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Review

Laccase in Biorefinery of Lignocellulosic Biomass

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Abstract: Biorefinery has emerged in recent years as an alternative to petrorefinery, as biofuels have all the potential to replace fossil fuels for the sustainable development of human society. From this aspect, lignocellulosic biomasses are the most important, since these are the most abundant ubiquitous most raw material on earth, which can be converted into biofuels such as bioethanol, biobutanol, biohydrogen, biogas, etc. There are several strategies for conversion, such as biochemical, thermochemical, and microbial conversions of biomasses to biofuels; however, each of the strategies has its own consequences. Enzymatic conversion of biomasses into sugars, and thereby into bioethanol, is considered as the most sustainable way. However, biomass recalcitrance to enzymatic hydrolysis is the biggest challenge, as cellulose, hemicellulose, and lignin are intricately attached to each other making their separation a tedious task. Pretreatment is necessary to partially remove or change the form of lignin to make cellulose and hemicellulose accessible to enzymes. Most of the pretreatment methods are designed to target lignin, as it is the major component responsible for recalcitrance nature of biomasses. Laccase is a versatile lignin-degrading or lignin-modifying enzyme which is secreted by filamentous fungi and bacteria, and is reported for the biological pretreatment of biomasses, which is the most sustainable way of pretreatment. However, the rate of the reaction is extremely slow making it less attractive. This article will give an insight into the biorefinery of biomasses, with the special significance to laccase.

Keywords: biorefinery; biomass; fungi; laccase; biological pretreatment

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1. Introduction

Lignocellulosic biomasses contain approximately 40–50% cellulose, 20–30% hemicellulose, and 20–30% lignin, as well as other minor components, such as ash and extractives [1]. Cellulose is a linear polymer of glucose molecules that are interconnected by β-1,4-glycosidic bonds. Hemicellulose is a heteropolymer composed of different sugar units that vary depending on the source of the biomass [2]. Lignin is a highly branched and cross-linked polymer that provides mechanical strength and protection against biotic and abiotic stresses. Lignin is a major component of plant biomasses, comprising up to 30% of the dry weight of woody plants. It plays an important role in providing rigidity and structural support to the cell walls, as well as protecting the plant from pests and pathogens. However, lignin is also responsible for the recalcitrance of plant biomasses, which makes it difficult to break down into its constituent parts [3]. Thus, the enzymatic breakdown of biomasses, as well as lignin, is a tedious and slow process, as nature has created them

to provide resistance to the erected plants with a microbial attack. This has been a major challenge in the development of biofuels and other applications that require the use of plant biomasses as a feedstock. The conversion of plant biomasses into useful products via biorefinery has been the subject of intensive research in recent years [4]. Enzymatic conversion of biomasses into their monomeric components has been regarded as the most sustainable way. One of the main challenges in biomass conversion is the recalcitrance of lignin, which makes it difficult to break down into its constituent sugars. Laccase has been shown to play an important role in the conversion of lignin to its constituent parts. Additionally, lignin peroxidase, manganese peroxidase, and versatile peroxidase are known to act on lignin in synergy [5]. These enzymes are usually produced along with laccase by fungi, such as *Phanerochaete chrysosporium*, which enables the fungi to degrade lignin completely. The action and mechanism of each of these enzymes have been discussed by Singhania et al., 2022 [1]. Laccase is a type of enzyme that is widely distributed in nature, being produced particularly by fungi and bacteria. It catalyzes the oxidation of a wide range of substrates, including phenols, polyphenols, and aromatic amines [6–8]. Laccase is classified as an oxidoreductase enzyme because of its catalytic property allowing it to transfer the electrons from a substrate to an oxidant. It has been shown to be an efficient catalyst for the degradation of lignin [9]. This makes it an attractive target for the development of new technologies for the conversion of plant biomasses into useful products.

Laccase acts by oxidizing lignin, which breaks it down into smaller molecules that can be more easily degraded by other enzymes. Laccase has also been shown to play a role in the conversion of other components of plant biomasses, including cellulose and hemicellulose [10]. In recent years, there has been a growing interest in using laccase-based technologies to break down lignin and other components of plant biomass [11]. This article will provide insight into the significance and contribution of laccase in biorefinery. Biomasses need to be pretreated first to undergo enzymatic treatment for any other biorefinery process. Biological pretreatment of biomasses by laccase is considered the most sustainable one. Laccase production via different modes of bioprocesses has been presented. This is a hot subject, as lignin valorization is much needed for an economically efficient biorefinery process of lignocellulosic biomasses. Laccase plays an extremely important role in this area.

2. Pretreatment of Lignocellulosic Biomasses

Lignocellulosic biomasses are an abundant and renewable source of bioenergy, which have the potential to reduce dependence on non-renewable resources. However, the complex structure of lignocellulose and its resistance to enzymatic hydrolysis present a major challenge to its utilization [12]. The removal or modification of lignin is necessary to increase the accessibility of the cellulose and hemicellulose fractions, which can be converted into biofuels, chemicals, and other valuable products. Pretreatment is a crucial step, regarded as one of the most cost-intensive steps in the process of lignocellulosic biomass conversion [13]. There are various techniques for biomass pretreatment which need to be selected wisely based on the nature of feedstock, process, end product, and its end application. Table 1 shows advantages and disadvantages of each pretreatment methods applied which is helpful to decide which one to use for particular biomass and process. Figure 1 shows a schematic of the effect of pretreatment on biomasses.

Table 1. Table showing different pretreatment techniques and their advantages and disadvantages.

Pretreatment Technique	Principle	Advantages	Disadvantages
Biological Pretreatment	Most biological pretreatment methods utilize the ligninolytic enzyme system, which consists of oxidoreductases that can break down lignin, thereby enhancing biomass degradation.	Environmentally friendly and sustainable; can be conducted at low temperatures and pressures; generates fewer inhibitor compounds	Slow process; may require multiple stages; limited applicability to certain types of biomasses; high enzyme costs
Hydrothermal Pretreatment	Water at subcritical temperature acts as a catalyst as it changes into the hydronium ion, the pH becomes acidic and liquifies the cellulose and hemicellulose to an extent.	Environmentally friendly and sustainable; can be conducted without chemicals or solvents; can improve the accessibility of cellulose; can produce high sugar and ethanol yields	Requires a significant energy input; may generate significant amounts of inhibitor compounds; limited effect on lignin
Organosolv Pretreatment	Dissolves lignin and hemicellulose, leaving behind solid crystalline cellulose.	Can selectively extract lignin and hemicellulose; yields high-quality lignin that can be used for high-value products; can be conducted at low temperatures and pressures; generates fewer inhibitor compounds than acid pretreatments	Requires the use of organic solvents, which can be expensive and hazardous; may generate significant amounts of waste; can be energy-intensive; limited scalability
Dilute Acid Pretreatment	Uses dilute acid to selectively remove hemicellulose and increase the accessibility of cellulose.	More environmentally friendly than concentrated acid pretreatments; can selectively remove hemicellulose and increase the accessibility of cellulose; can produce high sugar and ethanol yields	Generates significant amounts of inhibitor compounds; requires a large amount of water and energy input; limited applicability to certain types of biomasses; may require multiple stages
Alkali Pretreatment	Alkali pretreatment changes the lignocellulosic structure by causing cellulose swelling, reducing crystallinity, and increasing polymerization. The internal surface area is enhanced due to the removal of acetyl groups and uronic acids from hemicellulose.	Can be effective at breaking down lignin and hemicellulose; can increase accessibility to cellulose; can produce high sugar and ethanol yields	May require significant amounts of water

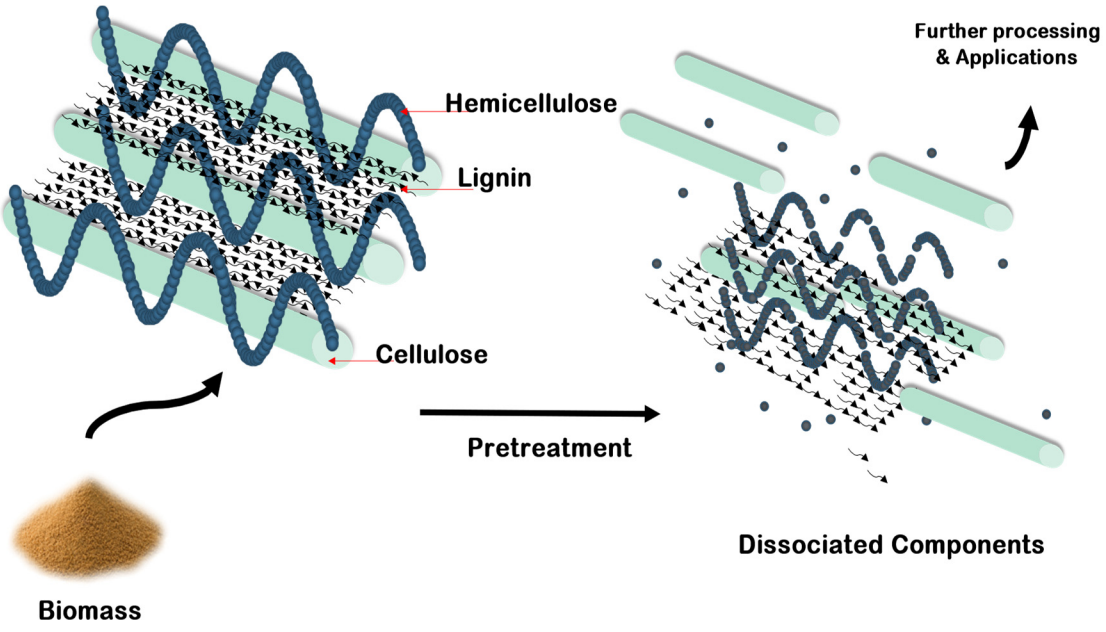

Figure 1. Effect of pretreatment on biomasses.

2.1. Acidic Pretreatment for Lignocellulosic Biomasses

Acidic pretreatment involves the use of acid at various concentrations, which acts on the hemicellulose fraction of the lignocellulosic biomass, thus, leaving behind a more amorphous cellulose fraction to be accessible to enzymes. When hemicellulose is removed from tightly packed biomass, lignin and cellulose become loosely packed. The most used acid for this purpose is sulfuric acid, although other acids, such as hydrochloric acid and nitric acid, can also be used [14]. The conditions for acidic pretreatment exhibit a temperature range of 120–200 °C and a pH range of 1–2.5. The disadvantages of acidic pretreatment include the generation of inhibitory compounds, such as furfural and 5-hydroxymethylfurfural (HMF), which can inhibit downstream enzymatic hydrolysis [15].

Various types, as well as various concentrations of acids may be employed for pretreatment methods that can be used to treat lignocellulosic biomasses, and can be categorized based on the concentration as dilute acid pretreatment, concentrated acid pretreatment, sulfite pretreatment, etc. Dilute acid pretreatment is a method that uses a low concentration of acid (usually sulfuric acid) at a moderate temperature (around 120–170 °C) for a short period (around 10–60 min) [16]. This method is effective in breaking down the hemicellulose component of the biomass and releasing monomeric sugars. Concentrated acid pretreatment is a method that uses a high concentration of acid (10–70% usually sulfuric acid) at a high temperature (around 180–220 °C) for a long period (around 1–5 h). This method is more effective in breaking down both the hemicellulose and lignin components of the biomass, making the cellulose component more accessible [17].

There is a sulfite pretreatment that uses a mixture of sulfite and acid to pretreat the biomass, which is effective in breaking down the lignin component of the biomass, and also lowers the hydrophobicity of lignin by sulfonation, making the cellulose component more accessible [18].

2.2. Alkali Pretreatment for Lignocellulosic Biomasses

Alkali pretreatment involves the use of alkali to break down the lignin and hemicellulose fractions of lignocellulosic biomasses. The most commonly used alkali for this purpose is sodium hydroxide (NaOH), although other alkalis, such as potassium hydroxide (KOH) and calcium hydroxide ($Ca(OH)_2$), have also been used. For alkali pretreatment the range of temperature varies from 70 to 120 °C, with a pH of 10–12. Alkali pretreatment is highly efficient in the removal of lignin and thereby enhances enzymatic hydrolysis. However, the generation of inhibitory compounds, such as phenols, is a major disadvantage which can inhibit downstream enzymatic hydrolysis [19].

2.3. Hydrothermal Pretreatment for Lignocellulosic Biomasses

Hydrothermal pretreatment is a process that involves the treatment of lignocellulosic biomasses with hot pressurized water or steam. The process typically takes place at high temperatures, mostly in the range between 160 and 260 °C, and pressures between 5 and 30 bar [20]. There are several types of hydrothermal pretreatments, including steam explosion, and liquid hot water [21]. Steam explosion is a process that involves the rapid depressurization of hot pressurized water, which causes the explosion of the biomass fibers and the release of soluble sugars [22]. Liquid hot water pretreatment is a process that involves the treatment of biomass with hot pressurized water, without any explosive decompression [23]. The high temperature and pressure conditions cause the breakdown of hemicellulose, which is the most labile component of lignocellulosic biomasses. The breakdown of hemicellulose results in the formation of soluble sugars, which can be easily converted into value-added products.

The process also leads to the partial depolymerization of lignin, which makes the remaining cellulose more accessible to enzymes for hydrolysis. The advantages of hydrothermal pretreatment include the removal of lignin, thereby making the biomass more amorphous, leading to an enhancement of enzymatic hydrolysis. The disadvantages include the generation of inhibitory compounds, such as acetic acid and formic acid, which

can inhibit downstream enzymatic hydrolysis [20]. Hydrothermal processes, coupled with solid catalysts, have been explored for biomass valorization [24].

2.4. Organosolv Pretreatment

Since the 1970s, organosolv pulping is known as an eco-friendly pulping method to extract lignin from lignocellulosic feedstock. One of the key advantages of organosolv pulping is that it enables the isolation of lignin as a solid material and carbohydrates as a syrup, both of which can be utilized as chemical feedstocks [25]. Furthermore, the solvents used in the process can be easily recovered by distillation and recycled for pretreatment purposes. Organosolv pretreatment is similar to organosolv pulping, but does not achieve the same degree of delignification as the latter. This approach allows for the utilization of all biomass components, but it comes with certain inherent drawbacks, such as the need for elaborate washing arrangements to prevent the re-precipitation of dissolved lignin [26]. Additionally, the use of organic solvents in organosolv pretreatment can be expensive and must be recovered as much as possible. This process must also be performed under tight and efficient control due to the hazardous nature of organic solvents, which are volatile. Organosolv pretreatment can be carried out in various solvents, with or without added catalysts, and at temperatures ranging from 100 to 250 °C [27]. Acid catalysts, such as mineral acids and some organic acids, can accelerate delignification and xylan degradation, leading to higher yields of xylose [15]. The pretreatment yields three separate fractions: dry lignin, an aqueous hemicellulose stream, and a relatively pure cellulose fraction. However, solvent removal is necessary to avoid inhibiting organism growth, enzymatic hydrolysis, and fermentation [28].

2.5. Biological Pretreatment for Lignocellulosic Biomasses

Biological pretreatment methods have gained attention in recent years as an eco-friendly and sustainable solution for waste management and environmental remediation. In this pretreatment method, primarily, lignin is depolymerized and in turn increases biomass accessibility to enzymes. During this process inhibitory compounds are not released being biological in nature, causing easy downstream processing. It involves the use of microorganisms, such as fungi and bacteria, or even free enzymes, such as laccase, to break down complex lignocellulosic compounds into simpler forms. This process enhances the biodegradability of the waste and reduces the time required for biodegradation. However, here we will discuss the fungi-, bacteria-, and enzyme-based biological pretreatment [29]. Microorganisms such as bacteria and fungi have been used for biological pretreatments of lignocellulosic biomasses. These microorganisms secrete ligninolytic enzymes that can break down lignin into smaller fragments. The most used microorganisms for this purpose are white-rot fungi, such as *Phanerochaete chrysosporium* and *Pleurotus ostreatus* [6,30]. Fungi can break down lignin into its constituent monomers, making it more accessible. The most commonly used fungi for lignin depolymerization are white-rot fungi, which secrete ligninolytic enzymes, such as laccase, manganese peroxidase, and lignin peroxidase [31]. Bacteria are also used for the biological pretreatment of lignocellulosic biomasses. Some bacteria secrete ligninolytic enzymes, while others can utilize lignin as a carbon source. Some common bacteria for this purpose are *Streptomyces* sp. and *Bacillus* sp. [32]. The major disadvantage is that it is quite slow; hence, it takes days and weeks to get biomasses pretreated by this process. It can also be done using enzymes directly rather than microorganisms. The common enzymes used for biological pretreatment are laccases, manganese peroxidases, and lignin peroxidases [33]. This is comparatively faster than the earlier one, where microorganisms are employed rather than the enzymes directly. These enzymes are mostly produced by filamentous fungi.

Most biological pretreatment methods utilize the ligninolytic enzyme system, which consists of oxidoreductases that can break down lignin, thereby enhancing the biomass degradation. The system comprises laccases and peroxidases that possess a high redox potential and can oxidize the lignin polymer either directly or via mediators, i.e., low-

molecular-weight organic compounds capable of attacking the lignin structure by diffusing into the cell wall pores [34]. Biological pretreatment can be carried out using microorganisms that secrete ligninolytic enzymes or the enzymes themselves to disrupt part of the lignin and its structure, thus enabling hydrolytic enzymes used in the biorefinery process to access holocellulose and release sugars [35]. Besides improving feedstock enzymatic digestibility, biological pretreatments have other potential biorefinery applications. The produced enzymes can be extracted and used in other processes, and the pretreatment may yield coproducts, including lignin derivatives and organic compounds that act as platform chemicals and enhance the process value [35]. Converting the nonfermentable constituents of lignocellulosic biomasses into value-added products is a crucial missing link for the biorefinery success [36]. Generally, ligninolytic microorganisms and enzyme extracts can serve as biocatalysts for many reactions aimed at producing biofuels and bioproducts within biorefinery.

3. Significance of Laccase in Biorefinery

In recent years, there has been a growing interest in lignin biorefinery for the production of valuable products. Lignin is the most abundant aromatic polymer available on Earth for utilization by human beings. For economic biomass biorefinery, it is necessary to utilize lignin for value addition rather than burning it for fuels. Generally, it is produced in large quantities in the pulp and paper industry as a waste byproduct, and is typically used as a fuel or disposed of as waste. Laccases are versatile enzyme which can act on lignin to break it into its individual components of monolignols: p-coumaryl, coniferyl, and sinapyl alcohols. Lignin has first emerged as a popular concept in biorefinery where lignin is extracted first and employed for various applications, as shown in Figure 2. The integration of lignin biorefinery in cellulose and hemicellulose biorefinery has several advantages over traditional biorefinery processes. It is the only way for making the overall process economic.

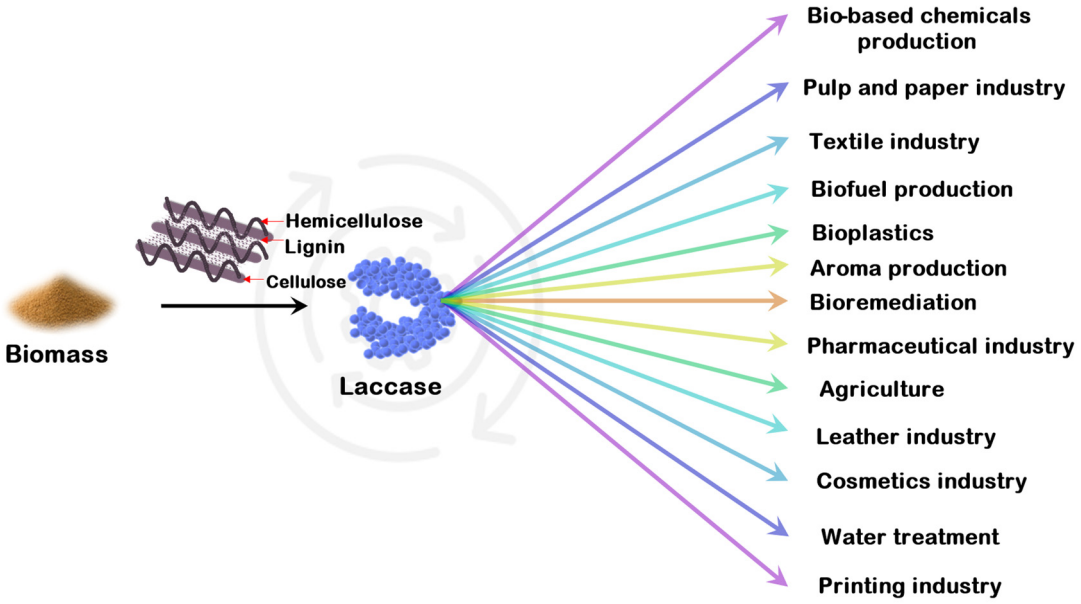

Figure 2. Figure showing the application of laccases in different industries and bioprocesses.

By using lignin as a substrate for laccase, integrated biorefinery can reduce the cost of production and increase the sustainability of the process. Lignin can be deconstructed into its components via lignin-degrading enzymes, including laccase. Figure 3 shows the chemical composition of lignin, and its structure and hydrolyzed components as well.

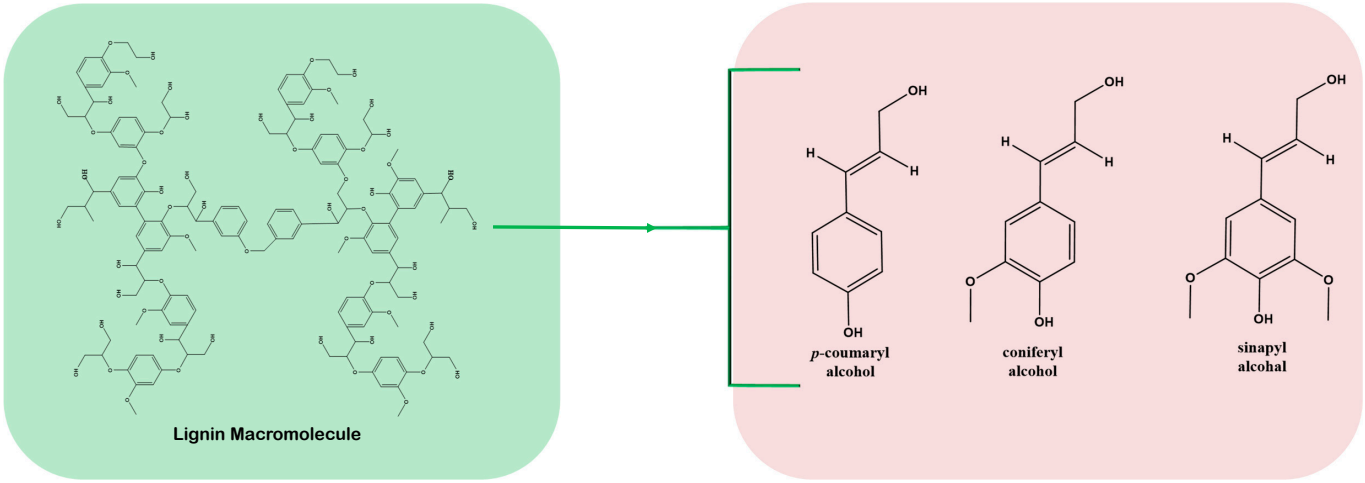

Figure 3. Structure and components of lignin.

3.1. Laccase Sources

Laccase has been gaining significant attention for its remarkable ability to oxidize a wide range of organic compounds. With its potential applications in various industrial and environmental fields, laccase has become a highly sought-after enzyme. However, its availability and cost have been significant limitations in its widespread use. Microorganisms are the primary source of laccase production, and several bacterial, yeast, actinomycetes, and fungal species have been reported to produce laccase.

3.1.1. Bacterial Laccase Production

Bacteria are among the earliest reported sources of laccase, with several species from different taxonomic groups known to produce laccase. Bacterial laccase production is influenced by several factors, including the type of bacterium, culture conditions, and substrate availability. Some of the commonly reported bacterial sources of laccase include *Bacillus*, *Pseudomonas*, and *Streptomyces* species [37]. Other than bacteria and fungi, few yeast species, including *Candida*, *Rhodotorula*, and *Saccharomyces* species [38], and actinomycetes, such as *Streptomyces*, *Nocardiopsis*, and *Micromonospora* species [39], have been reported.

3.1.2. Fungal Sources of Laccase

Fungi are the most widely studied and reported sources of laccase, with several fungal species from different taxonomic groups known to produce laccase. Fungal laccase production is influenced by several factors, including the type of fungus, culture conditions, and substrate availability. Some of the commonly reported fungal sources of laccase include white-rot fungi, brown-rot fungi, and ascomycete fungi [40]. White-rot fungi are the most extensively studied group of fungi known to produce laccase. White-rot fungi laccase production is influenced by several factors, including the type of fungus, culture conditions, and substrate availability. Some of the commonly reported white-rot fungal sources of laccase include the *Phanerochaete chrysosporium*, *Trametes versicolor*, and *Pleurotus ostreatus* species [41]. Brown-rot fungi are another group of fungi known to produce laccase. Brown-rot fungi laccase production is influenced by several factors, including the type of fungus, culture conditions, and substrate availability. Some of the commonly reported brown-rot fungal sources of laccase include the *Gloeophyllum trabeum*, *Fomitopsis pinicola*, and *Laetiporus sulphureus* species [42]. Ascomycete fungi are a group of fungi that produce ascospores during sexual reproduction. Several ascomycete fungi have been reported to produce laccases, including *Nectriella pironii*, *Aspergillus*, and *Penicillium*. Ascomycete fungi laccases have potential applications in the production of aromatic compounds and the degradation of lignocellulose [43].

4. Bioprocesses for Laccase Production

There are two main bioprocesses for microbial production of laccase: submerged fermentation (SmF) and solid-state fermentation (SSF). Table 2 gives an account of various microorganisms involved in laccase production, employing different modes of bioprocesses.

4.1. Solid-State Fermentation

Solid-state fermentation (SSF) is a fermentation method that involves the growth of microorganisms on the moist solid substrate or on the surface of a solid support material, which does not act as carbon source without the presence of free water. The solid support material used for fungal growth can be either inert or biomaterial, typically lignocellulose. SSF is regarded as superior bioprocess by many researchers for enzyme production via filamentous fungi, as it closely imitates its natural environment [44,45]. It can also be more environmentally friendly, as it can use agricultural waste as a substrate, which can reduce waste and improve sustainability.

Agricultural waste, being lignocellulosic in nature, can be used for laccase production, such as rice straw. Rice straw is produced in large quantities globally and could be a potential substrate for laccase production. Studies have shown that rice straw can be effectively used as a substrate for laccase production by fungi, which can be used for various industrial applications. When lignocellulosic material is used primarily as a carbon source and then as support, the fungal mycelium grows through the biomaterial, inducing laccase production due to the presence of lignin, and a slow nutrient release effect makes it better. Water alone can be used as a moisturizing agent, but solutions containing carbon and nitrogen stimulants may also be used to accelerate the initial stages of material colonization to improve laccase productivity [46]. The properties of the lignocellulosic material, such as particle size and composition, as well as the type of moisturizing agent, are critical factors that affect both the cost and efficiency of the production process [44].

Other lignocellulosic agricultural wastes that have been studied for laccase production include wheat straw, corn stover, and sugarcane bagasse. Limitations and challenges are also associated with SSF, such as difficulty to control and online-monitoring, which makes optimization difficult as compared to SmF [47]. There are several factors that can affect the production of laccase from lignocellulosic agricultural wastes, such as particle size of the substrate, moisture content or water activity, including the type of waste used and the type of fungus used. Optimizing these factors can improve the efficiency and yield of laccase production from lignocellulosic agricultural wastes [48].

4.2. Submerged Fermentation

Submerged fermentation (SmF) is a process in which microorganisms are grown in liquid media. This is the most common bioprocess for producing laccase, as it allows for the large-scale production of the enzyme and control, as well as online monitoring. In this process, the microorganisms are grown in a controlled environment, and the conditions (e.g., temperature, pH, nutrient levels) can be regulated to maximize the production of laccase [49]. In SmF, laccase production is typically enhanced by adding nutrients such as glucose, peptone, and inorganic salts to the culture medium. The pH of the medium is also carefully controlled along with aeration for laccase production. SmF has several advantages for laccase production, the best two being an easy scale-up in a relatively short period of time, and an easy recovery of the enzyme from the fermentation broth. However, SmF also has some limitations, including the need for a sterile environment and the risk of contamination by other microorganisms [50].

Table 2. An account of microorganisms producing laccase via different modes of bioprocesses.

Serial No.	Microorganisms	Bioprocess Type	Titers of Enzyme	Application	Reference
	Bacteria				
1.	*Bacillus sp.* PCH94	SmF	0.27 IU/mL	Lignin depolymerization	[51]
2.	*Aquisalibacillus elongatus*	SmF	8.02 U mL^{-1}	Biowaste delignification Biotreatment	[52]
3.	*Bacillus subtilis* LP2	SmF	140.4 U/mg	Bioremediation process	[53]
4.	*Bacillus tequilensis* SN4 MTCC 11828	SmF	18,356 nkats/ml	Degradation of residual lignin in kraft pulp	[54]
5.	*Bacillus safenis* DSKK5	SmF	10.51 U/mL	-	[55]
6.	*Geobacillus thermocatenulatus* MS5	SmF	1.52 U/mg	-	[56]
7.	*Pseudomonas aeruginosa*	SmF	0.038 U/ml	-	[57]
8.	*Pseudomonas desmolyticum* NCIM 2112	SmF	0.012 ± 0.0003 U/mg	-	[58]
9.	*Pseudomonas putida* F6	SmF	573 U/mg	-	[59]
10.	*Azospirillum lipoferum*	SmF	0.9 U/mg	-	[60]
11.	*Ceratorhiza hydrophila*	SmF	154 U/mL	Textile dyeing and printing processes	[61]
	Fungi				
12.	*Trametes versicolor*	SSF	77.88 ± 5.62 U/g	Pre-industrial procedures to produce laccases	[62]
13.	*Pycnoporus sanguineus*	SSF	130.95 ± 2.20 U/g	Pre-industrial procedures to produce laccases	[62]
14.	*Trametes gibbosa* An 360	SmF	55.83 ± 0.28 U/L	-	[63]
15.	*Vanderbylia fraxinea* An 369	SmF	77.96 ± 1.60 U/L	-	[63]
16.	*Perenniporia pyricola* Han 202	SmF	443.33 ± 15.49 U/L	-	[63]
17.	*Coriolopsis trogii* Han 474	SmF	686.57 ± 16.49 U/L	-	[63]
18.	*Trametes versicolor* Han 1504	SmF	162.04 ± 11.33 U/L	-	[63]
19.	*Pleurotus floridanus*	SmF	80.45 ± 0.132 U/mL	Bioprocess for de-oiled microalgal biomasses	[64]
20.	*Ganoderma leucocontextum*	SmF	855 U/L	-	[65]
21.	*Trichoderma harzianum* S7113	SmF	391.38 ± 9.51 U/L	-	[49]
22.	*Trametes trogii*	SSF	2.1 U/g	-	[66]
23.	*Pycnoporus cinnabarinus*	SmF	280 U/L	Degradation of the disazo dye	[67]
24.	*Trametes pubescens*	SmF	333,000 U/L	-	[68]
25.	*Neurospora crassa*	SmF	10,000 U/L	Bioremediation of phenols	[69]
26.	*Trametes versicolor*	SSF (Immersion, nylon sponge)	229	-	[70]
27.	*Trametes hirsuta*	SSF (Tray, grape seeds)	18,715 U/L	-	[71]

5. Applications of Laccases

Laccases are versatile, nonspecific enzymes involved in a wide range of biological processes, including lignin degradation, pigment synthesis, and the detoxification of toxic compounds. Lignin is a complex polymer found in plant cell walls that gives plants their structural support. Laccases are involved in the breakdown of lignin, which is important for the recycling of plant material and the release of components back into the environment [6].

In pigment synthesis, laccases are involved in the production of pigments, such as melanin, by endogenous substrates such as dihydroxy-naphthalene, and exogenous substrates such as homogentisic acid and dihydroxyphenylalanine [72]. These pigments play important roles in various functions, such as protection from UV radiation and production of antibiotic compounds. Laccases are also involved in the detoxification of toxic compounds. They help convert toxic compounds into less harmful forms, making them less harmful to the organisms that produce them to the environment in which they are found [7]. In addition to their biological roles, laccases have also been studied for their potential use in bioremediation. They have been shown to be effective in removing contaminants such as pesticides and dyes from water and soil. Laccases have also been explored for their potential use in the production of paper, textiles, and other products. The study of laccases has led to a better understanding of their structural and functional properties, and this knowledge has been used to improve its production and use in various applications. For example, researchers have developed methods to improve laccase production in microorganisms and have explored the use of genetically modified organisms to produce laccases with enhanced production titers and superior properties [6].

There are many potential applications of laccases in biorefinery, ranging from the production of biofuels and bio-based chemicals to the treatment of wastewater and the production of food, feed, and other consumer products. Some of the key areas where laccase has been studied for its potential applications is in the deinking of recycled papers where it can remove ink from recycled paper, making it possible to produce high-quality, recycled paper products that are comparable to those made from virgin fibers. Laccase has also been shown to be effective in removing other contaminants, such as dyes and resins, from recycled paper, making it a promising enzyme for use in the deinking process [73].

Laccase can be used in combination with other enzymes to bleach pulp, resulting in brighter and higher-quality paper products. When laccase is used for this purpose, it typically works by breaking down lignin that can make paper yellow or brown in color. By breaking down the lignin, laccase helps to whiten the pulp and improve the brightness of the finished paper product. Laccase is often used in combination with other enzymes, such as peroxidases, to enhance the bleaching effect and produce higher-quality paper products [74].

Recently, the most important application of laccase was determined to be in biorefinery of biomasses, and more specifically, lignin. Lignocellulosic biomasses are difficult to break down because they contains complex lignin, polymeric materials that are resistant to decomposition. Laccase comes handy here to help the break down lignocellulosic biomasses by breaking down lignin. When laccase breaks down lignin, it creates smaller, more reactive molecules that can be further decomposed by other enzymes. This process helps make the lignocellulosic biomass more accessible to other polymer-degrading enzymes, such as cellulase and xylanase, which breaks polymers into simple fermentable sugars which can be converted into ethanol and butanol, and be employed for biofuel applications [46,47,75].

Laccase can also be used to produce a wide range of bio-based chemicals, including vanillin, syringol, and catechol, which have applications in the food, flavor, and fragrance industries [76]. Vanillin, for example, is used as a flavor and fragrance in a wide range of products, including food, beverages, and personal care products [77]. It is also used as a flavor enhancer in the tobacco industry. Syringol is a compound with a woody, smoky aroma, and it is used as a flavor and fragrance in a variety of products, including perfumes, candles, and air fresheners. Catechol is a compound with a sweet, woody aroma, and it is used as a fragrance in a variety of products, including perfumes, candles, and personal care products [78]. In addition to their use in the food, flavor, and fragrance industries, these bio-based chemicals also have several other potential applications. For example, vanillin has been shown to have antioxidant properties, and it has been suggested that it may have potential as a natural food preservative [79]. Similarly, catechol has been shown to have antimicrobial properties, and it has been suggested that it may have potential as a natural food preservative or as an antimicrobial agent in the medical field [80].

Laccase can be used to produce bio-based plastics, such as polyhydroxyalkanoates (PHAs), which are biodegradable and can be used as a substitute for traditional plastics. They are of interest because they are sustainable and environmentally friendlier than traditional fossil-fuel-based plastics [81]. Laccase can catalyze the oxidation of lignin, making it easier to extract and utilize the sugars and other components of lignocellulosic materials for the production of bio-based plastics [82]. Laccase has also been shown to have potential for use in the production of bio-based plastics through the modification of existing plastics. For example, laccase has been used to modify the properties of polyethylene, a widely used plastic, to make it more biodegradable [83].

Laccases have been used to remove dye pollutants from wastewater, making it possible to recycle and reuse the water [84]. Some dyes that have been successfully decolorized using laccase include azo dyes, which are a commonly used class of synthetic dyes in textiles, leather, and paper [30]. Laccase has been shown to be effective at decolorizing azo dyes, such as Acid Red 18 [85] and Direct Blue 1 [86].

Reactive dyes that contain a reactive group, such as a halogen or a double bond, can covalently bond to the fiber. Laccase has been shown to be effective at decolorizing reactive dyes, such as Rem Blue RR, and Dylon Navy 17 [87]. Laccase has been shown to be effective at decolorizing disperse dyes, such as Blue 2BLN, Yellow SE-4GL, and Red 3B-KH2015 [88]. Indigo is a natural dye derived from the indigo plant, and it is used to color denim and other textiles. Laccase has been shown to be effective at decolorizing indigo [89].

6. Laccase-Based Biological Pretreatment Lignocellulosic Biomasses

Laccase biological pretreatment plays a critical role in biomass pretreatment by efficiently degrading the lignin component of lignocellulosic biomasses. It involves the use of laccase enzymes to oxidize phenolic compounds in lignocellulosic biomasses. The phenolic compounds act as natural cross-linking agents that bind lignin to the cellulose and hemicellulose fibers in the biomass, making it difficult to break down. The lignin component acts as a barrier that prevents the efficient hydrolysis of cellulose and hemicellulose components by the enzymatic hydrolysis. Oxidizing these compounds, laccase breaks the cross-links and makes the lignocellulose more accessible to the enzymatic hydrolysis step. By degrading the lignin, laccase enhances the accessibility of the cellulose and hemicellulose components, resulting in higher sugar yields during the subsequent hydrolysis step. The optimal conditions for laccase biological pretreatment include a temperature range of 30–60 °C and a pH of 4.5–5.5. The advantages of laccase biological pretreatment include the selective removal of lignin and the enhancement of enzymatic hydrolysis. The disadvantages include the high cost of the enzyme and the need for additional steps to remove the residual enzyme [5].

7. Challenges and Future Directions

Despite the many advantages of laccase-based biorefinery approaches and other applications, there are several challenges that need to be addressed. The main challenges include the high the cost, availability and slow reaction of laccase enzymes, which can limit the scalability of the process.

Laccase low activity and stability under industrial conditions are a big limit to its commercialization. Laccase is highly sensitive to temperature, pH, and inhibitors; hence, robust laccase production is crucial for its commercialization [90]. Its production is expensive and requires specialized equipment and skilled personnel. The high cost of laccase production often limits its use in lignocellulosic biomass conversion [91]. However, immobilization of laccases has been explored to improve their effectiveness and reduce the cost to some extent [92].

Laccase has a narrow substrate specificity, which limits its ability to degrade a wide range of lignocellulosic biomasses. This can result in the incomplete degradation of lignin and limited access to cellulose and hemicellulose. Improving the substrate specificity of laccase can enhance its efficiency and effectiveness in lignocellulosic biomass conversion [93].

Lignin-derived phenols, such as vanillin, syringaldehyde, and coniferyl alcohol, lignin-derived lignans, such as pinoresinol and lariciresinol, lignin-derived flavonoids include compounds such as kaempferol and quercetin, lignin-derived stilbenes such as resveratrol and piceatannol, lignin-derived cinnamic acids such as p-coumaric acid and ferulic acid are known to inhibit laccase and negatively affect the biorefinery process [94].

However, with continued research and development, laccase-based biological processes are becoming the key technology for the production of bio-based products from lignocellulosic biomass. Laccase-based biological pretreatment can be integrated with other pretreatment methods, such as steam explosion, microwave-assisted pretreatment and acid hydrolysis, to enhance its efficiency and effectiveness [95]. However, the integration of different pretreatment methods requires careful optimization to avoid negative interactions and maximize the benefits of each method [96]. Several future directions can be explored to enhance its efficiency and effectiveness.

The engineering of laccase can improve its activity, stability, and substrate specificity. This can be achieved through genetic modification or protein engineering to enhance its properties and make it more suitable for industrial applications [97]. Recent studies have used various techniques, such as directed evolution, rational design, and protein engineering, to develop novel laccase variants with improved properties. In addition, the identification of new laccase enzymes from different sources, such as bacteria and fungi, can expand the diversity of laccase enzymes and provide new opportunities for biorefinery [98]. The use of statistical experimental designs, such as response surface methodology, can facilitate the optimization process even for engineered microbes [99].

Understanding the mechanisms of lignin degradation by laccase is necessary, which involves the cleavage of various types of bonds and the release of different types of products. Laccase plays a critical role in the oxidation of lignin, which can lead to the depolymerization of lignin and the release of valuable aromatic compounds. Understanding the mechanisms of lignin degradation by laccase can help design more efficient biorefinery processes. Recent studies have used various techniques, such as NMR spectroscopy, mass spectrometry, and computational modeling, to elucidate the mechanisms of lignin degradation by laccase [100].

Scale-up of the laccase-based biorefinery process is crucial for industrial applications. The development of scalable and cost-effective biorefinery processes can promote the utilization of lignocellulosic biomasses as a sustainable feedstock. Recent studies have focused on the scale-up of laccase-based biorefinery processes, such as the use of bioreactors and continuous flow systems. In addition, machine learning and big-data-based technologies can be used to efficiently model the industrial process [101,102].

8. Conclusions

After cellulose and hemicellulose, lignin has attracted the attention of researchers Worldwide as the renewable feedstock for various green processes. Laccase is a lignin-modifying and lignin-degrading enzyme, and is known for its versatile and nonspecific nature. Though laccase action on lignin is quite slow, pretreatment of biomasses via fungi producing laccase is the most sustainable approach, as none of the chemicals are required and it is an extremely natural process.

Although the action of laccase on lignin is slow, pretreatment of biomasses, using fungi that produce laccase, has been identified as the most sustainable approach, as it does not require any chemical treatments, making it an extremely natural process. This approach has been shown to significantly enhance lignin degradation and improve the efficiency of subsequent processes. In the "lignin first approach", laccase can be used to selectively degrade lignin, which can then be utilized as a feedstock for various applications. The products obtained from lignin degradation using laccase can be used to produce a variety of value-added products, including biofuels, chemicals, and materials. These products can potentially replace fossil-based materials and contribute to the development of a more sustainable economy. However, research into laccase and lignin degradation is still in

its early stages. This direction has the potential to pave the way for green solutions to environmental challenges. The development of efficient and sustainable lignin biorefinery processes could contribute to reducing greenhouse gas emissions, mitigating climate change, and promoting a circular bioeconomy.

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