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Review

Biocatalysis for Lignin Conversion and Valorization: Driving Sustainability in the Circular Economy

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Abstract: In recent years, lignin derived from lignocellulosic biomass has emerged as a critical component in modern biorefinery systems. The production yield and reactivity of lignin are critical factors for advancing the research and development of lignin-derived biochemicals. The recovery of high-purity lignin, along with carbohydrates, is accomplished through the application of various advanced pretreatment techniques. However, biological pretreatment using lignin-degrading enzymes to facilitate lignin depolymerization is an environmentally benign method for the sustainable production of valuable products that occurs under mild conditions with high substrate specificity. The current review presents the role of biocatalysis in lignin valorization, focusing on lignin-degrading enzymes that facilitate different bond cleavage in the lignocellulosic biomass. The review also highlights the recent advancements in enzyme engineering that have enabled the enhancement of enzyme stability and catalytic efficiency for improving lignin valorization processes. Furthermore, the integration of omics technologies that provide valuable insights into the microbial and enzymatic pathways involved in lignin degradation is presented. The challenges and future prospects in this emerging field of study for a biorefinery concept are also outlined for improving lignin depolymerization efficiency.

Keywords: circular economy; biorefinery; lignin; lignin-degrading enzymes; omics technologies

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1. Introduction

Overreliance on fossil fuels and their combustion as a primary energy source, with approximately 82% dependency in 2020, is a major driver of climate change, a global crisis that has created significant challenges across the world [1]. Natural resource scarcity is an escalating concern worldwide, which is driven by rapid population growth and increasing industrialization and is exerting considerable pressure on and straining the finite resources of the world, ultimately causing shortages in many areas [2]. Moreover, by 2040, it is anticipated that the worldwide consumption of natural gas and oil will rise by 22% and 16%, respectively [3]. Global warming, and environmental warming and environmental

degradation, are mostly caused by carbon dioxide emissions, which make up around 75% of greenhouse gas emissions [1,4]. By 2030, greenhouse gas emissions are predicted to increase by as much as 50% [5]. Apart from greenhouse gas emissions, the accumulation of solid waste is also a major threat to the environment. Global waste generation is projected to reach 3.40 billion tons by 2050, outpacing population growth by more than double during the same period. By 2050, daily per capita waste production in high-income countries is projected to increase by 19%, compared to at least a 40% increase in low- and middle-income countries [6].

In light of the current scenario, the effective processing of solid waste for energy production, coupled with the utilization of renewable, eco-friendly energy sources, offers a viable replacement for fossil fuels. This shift can contribute to reducing global greenhouse gas emissions and advancing sustainable development goals [7]. Globally, lignocellulosic biomass (LCB) contributes 181.5 billion tons to total waste production, but merely 8.2 billion tons are used across different applications, leaving the majority untapped. LCB has enormous potential to forage the constantly rising energy needs of various sectors [8,9]. The main structural components of LCB are cellulose (35–50%), hemicellulose (20–35%), and lignin (15–30%), which is a polyphenolic aromatic polymer [10]. The polysaccharide components of LCB, i.e., cellulose and hemicellulose, have been effectively utilized for the production of biofuels to substitute conventional oils in the transportation section and other valuable products such as levulinic acid, furfural, pyrones, and furfuryl alcohol, leaving behind lignin as a waste [11,12]. However, lignin has historically been considered a roadblock in the conversion of LCB into useful products due to its rigid and recalcitrant nature. Its strong binding to cellulose and hemicellulose has frequently been seen as a significant barrier to the saccharification enzymes' easy access to complex carbohydrates [13]. The perception of lignin has evolved from being considered a "waste" polymer to a valuable resource. Its potential as a critical raw material for industrial use lies in its ability to replace a variety of products derived from diminishing fossil-based resources [14]. The natural abundance of lignin and the fact that it is produced as a byproduct, particularly in the pulp and paper industry and cellulosic ethanol biorefinery, suggest that it may be used as a raw material for valuable bioproducts with a variety of commercial uses. The lignin reserves in the biosphere grow by an estimated 20 billion tons each year, amounting to a total of approximately 300 billion tons. Meanwhile, the paper and pulp industry produces approximately 50 million tons of lignin annually as a byproduct. Moreover, aside from established fossil-based chemical processes, lignin stands as the sole natural source of aromatic chemicals [15].

Traditional lignin valorization techniques limit the use of lignin as a low-quality solid fuel to generate heat and electricity. Instead of burning or disposing of the lignin, which exacerbates environmental issues, its valorization can be implemented through the circular economy approach [12,16]. Lignin is a versatile raw material that can be converted into valuable compounds, including p-hydroxybenzoic acid, ferulic acid, adipic acid, pyrogallol, guaiacol, benzene, xylene, and polyhydroxyalkanoates. Its applications extend to pharmaceuticals, drug delivery systems, agrochemicals, carbon fibers, and nanocomposites. Additionally, lignin acts as an absorbent for toxic metal ions, is used in dye manufacturing, aids in biosensor synthesis, and contributes to flavoring agents such as eugenol, guaiacol, and catechol [15,17,18]. The circular economy promotes sustainable growth and development and facilitates the effective achievement of sustainable development objectives [19]. The concept of a circular economy can be realized through an integrated biorefinery approach, which enables the efficient valorization of lignin from LCB for the production of various useful products. The integrated biorefining of LCB can also help in its efficient channelizing to produce chemicals, heat, electricity, and biomaterials in

addition to biofuels, making it a desirable energy source [3]. The four main stages of most integrated biorefineries are biomass collection and storage, pretreatment, enzymatic hydrolysis, and conversion.

The pretreatment stage is important for the breakdown of linkages between cellulose, hemicellulose, and lignin, enabling the extraction of each component followed by conversion into useful bioproducts [20]. However, most of the lignin depolymerization methods operate under extreme conditions, including high temperatures, substantial energy requirements, and the use of costly and corrosive chemicals, leading to additional waste disposal costs and also loss of certain valuable linkages in the lignin structure [21]. Consequently, the use of lignin-degrading biocatalysts provides a sustainable and efficient alternative to mitigate the challenges associated with conventional pretreatment approaches. Enzymes are highly specific for their substrates, work under milder conditions, and produce no or fewer byproducts [22]. However, there are still a number of obstacles that must be overcome in order to bridge the gaps between scientific discoveries and practical applications. Many lignin valorization methods have been applied only at the laboratory scale, with limited studies addressing scalability and cost-effectiveness. Furthermore, rather than high-value uses like pharmaceuticals, specialty chemicals, or innovative materials, the emphasis has mostly been on low-value products (such as fuels or bulk chemicals). These obstacles call for creative solutions and interdisciplinary cooperation.

This review explores the immense potential of integrated biorefineries in efficiently converting LCB, particularly lignin, into valuable products. It delves into lignin sources, various lignin-degrading biocatalysts, and the microorganisms involved in the process. The review also highlights the advancements in omics technologies and protein engineering for improved lignin degradation. Additionally, it outlines the challenges and discusses future opportunities in lignin utilization.

2. Integrated Biorefineries in a Circular Economy for Lignin Valorization

The linear economy has historically been used to produce biofuels and biochemicals, which are promising substitutes for their traditional petroleum-based counterparts. The linear economy is a model based on the "take-make-dispose" approach. In this system, raw materials are extracted (take) from the environment, and used to form products (make), and once these products reach the end of their life, they are discarded as waste (dispose). This model does not account for the sustainable use or recycling of materials leading to resource depletion, increased waste generation, and environmental pollution. However, in opposition to this, the concept of the circular economy has gained tremendous attention over the last few years [23,24]. The cornerstone of the circular economy system is the 5R principle of sustainable development, which represents reduce, reuse, recycle, recovery, and restore. The circular economy offers a logical waste disposal method for a holistic approach to economic development by integrating the flow of bio-waste from one industry as a raw material for another industry, to produce value-added products [25]. A circular economy offers the advantages of eco-efficiency and improved resources, reduced dependence on fossil reserves, valorization of waste, and lower greenhouse gas emissions. The circular economy has the potential to develop a more sustainable and environmentally friendly environment and, therefore, can be considered a low-carbon economy [26,27]. For LCB, efficient fractionation and utilization of each key component are essential for establishing a successful circular bioeconomy. Biorefining is a strategic approach that aligns perfectly with circular economy principles by converting biomass into value-added products.

The concept of "biorefinery" garnered substantial attention in the 21st century due to challenges associated with the petrochemical refinery, such as negative effects on the environment. The focus of policymakers has shifted to biorefineries, which utilize renew-

able sources that are greener and cleaner to meet the energy demand in a sustainable manner [28]. The word "biorefinery" is derived from two words: "bio", meaning life, and "refinery", which signifies a processing facility. A biorefinery involves the sustainable conversion of biomass into a range of useful products, including food, fuels, and chemicals [29]. A biorefinery incorporates a wide range of sustainability factors, including environmental, societal, and economic effects, and operates on two basic tenets: first, the sustainable processing system; and second, the production of energy and chemicals that may be commercialized [30,31]. A modern biorefinery relies heavily on residual and inedible LCB as a feedstock because of its comparatively low cost and large supply [27]. LCB consists of cellulose, hemicellulose, and lignin. Historically, the focus has been on the conversion of cellulose and hemicellulose for product formation, while lignin was generally considered a roadblock due to its tendency to cause biomass recalcitrance [32]. While the value-added utilization of cellulose and hemicellulose is equally important, lignin can also be exploited for producing green aromatic compounds due to its phenolic composition. For this reason, significant attention has shifted to the depolymerization of lignin in recent years. Therefore, generally, biorefinery ideally requires lignin extraction in the first stage, and in the second stage, involves product formation. In the first or primary stage, aromatic compounds such as phenol, benzene, toluene, and xylene are extracted by removing the functional groups from the lignin polymer. In the second or secondary stage, highly selective catalysts break specific linkages within the biomass, facilitating the production of value-added chemicals [20,33]. Utilizing LCB as a biomass for biorefineries for the production of alternative fuels and chemicals has several advantages, such as reducing the detrimental effects of fossil fuel consumption and the disposal of lignocellulosic waste on the environment, reducing greenhouse gases, and sustainable management of lignocellulosic waste [34]. Products from biorefineries are renewable sources of carbon that help to meet the endless need for energy, materials, and fuels, while also reducing the threat of climate change.

In the traditional biorefinery, the production of valuable products is minimal due to the restricted use of substrates and technologies. This shortcoming of a conventional biorefinery can be overcome by integrated biorefineries. In an integrated biorefinery, different technologies of biomass conversion are combined to produce different products. An integrated biorefinery can enable the complete valorization of all three components of LCB (cellulose, hemicellulose, and lignin) into a wide range of value-added products, including biofuels, bioplastics, drug delivery agents, lignin hydrogels, food additives, prebiotics, vanillin, biocomposites, furfural, and antimicrobial and antioxidant agents, as well as applications in nutraceuticals and cosmetics (Figure 1) [7,32]. The steps in integrated biorefinery include biomass pretreatment, product extraction, processing, and recycling of the waste or byproducts generated to accomplish near-zero waste generation [20,35]. The efficient separation of each polymer (cellulose, lignin, and hemicellulose) in the biomass to facilitate their subsequent value-adding depends on biomass pretreatment [36]. This should also make it easier to recover the fractions with the fewest purification stages [37]. Integrated biorefinery employs different conversion processes (for example, thermochemical, biochemical, physical, and biocatalytic conversion technologies) to boost the yield of targeted products. Utilization of several conversion technologies not only increases process efficiency but also offers the advantage of using lignocellulosic feedstocks of different seasons and geographical areas, ultimately expanding the spectrum of raw materials and ensuring year-wide production. Moreover, the ability of integrated biorefinery to completely use LCB may improve the cost-effectiveness of the whole process [20]. Technological advancements and the optimization of mass and energy flow in all the processes, from pretreatment and extraction, to byproduct processing, are highly crucial for increasing product valorization in

the integrated system [38]. While setting up an integrated biorefinery illuminates the path of LCB valorization, ensuring its success and sustainability requires attention to various factors, such as minimal consumption of power and energy, minimal negative effect on the environment, socioeconomic impact, near-to-zero waste generation, maximal product yield, and high-quality production [39].

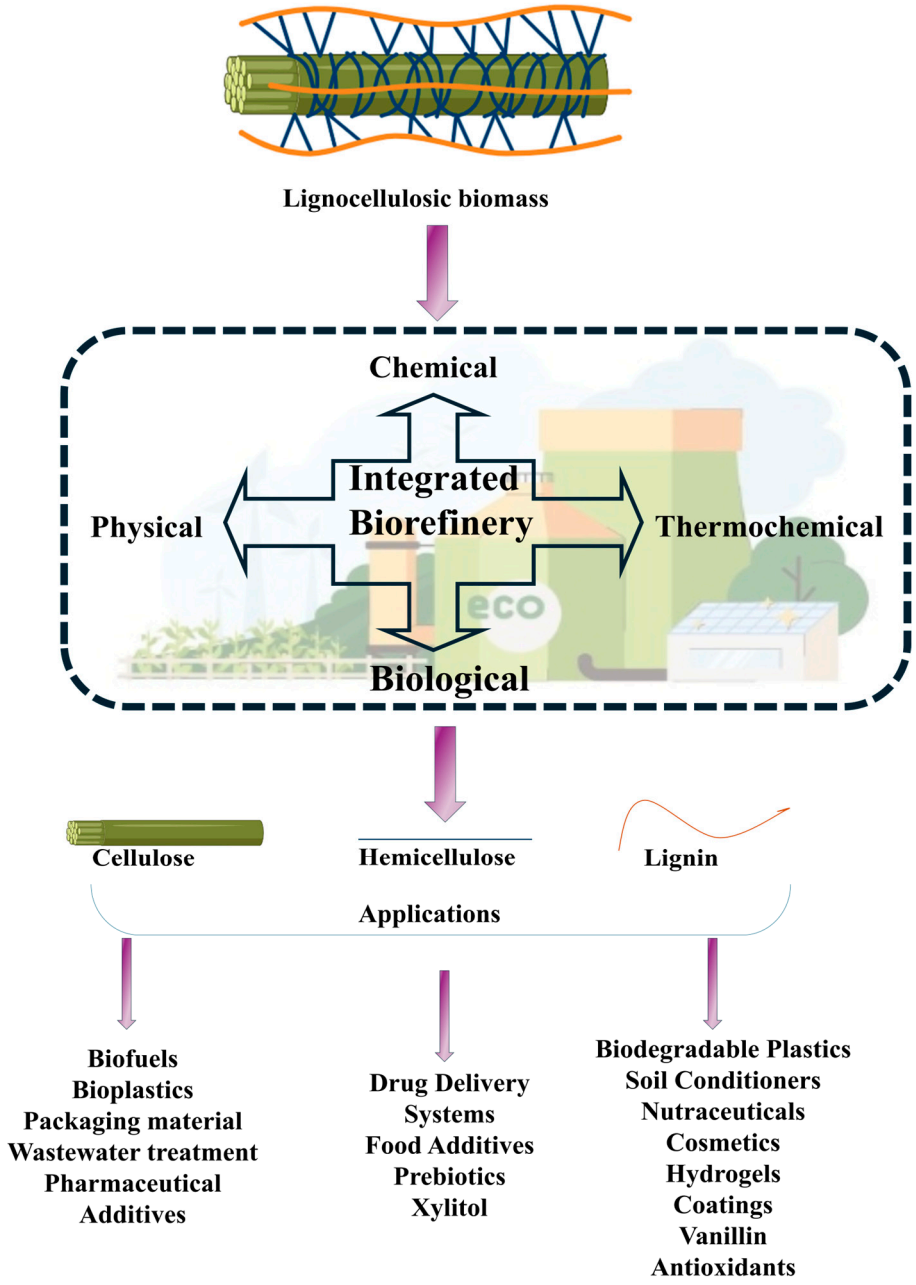

Figure 1. Bioconversion of lignocellulosic components into diverse value-added products through an integrated biorefinery approach.

The concept of an integrated biorefinery for LCB valorization has been extensively explored by researchers. The production of 1,4-butanediol has been reported from a 60 kton/y cardoon LCB using an integrated biorefinery system. The energy recovery system and thermal energy recovery system were able to cover approximately 78% of the total electricity demand and 83% of the total thermal demand, respectively. Moreover, the carbon footprint value of kg CO_2eq/kg 1,4-butanediol was reported for the entire production process of BDO [40]. An integrated approach was used for the coproduction of ethanol

and 1,5-pentanediol from corn stover biomass via organosolv fractionation technology, combining biomass fractionation with the concurrent conversion of hemicellulose into 1,5-pentanediol and cellulose into ethanol [41]. In another study, plug-in processes for lignin were analyzed for their role in producing polyhydroxyalkanoates as a coproduct with cellulosic ethanol. The findings indicate that this biorefinery model holds promise for fostering sustainability by boosting carbon efficiency and streamlining overall capital costs [42]. A simple pilot-scale method was adopted to successfully recover lignin from a black liquor made by *Miscanthus x giganteus* treated with mild soda [43]. This produced high-quality native-like lignin that may have a promising future in a variety of high-value applications. A pilot-scale process mimicking the continuous full-scale industrial production was used to create fiberboards combined with laccase-catalyzed oxidation and through lignin cross-linking [44]. This study exhibited that it is possible to see lignin cross-linking in enzyme-bonded boards. It is evident from the reported case studies and pilot-scale lignin use that lignin could well be explored for industrial scale applications through integrated biorefinery systems that enhance yield, produce multiple products, and reduce wastage.

3. Lignin, Sources, and Valorization

3.1. Lignin and Its Sources

Lignin, a heteropolymer, is the second-highest predominant biopolymer, which is present in the plant biomass and is an integral constituent of LCB alongside cellulose and hemicellulose [32]. The outer cell wall of plant biomass contains lignin, which forms a complex three-dimensional network that firmly encircles the polymeric sugars and gives LCB its extremely resistant characteristics [32,45]. It accounts for 40% of the calorific value of LCB and is bound to polysaccharides through benzyl-ether linkage, which occurs between the primary/secondary hydroxyl groups of monomeric sugars of cellulose and hemicellulose and the α-position of lignin to form a lignin carbohydrate complex. The bonding is further strengthened as the lignin carbohydrate complex's benzyl-ester bond joins the lignin's hydroxyl group with the uronic acid group of carbohydrates [46,47]. Along with giving structural strength to the plant, the lignin also prevents the deterioration of the plant and seals the water conduction system between the roots and leaves. Depending on the type of LCB, the lignin content varies: 25–38% is found in softwood (gymnosperms), 20–30% is found in hardwood (angiosperms), 8–15% is found in herbaceous plants, and 11–20% is found in agroindustrial waste [47].

In all types of LCB, lignin is composed of aromatic polymers resulting from the oxidative coupling of 4-hydroxyphenylpropanoids. It is naturally synthesized through the controlled polymerization of three main monomeric subunits, syringyl (S), p-hydroxyphenyl (H), and guaiacyl (G), originating from sinapyl alcohol, p-coumaryl alcohol, and coniferyl alcohol, respectively. The physicochemical and biological properties of lignin polymer are determined by the linkages in the lignin, such as alkyl-aryl, alkyl-alkyl, and aryl-aryl group linkages [11,48]. The variation in the lignin composition also plays a role in the categorization of different woods; for example, guaiacyl lignin is present in high concentration in softwood, whereas hardwood has a shared composition of guaiacyl lignin, syringyl lignin, and a minor proportion of p-hydroxyphenyl lignin. Herbaceous wood contains guaiacyl lignin and a small amount of p-hydroxyphenyl lignin. A key consideration in lignin valorization is the diversity in lignin composition among various kinds of wood, which helps define the technology to be used for lignin fractionation in order to produce value-added products [49,50].

The two types of lignin sources are known as native lignin and industrial or technical lignin. Native lignin is a component of LCB and is widely distributed within a complex

network of cellulose and hemicellulose. However, after undergoing multiple rounds of biomass processing, technical lignin is primarily produced in the pulp and paper industries [12]. With over 85% of the world's total lignin production, kraft lignin is the most widely produced technical lignin among all forms of lignin, including lignosulphonates, soda lignin, organosolv lignin, and hydrolysis lignin [45]. Lignin from the pulp and paper industry has been the subject of the majority of studies on lignin valorization. When compared to other possible sources, the benefit of using the lignin from those facilities is that the resource is already consolidated, and the transportation expenses to further process it are substantially lower. However, profusely present LCB, primarily in the form of agricultural and forestry residues, has the greatest potential for lignin usage in future circular economies through biorefineries. The current sources of LCB are roughly 1.3 billion tons from agricultural biomass, 3.7 billion tons from grasslands, and less than 1 billion tons from specialized crops. Moreover, the generation of agricultural waste differs according to the geographical location and the primary crops grown there. A significant amount of agricultural waste is also produced in developing nations like India and China. India, for instance, produces 500 million tons of agricultural residues, whilst China produces almost 900 million tons of agricultural straw [49,51,52]. Therefore, the valorization of all components of LCB, especially lignin, evolves into an intriguing responsibility.

3.2. Lignin Valorization Routes

Despite the generation of a large amount of LCB worldwide, most of it is discarded without proper valorization. Alongside cellulose and hemicellulose, the lignin in biomass can be efficiently valorized for the sustainable production of value-added components through the establishment of a successful biorefinery, which might greatly alleviate the energy crisis and environmental restrictions. However, the major bottleneck in the valorization of lignin is its recalcitrant nature [51]. Different linkages, including β-O-4, β-5, β-1, 5-5, β-β, α-O-4, and 4-O-5, bond the lignin monomers. Within the native lignin structure, the major inter-unit bonds are the β-aryl ethers (β-O-4), which account for approximately 50% of linkages. The subunits contribute to recalcitrance by being cross-linked with the polysaccharides found in the xylem and phloem tissue [53]. The recalcitrance of the lignin can be reduced by the biomass pretreatment approach, which can be achieved by lignin depolymerization. Targeting the β-O-4 bonding could result in effective depolymerization, since it contributes to the highest number of linkages in lignin and the C-O bond in the β-O-4 linkage has a relatively low bond dissociation energy (70 kcal/mol) [54]. The dissociation of linkage β-O-4, however, may produce aromatic monomers that are vulnerable to further repolymerization [55]. Various methods such as thermochemical, chemical, and physiochemical, can be employed for the fractionation of lignin, which can later be utilized for producing high-end products. In terms of efficiency, processing time, and yields, the thermochemical biomass fractionation for lignin valorization is a more effective method than the biological method involving microbes and their enzymes. The breakdown of lignin in nature usually takes a long time and involves a variety of microbes, such as fungi, yeasts, and bacteria. Different enzymes that enable metabolic pathways to fully break down lignin are contributed by microbial communities. Moreover, biological processes produce yields and productivity that are far less than what industry demands [56]. However, the need for significant volumes of acids and organic solvents is the main disadvantage of lignin precipitation by in thermochemical lignin valorization by acids. These methods may have certain disadvantages; for example, use of harsh chemicals, high energy inputs, and non-selective degradation of lignin [57]. The commercialization of lignin-based biorefineries requires addressing the ongoing challenges of target phenolic separation and purification. The capacity of metabolically modified microbial cells to sequentially utilize particular

substrates in mixed products makes the biological conversion of complicated degradation products a viable option to circumvent this constraint. Among the various cutting-edge methods currently in use, lignin deconstruction by biological means is superior. While breaking down lignin, enzymes or microbes that contain the lignin-degrading enzymes function under mild reaction conditions and show strong regioselectivity and stereoselectivity. Nonetheless, biological lignin valorization remains difficult, producing few valuable products despite numerous advancements. Therefore, different methods can be combined for the increased efficiency of lignin valorization and subsequent production of valuable products [51]. The utilization of lignin for the development of various valuable products is summarized in Table 1.

The solubilization of lignin in water without the addition of any other solvent can be greatly aided by mechanical techniques such as sonication, ultra-turrax churning, microwave irradiation, and steam activation [58,59]. These techniques are essential for overcoming lignin's natural resistance and increasing its accessibility for enzymatic and microbial breakdown into chemicals with added value. Ultrasonication is the process of creating high-frequency vibrations in a liquid medium using ultrasonic waves. Cavitation is produced by this process, resulting in microbubbles that violently collapse, producing strong shear forces and localized high temperatures [60]. The lignin structure is upset by these effects, which cause it to break up into smaller pieces and become more soluble in water. Through this method, the surface area of lignin is increased as it breaks down into smaller molecules, which enhances its accessibility to microbial enzymes. Furthermore, lignin's molecular weight can be decreased by sonication, which promotes the breakdown of lignin into phenolic monomers. Similarly, lignin's intermolecular interactions, including van der Waals and hydrogen bonds, are weakened by the localized heating brought on by microwave radiation [61]. This alteration makes lignin more soluble in water and more vulnerable to microbial and enzymatic breakdown.

Table 1. Different value-added products derived from lignin extracted from various lignocellulosic biomass with their characteristics.

Lignocellulosic Biomass Source	Extraction Method	Product and Yield	Product Characteristics	Reference
Corn stover	Anhydrous ammonia gas pretreatment (low-moisture, flow rate = 6.9 L/min, 30 min); heating (90 °C, 24 h); enzymatic hydrolysis (cellulase CTec2, 0.5 mL/g cellulose and hemicellulase HTec2 0.25 mL/g cellulose)	Lignin extract (33.92 g lignin/100 g corn stover)	Inhibited *Listeria innocua growth* after 48 h of incubation; antioxidant values ranged from 1875.22 to 2422.52 µmol TE/g sample	[62]
Eucalyptus grandis × *Eucalyptus urophylla*	Alkaline nitrobenzene oxidation (100 mg lignin sample mixed with 7 mL sodium hydroxide (2 M) and 0.4 mL nitrobenzene); heating (170 °C, 3 h, continuous agitation)	Vanillin (13.39% yield)	-	[63]
Corncobs/pines	100 mL enzymatic hydrolysis lignin solution (0.1 g/L Enzymatic hydrolysis lignin with acetone/water, 8:1, *v*/*v*) solution mixed with 400 mL deionized water at continuous stirring at room temperature, concentrated at 40 °C to recycle acetone, freeze-drying	Lignin-based sunscreen	Better UV blocking performance; SPF value of 9.10	[64]
Phyllostachys, *Miscanthus*, *Pinus densiflora*	Ammonia water treatment (autoclaving, 120 °C, 20 min)	Lignin extract	High antioxidant capacity, Low IC_{50} value (14.6 mg/mL). High residual superoxide dismutase-like activity (94.2%)	[65]

Table 1. *Cont.*

Lignocellulosic Biomass Source	Extraction Method	Product and Yield	Product Characteristics	Reference
Wheat straw	Alkali extraction (dewaxed using toluene-ethanol with 2:1 proportion; 2% NaOH solution, 120 °C, 45 min)	Lignin-based silver nanoparticles; total phenolic content in lignin-140.6 ± 5.6 µg GAE	Long-term stability, substantial DPPH radical scavenging potential (IC_{50}-62.5 µg/mL), 70% inhibition against SKOV3 ovarian cancer cell lines inhibited *Staphylococcus aureus* (MIC-25.0 µg/mL) and *Escherichia coli* (MIC-20.0 µg/mL)	[66]
Wheat straw	Hydrothermal/alkaline treatment; 7.0 g dewaxed powders heated (170 °C, 30 min); neutralized (6 M NaOH); 2% NaOH solution (90 °C, 120 min, solid-to-liquor ratio of 1:10 g/mL)	Lignin-90.3% yield, purity-97.4% M_w of the lignin-1210 g/mol, S/G/H-41/55/4,	Relatively high antioxidant activity (IC_{50}-0.07 mh/mL)	[67]
Rice husk	Acid-Alkali treatment (1 mol/L HCl, solid–liquid ratio −25 mg/mL, stirred-24 h, washed until neutral and dried, 12.0 g rice husk powder dispersed in 48 g 14 wt% NaOH, pre-cooled 0 °C, stirred-1 min, added pre-cooled 48 g 24 wt% urea, stirred-2 min, and separated the supernatant)	Hydrogel adsorbent (RH-CTS/PAM gel)	Macroporous network structure, high buried water content, 96.8% moisture content, stable at pH 1.00 to 13.00, more than 90% lead removal efficiency, theoretical maximum adsorption capacity of 374.32 for lead	[68]
Corn stover	Dilute sulfuric acid (1% H_2SO_4, 120 °C, 30 min, 10% solid loading); sodium hydroxide (1% v NaOH, 120 °C, 60 min, 10% solid loading); Laccase (50 °C, 200 rpm, 48 h, loading-15-mg/g substrate	Lipids, concentration-1.83 g/L	-	[69]
Alamo switchgrass (*Panicumvirgatum* L.)	soaked 1 wt% switchgrass, 24 h, room temperature, stirred- 2 min; hydrothermal pretreatments (180 °C, 120 min); mechanical fibrillation (constant rotation speed-1500 rpm)	Chitosan/lignin-containing cellulose nanofibrils biocomposites for food packaging application	Excellent flexibility and transparency, good dispersion, visible light transmittance (94.41%), lower UV transmittance (12.28%), higher tensile strength, higher onset degradation temperature (T_{onset}) and the maximum decomposition temperature (T_{max}), lower oxygen permeability rate (0.20 cc/(m^2 day))	[70]
Olive pomace	Alkali method (200 g of ground biomass immersed in 1.5 L of water with 0.16 M NaOH); heated 70 °C, 300 rpm, 2 h; centrifugation (10 min, 4000 rpm)	Lignin-carrageenan composite for water-soluble triple superphosphate (TSP) granular fertilizers	Tensile modulus-1191.51 Kpa, tensile strength-3674.11 Kpa, elongation at break-9.37%, highest hygroscopicity of coated fertilizer, slow release	[71]

4. Biocatalysis in Lignin Valorization

As discussed above, the physical, chemical, and thermochemical pretreatments used for the fractionation of biomass have their own limitations, which can be overcome by adopting the biological mode of pretreatment [72,73]. Biological pretreatment is done by either using microbes that secrete lignin-degrading enzymes or directly, using the extracted lignin-degrading enzymes. Enzymes are environmentally friendly biocatalysts and carry out the breakdown of complex polymers in a mild milieu with high specificity [11,32,74]. With these enzymes, depolymerization of lignin can be effectively achieved and the extracted lignin can be used for the production of various chemicals after separation and purification (Figure 2). A wide range of biocatalysts are involved in lignin degradation, including laccase, lignin peroxidase, manganese peroxidase, versatile peroxidase, dye-decolorizing peroxidase, β-etherase, biphenyl bond cleavage enzyme, aryl alcohol oxidase, glyoxal oxidases, etc. [25,50].

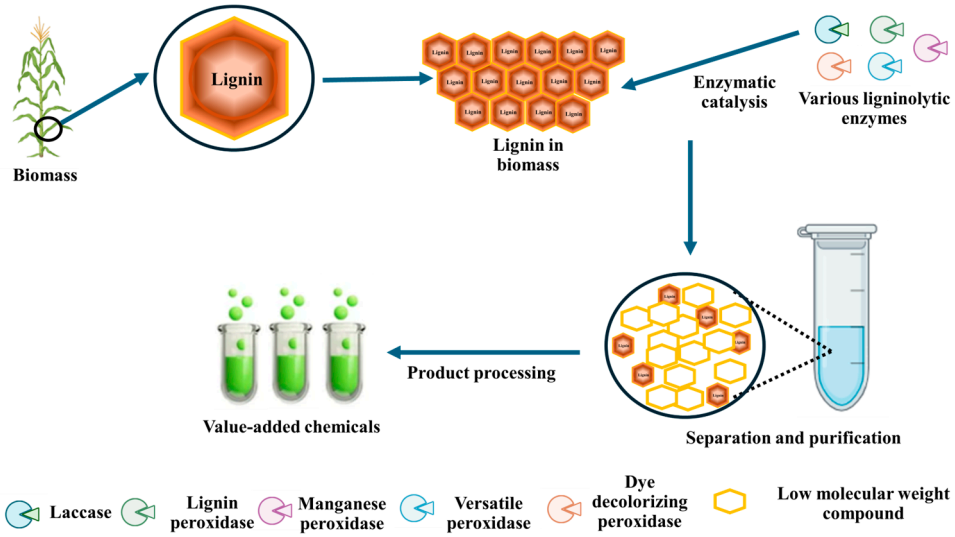

Figure 2. Overview of lignin depolymerization by ligninolytic enzymes for production of value-added chemicals.

4.1. Mechanism of Lignin Fractionation by Enzymes

The advantages of biological lignin processing include the ability to express target molecules with a specificity that outperforms synthetic chemistry, and the utilization of renewable carbon sources as feedstock to maintain cellular development for the generation of diverse intermediates. It has been discovered through studies that bacteria and fungi, using a variety of enzymes and related small molecule co-factors, may break down lignin in vitro and/or in vivo [75]. Various natural biomass usage systems have also been used to study the lignin and aromatics degradation pathways. Among the extracellular oxidoreductases that aid in the breakdown of native lignin are lignin peroxidase, manganese peroxidase, versatile peroxidase, laccase, and dye-decolorizing peroxidase. These enzymes are essential for the breakdown of the lignin because they generate intermediate phenoxy radicals. Although bacterial lignin breakdown may be less effective than fungal degradation, bacterial delignification has several advantages, such as resistance to a greater range of pH levels, temperature changes, and oxygen level variations, which makes bacteria easier to control than fungi [76].

The oxidative cleavage of lignin β-O-4 units produces vanillin, where the lignin's β-O-4 aryl ether bond can be selectively broken by microbial β-etherases. *Chaetomium*, *Novosphingobium* sp., and *Sphingobium paucimobilis* SYK-6 are the primary hosts of these enzymes [75]. Although the mechanisms of lignin depolymerization in fungi have been studied, it is far more difficult to overexpress ligninolytic enzymes because of difficulties with genetic modification and the recombinant expression of functionally active proteins. The lignin and phenolic compounds can also be catabolized by bacteria, but ligninolytic bacteria are far less effective at degrading lignin than fungal systems. Therefore, to increase biological lignin value and the viability of biorefineries from an economic standpoint, the molecular pathways behind microbial lignin degradation should be carefully uncovered. In order to efficiently degrade kraft lignin and produce the most vanillic acid possible, a previous study concentrated on isolating promising ligninolytic strains from soil combined with decaying wood [77]. Out of the fifteen isolated strains, five possible ligninolytic strains were found, with RW-B15 being the most prevalent with an increasing vanillic acid concentration from 60.1 to 199.9 mg/L obtained from kraft lignin concentration (500–2000 mg/L).

Peroxidase and laccases have the ability to hydrolyze both phenolic and nonphenolic lignin when free radicals such as the hydroxyl group are present. Several redox oxidation

pathways, in which H_2O_2 is an oxidant, regulate the enzymatic and chemical breakdown of lignin from LCB [78]. These enzymes are produced by a variety of bacteria and fungi. A single microorganism does not express all the enzymes; rather, different microorganisms produce different enzymes. There are two main categories of lignin-degrading enzymes comprised of lignin-modifying enzymes; these are fundamental lignin-degrading enzymes, and lignin-degrading auxiliary enzymes, which are necessary to complete the lignin degradation process even though they are unable to degrade lignin on their own [79]. Rather than being hydrolytic (ionizing the water molecule), lignin-modifying enzymes have an oxidative (electron withdrawing) enzymatic action [78]. Auxiliary enzymes facilitate the lignin depolymerization process by sequentially acting on different proteins, including the oxidative production of H_2O_2. Numerous enzymes, including cellobiose dehydrogenase, glyoxal oxidase, aryl alcohol oxidases, glucose oxidase, and pyranose 2-oxidase, are included in this group [11]. A comparative analysis of various lignin-degrading enzymes is represented in Table 2 [80–82].

Table 2. Comparative analysis of biochemical properties, structure, and substrates of various lignin-olytic enzymes.

Enzyme	Source	Optimal Temperature/pH Range and Molecular Weight	Structure and Catalytic Center	Cofactor	Substrate	Hydrogen Peroxide Dependence
Lignin peroxidase	White-rot fungi	30–60 °C; 2.0–8.0; 38–47 kDa	Monomer, glycoprotein; Heme (Fe-protoporphyrin)	H_2O_2	Phenols, aromatic amines, aromaticethers, polycyclic aromatics and G-, S-, and H-lignin	Yes
Manganese peroxidase	White-rot fungi	30–70 °C; 2.0–6.0; 38–50 kDa	Monomer, glycoprotein; Heme (Fe-protoporphyrin)	H_2O_2	Phenolic compounds and lignin	Yes
Laccase	Fungi and bacteria	30–85 °C; 2.0–8.5; 37–180 kDa	Mono-, di-, or tetramer, glycoprotein; Four Cu atoms	O_2	Phenolic compounds, aromatic amines, polyphenols, catechol anddye molecules	No
Versatile peroxidase	White-rot fungi	30–50 °C; 3.0–5.0; 40–50 kDa	Monomer, glycoprotein; Heme (Fe-protoporphyrin)	H_2O_2	High redox potentialaromatic compounds and recalcitrant dyes	Yes
Dye-decolorizing peroxidase	Fungi and bacteria	30–50 °C; 2.0–5.0; 40–50 kDa	Monomer, dimer, hexamer; Heme (Fe-protoporphyrin)	H_2O_2	Dye compounds, carotenoids, and phenolics	Yes

4.2. Lignin-Degrading Enzymes

4.2.1. Lignin Peroxidase

Lignin peroxidases (LiP) were first discovered from basidiomycetous fungi *Phanerochaete chrysosporium* in 1983 by Tien and Kirk. These enzymes belong to the family of oxidoreductases and degrade both phenolic and nonphenolic components of lignin with or without mediators [25,78,79]. LiP are glycosylated monomeric enzymes that have an iron protoporphyrin prosthetic group with a heme group in the active center and the catalytic activity of LiP is dependent on the conserved trp171 residue found in LiP sequences [80,83]. These enzymes are produced by various bacteria and fungi. The molecular weight of LiP falls in the range of 38–48 kDa, with an isoelectric point between 3.2 to 4.7 and an acid optimum pH. These are globular proteins that are made up of eight α-helices grouped into two domains, with two calcium ions and disulfide bridges formed between eight cysteine residues stabilizing the structure [78,83]. LiP is a hydrogen peroxide (H_2O_2)-dependent enzyme that causes the cleavage of C-O-C and C–C linkages of lignin by the process of

oxidation, resulting in the breakdown of lignin, and does not act on polysaccharides, leaving them unharmed [84]. The activity of LiP is also increased in the presence of veratryl alcohol, which acts as an electron donor and cofactor compared to other enzymes in the same family; LiP has a larger redox potential, which confers excellent lignin disruption capabilities, making it the most prevalent kind of peroxidase [25,80]. LiP is useful not only for depolymerizing lignin but also for its strong delignification potential during the pretreatment of LCB for the production of value-added products such as biocomposites [85]. There are four steps in the catalytic cycle of LiP. The enzyme is oxidized by H_2O_2 in the first step, producing an intermediate Compound I. This is followed by a two-step reduction process that produces Compound II and a radical cation by reducing the electron donor substrate. The final and fourth step includes the oxidation of the second substrate molecule by Compound II following the initiation of the next catalytic cycle as the enzyme returns to its native ferric state [81].

In a previous study, six bacterial lignin-oxidizing enzymes and five accessory enzymes were employed for the improved biocatalytic degradation of lignin. Two novel DyP-type peroxidase enzymes from *Comamonas testosteroni* and *Agrobacterium* sp. were included that exhibited Mn^{2+} oxidation activity [86]. The findings demonstrated that the inclusion of auxiliary enzymes increased the activity of lignin-oxidizing enzymes (peroxidases, laccases, and manganese peroxidases). The depolymerization of technical lignin was examined in relation to the combined effects of quinone reductase and lignin peroxidase that lowered the molecular weights and thermal stability of different technical lignins, showing that the synergistic approach is promising for lignin depolymerization [87].

4.2.2. Laccase

Laccases belong to the polyphenol oxidases group and are a blue multicopper enzyme that can oxidize a broad range of organic and inorganic compounds. These are commonly found in bacteria, fungi, plants, and insects and have a redox potential of approximately 800 mV [12,80,88]. By using molecular oxygen as an electron acceptor and producing water, laccases oxidize a wide array of compounds, including mono-, di-, and poly-phenols, aminophenols, methoxyphenols, aromatic amines, ascorbate, as well as phenolic and nonphenolic lignin compounds. Laccases are classified into two groups based on their ability to collect electrons, or the redox potential of the reaction they catalyze: low redox potential laccases, which are primarily found in bacteria and plants, and high redox potential laccases, which are found in fungi [83]. Laccases have an average molecular weight of 50–300 kD and have three domains that are sequential, with a Greek key β barrel structure that circulates inside a single molecule. There are two disulfide bridge bonds connecting domains I and II that stabilize the structure in general, and domains I through III are also present [83,89]. Laccase uses four copper ions in its active sites: a trinuclear copper cluster and a blue type 1 copper core. The four copper ions found in laccase's molecular structure can be categorized into three groups based on their spectroscopic and magnetic characteristics. Type I and type II Cu^{2+} are paramagnetic acceptors with a single electron, whereas type III Cu^{2+} is a coupled-ion diamagnetic pair with two electrons. Following oxidation of the substrate being reduced at the T1 site, the electrons are moved to the T2/T3 site, where oxygen is reduced to water [81,90]. A His–Cys–His tripeptide route facilitates the transport of four electrons to the trinuclear center in the reaction that the type 1 laccase catalyzes [80].

Laccases can oxidize lignin's phenolic subunits directly by using a process that produces cationic radicals in the substrate, which then causes lignin to depolymerize through the cleavage of an aromatic or aliphatic bond. They utilize the redox properties of copper to oxidize aromatic molecules. The oxidation of phenolic substrates by laccase results in the production of the unstable intermediate phenoxyl free radical, which subsequently

promotes Cα oxidation and polymer cleavage, including Cα-Cβ and alkyl-aryl cleavage [80,88]. The oxidation of nonphenolic compounds by laccases cannot be completed without the presence of mediators. Mediators, including 3-hydroxyanthranilic acid (HAA), 1-hydroxybenzotriazole (HBT), and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate), are small molecules that can be employed to rectify difficulties by moving electrons between the enzyme and the substrate, raising the enzyme's redox potential and allowing the substrate to oxidize. Therefore, when a mediator is present, laccase oxidizes it first, followed by lignin's nonphenolic units, enhancing the enzyme's substrate range [11,91]. When paired with mediators, the oxidized nonphenolic substrates can promote Cα-Cβ cleavage, aromatic ring cleavage, β ether cleavage, and Cα oxidation. Mediators increase the oxidation potential of laccase by helping it to establish a stable intermediate with the substrate, which helps to overcome the steric barrier between the enzyme and substrate. Approximately 80–90% of the lignin can be broken down by laccase with the aid of mediators [25,80,91]. Laccases are generally regarded as the "green catalysts" enzyme because of their key characteristic of utilizing for oxidation and releasing only water as a byproduct [12]. They are the sole enzyme capable of breaking down the lignin on its own and therefore important in lignin valorization [90].

4.2.3. Manganese Peroxidase

Manganese peroxidases (MnP) are another heme group containing peroxidases that play a vital role in the breakdown of lignin and are commonly found in basidiomycetes. The heme group is located between two domains. These ubiquitous enzymes are monomeric glycosylated and globular proteins composed of 11–12 α-helices, which are actively maintained by 2 Ca^{2+} binding sites and 5 disulfide bonds, one of which is associated with Mn coordination [83,89,92]. Their pI ranges from 2.9 to 7.1, and their molecular weight, which includes 4–18% glycans, spans from 38 to 62.5 kDa [79]. Apart from H_2O_2, it is also dependent on manganese ions for its catalytic activity and oxidizes Mn^{2+} to extremely reactive Mn^{3+}, hence the name manganese peroxidase. Chelators like oxalic acid in fungi help to stabilize Mn^{3+}. The binding site of Mn^{2+} in an enzyme is situated near the porphyrin macrocycle and is made up of two glutamates and one aspartate γ-carboxylic group [12,79,81]. Both oxidation and reduction steps are involved in the lignin degradation catalyzed by MnP. During the catalytic cycle of MnP, H_2O_2 initiates the catalytic cycle by forming an iron-peroxide complex with the resting ferric enzyme. In order to cleave the peroxide enzyme's O-O bonding, two electrons are transferred from the heme-porphyrin, which results in the formation of the unstable intermediate MnP Compound I (Fe^{4+} oxo-porphyrin radical cation). This leads to the hydrolysis of dioxygen bond heterolytically and the release of one water molecule. Subsequently, oxidation of Mn^{2+} ion to Mn^{3+} takes place which acts as a one-electron donor for porphyrin to form unstable intermediate Compound II. This intermediate Compound II is reduced in a manner similar to that of another Mn^{3+}, being generated from Mn^{2+}, which results in the enzyme returning to its resting state and release of the second water molecule [80,89]. Nonphenolic compounds, which make up to 90% of the lignin, are difficult to oxidize by MnP, unlike LiP. However, small mediators such as thiols, unsaturated fatty acids, and their derivatives may aid MnP to hydrolyze the nonphenolic components of lignin [11].

4.2.4. Versatile Peroxidase

Another heme group containing peroxidases used in the lignin hydrolysis is versatile peroxidases (VP). These are also called hybrid peroxidases since their molecular structure is a combination of the molecular structure of both LiP and MnP and, therefore, exhibit catalytic attributes of LiP and MnP [78]. These glycosylated monomeric proteins have a

molecular weight and isoelectric point between 40 to 50 kDa and 3.4 and 3.9, respectively. Structurally, VP consists of 11–12 helices, 2 structural Ca^{2+} sites, 4 disulfide bridges, and an Mn^{2+} binding site [83]. VP can oxidize both phenolic and nonphenolic substrates without the need for mediators and reacts with H_2O_2 to form oxo-ferryl intermediates. The three active substrate oxidation sites of VP, a Mn^{2+} to Mn^3 oxidation site, which acts as the diffusible mediator, a high-redox potential surface-reactive tryptophan radical, and a low-redox potential heme-containing binding pocket, allow it to oxidize Mn^{2+} to Mn^{3+} through a long-range electron-transfer channel that links to the heme molecule [93]. The catalytic cycle of VP involves two oxidation steps, one is ferryl-oxo iron (Fe^{4+} = O) and another is delocalized as a porphyrin/tryptophanyl radical and the formation of intermediates Compound I and Compound II. The substrate direct oxidation is catalyzed by Compound I, where an electron interacts with the heme through tryptophanyl radical and forms Compound II. This intermediate step transfers the remaining oxidation that is equal to the tryptophan in chemical I and retrieves the Fe^{4+} = O state. Additionally, intermediate Compound II has the ability to produce one-electron oxidation of substrates directly and exhibits interaction with the tryptophanyl radical or heme to return to the initial state of the enzyme. Under conditions of high H_2O_2, compound III, a superoxide anion, can be formed, which follows distinct mechanisms of decomposition. Porphyrin amino acid side chains can be oxidized by these reactive oxygen species prior to enzyme deactivation [89].

4.2.5. Dye-Decolorizing Peroxidase

Dye-decolorizing peroxidases (DyP) are recently discovered peroxidases for the degradation of lignin through the radicals-mediated process of oxidation and were named for their remarkable ability to decolorize a wide range of dye molecules [79]. DyP is different from other peroxidases containing class II heme in sequence and structure but utilizes H_2O_2 for oxidation and exhibits a mediator-dependent catalytic cycle [94]. On the basis of primary structural homologies, these are categorized into four types: A, B, C, and D. Types A through C are primarily composed of bacterial enzymes where Type A enzymes contain a Tat-dependent translocation signal sequence, and Types B and C are cytoplasmic enzymes, while Type D is primarily associated with fungal DyPs. Type A and Type B DyP are smaller and less active compared to Type C and Type D DyP which exhibit better substrate oxidation activity. Although peroxidase activities are present in all types of DyPs, their substrate specificity values vary [81,91,95]. DyP-type peroxidases exhibit a structural organization of two domains containing α-helices and anti-parallel β-sheets, with the heme cofactor positioned in the interdomain cavity. The molecular weight of DyP falls in the range of 40 and 60 kDa [83]. The active site of these enzymes includes an aspartic acid residue believed to function as a proton donor, facilitating the formation of the Compound I intermediate required for substrate oxidation [94]. Unlike other peroxidases, these enzymes are known to degrade carotene, aromatic sulfides, 2,6-dimethoxy-phenol, guaiacol, and veratryl alcohol, Mn^{2+}, anthraquinone dyes owing to their broad substrate specificity [21].

4.3. Other Enzymes

Apart from the above-discussed enzymes, many other biocatalysts play an important role in lignin degradation. Enzymes such as β-etherase and biphenyl bond cleavage enzymes are also involved in lignin degradation [25]. β-etherase is a glutathione-dependent enzyme that attacks glutathione at the β position of an oxidized aryl unit with an α ketone group, catalyzing the reductive breakage of the β-aryl ether bond. These enzymes are known to in vivo hydrolyze lignin fragments by attacking the predominately present β-O-4 ether linkages in lignin and breaking β-aryl ether and biphenyl linkages within lignin molecules [80,96]. A Cα-dehydrogenase, LigD initiates the cycle of β-O-4 degradation

by oxidizing the hydroxyl group at Cα position with the utilization of NAD^+. This is followed by the hydrolysis of the the β-O-4 ether linkages by β-etherase LigE or LigF, resulting in the production of vanillin and α-glutathionyl-β-hydroxypropiovanillone (GS-HPV) as intermediate. In the last step, glutathione lyase LigG oxidizes GS-HPV to oxidized glutathione (GSSG) and results in the release of β-hydroxyproppiovanillone (HPV) as the final product [80,91,97].

In softwood, other prevalent linkages in lignin are biphenyl linkages, which contribute about 10% of the total linkages. These linkages are mostly formed between two guaiacyl units [98,99]. Therefore, biocatalysts that cleave biphenyl bonds also influence lignin degradation. During the catalytic cycle, oxidation-mediated cleavage of the C3 side chain attached to the biphenyl group results in the generation of 2,2′-dihydroxy-3,3′-dimethoxy-5,5′-dicarboxy-biphenyl. A non-heme iron-dependent demethylase LigX demethylates one of the methoxy groups from 5, 5′-dehydrodivanillate and converts it to the hydroxyl group. LigZ, OH-5, 5′-dehydrodivanillate dioxygenase, uses the LigX product as the substrate for oxidative meta-cleavage. Subsequently, the C–C hydrolase LigY hydrolyzes the products from LigZ to form 4-carboxy-2-hydroxypentadienoic acid and 5-carboxyvanillic acid. Lastly, 5-carboxyvanillic acid is converted to the metabolic core intermediate vanillic acid or vanillate for the manufacture of bioproducts by the decarboxylases LigW and LigW2 [80,91,99]. Considering the role of various ligninolytic enzymes, the combined action of multiple lignin-degrading enzymes facilitates efficient lignin depolymerization and its subsequent valorization. Table 3 summarizes the application of various lignin-degrading enzymes on different biomasses.

Table 3. Different enzymes from microbial sources involved in lignin degradation derived from lignocellulosic biomass.

Microorganism	Enzyme	Enzyme Activity	Biomass	Reference
Myrothecium verrucaria	Laccase	6.61 U/g dry biomass	Corn stover	[100]
Klebsiella pneumoniae	Laccase and manganese peroxidase	Laccase-92.54 U/mL and manganese peroxidase-70.62 U/mL	Wheat bran and rice bran	[101]
Pycnoporus sanguineus	Laccase	11.901 U/mL	Oil palm empty fruit bunch	[102]
B. ligniniphilus L1	Laccase	201.57 ± 0.53 U/mg	Rice straw	[103,104]
Aspergillus terrus	Laccase	4.12 U/mL	Sugarcane bagasse	[105]
Gloeophyllum trabeum	Manganese peroxidase and laccase	Manganese peroxidase-141.5 U/g and laccase-30.1 U/g	Wheat straw (WS) and fresh swine manure (SM)	[106]
Aspergillus oryzae CGMCC5992	Lignin peroxidase	652.34 U/L	Corn stalk	[107]
Ganoderma lucidum IBL-05	Lignin peroxidase	2492.4 U/mL	Wheat straw	[108]
Amauroderma rugosum	Lignin peroxidase	106.32 U/mL	Saw dust	[109]
Irpex lacteus F17	Manganese peroxidase	950 U/L	Agroindustrial residues	[110]
Thermothelomyces thermophilus	Lignin peroxidase	1645 mU/L	Corn steep liquor	[111]
Halopiger aswanensis	Lignin peroxidase, manganese peroxidase, and laccase	Lignin peroxidase-215.4 ± 1.57 IU/gds, manganese peroxidase-36.8 ± 2.38 IU/gds and laccase-8.34 ± 0.21 IU/gds	Wheat bran	[112]
Moniliophthora roreri	Manganese peroxidase	-	Locust bean gum and wheat bran	[113]
I. lacteus 7B	Manganese peroxidase	40.89 U/L	Corn stover	[114]
-	Manganese peroxidase	75.0 U/mL	Poplar chips prehydrolysis liquor	[115]
Physisporinus vitreus	Versatile peroxidase	17.5 U/g	Corn stover	[116]
Lentinus squarrosulus	Versatile peroxidase	~8 U/mL	Poplar wood chips	[117]

5. Fungal and Bacterial Biocatalysts for Lignin Breakdown

Lignin-degrading biocatalysts are widely present in plants, insects, and microbes; however, due to economic reasons, these biocatalysts are majorly produced from microorganisms. Although ligninolytic enzymes have been reported in both bacteria and fungi (Table 4), fungal enzymes are generally more effective at degrading lignin [118]. Fungi are considered the most efficient microbes for breaking down lignin because of their very effective enzymatic system, which includes hydrolytic and oxidative enzyme systems for LCB degradation. There are three primary categories of lignin-degrading fungi based on the process of lignin degradation: white-rot, brown-rot, and soft-rot fungi [80,119]. Among the three fungal groups, only white-rot basidiomycetes can completely decompose all major components of LCB, including cellulose, hemicellulose, and lignin. In contrast, soft-rot fungi metabolize polysaccharides only in the surface layers of plant material, while brown-rot fungi rapidly degrade cellulose and hemicellulose but only slightly modify lignin. The constrained lignin degradation by brown-rot fungi is due to the lack of lignin-degrading enzymes, depending instead on small reactive molecules for lignin depolymerization. White-rot fungi are distinctively capable of entirely mineralizing lignin into CO_2, making them more effective than brown-rot and soft-rot fungi at lignin breakdown [120]. The noteworthy ability of white-rot fungi to break down lignin may be closely associated with their production of lignin peroxidases, manganese peroxidases, laccases, and versatile peroxidases, among other extracellular oxidases. Additionally, excessive production of these oxidases may also contribute to the higher lignin breakdown capabilities of white-rot fungi [91,121]. *Phanerochaete chrysosporium*, a well-known white-rot fungus, serves as a model for lignin degradation, presenting the remarkable ability to degrade one gram of lignin per gram of mycelium per day [119]. Due to the exception lignin degradation quality of white-rot fungi, they have been explored for various industrial applications, including biomass de-lignification, bio-bleaching of cotton, phenolic compounds removal from pollutants, biomethane production, wastewater remediation, textile dyes degradation, clarification of numerous fruit juices, wines and musts, and bio-stoning of denim fabric in denim industries, as well as in pulp and paper industries [12,121,122].

White-rot fungi break down lignin in selective and non-selective ways depending on their ability to secrete polysaccharide and lignin-degrading enzymes, and hence, may be categorized as selective and non-selective delignifiers. Species such as *Phanerochaete chrysosporium* simultaneously hydrolyze cellulose, hemicellulose, and lignin and thus belong to a non-selective group. Conversely, organisms known as selective lignin degraders, such as *Obba rivulose*, break down lignin before polysaccharides [123,124]. Numerous studies are available on the degradation of lignin by white-rot fungi. A study reported the use of two white-rot fungi namely *Trametes hirsuta* S13 and *Pleurotus ostreatus* S18 for lignin degradation in tobacco stalk. The addition of fungal cultures increased lignin degradation by approximately 2-fold from 23.7 to 41.1% [125]. Xie et al. [126] studied the lignin degradation ability of *Cunninghamella echinulata* FR3 during the conversion of sorghum biomass into lipids. The results of the study indicated that lignin and polysaccharides were simultaneously consumed during the sorghum biomass conversion. In a novel fungal pretreatment method, *P. sajor-caju* and *T. versicolor* were used to improve the production of fermentative volatile fatty acids from solid digestate. *P. sajor-caju* pretreatment of solid digestate in 6 weeks resulted in a maximum volatile fatty acid yield of 240 mg COD/g volatile solids, which was 1.17 and 1.24 times greater as compared to the autoclaved group and raw substrate, respectively. It was indicated that the fungal strains could secrete lignin-degrading enzymes including laccase and manganese peroxidase [127]. In a study co-overexpression of three peroxidases and one laccase was attempted from *Phanerochaete chrysosporium* exposed to shock wave-induced acoustic cavitation for the disruption of sugarcane bagasse

and wheat bran. Lignin depolymerization using recombinant strains was enhanced by 25% as compared to wild types. Moreover, peroxidase and laccase activities were also increased by 2.6 and 4-fold, respectively, in comparison to the wild type [128]. Even though white-rot fungi have been shown to be more effective than bacteria at breaking down lignin due to the high efficiency of their ligninolytic enzymes, the catalytic effectiveness of fungi is significantly lower than that of chemical-based catalytic techniques. Furthermore, the potential for lignin fragments to repolymerize due to fungal oxidoreductases is another disadvantage of these enzymes [12].

Bacterial lignin metabolism is less thoroughly studied than its fungal counterpart, and bacteria are generally regarded as less effective in lignin degradation. However, they produce a range of ligninolytic enzymes that convert lignin into smaller aromatic compounds [21,129]. Bacteria can be used to break down lignin because of their remarkable environmental adaptability, varied biochemistry, and ability to produce enzymes. Various lignin-degrading bacteria have been identified from different niches, including soil, rotten wood, activated sludge, wastewater treatment plant, compost, and animal gut [130]. The main bacteria that break down lignin are *Actinobacteria*, *Proteobacteria*, and *Firmicutes*. The first bacterial lignin-oxidizing enzyme identified was peroxidase DypB from *Rhodococcus jostii* RHA1, a member of the dye-decolorizing peroxidase family, found in both bacteria and fungi. It has been demonstrated that *R. jostii* RHA1 uses dye-decolorizing peroxidase (DyP) to cleave β-O-4 links in lignin, yielding vanillin as the end product [96,131]. Additionally, *Rhodococcus jostii* has also been reported to disrupt wheat straw and kraft lignin to produce aromatic dicarboxylic acids and vanillin [132]. In addition to *R. jostii,* the ability of the bacterium *Pseudomonas putida* to degrade lignin is also being studied in great detail. Both *Rhodococcus jostii* and *Pseudomonas putida* break down lignin and have demonstrated the capacity to withstand and break down toxic intermediates [50,80]. Polyhydroxyalkanoate from combined lignin and glycerol-based substrates was attempted using *Pseudomonas putida*. The results indicated an increase in the yield of cell dry weight (9.4–16.1%) and polyhydroxyalkanoate (29.0–63.2%) as compared with using glycerol feedstock alone [133]. Another study reported the valorization of toxic compounds by employing *Pseudomonas putida KT2440*. The isolate demonstrated the ability to degrade 30% lignin (1.60 $g \cdot L^{-1}$), 45 mM benzoate, 40 mM p-coumarate, 35 mM ferulate, 10 mM phenol, 10 mM pyrocatechol, and 8 mM of an aromatic mixture, while also synthesizing biopolymers from these compounds under feast and famine conditions [134].

In addition to aerobic bacteria, anaerobic bacteria also break down and convert lignocellulosic materials. The main lignin degraders are often fungi, although their breakdown is inhibited by low oxygen concentrations. On the other hand, bacteria exhibit remarkable adaptation to anaerobic environments. Most of the anaerobic bacteria are known to act on the methoxyl group and cleave the aromatic ring, breaking down lignin and producing carbon dioxide and methane. The process of lignin breakdown, converting aromatic chemicals into methane, takes three steps. The first step involves the removal of the methyl group from the methoxylated aromatic molecule, which is known as de-methylation. The de-methoxylated aromatic structure is opened in the second stage, producing acetate, hydrogen, and carbon dioxide, which are then transformed into methane and carbon dioxide in the third step [135]. An anerobic bacterium, *Clostridium thermocellum*, was reported to be utilized for the degradation of *Populus* cell wall. It was demonstrated that with the action of *C. thermocellum*, the content of β -O-4 linkage was reduced, and increased the lignin S/G ratio [136]. *Acetoanaerobium* sp. WJDL-Y2, isolated from the sludge of a pulp and paper mill, was utilized for the biodegradation of kraft lignin, and a maximum degradation of 24.9% on a COD basis was achieved. The strain was able to oxidize p-hydroxyphenyl (H) units, guaiacyl (G) units, and syringyl (S) of lignin [137]. Anaerobic ligninolytic bacteria can be

used to produce biofuels, wastewater treatment, and perform downstream hydrolysis and fermentation in ways that are both economically viable and compatible.

Table 4. Fungal and bacterial ligninolytic enzymes with their properties, characteristics, and major findings.

Enzyme	Source Organism	Properties and Characteristics	Study Outcomes	Reference
		Bacteria		
Laccase	*Geobacillus* sp.	Optimal activity at pH 6.0 and temperature 50 °C (100%), Km = 0.146 mM, Vmax = 1.52 U/mg, Kcat = 1037 s^{-1} and Kcat/Km = 7102.7 s^{-1} mM^{-1}, molecular weight ~30 kDa	• Highest laccase production (37 U/L) using untreated corn stover as substrate • Adding recombinant laccase to Accellerase 1500 improved the enzymatic hydrolysis of corn stover by 1.44-fold • 1.22-fold-enhanced sugar yield using in-house laccase doped enzymes as compared to commercial enzymes	[138]
Manganese peroxidase	*Ganoderma lucidum*	Mamixmum activity = 670 U/L, yield = 126 mg/L, molecular weight ~37.72 kDa, specific enzyme = 524.61 U/L	• Maximum decolorization rates (70%) of the four dyes (Drimaren Blue CL-BR, Drimaren Yellow X-8GN, Drimaren Red K-4Bl) • Phenol degradation rate of 8.079% at 10% crude enzyme concentration	[139]
Lignin peroxidase and manganese peroxidase	*Acinetobacter* sp. B213	Lignin peroxidase = 219.78 U/L, manganese peroxidase = 83.18 U/L	• Delignification efficiency of lignin in whole-cell pretreated straw = 12.02% and in chemical pretreated straw = 32.38%	[140]
Lignin peroxidase, laccase, and manganese peroxidase	*Aneurinibacillus* sp. LD3	Lignin peroxidase = 3117.25 U/L, laccase = 1484.5 U/L, manganese peroxidase = 1770.75 U/L	• Higher alkali lignin degradation rate (61.28%) • Formation of 4′-hydroxy-3′-methoxy acetophenone, vanillic acid, 1-(4-hydroxy-3,5-dimethoxyphenyl), benzoic acid, and octadecanoic acid after alkali lignin degradation	[141]
Dye-decolorizing peroxidase	*Bacillus* sp. BL5	Molecular weight = 46 kDa, optimum activity at 35 °C and pH 5.0, Km = 1.06 mM, Vmax = 519.75 µmol/min/mg, Kcat = 395 s^{-1}	• 26.04% reduction of lignin contents • Formation of various low molecular weight products including butyl alcohol, dihydroxy dimethylsilane, acetic, vanillin, valeric acid, 2-methyoxyphenol, 3,4-dimethoxybenzyl alcohol, 3,4-dimethoxybenzyl alcohol, methoxy-3,5-dihydroxybenzoic acid, and p-Hydroxybenzoic acid	[142]
Laccase	*Bacillus altitudinis* SL7	Optimum activity at 55 °C and pH 5.0, Km = 0.4 mM, Vmax = 2777 µmol/min/mg and kcat = 5194 s^{-1}, isoelectric point = 5.7, molecular weight ~56 kDa, specific activity = 2530 U/mg	• Reduced lignin content by 31% • Formation of low molecular weight compounds including glutamic acid, succinic acid, oxalic acid, vanillin, 3-methyl phenol, 2-methoxyphenyl, ethyl vanillin, ferulic acid, styrene, phthalic acid, 3,5-dihydroxy-benzoic acid, and benzoic acid	[143]

Table 4. *Cont.*

Enzyme	Source Organism	Properties and Characteristics	Study Outcomes	Reference
		Fungi		
Laccase	*Gymnopus luxurians*	Specific activity = 118.82 U/mg, molecular weight = 64 kDa, optimum temperature range = 55–65 °C, pH 2.2, high thermostability and pH stability, Km = 539 µM, Vmax = 3.1 µmol/min, kcat = 75.4 s^{-1}, and kcat/Km = 140 $mM^{-1} \cdot s^{-1}$	• Dye decolorization rates = ~60–90% towards 11 dyes • Efficient degradation towards Evans blue with decolorization rates 90.4% and 93.6% in the presence of mediators	[144]
Lignin peroxidase and aryl alcohol oxidase	*Aspergillus nidulans*	Optimal temperature = 50 °C, pH = 6.0, lignin peroxidase relative activity = 92% in 1% cholinium glycinate	• 16.0% lignin oil yield • Presence of monomers including phenol and 1,2-dimethoxybenzene • Decrease in lignin molecular weight as compared to untreated	[145]
Lignin peroxidase	*Aspergillus oryzae* CGMCC5992	Maximum µa,max = 1.68 (mg/mL)/min, ka,m = 0.37 mg/mL, enzymatic activities range = 1500 to 1800 U/mL	• In a system of diluted solution, the Michaelis-Menten analogous model was followed by the oxidation degradation of lignin in lignocellulosic biomass	[146]
Manganese peroxidase	*Aspergillus flavus*	Maximum catalytic potential at 50 °C, pH 5.0, Vmax = 0.140 U/mg and Km = 0.704 mM, specific activity = 732 Ul mg^{-1}	• 100% decolorization of dye Direct red 31 • 92% decolorization of dye Direct red 31 Acid black 234	[147]
Manganese peroxidase	*Echinodontium taxodii* 2538	Maximum activity = 10 U g^{-1} culture, highest activity at pH 3.5 or 55 °C, specific activity = 46.52 U g^{-1}	• 4.1% degradation ratio for 4-Hydroxy cinnamic acid • 30.03% degradation ratio for 3-Methoxy cinnamic acid	[148]

6. Omics Technology and Enzyme Engineering for Enhanced Lignin Utilization

6.1. Omics Technology

Lignin degradation efficiency and its bioconversion into value-added products can be improved with the assistance of various genetic and pathway engineering approaches. The development of next-generation sequencing technology has completely changed insights into lignin degradation by offering a thorough multi-omics analysis that looks at the mechanisms at the molecular and systemic levels. This holistic approach leverages genomics, metagenomics transcriptomics, and proteomics, to reveal the intricate dynamics of gene expression, protein interactions, and metabolite changes throughout lignin metabolism [149,150]. Genomics has proven helpful in analyzing the genes of microorganisms involved in the synthesis of lignin-degrading enzymes. Genomic analyses of lignin-degrading microorganisms offer valuable insights into their lignin degradation capabilities through genome sequencing, assembly, and annotation, allowing for the identification of key genes, enzymes, and pathways that play a role in lignin metabolism [78]. For instance, genomic analysis of *Bacillus amyloliquefaciens* MN-13 was performed, showing that *B. amyloliquefaciens* MN-13 contained a large number of lignin-degrading enzymes and a pathway for the breakdown of aromatics, such as benzoate and aminobenzoate [151]. In another study, genome sequencing of *Rhizobium* sp. strain YS-1r and *Pseudomonas* sp. strain YS-1p showed many genes encoding for laccase, DyP-peroxidase, β-etherase, vanillate O-demethylase, feruloyl esterase, 4-hydroxybenzoate, catechol-2,3-dioxygenase, protocatechuate 3, 4-dioxygenase, and gentisate 1,2-dioxygenase, which are involved in the degradation of lignin and aromatic compounds, confirming the lignin-degrading ability of these microorganisms [152]. The genome of the anaerobic bacterium *Tolumonas lignolytica*

BRL6-1 T sp. was also sequenced and analyzed for the characterization of putative pathways involved in the degradation of lignin. The analysis revealed the presence of genes encoding for extracellular peroxidase, which is involved in lignin depolymerization, other enzymes involved in β-aryl ether bond cleavage, and other key enzymes involved in the pathway of lignin degradation [152]. With the help of whole genome sequencing, genes involved in the hydrolysis of biaryls and monoaryls derived from lignin were reported in *Sphingobium* sp. SYK-6 [153]. Comparative genome analysis of *Phanerochaete chrysosporium* and *Ceriporiopsis subvermispora* revealed that the genome of *C. subvermispora* contains 13 and 7 genes for manganese peroxide genes and laccase, respectively. While no laccase gene and five manganese peroxide genes were present in the genome of *P. chrysosporium.* Moreover, Microarray-based transcriptome study also showed the upregulation of manganese peroxide genes [154].

Metagenomics is another technology that is also helpful in analyzing the key gene coding for lignin-degrading enzymes. Metagenomics circumvents the drawbacks of laborious and conventional genome analysis techniques by enabling the comprehensive analyses of whole genetic material extracted directly from any environmental sample [155]. This approach directly unveils the bioactive capabilities of microbial consortia without relying on pure cultures. It also sheds light on the metabolic and cellular mechanisms behind the production of innovative bioactive metabolites [156]. Metagenomics has been extensively used for understanding lignin degradation by microorganisms. For instance, the functional traits of fungi and bacteria from coniferous forest soils across North America capable of degrading lignin, cellulose, and hemicellulose were identified and characterized using stable isotope probing coupled with amplicon and shotgun metagenomics. The results revealed overall high lignin degradation by gram-negative bacteria (*Comamonadaceae* and *Caulobacteraceae*) and cellulose degradation by fungi. Moreover, members of *Caulobacteraceae* were able to degrade all three components of LCB, demonstrating their significance in the breakdown of lignocellulose. Shotgun metagenome assembly was greatly improved by stable isotope probing, which resulted in the recovery of approximately 7500 contigs with distinct clusters of carbohydrate-active genes and many high-quality draft metagenome-assembled genomes. This research provides valuable insights into the organisms, environmental conditions, and associated functional genes that drive lignocellulose decomposition [157]. Metagenomics and metasecretomics-based tools were used to characterize microbial consortia capable of degrading three different plant biomass, including wheat straw, switchgrass, and corn stover. The biomass favored members of the *Bacteroidetes* (wheat straw) and *Proteobacteria* (switchgrass and corn stover). Moreover, a high lignin depolymerization of approximately 59% was observed in the case of switchgrass [158]. Another study extracted a lignin-degrading consortium from the soil of a sugarcane plantation. *Actinobacteria*, *Firmicutes*, and *Proteobacteria* were the most common taxa according to 16S rRNA taxonomy analysis. Numerous laccases, carbohydrate esterases, peroxidases, dye-decolorizing peroxidases, and lignocellulosic auxiliary (redox) activities were exhibited, and the main pathways associated with aromatic degradation were determined. The metagenomic dataset also yielded novel gene sequences encoding an enoyl-CoA hydratase/aldolase and a feruloyl-CoA synthetase, which were used to design a recombinant pathway for the production of vanillin [159]. These studies exhibit a targeted metagenomic discovery platform that may be useful in identifying microorganisms and gene sets that are optimal for initiatives based on synthetic biology principles to create high-value compounds from LCB.

Another versatile omics technology that enables comprehensive analysis of gene expression and regulatory pathways in specific tissues, conditions, or stages of development in an organism is transcriptomics [78]. This method aids in identifying the genes responsible

for regulation, metabolic pathways, and enzyme coding that are implicated in the degradation process. Through the analysis of the gene expression patterns of microorganisms that break down lignin, it is possible to pinpoint the precise gene groups that are active at various phases of lignin decomposition, ultimately identifying the crucial genes needed for the breakdown of lignin [76]. A more thorough understanding of the mechanisms at play can be obtained by comparing transcriptional data from other microbes to find similarities and differences in degradation pathways. Additionally, transcriptomic analysis may also help to study the impact of distinct microorganisms and their growth circumstances in diverse habitats on the potential for lignin breakdown. It has been widely used to study the genes, pathways, enzymes, and microorganisms related to lignin degradation. Transcriptomics analysis of white-rot fungus *Phanerochaete sordida* YK-624 was performed by studying the gene expression of YK-624 after growth under ligninolytic and nonligninolytic conditions and comparing the difference in gene expression with the model *Phanerochaete chrysosporium*. The results revealed the upregulation of ligninolytic and lignin-degrading auxiliary enzymes in YK-624. Moreover, under ligninolytic conditions, the genes implicated in the TCA cycle, lipid metabolism, carbon metabolism, and glycolysis were also upregulated in the case of YK-624 [160]. In another study, transcriptomics analysis of *Trametes gibbosa* indicated high laccase activity. These genes were associated with biological functions, such as the cell cycle, citrate cycle, nicotinate and nicotinamide metabolism, succinate dehydrogenase activity, flavin adenine dinucleotide binding, and oxidoreductase activity, all of which are closely linked to the laccase synthesis pathway [161]. The enzymes responsible for lignocellulose degradation by *Trametes versicolor* were identified by transcriptome analysis. Out of 853 differently expressed genes, 360 were upregulated and 493 were downregulated on poplar wood. Genes involved in lignin degradation, especially eight genes responsible for lignin peroxidase, were upregulated [162]. Metatranscriptomics and metagenomics techniques are frequently combined in research to advance knowledge and the investigation of microbial lignin breakdown. A combined metagenomics and metatranscriptomics study of the breakdown of lignin-based aromatics in thermal swamp sediments was conducted. The shotgun metagenome library was assembled using 351 distinct genomes that were resolved from mesophilic and hot spring sediments. An analysis of 39 refined draft genomes showed oligotrophy-like metabolism, including pathways for the breakdown of aromatic chemicals, including phenol, p-hydroxybenzoate, vanillate, and syringate. The extensive metabolic capacity of thermotolerant *Burkholderiales*, such as *Rubrivivax* ssp., was highlighted by their involvement in a variety of biogeochemical and aromatic transformations. Metatranscriptomics of sediment treated with kraft or milled lignin at 45 °C was used to further study lignin catabolism. Over the course of 148 h, aromatic compounds were reduced in the lignin-amended sediment. The metatranscriptomic data showed that the sphingomonads *Altererythrobacter* ssp. and *Novosphingobium* ssp, and *Burkholderiales* genus *Rubrivivax*, had upregulated des/lig genes that were predicted to specify the catabolism of syringate, vanillate, and phenolic oligomers [163].

An in-depth understanding of enzymes and proteins involved in the process of lignin degradation compared to genomics and transcriptomics can be obtained by using a proteomics approach. Proteomics focuses on understanding the complete protein complement (proteome) of a biological system under defined conditions or time periods. Unlike genomics, which deals with a static genome, proteomics is more intricate due to the dynamic and ever-changing nature of the proteome. Through mass spectrometry (MS)-based techniques, proteomics facilitates protein identification, quantification, localization, and the study of post-translational modifications and interactions [164]. Proteomic analysis of *Thermomyces lanuginosus* and *Thermobifidafusca* was performed to get insights into the collaborative mechanism for lignocellulose degradation by using different substrates. The

results showed the xylanolytic ability of *T. fusca* was inhibited by high concentrations of xylo-oligosaccharide or xylose. However, *T. lanuginosus* favored its growth by effectively consuming xylo-oligosaccharide and xylose [165]. The production of different proteins by *Aspergillus fumigatus* G-13 during the degradation of various lignin models (ferulic acid, sinapic acid, and *p*-coumaric acid) was analyzed by proteomics approach. It was observed that among 1447 identified peptides, 134 proteins changed significantly while 73 and 61 proteins were upregulated and downregulated, respectively. The significant proteins responsible for the degradation of lignin compounds were catechol dioxygenase, glutathione reductase, dextranase, isoamyl alcohol oxidase, glyceraldehyde-3-phosphate dehydrogenase and superoxide dismutase [166]. Genomic studies and proteomics of *Pandoraea* sp. ISTKB were conducted to identify key enzymes involved in the utilization of polymer kraft lignin and vanillic acid. The results revealed the presence of various lignin-degrading enzymes, including DyP-type peroxidase, peroxidases, glycolate oxidase, aldehyde oxidase, GMC oxidoreductase, laccases, quinone oxidoreductase, dioxygenases, monooxygenases, glutathione-dependent etherases, dehydrogenases, reductases, methyltransferases, and superoxide dismutases or catalase–peroxidase. The study revealed a significant stress response and detoxification system, identifying two critical lignin-degrading gene clusters and three polyhydroxyalkanoate polymerase-associated gene clusters, all of which demonstrated activity on KL–VA [167]. The metabolic adaptation of *Arthrobacter phenanthrenivorans* Sphe3 while using different substrates was investigated by employing a comparative quantitative proteomics approach. The involvement of various proteins in the degradation of aromatic substrates was confirmed. The quantity of proteins implicated in stress response, detoxification, membrane, and cell wall metabolism, and the metabolism of substrates and amino acids, were altered when aromatic substrates were present. The presence of aromatic compounds also resulted in the activation of a glyoxylate shunt due to the upregulation of crucial enzymes involved in its pathway [168]. Based on the above studies, it is evident that these findings are pivotal for advancing lignin biotransformation strategies and hold significant potential for applications in environmental biotechnology and the production of valuable products.

6.2. Enzyme Engineering

The function of lignocellulose-degrading enzymes may further be improved using enzyme engineering technology. Tailor-made enzymes can be designed to enhance their catalytic efficiency for the breakdown of all three major components of LCB. One of the key elements influencing the adoption of catalytic bioprocesses for industrial-scale biotransformation is believed to be protein engineering [169]. Enzyme properties have been improved through engineering techniques that utilize rational design or directed evolution (Figure 3), enabling the production of highly efficient biocatalysts that exhibit potential for various industrial applications [170,171]. As shown in Figure 3, engineered enzymes exhibit enhanced catalytic characteristics, kinetics, thermostability, pH stability, substrate specificity, regioselectivity, stereoselectivity, enantioselectivity, and resistance to organic solvents [172,173]. Directed evolution is a random mutagenesis technique that involves iterative mutagenesis cycles to produce a large library and high-throughput screening, followed by the identification of the better variants [174]. Directed evolution or molecular evolution is a promising technique that can create effective biocatalysts without detailed knowledge of enzyme sequence or three-dimensional structure [172]. Contrary to directed evolution, the rational design approach of protein engineering involves targeted mutagenesis computational techniques and a de novo design. It relies on the structure, function, and catalytic action of the enzyme of interest to induce specific site-directed mutagenesis-mediated modifications in the amino acid sequence [172,173,175,176]. A study reported

the application of a directed evolution approach for improving the catalytic efficiency of enzyme DyP from *Pseudomonas putida* MET94 for phenolic compound disruption. The error-prone polymerase chain reaction was used for three rounds of random mutagenesis. The results indicated a 100-fold enhancement in catalytic efficiency for substrate 2,6-dimethoxyphenol, better effectiveness for syringyl-type phenolics, guaiacol, aromatic amines, kraft lignin, and the lignin phenolic model dimer guaiacylglycerol-β-guaiacyl ether. The mutations were seen in three amino acids (E188K, A142V, and H125Y), all present on the enzyme surface [177]. Dye-decolourizing peroxidase Dyp1B from *Pseudomonas fluorescens* Pf-5 was engineered using directed evolution to improve its oxidation ability of lignin and phenolic compounds. An enhanced k_{cat}/K_M for Mn(II) oxidation up to 4–7 fold, thermostability, and product release were observed in the case of mutant enzymes as compared to wild type [178]. The native signal peptide of fungal laccases was replaced with the preproleader sequence from the α-factor of *S. cerevisiae.* Laccase activity in the extracts of *S. cerevisiae* cultures was enhanced using protein engineering approaches [179]. Lignin-based compounds were converted into *cis*,*cis*-muconic acid, for subsequent hydrogenation to adipic acid by metabolically engineering *Pseudomonas putida* KT2440. The engineered organism resulted in a *cis*,*cis*-muconate yield of 13.5 g L^{-1} in 78.5 h from a model lignin-based compound. The *cis*,*cis*-muconic acid was later hydrogenated to adipic acid with a conversion rate of more than 97% using Pd/C catalyst [180]. Considering the above-mentioned studies, it is evident that using semi-rational design methodologies that combine artificial intelligence with knowledge of protein structures and sequences, these techniques may aid in producing diverse enzymes with higher specificity and functionality tailored for various industrial applications.

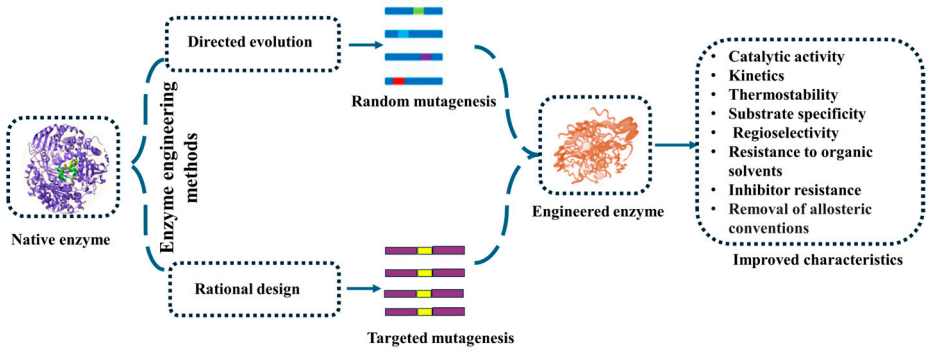

Figure 3. Various enzyme engineering approaches for the development of engineered enzymes with advanced properties.

Artificial intelligence (AI) and CRISPR-Cas gene editing are two cutting-edge techniques that have recently become effective instruments for enzyme engineering technology in lignin valorization. Machine learning (ML) approaches are required for enzyme biosystem modeling due to the enormous volumes of multi-omics data that are available and the increasing high throughput. ML models have the potential to uncover patterns in complex biological data, including genotype at various scale analyses and phenotype at metabolic status. By forecasting novel candidates or procedures for the production of valuable products from lignin, pattern recognition, and statistical models facilitate the design applications of biosystems [181]. Even though genomics, metabolomics, and gene alterations for lignin valorization strategies are going ahead with distinct achievements, integrated data-driven prediction models of microbial strains/consortia with improved enzymatic efficiencies for lignin valorization are still needed. Understanding the integrated operation and compatibility of novel enzyme systems within microbial communities or microbes is also essential. In order to mimic microbial systems, synthetic biology is needed,

along with investigation in non-model organisms and robust experimental validation of the metabolic, biochemical, regulatory, and genetic underpinnings of certain products and defined processes in known microorganisms.

7. Challenges and Future Perspectives

Lignocellulosic biomass is a viable substitute for producing fuels and fine chemicals, where cellulose and hemicelluloses, as alternative carbon sources, have been extensively investigated. However, research on lignin valorization to bioproducts development as an essential component of modern biorefineries has picked up pace in recent years only. Despite significant advancements, there are still many challenges with lignin depolymerization using different lignin-degrading enzymes. The limited specificity and efficacy of natural lignin-degrading enzymes is one of the main obstacles observed. These enzymes frequently have low catalytic activity, are unstable at pilot-scale or in industrial environments, and are easily inhibited by lignin breakdown products. Furthermore, enzyme performance varies due to the structural complexity and diversity of lignin generated from various biomass sources. Large-scale industrial uses of these enzymes are also restricted by the continued high cost of downstream processing and enzyme production. In addition to this, certain end products generated via lignin degradation may inhibit enzyme activity, reducing the overall efficiency of the process.

For years, researchers have investigated lignin production or valorization with different lignin extraction methods, and more recently, they have used genetic engineering tools to tailor the biological pathways to boost pulping efficiency or saccharification yield in a biorefinery. Although it has been demonstrated that these feedstocks with engineered lignins fulfill the objective function of raising saccharification yields, they have not yet been thoroughly investigated with the goal of generating lignin degradation products that microorganisms may readily catabolize.

Additionally, some naturally occurring lignins found in specific plant species have unique chemical compositions that could result in much more uniform slates of lignin breakdown products that are better suited for microbial catabolism. A potential illustration would be the recently identified C-lignin, which is made up of caffeyl alcohol and found in the seed coats of certain species of *Cactaceae* and vanilla. Moreover, to measure and monitor the conversion of lignin degradation products within actual lignin-enriched process streams, significant developments in analytical technology are also required. These tools are essential for determining how well microorganisms convert lignin overall, guaranteeing process consistency, and comprehending the metabolic patterns that each microbe displays in a chemically complicated matrix. Promising innovative methods to improve enzyme stability, catalytic efficiency, and substrate specificity have been made possible by recent developments in enzyme engineering, such as directed evolution and rational protein design. Finding new lignin-degrading enzymes and better understanding the metabolic pathways involved in lignin catabolism could be made easier by the integration of omics technologies, including proteomics, metabolomics, and genomes. In order to create microbial consortia that can break down lignin cooperatively, synthetic biology techniques should also be investigated.

Analyzing the impact of lignin on enzymatic hydrolysis is made more difficult by the composition and structural differences of lignin from various biomass and pretreatment methods. For the effective analysis of lignin, it is imperative to speed up the development of analytical tools and techniques, such as high throughput, and multivariate analysis modeling, combining several analytical techniques to create a comprehensive range of lignin structural features. In the future, industrial scalability will depend on the creation of reliable enzyme cocktails suited for certain lignin feedstocks as well as affordable enzyme

production technologies. The efficiency of lignin valorization can be further increased by combining biocatalysis with physical and chemical "green" pretreatment techniques. The genetically engineered microbial hosts (bacteria, fungi) could be used for high-yield, cost-effective enzyme production. Moreover, developing enzymes with reduced sensitivity to inhibitors through rational design or mutagenesis is a promising and viable solution for better efficacy of the lignin degradation process. Furthermore, there are new opportunities to advance this sector more quickly thanks to the use of artificial intelligence and machine learning in enzyme design and pathway optimization.

In order to maintain the value of the resources in the cycle, a lignin-based circular bioeconomy uses waste and lignin byproducts as feedstocks (Figure 4). The cradle-to-cradle concept states that since lignin is depolymerized by extracellular oxidative enzymes to produce low molecular weight aromatic compounds, a lignin-based circular bioeconomy allows the incorporation of technical materials into the biological cycle. The market value of lignin-based products has been rising, and new knowledge on technologies that use lignin for various products and uses has been clarified with the circular economy element, with an emphasis on creating a practical, affordable lignin valorization process. From a commercial perspective, the potential applications of lignin will be determined by the selling price range of lignin from biorefineries in the circular economy. The degree of standardization of lignin as the primary substrate and the level of purity of the lignin-derived products using enzymatic methods must be determined by markets in order for them to be more readily accepted. More lignin is anticipated to be produced as a byproduct by different industrial sectors as more regulations are implemented in the direction of a structured bioeconomy. In light of this, the studies concentrating on biocatalysis, sourcing, durability, separability, and recyclability will significantly advance the creation of circular lignin materials in the years to come.

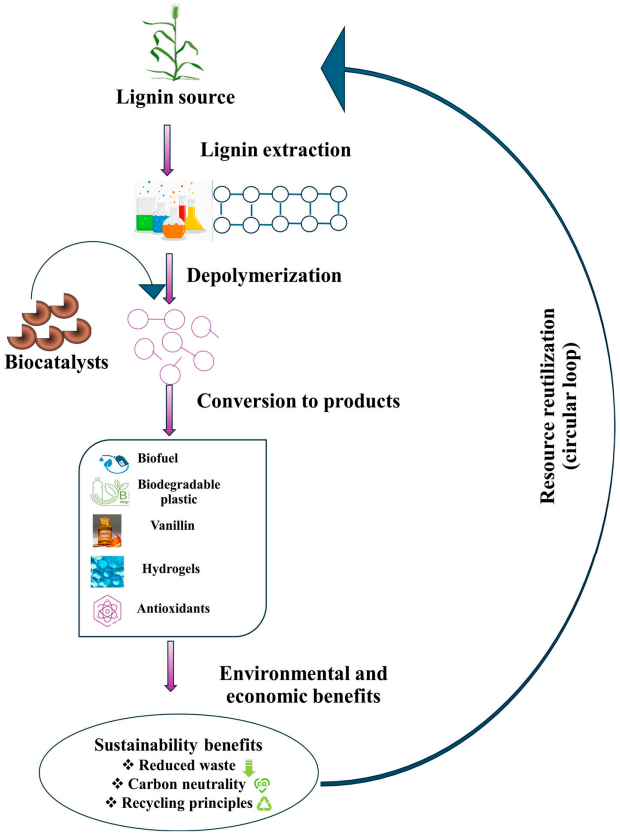

Figure 4. Flowchart depicting lignin valorization into value-added products and sustainability benefits in a lignin-based circular economy.

8. Conclusions

Lignin is the most prevalent aromatic heteropolymer that represents a significant renewable resource for producing high-value chemicals, fuels, and biomaterials. Recent developments in the use of lignin across various industrial sectors highlight its potential as a crucial element in high-performance, eco-friendly materials of the future. The current review outlines the valorization of lignin through an environment-friendly biological process involving microbial enzymes. The use of lignin-depolymerizing enzymes has demonstrated remarkable potential in selectively breaking down lignin's complex structure under mild reaction conditions. Microbial systems, including engineered strains, further contribute to enhancing lignin depolymerization and product specificity. When combined with cutting-edge bioprocess techniques like multi-enzyme cascade reactions and immobilized enzyme systems, lignin bioconversion's scalability and viability have significantly increased. Additionally, combining biocatalytic methods with other valorization techniques, including solvent extraction or chemical catalysis, has demonstrated synergistic benefits, increasing the conversion efficiency of lignin. However, to enable the sustainable and economical use of lignin for the production of biofuels, platform chemicals, and advanced biomaterials, future research should emphasize overcoming present constraints through interdisciplinary approaches.

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