosystems **[8]**. By converting this plentiful polymer into high-value compounds, we can obtain considerably more value from it. This review describes different methods of lignin degradation, highlights environmentally friendly ones, and the different applications of this promising field.

## 2. Approaches of lignin degradation

### 2.1. Thermal and chemical depolymerization

The key feature of thermal and chemical depolymerization reactions is the requirement of heat to decompose the lignin structure into smaller molecules in the presence or absence of catalysts **[9]**. Hydrolysis, alkaline oxidation, rapid pyrolysis, and hydrogenolysis are some examples of thermochemical reactions. Depolymerization is partially assisted in the presence of base, metal, or acid catalysts, with heating reactions that can reach up to 600 °C **[10]**. These techniques produce different substances which vary depending on the process parameters and the variety of lignin used **[6, 11]**. About 30 to 45% of the originally depolymerized lignin can undergo undesirable repolymerization which has a detrimental impact on the yield of such processes and remains to be optimized **[5]**. Thermochemical processes have benefits such as quick reaction times and high monomer yields. However, these methods have several disadvantages and obstacles that must yet be solved which include high char production, harsh reaction conditions, undesirable repolymerization, and low selectivity of depolymerization. These disadvantages make these procedures more difficult to obtain the resulting products **[12]**.

### 2.2. Biological depolymerization

As demonstrated, due to difficulties in the thermal and chemical depolymerization of lignin **[13]**, it is thought that lignin depolymerization using enzymes and microbes could be beneficial **[14]**. Initial research showed that bacterial consortia **[15]** and individual strains **[16]** could depolymerize lignin. Undoubtedly, it is still a great challenge to gain effective enzymes for lignin depolymerization. The use of biological processes for lignin depolymerization with the aid of microorganisms and enzymatic systems is a trustworthy, functional, and effective technique when compared to chemical processing. Typically, lignin is broken down into smaller units or aromatics by microbial systems and then further transformed into substances with a high value through different metabolic pathways during lignin bioprocessing **[17]**.

## 3. Mechanisms of lignin biodegradation

The breakdown of lignin by microorganisms occurs mainly in two main stages. Firstly, the depolymerization or decomposition of the lignin polymer results in the production of a variety of phenolic compounds. Secondly, the modification or degradation of those phenolic substances derived from lignin. Microorganisms that can degrade lignin can take part in both processes; however, participation in any step of them may be also viewed as having lignin-degrading potential **[18]**. Several mechanisms of lignin biodegradation have been reported, including hydroxylation, oxidation, reduction, and methylation **[19, 20]**.

### 3.1. Enzyme-based depolymerization of lignin

The depolymerization process by microorganisms depends on the presence of several oxidative enzymes including peroxidases, laccases, oxidoreductases, and oxidases **[21]**. These enzymes can produce free radicals or reactive intermediates that can begin depolymerization by penetrating the resistant structure of lignin converting polymeric lignin into various aromatic compounds or monolignin such as ferulate, phenol, caffeate, benzoate, and cresol (**Fig. 1**) **[6]**. Those tiny fragments may be interesting chemicals on their own or even after minor modification and this is considered one method of the lignin valorization process to create substances of high value.

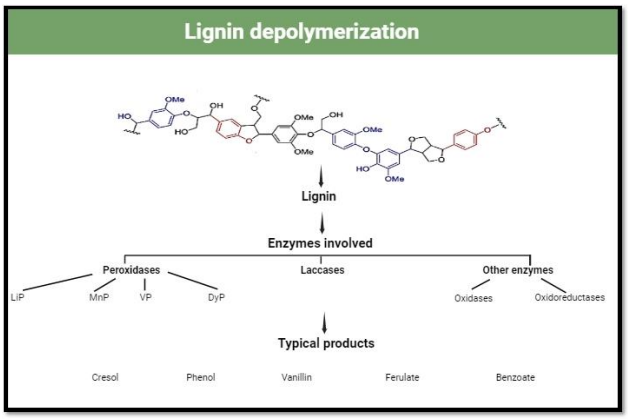


**Fig. 1**. The most probably involved ligninolytic enzymes in the initial step of lignin breakdown. LiP: lignin peroxidase, VP: versatile peroxidase, MnP: manganese peroxidase, DyP: dye decolorizing peroxidase.

### 3.2. Enzyme-based catabolism of lignin-derived compounds

The second step of lignin degradation is the catabolism of the produced aromatics. In this step, the microorganisms have a collection of converging catabolic pathways that they use to convert the mixture of aromatic chemicals produced during the initial stage of lignin breakdown into central intermediates. As shown in **Fig. 2**, those catabolic pathways also include two steps. Firstly, a wide variety of enzymes are used in the processing of phenolic products to produce central intermediates such as protocatechuate and catechol. Followed by the deed of other enzymes which are accountable for aromatic ring cleavage of these intermediates by different modes to produce various metabolites that provide carbon through different routes to microbial systems for energy production, growth, and synthesis of different products **[22]**. Several beneficial products may also be retrieved from this step including cis, and cis-muconate which is considered a promising functional substitute chemical **[23]**. This substance is a direct precursor to the industrial mass chemicals adipic acid and terephthalic acid, which are used in the production of commercial plastic **[13]**.

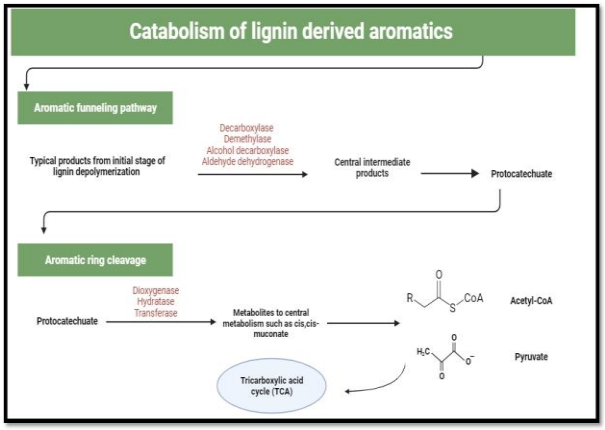


**Fig. 2.** The second step of lignin breakdown (catabolism of the produced aromatics)

## 4. Lignin degrading microorganisms

### 4.1. Lignin degrading fungi

The primary studied originators of ligninolytic enzymes are White-rot fungi. They have a high capacity for lignin depolymerization by their various enzymes mainly peroxidases and laccases **(Table 1)**. However, few investigations have succeeded in lignin breakdown by fungi on a commercial scale due to their complicated biological processes **[24]**. They are challenging to grow and have complex and intricate synthetic metabolic mechanisms which make the manufacture of their enzymes an uncertain industry **[6, 25]**.

**Table 1.** Examples of well-known microorganisms that produce ligninolytic enzymes

| Microorganism | Enzymes | Substrate | Reference |
|---|---|---|---|
| **Fungi** | | | |
| *Phanerochaete chrysosporium* | Peroxidase | Lignin | **[30]** |
| *Fusarium oxysporum* | Peroxidase, Laccase | Kraft black liquor | **[1]** |
| *Trichoderma reesei* | Laccase, Peroxidase | Lignin | **[31]** |
| *Lenzites betulina* | Laccase, peroxidase | Alkaline lignin | **[32]** |
| *Trametes versicolor* | Laccase, peroxidase | Alkaline lignin | **[32]** |
| **Bacteria** | | | |
| *Amycolatopsis* sp. *75iv2* | DyP | Lignin model compounds | **[33]** |
| *Rhodococcus erythropolis* | Laccase, peroxidase | Kraft lignin | **[1]** |
| *Rhodococcus jostii* | Laccase, peroxidase | Kraft lignin, lignin model compounds | **[30]** |
| *Bacillus atrophaeus* | Laccase | Kraft lignin | **[34]** |
| *Enterobacter lignolyticus* | Laccase, DyP | Alkali lignin | **[35]** |
| *Oceanimonas doudoroffii* | Peroxidase | Lignin | **[36]** |
| *Ochrobactrum tritici* | Laccase, peroxidase | Kraft lignin | **[37]** |

DyP; Dye decolorizing peroxidase

### 4.2. Lignin degrading bacteria

Bacteria are expected to have additional types of ligninolytic enzyme groups that are not present in fungi **(Table 1)**. In comparison to fungi, bacteria are easier to genetically modify and can endure a larger range of pH, temperature, and oxygen levels with enormous stability against various inhibitory agents, cost-effective production in a short period, broad substrate specificity, and ease of cloning and expressing in the host with the proper manipulation **[26-28]**. The commercial manufacturing of MetZyme® LIGNO™ (Finnish company MetGen Oy), was a significant step towards enzymatic lignin breakdown. This laccase enzyme can operate in high temperatures and extreme alkaline pH levels, providing an important role in the lignin valorization process in biorefineries. The recombinant synthesis of enzymes that function in challenging environments becomes highly desirable in the future, and extending the range of enzymatic actions may accelerate the integration of these enzymes into industrial use **[29]**.

### 4.3. Examples of naturally occurring lignin-degrading bacteria

#### 4.3.1 *Pseudomonas putida*

Similar to other soil bacteria, *P. putida* makes use of lignin by secreting laccase and peroxidase. A certain strain of *P. putida* was engineered to use the protocatechuate meta-cleavage pathway to convert lignin monomers into pyruvate. This genetic modification resulted in the production of 1.4 g/L of pyruvate **[22]**. However, the release of toxic intermediates and products during this process is still challenging to get rid of in *P. putida* by metabolic engineering. There is a lot of promise for lignin valorization in

*P. putida*, but little study has been done on the metabolic engineering of this organism to use lignin **[38]**.

#### 4.3.2. *Rhodococcus* sp

*Rhodococcus jostii* was identified as a natural lignin-degrading bacteria by the production of the DyP type B enzyme. The metabolic engineering of *R. jostii* RHA1 was applied to convert lignin to value-added products and this resulted in the production of 2,5-dicarboxylic acid in a yield of 80-125 mg/L from lignin [39]. Another species of *Rhodococcus* called *Rhodococcus opacus* was identified for generating lipids from KL. When *R. jostii* and *R. opacus* PD630 were combined, there was synergistic activity during the conversion of aromatic compounds, lignin breakdown, and lipid production. These strains co-fermentation, producing lipids that account for 39% of the dry cell weight **[40]**. This outcome demonstrates that, under some circumstances, the co-fermentation of strains in lignin biorefinery is promising. *Rhodococcus* strains demonstrate sufficient tolerance and activity for lignin consumption in light of these findings.

#### 4.3.3. *Streptomyces* sp

*Streptomyces* are among the identified bacteria capable of degrading lignin. Several *Streptomyces* strains have been reported to degrade lignin such as *Streptomyces viridosporus* T7A and *Streptomyces setonii* 75Vi2 **[41]**. Recent studies have been conducted for genomic characterization of *Streptomyces* sp. which proved the ability to use KL as the carbon source in preliminary screening experiments **[41, 42]**. The genomic sequences of *Streptomyces* sp. revealed the presence of several genes encoding enzymes responsible for lignin depolymerization and catabolism of lignin-derived aromatics providing more indication for the ability of *Streptomyces* sp. in potential application in lignin degradation **[42]**.

#### 4.3.4. *Bacillus* sp

Even though it has been demonstrated that some *Bacillus* strains may disintegrate lignin, their lignin-degrading mechanism is still unknown, and their rate of lignin degradation is lower than that of fungi. Most *Bacillus* sp. barely degraded lignin at a general rate of 15–18% **[43]**. Numerous investigations are underway to enhance the degradation of lignin by *Bacillus* species through the utilization of statistical optimization methods like Response Surface Methodology (RSM) **[44, 45]**. To increase our understanding of this bacterium, it is therefore necessary to investigate more effective ligninolytic *Bacillus* strains **[43]**.

## 5. Bacterial enzymes involved in lignin degradation

The use and application of bacterial ligninolytic enzyme systems have expanded rapidly in recent years due to their numerous exceptional characteristics compared to fungi from an industrial point of view. Comparatively less research has been done on bacterial ligninolytic enzymes than on their fungal counterparts up until this point **[26]**.

### 5.1. Lignin depolymerizing bacterial enzymes

Unlike fungal lignin-depolymerizing oxidases, bacterial lignin depolymerization is dominated by dye-decolorizing peroxidase (DyP) and laccase.

#### 5.1.1. Dye-decolorizing peroxidase (DyP)

In the biochemical research on ligninolytic bacteria, homologs of the most prevalent fungal ligninolytic peroxidases, lignin peroxidase (LiP), manganese peroxidase (MnP), and versatile peroxidase (VP), have not been found. Additionally, no homologs are found when analyzing the sequenced genomes or proteomes of ligninolytic bacteria. These lignin-degrading peroxidases appear to be unique to fungi.

However, it has recently been discovered that bacteria have rather high levels of a different form of peroxidase, known as Dye decolorizing peroxidase (DyP) **[46]**. DyPs is a newly identified family of heme-containing peroxidases that have gained attention recently for their capacity to break down lignin and other high-redox synthetic dyes such as anthraquinone and azo dyes **[47-49]**. This family of peroxidases was named as a consequence of research on the enzyme's activity with synthetic anthraquinone and azo dyes. Even though DyPs are distinct in structure from the typical fungal peroxidases, they share almost the same catalytic characteristics, redox potentials, and reactivities. The PeroxiBase database has categorized DyPs in to four classes based on sequence features **[50]**. Class A, B, and C of DyPs are primarily found in bacteria, while class D DyPs are found in fungi. Class A DyPs are generally secreted because they have a Tat-signal sequence. Class B and C DyPs, in contrast, lack any secretion signal peptides in their DyP protein sequences. However, class B- and C-type DyPs normally don't have a secretion signal, this might not rule out an external enzyme function. The extracellular fraction of the *Rhodococcus jostii* RHA1 DyPB mutant displayed great activity towards nitrated lignin, indicating that DyPB is located extracellularly. Therefore, it has been suggested that this enzyme may be exported via a different method, maybe through the encapsulation and subsequent secretion of DyPB. The encapsulation gene is found together in 14% of the B-type DyPs' operon. These DyPs often include a C terminal extension of 30-40 amino acids, making these enzymes targets for encapsulation by protein-based cages, known as encapsulins **[51]**. In comparison to DyPB alone, the encapsulated form showed an eight-fold increased activity against nitrated lignin. This suggests that encapsulation somehow facilitates DyP-mediated lignin breakdown **[52]**. Currently, there are around 8318 DyP sequences in the InterPro database of these enzymes, and only about thirty have been isolated and described **[53]**. DyPs don't require calcium binding sites, glycosylation, or disulfide bonds, are less complex in terms of their protein structure, and are found widely in bacteria. Recently, several bacterial DyP-type peroxidases have been attributed to the lignin and lignin model compound degradation. Many bacterial DyPs have been demonstrated to work on KL, a waste product of the paper industry, thus making them an attractive option for industrial applications **[54, 55]**. Indeed, via oxidizing redox mediators, DyPs appear to be able to induce lignin breakdown similarly to the normal fungal LiPs, MnPs, and VPs. Although the obtained compounds haven't been described, HPLC examination has shown that there are many breakdown products **[55]**. In contrast to the expression of fungal peroxidases, recombinant expression of several DyPs in *E. coli* often produced high levels of expression. This makes these bacterial peroxidases intriguing candidates for industrial applications **[56]**.

### 5.1.2. Laccase

Laccase, also known as multicopper oxidase, is a copper-containing enzyme that oxidizes a wide variety of compounds **[49]**. In nature, laccases are found in plants, insects, fungi, and bacteria. However, most laccases known are of fungal origin, recently, bacterial laccases have attracted a lot of attention regarding their potential function in the breakdown of lignin and other biotechnological applications. Recent developments in genome analysis and other techniques have made it possible to identify a large number of laccases in bacteria. Actinomycetes, specifically species of *Streptomyces*, are the actinomycetes whose bacterial laccases in the breakdown of lignin have received the most research **[57]**. According to a recent comprehensive study, different bacterial

laccases can be created in *E. coli* by adjusting the expression factors **[58]**. Two possible types of mechanisms are catalyzed by laccase: direct substrate oxidation and indirect substrate oxidation. In the direct process, the reaction occurs due to direct contact of the substrate with the enzyme resulting in the oxidation of the substrate into its radical form. The indirect reaction involves a two-step process in which an enzyme oxidizes the mediator first then the mediator oxidizes the substrate. A mediator is a small substance that is continually oxidized by the enzyme and reduced by the substrate **[19]**. Because of its ability to oxidize a wide range of chemicals utilizing $O_2$ and only produce $H_2O$ as a byproduct, laccase is regarded as a perfect "green catalyst". It does not result in the hazardous byproduct of typical oxidases, hydrogen peroxide **[19]**. The created radical products can go through additional oxidation or different processes including hydration, or disproportionation.

### 5.1.3. Other enzymes involved in lignin depolymerization

Other enzymes take part in lignin depolymerization. According to previous reports, oxidoreductase enzymes are involved in the depolymerization of lignin. They produce reactive intermediates and free radicals as part of their function to disintegrate lignin and aromatic compounds **[59]**. The enzymes needed to supply the necessary hydrogen peroxide to the bacterial peroxidases represent one family of enzymes that have been identified. Similar to how these oxidases have been identified for fungal peroxidases, it is anticipated that bacteria also release oxidases **[55]**.

## 5.2. Enzymes involved in catabolism of lignin-derived aromatics

This step is dominated mostly by bacterial sp. compared to fungi. Several converging catabolic pathways and different enzyme types are used in this step to transform the variety of aromatic compounds created during the first stage of lignin breakdown into other products. The simple carbon molecules can then enter the microorganisms' metabolic and growth cycles. Therefore, these substances are utilized for cell development and product synthesis. Following depolymerization, lignin monomers can be processed by bacteria through two frequent processes: biological funneling and ring cleavage. The resulting substances can be absorbed via the common metabolic routes following ring cleavage, such as the citric acid cycle and the Krebs cycle **[60]**. Biological funneling is a complex set of different pathways involving many different enzymes, such as decarboxylases, alcohol, and aldehyde dehydrogenases. The most created intermediate product during the funneling process is protocatechuate. The final products can then proceed to the ring cleavage pathway for the conversion of funneling products into compounds that can be taken up by the cellular metabolic pathways, which is the second stage of catabolism. Oxygenase enzymes are the most common types of enzymes for ring cleavage in an aerobic environment. A thorough analysis identified a group of secreted enzymes called dioxygenases initially isolated from a *Streptomyces* sp. involved in aromatic ring cleavages of produced aromatics. The protein sequence suggested that it was produced by the union of an intraradiol dioxygenase and a carbohydrate-binding module. It was interestingly found that the carbohydrate-binding module showed an affinity for synthetic lignin polymers **[61]**. The cleavage may occur in either the ortho (between the two hydroxyl groups) or Meta (near the hydroxyl groups) positions. Two enzymes, protocatechuate dioxygenase, and catechol dioxygenase, are implicated in the ortho-cleavage routes. Both oxygen atoms are

incorporated into the ring-cleavage product by these enzymes. On the other hand, estradiol dioxygenases are the names of the enzymes responsible for meta-cleavage. Some transferase and hydratase encoding genes were also found to play a part in the breakdown of the aromatic molecule benzoate **[62]**. The intermediate products can be further processed into the products of the tricarboxylic acid cycle (TCA). The destiny of the ring-cleavage products is unknown at this time. Depending on the types of microbes using these products **[63]** For instance, acetyl-CoA is utilized by lysates in the oleaginous species to create fatty acids **[64]**.

## 6. Examples of lignin-derived valuable products

The enzyme-based degradation of lignin produces several aromatic compounds or substances that can be used either directly or can be transformed into other substances of high value. Therefore, lignin serves as a feedstock of valuable products. There are two approaches for obtaining value-added products: one involves depolymerizing lignin, and the other involves the degradation or biotransformation of compounds generated from lignin. Enzymatic processes might be used to depolymerize lignin to obtain aromatic lignin-derived chemicals, which would then be converted by the metabolic pathway. As shown in **Table 2**, different hosts could produce a variety of value-added products through the degradative pathways **[65]**. Additionally, genetic engineering could be applied to produce products with added value by introducing various important genes into the host cells. For instance, by expressing a heterologous route from *Pseudomonas putida*, an engineered *E. coli* strain could utilize protocatechuate as the only source of carbon and energy, offering a useful method for improving the biotransformation of lignin-derived aromatics to valuable products **[66]**. Likewise, *Pseudomonas putida* KT2440 may handle several substances originating from lignin, such as transforming guaiacol into catechol, by expressing an exogenous O-demethylase gene from *Amycolatopsis* sp **[67]**. It implies that genetic engineering may help increase the production of lignin-derived compounds with added value. Additionally, we can treat the substances produced from depolymerization processes as substrates for further valorization processes. When ferulic acid was provided as substrate in *Amycolatopsis* sp. ACTT 39116 with knocked out vanillin dehydrogenase gene, an increase in vanillin accumulation was achieved **[68]**. In engineered *E. coli*, vanillin can be employed as a substrate for catechol and vanillic acid production **[69]**. To valorize lignin successfully, it is essential to choose the right substrates, and host cells and necessitate careful thought to lower value-added production costs. Here are some examples of valuable compounds which can be gained through lignin biodegradation.

### 6.1. Fuels

As an example of biofuel production, *Rhodococcus opacus* has been employed for generating lipids from technical lignin, including kraft lignin, and has gained attention in this regard as a possible biofuel source **[71]**. The high output was most likely caused by enhanced lignin release of aromatic monomers, less inhibition through lignin stream detoxification, and a favorable reaction environment such as sufficient oxygen supply and pH optimization during the fermentation process. In an efficient fed-batch fermentation, Liu et al. **[81]** produced a high lipid titer (1.83 g/L) upon optimization of the fermentation process while using corn stover as a substrate.

**Table 2.** Valuable substances produced from the biodegradation of lignin or lignin-derived compounds through various microorganisms

| Microorganism | Substrate | Product | Reference |
|---|---|---|---|
| *Rhodococcus opacus* PD630 (WT) | Kraft lignin | Lipid | **[70]** |
| *Rhodococcus opacus DSM1069* | Kraft lignin | Lipid | **[71]** |
| *Pseudomonas putida* A514 | Lignin/vanillic acid | PHAs | **[72]** |
| *Pseudomonas putida* | Ferulate | PHAs | **[73]** |
| *Pandoraea* sp. ISTKB | Kraft lignin | PHAs | **[74]** |
| *Pandoraea* sp. ISTKB | Vanillate | PHAs | **[74]** |
| *Pseudomonas* sp. HR199 | Eugenol | Vanillin | **[75]** |
| *Escherichia coli* | Eugenol | Vanillin | **[76]** |
| *Staphylococcus lentus* (SB5) | Kraft lignin | Vanillin | **[77]** |
| *Sphingobacterium* sp. M4115 | Benzoate | cis,cis-muconate | **[78]** |
| *Pseudomonas putida* KT2440-MA9 | Lignin | cis,cis-muconate | **[79]** |
| *Pseudomonas putida* IDPC/pTS110 | Vanillate | cis,cis-muconate | **[80]** |

PHAs; polyhydroxyalkanoates

### 6.2. Phenolic compounds

Through the process of depolymerization, lignin can yield multiple phenolic compounds. One of the most important aromatic compounds is phenol which is used in oral preparations to treat pharyngitis. Additionally, it is used as a precursor of several products including, bioplastics, resins, herbicides, and medications (most notably aspirin by heating phenol with NaOH & $CO_2$ in an acidic medium) **[82]**.

### 6.3. Ferulic acid (FA)

Ferulic acid from natural sources has been discovered to possess some significant biological and therapeutic characteristics **[83]**. Under its antioxidant properties, it has a neuroprotective and antiaging effect. Besides, it has antibacterial, anti-inflammatory, and anticarcinogenic attributes. Ferulic acid is essential for curcumin, coniferyl alcohol, sinapic acid, vanillin, and other significant chemical compounds synthesis **[84]**. A study was conducted on *Streptomyces* sp and successfully increased the enzymatic production of FA from defatted rice bran **[85]**. They proposed that these enzymes could be employed to extract FA from various sources of lignin-containing plants. There are several reports of biotransformation of FA into vanillin by *Sphingomonas paucimobilis* SYK-6. This was accomplished by the action of several enzymes, including feruloyl-CoA synthetase (FerA) and feruloyl-CoA hydratases/lyases (FerB and FerB2). It then produces vanillin, and vanillin may also be converted into pyruvate and oxaloacetate, both of which are regarded as useful by-products **[86]**.

### 6.4. Organic acids

Another example of lignin valorization includes the production of pyridine-2,4-dicarboxylic acid (2,4-PDCA) and pyridine-2,5-dicarboxylic acid (2,5-PDCA) by a naturally occurring lignin-degrading bacterium, *Rhodococcus jostii* RHA1 after applying genetic engineering technique. These dicarboxylic acids can be used as constituent parts of aromatic polymers like polyesters and polyamides. The two alternative meta-cleavage pathways were added to protocatechuate's natural ortho-cleavage pathway for achieving these organic acids synthesis. The recombinant genes for protocatechuate's aromatic ring cleavage were inserted to carry out the design. The two genes were encoding for protocatechuate 4,5-dioxygenase and protocatechuate 2,3-dioxygenase from *S. paucimobilis*, and *Paenibacillus* sp, respectively. After recombination and upon culturing this strain in a medium containing wheat straw, it produced 80 mg/L of 2,4-PDCA and 125 mg/L of 2,5-PDCA **[39]**.

### 6.5. Polyhydroxyalkanoates (PHAs)

Polyhydroxyalkanoates are polyesters that are highly valuable for use in a variety of application domains, such as tissue engineering, medication delivery, or environmentally friendly packaging due to their biodegradability and biocompatibility **[87]**. Many microorganisms were tested for their capacity to utilize lignin for the production of valuable products like PHAs [88]. In the study of K. Numata et al. **[89]**, six types of marine bacterial strains were screened for their ability to synthesize PHAs from lignin derivatives. Their results exhibited that *Oceanimonas doudoroffii* could directly synthesize PHA from lignin derivatives. Additionally, Yan Shi et al. **[90]** improved the production of PHAs by 319.4 mg/L from *Cupriavidus basilensis* B-8 in a fermentation medium supplied with KL as the sole carbon source. Other bacteria were also able to produce PHA from lignin or lignin-related aromatic compounds, including *Pandoraea* sp **[91]** and *Pseudomonas putida* **[92]**.

### 6.6. Vanillin

Vanillin is commonly utilized in the food, beverage, perfumery, cosmetic, and pharmaceutical industry. It is considered the most popular aromatic flavoring substance **[26, 93]**. Vanillin can be obtained via bioconversion of various natural substrates, such as lignin. Several studies found that bacterial strains such as *Pseudomonas* sp., *Escherichia coli*, *Bacillus* sp., and *Streptomyces* sp. can manufacture vanillin from FA. However, getting a good yield is still difficult **[93]**. Recently, Bachel et al. **[77]** screened 5 bacterial strains for their ability to synthesize vanillin from KL. Their study showed that maximum vanillin production was achieved by mixed culture after incubation with KL for 6 days. In their study, increasing KL concentration from 0.25 to 2 g /L resulted in a rise in vanillin production from 6.62 to 72.55 mg/L. Another study was reported on the production of vanillin by *Bacillus pumilus* ZB1 using guaiacyl lignin monomers as the substrates **[94]**.

## 7. Applications of lignin in the medical field

Besides being a source of value-added products, lignin itself has been used for a variety of biomedical applications in multiple reported works, either directly or as composite materials. The fact that lignin is biocompatible, and biodegradable makes it beneficial to use in this industry. Since lignin is regarded as an antioxidant, anti-bacterial, and anti-viral substance, it can be utilized as a medicine to treat a variety of illnesses. It has also been utilized for tissue engineering and medicinal delivery **[95]**. In the biomedical industry, lignin nanoparticles are frequently used, particularly for covering

biomedical devices, gene delivery, and personal care **[96]**. In particular for anticancer medications, many researchers have employed lignin and lignin nanoparticles for drug delivery. For instance, the use of lignin nanoparticles in the delivery of anticancer medicines was extensively discussed by Garg et al. in 2022 **[97]**. However, research is still ongoing to fully harness its capabilities in this domain.

## 8. Industrial applications of ligninolytic enzymes

It is approved that ligninolytic enzymes possess a vast array of industrial uses. Besides, the production of numerous fine compounds from lignin and detoxification of pulp produced from paper industry processes, they also have applications in food processing, bioremediation, and textile dye effluent decolorization. The transformation of waste lignocellulose into valuable substances like biofuels and fine chemicals has been reported as the most significant application **[98]**. They can be used, for instance, to transform lignin into aromatic compounds, which are used to make chemicals, polymers, and fuels. Employing them to remove lignin from pulp fibers instead of harsh chemicals like chlorine will have a smaller negative impact on the environment. Ligninolytic enzymes can be utilized to break down a variety of resistant pollutants, such as insecticides, and aromatic hydrocarbons. They help clean up the environment since they may transform toxic substances into less dangerous forms **[19]**. In the food industry, certain procedures that improve the color look of food and beverages and get rid of unwanted phenolic compounds can be done with laccase enzymes. Additionally, peroxidases may contribute to the generation of aromatic flavors **[99]**. Moreover, they have significant applications in textile dye effluent decolorization due to their ability to remove and degrade dyes and other textile pollutants in wastewater treatment. They can efficiently break down and remove colors, reducing the amount of water pollution caused by the textile industries **[18, 100, 101]**.

## 9. Various optimization strategies for lignin-degrading enzymes

Many conventional and contemporary technologies have been employed to improve the ligninolytic enzymes' ability to degrade lignin and to increase yields with better performance for usage in the industrial field **(Table 3)**. The conventional processes are mostly focused on the selection of enzyme-producing organisms, enzyme optimization, and biochemical characterization. Contemporary technologies depend on multiple strategies such as genetic and metabolic engineering of organisms in which DNA can be altered, protein engineering to improve strategies for the specificity, stability, and economy for chemically modifying enzymes, **[19]** and utilization of statistical optimization strategies for the creation an applied model of optimization **[45, 102]**.

## 10. Challenges and future perspectives

Despite substantial advancements in lignin biodegradation, research conducted over the past 10 years has revealed some challenges with biological lignin valorization such as low enzyme or product yield because of the lack of knowledge about the intricate mechanisms underlying lignin biodegradation. In the future, the following aspects will be taken into account for the improvement of biological lignin degradation:

First, to increase biological lignin processing for producing value-added products, bioprospecting needs to be broadened to utilize efficient ligninolytic microorganisms and enzymes. It is promising to keep looking for new lignin-degrading enzymes and to tailor them for greater selectivity and efficiency.

**Table 3.** Approaches for optimization of ligninolytic enzymes

| Improvement strategy | Improved Characteristics | Reference |
|---|---|---|
| Genetic engineering and solid-state fermentation | Improved recombinant protein production | **[103]** |
| Immobilization of laccase enzyme on the surface of yeast cells using synthetic biology techniques. | Improved laccase activity and stability | **[104]** |
| Substrate binding-pocket mutagenesis | Narrowing substrate specificity and improved catalytic efficiency | **[105]** |
| Heterologous expression, recombinant expression, and affinity chromatography | Improvement in production, time, and cost | **[56]** |
| Solid-state and submerged fermentation | Production cost and optimization | **[106]** |
| Statistical optimization by using Response surface methodology | Improvement in ligninolytic enzyme production | **[107]** |

Second, only a few studies have been published recently that describe the function of recombinant enzymes in the breakdown of lignin **[56]**. To find the optimum pathways for targeted compound production from lignin using ligninolytic microorganisms, it is important to fully comprehend the genomes of lignin degrading organisms and metabolic pathways for lignin breakdown. High-throughput omics techniques, such as genomics, proteomics, and metabolomics, could provide deeper insights into lignin degradation pathways and mechanisms.

Third, to improve the catalytic property and speed up the processing of lignin, innovative synergistic systems of ligninolytic microbes and enzymes must be developed **[108]**. Investigating the synergistic interactions between several microbes using microbial consortia with complementary functions or by combining particular lignin-degrading enzymes, will allow for the efficient and targeted lignin feedstock source degradation to produce particular value-added products **[56]**.

Finally, the most accurate prediction is that industries will warmly embrace an increase in the efficiency of lignin conversion into bioplastics, biofuels, food additives, and other biochemical components. Consequently, the combination of a synergistic enzyme mixture with a metabolically designed microbe may become the leading lignin biorefinery **[38]**.

In the future, lignin biodegradation and value-added product production will depend on multidisciplinary collaboration, innovative technologies, and sustainable practices, contributing to a more environmentally friendly and resource-efficient future.

## 11. Conclusion

In recent years, lignin research has made great progress due to its potential to act as a renewable source of alternative fuels. In the chemical industry, the importance of producing aromatics from lignin is growing due to the current scarcity of petroleum and its high price. Several studies have indicated that biological lignin processing is a potential approach for achieving higher lignin conversion efficiency and can improve biorefinery sustainability by reducing the chemical used for lignin breakdown and the process costs in biorefineries. Thus, bio-based techniques have been suggested for each step of the lignin degradation and valorization process. Recent research on ligninolytic bacteria has identified a broad arsenal of enzymes for the degradation of the linkages connecting lignin building blocks, and they also have several mechanisms for transforming lignin-derived monomers into central intermediates and value-added products with various applications in several fields. Lignin-derived compounds might find applications in industries such as cosmetics, pharmaceuticals, and agriculture. Therefore, biological lignin valorization is anticipated to have tremendous market growth. Consequently, it is assumed that this field has undergone substantial research and is still being investigated currently and, in the future, by collaboration of several innovative technologies to unlock the full value of lignin as a renewable resource.

## Declarations

### Consent to publish

All authors have read and agreed to the published version of the manuscript

### Ethics approval and consent to participate

Not applicable

### Availability of data and material

All data generated or analyzed during this study are included in this published article in the main manuscript.

### Competing interests

The authors have no financial or non-financial benefits to relate.

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### Author contribution

Sarah Milad has collected the data for the manuscript under the supervision and guidance of authors; Sarra E Saleh, Mohammad M Aboulwafa, and Nadia A. Hassouna. Sarra E Saleh and Mohammad M Aboulwafa have helped in writing and revising this manuscript. All authors have read and approved the final manuscript.

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