Korean J. Chem. Eng., 37(3), 387-401 (2020)
DOI: 10.1007/s11814-019-0458-9

pISSN: 0256-1115
eISSN: 1975-7220

INVITED REVIEW PAPER

Biological conversion of lignin and its derivatives to fuels and chemicals

Pritam Kumar Dikshit*, Hang-Bae Jun, and Beom Soo Kim*,†**

*Department of Chemical Engineering, Chungbuk National University, Cheongju, Chungbuk 28644, Korea
**Department of Environmental Engineering, Chungbuk National University, Cheongju, Chungbuk 28644, Korea
(*Received 26 October 2019 • accepted 7 December 2019*)

Abstract−Lignocellulosic biomass, which is one of the most abundant and renewable sources for the production of clean fuels and chemicals, consists mainly of cellulose, hemicellulose and lignin. The conversion of cellulose and hemicellulose to value added products has been extensively carried out over the last few decades. However, the direct conversion of lignin, the second most abundant aromatic polymer on earth, is challenging due to its heterogeneity and low reactivity. Most of the lignin produced in the pulp and paper industry is used as a fuel to generate heat and electricity. Recently, the chemical or biological conversion of lignin is considered one of the most promising technologies for the production of high-value products. The biological conversion of lignin has several advantages over the chemical conversion route in terms of low operating costs, high specificity, and the absence of harsh operating conditions and hazardous chemicals. The present review summarizes recent studies on biological valorization of lignin to value-added products. Additionally, this review emphasizes the various lignin extraction techniques, catabolic pathways involved, necessary enzymes, and the major challenges of this process.

Keywords: Lignin, Extraction Method, Biological Conversion, Enzymes, Value-added Products

INTRODUCTION

Its abundant availability and renewability make lignocellulosic biomass one of the potential sources for the production of clean energy and high-value products [1,2]. As per the report, lignocellulosic biomass shares approximately 10% of the global primary energy with an annual production of 170 billion tons worldwide [3]. Due to its high productivity, lignocellulosic biomass is the fourth largest source of energy in the world after oil, coal, and natural gas [4]. It primarily consists of cellulose, hemicellulose, and lignin, along with some minor concentrations of minerals, acetyl group, and phenolic substituents. The composition of primary components in the lignocellulose varies significantly with the source of biomass, as given in Table 1 [5]. Fig. 1 shows the structural and chemical composition of lignocellulosic biomass. Cellulose consists of a linear chain of glucose molecule linked by β-1,4 linkages. Hemicellulose is mainly composed of xylose and mannose monomers. Lignin is a complex polymer of phenylpropane units. Utilization of cellulose and hemicellulose for the production of various chemicals, biofuels, papers, and sugars after adopting suitable pretreatment methods has been accomplished in the past several years. In contrast, the effective utilization of lignin from these activities has not been explored in detail so far. The carbon-carbon and aryl ether bonds in the aromatic rings contain higher bond energy, which further hinders its direct conversion and requires prior depolymerization for its valuable utilization. Most of lignin reported to be produced from the pulp and paper industry (>95%; approximately 50 million tons annually on a weight basis) is being utilized as a solid fuel for the generation of heat and electricity in the biorefinery industry [6,7].

Table 1. Lignocellulosic biomass composition

Wood types	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwood	45-50	20-25	20-25
Softwood	35-40	25-30	27-30
Nonwood	30-50	10-40	5-20

In the past few decades, several methods, such as biological, chemical, or thermal pretreatment, have been successfully adopted to depolymerize lignin for the production of monomers and oligomers [8-11]. These monomers and oligomers are further used as starting materials for the production of various fuels and chemicals [12,13]. Thermochemical conversion of lignin and its derivatives to several high-value products is carried out by adopting several techniques such as gasification, liquefaction, combustion, pyrolysis, and carbonization [14,15]. The liquefaction section is further divided into four subsections: acidolysis, base-catalyzed depolymerization, hydrogenolysis, and oxidation [15]. The combustion of lignin generates a large amount of heat, which can later be used to generate electricity. The major gaseous product (syngas) generated from all of these processes can be further converted to various fuels and chemicals by catalytic conversion [16,17]. Apart from syngas, solid char is another major product produced from all the above categories, which can be converted for the generation of electricity. Bio-crude oil is the main product formed during liquefaction and pyrolysis process, mainly composed of several phenolic compounds [18-21]. These phenloic compounds undergo further purification process prior to their application in several sectors. Alternatively, hydrodeoxygenation is required for the conversion of these oxygen-rich phenolic mixtures to liquid fuels such as gasoline and die-

†To whom correspondence should be addressed.
E-mail: bskim@chungbuk.ac.kr

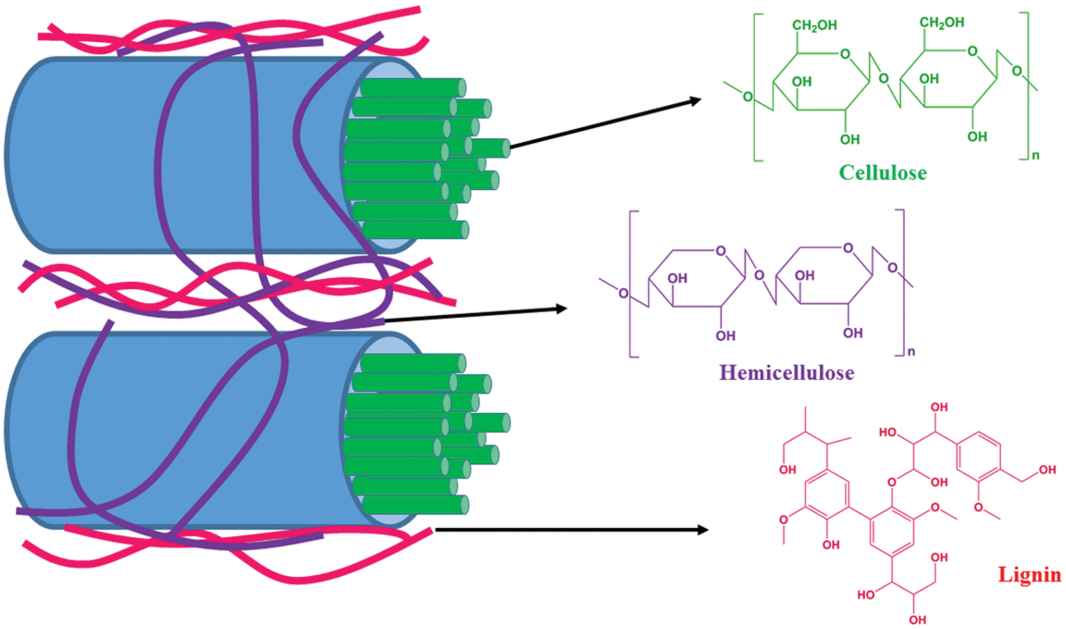


Fig. 1. Schematic representation of lignocellulosic biomass and its components (cellulose, hemicellulose, and lignin).

sel fractions.

In addition to thermal and chemical methods, biological lignin processing has gained major research attention with the discovery of various fungi and bacteria from ligninolytic environments [22-25]. The high enzymatic activity of these microorganisms aids in the degradation of lignin and further conversion to possible end products [26]. The present review mainly focuses on the biological conversion of lignin and its derivatives to high-value products using bacteria, fungi, and enzymes. The methods of lignin extraction, the main metabolic pathways involved in the degradation of lignin using microorganisms, and strategies for improving the conversion process, are also discussed in detail.

LIGNIN EXTRACTION

Successful extraction of lignin from lignocellulosic biomass and its purity are two important aspects of lignin valorization. The formation of lignin-carbohydrate complex (LCC) with hemicellulose component of biomass (mainly xylan and glucomannan) by stable covalent bonds makes the lignin extraction process more challenging [9,27]. Prior to extraction, breakage of LCC bonds to disengage lignin from carbohydrates and further depolymerization is required to reduce the size into smaller fractions for solubilization from the main biomass components. Repolymerization of lignin and its fractions, leading to the synthesis of various intermediates, is often associated with the extraction process due to the high reactivity nature of lignin and its derivatives [27]. Therefore, the structure of isolated lignin depends primarily on the feedstock source, the extraction method, and the operating conditions of the extraction process. A detailed study of the lignin extraction process from the source was previously described by Wang et al. [14]. The majority of lignin is produced as a co-product in the pilot-scale lignin extraction process, while others are produced as a byproduct in the laboratory-scale process: biomass pretreatment and anaerobic fermentation. In the last few years, large amounts of lignin recovered during the processing of lignocellulosic biomass in the pulp and paper industry have not been utilized effectively and still remain unexplored. For a long time, lignin generated from the pulp and paper industry was discarded, causing not only waste of resources but also environmental pollution. Recent major research has focused on the possible utilization of lignin and its derivatives for the production of value-added products in various sectors, including cosmetics, food, chemistry, pharmaceuticals, and textiles.

Based on the technique adopted, the extraction of lignin from lignocellulosic biomass is broadly categorized into two groups. In the first category, the treatment process is targeted to hydrolyze or solubilize the cellulose and hemicellulose fractions of biomass while recovering the lignin as insoluble residues. Conversely, in the second category, the treatment method is designed for dissolution and further recovery of lignin from waste liquor while keeping the cellulose and hemicellulose fractions insoluble [28]. The lignin extraction process is mainly divided into industrial-scale extraction process and laboratory-scale extraction process. Here, a brief description of various lignin extraction processes and its important features is presented. The various pretreatment processes adopted for the extraction of lignin from lignocellulosic biomass and its operating conditions are summarized in Table 2.

1. Industrial (Pilot) Scale Extraction Process

Industrial or pilot scale lignin extraction processes are more challenging than laboratory scale processes. Kraft pulping, sulfite pulping, soda pulping, organosolv pulping, and steam explosion are the major processes adopted by the pulp and paper industry to recover lignin from lignocellulosic biomass. The structural and chemical composition of the extracted lignin depends on the extraction process adopted and differs from native lignin [29].

Kraft pulping is the most widely used method in the pulp and

Table 2. Summary of lignin extraction methods and their operating conditions

Extraction methods		Operating conditions
Lignin from pulp and paper industry (Industrial extraction process)		
Kraft pulping		Aqueous soda (NaOH) and sodium sulfide (Na_2S); High temperature: 150-180 °C; Duration: 2 h
Sulfite pulping		Aqueous sulfur dioxide (SO_2) and sulfite base salts ($CaSO_3$, Na_2SO_3, $MgSO_3$, $(NH_4)_2SO_3$); Temperature 120-150 °C; Duration: 2-7 h
Soda pulping		Sodium hydroxide: 1 M; Temperature: ~170 °C; Pressure: 10 psi
Organosolv pulping		Organic solvents (methanol, ethanol, acetic acids, esters, acetone, or a mixture of water/organic solvents); Temperature: 100-250 °C
Steam explosion		Hot steam: 180-240 °C; Pressure: 1-3.5 MPa
Laboratory scale extraction process		
Physical pretreatment		Milling, extrusion, microwave treatment, ultrasonication
Acid hydrolysis	Dilute acid hydrolysis	0-5 wt% of HCl, H_2SO_4, H_3PO_4, HF; Temperature: 120-300 °C
	Concentrated acid hydrolysis	Concentrated acids: HCl, H_2SO_4, HF; Temperature: 18-25 °C
Alkaline hydrolysis		Solvents: NaOH, $Ca(OH)_2$, ammonia (aqueous, liquid, gaseous); Temperature: 40-120 °C
Enzymatic hydrolysis		Enzymes: cellulase, hemicellulase with additional pretreatments (e.g. dilute acid, steam explosion, ammonia fiber explosion); Temperature: 30-60 °C
Combined physiochemical		Solvents: H_2O or organic solvents, e.g. acid hydrolysis with mechanical milling, Temperature: 25-200 °C

paper industry to recover cellulose from wood materials while leaving lignin in the waste stream. At present, about 80% of the world's chemical pulp production relies on the Kraft process to isolate lignin from lignocellulosic biomass [30]. In this process, lignocellulosic biomass is treated for 2 hours at high temperature (150-180 °C) in the presence of high concentrations of aqueous soda (NaOH) and sodium sulfide (Na_2S). Lignin fraction from lignocellulosic biomass is solubilized in the pulping liquor and collected in the spent liquor, also known as black liquor. The black liquor also contains a certain amount of hemicellulose fraction. Conventionally, black liquor containing 90% of wood lignin is used in boilers for the generation of steam and electricity. However, lignin from black liquor can be recovered by acidification to pH 5.0 or below, which precipitates lignin in the liquor.

The sulfite process is another old lignin extraction method that produces about 7 million tons of lignin per year as a by-product of this process [31]. In this process, aqueous sulfur dioxide (SO_2) and sulfite bases of calcium, magnesium, sodium or ammonium are used for 3 to 7 hours in the pulping process at high temperature (120-150 °C). During this process, large amounts of sulfate are incorporated into the lignin to form lignosulfonates. Compared to Kraft lignin, lignin recovered during this process contains higher amounts of sulfur, carbohydrates, and inorganic impurities [32].

Soda process, which was discovered a few years before the Kraft process, uses a high concentration of sodium hydroxide (1 M NaOH) in the presence of high temperature (~170 °C) and pressure (10 psi). This process primarily targets α-ether linkages of lignin structures and is mainly applied to non-wood lignocellulosic biomass (agricultural and aquatic waste).

Organosolv lignin is recovered from lignocellulosic biomass by application of an organic solvent. In this process, solvents such as methanol, ethanol, acetic acids, esters, acetone, or mixtures of water/organic solvents are used under specific operating conditions (temperature 100 to 250 °C). The addition of acid catalysts such as sulfuric acid, hydrochloric acid, formic or acetic acid, and phosphoric acid can improve the cleavage of ether linkages, further increasing the extraction efficiency [33]. This method is reported to produce high purity lignin from biomass compared to other lignin extraction processes.

The usage of chemicals in the pretreatment process is often associated with additional costs in terms of neutralization and recycling of these chemicals. These chemicals also cause environmental hazards. Given this, steam explosion is a thermochemical process that lacks the use of additional chemicals in the extraction process. High pressure steam penetrates into the biomass structure due to high vapor phase diffusion, causing the breakdown of biomass structure. This process also leads to the formation of organic acids that further aid in the hydrolysis of glycosidic bonds [31,34]. Two phases such as steam cracking involved in structural breakdown and explosive decompression take place during this process. Steam explosion solubilizes small fractions of lignin, while steam explosion combined with alkaline delignification is often used for complete fractionation of biomass.

2. Laboratory Scale Extraction Process

The laboratory-scale lignin extraction process is alternatively known as a pretreatment process. Extraction of lignin from different lignocellulosic biomass is carried out by adopting various pretreatment methods or a combination of these methods. These pretreatment methods are broadly classified into four different categories such as physical, chemical, physicochemical, and biological. Due to the various operating conditions and severity of the chemicals used in the process, changes in lignin composition, purity, and yield can be observed from the same feedstock.

Physical pretreatment of lignocellulosic biomass is essential before

moving to other pretreatment methods. This pretreatment process reduces the particle size of the biomass by increasing the surface area and decreases the degree of depolymerization and crystallinity [35]. This helps the subsequent treatment process be easier and effective. Physical pretreatment methods such as milling, extrusion, microwave treatment, and ultrasonication are commonly used in the laboratory scale.

Chemical pretreatment of biomass mainly includes acid hydrolysis and alkali hydrolysis. The acid hydrolysis of biomass is further divided into two categories: (1) dilute acid hydrolysis (0-5 wt% H_2SO_4, HCl, H_3PO_4, 120-300 °C) [36,37], (2) concentrated acid hydrolysis (70% H_2SO_4, HCl, HF, 18-25 °C) [38]. This method is mainly targeted to depolymerize the cellulose and hemicellulose fractions, while some amounts of lignin are also removed during this process. The formation of hydronium ions during the pretreatment process causes the breakdown of long-chain cellulose and hemicellulose, resulting in the formation of sugar monomers [39]. A highly condensed form of lignin is obtained during this process which is otherwise called Klason lignin. The Klason lignin is also used as a standard for estimation of lignin content in biomass sample [40]. Alkaline hydrolysis is similar to acid hydrolysis method using alkaline reagents such as NaOH, Na_2CO_3, $Ca(OH)_2$, and ammonia (aqueous, liquid, and gaseous) instead of acids. The operating conditions of alkaline hydrolysis are milder than those of acid hydrolysis. The intermolecular ester linkage between cellulose and hemicellulose is disrupted by the saponification reaction caused by alkali reagent. This causes solubilization or depolymerization of lignin and hemicellulose into the solvent from the biomass. Ammonia and its aqueous solutions are the most extensively used solvents in the pretreatment of biomass. Some of these technologies include ammonia fiber explosion/expansion, ammonia recycle percolation, low-liquid ammonia, soaking in aqueous ammonia, and low-moisture anhydrous ammonia [41]. Due to the high volatility of ammonia, it can be easily recovered after the pretreatment process.

In biological pretreatment process like enzymatic hydrolysis, enzymes such as cellulases and hemicellulases are used to convert polysaccharides to oligo/monosaccharides, leaving lignin in solid residues. Before enzymatic hydrolysis, biomass undergoes additional chemical or physical pretreatment to reduce the rigidity and recalcitrant nature of the biomass, making it easier to access the enzymes [37]. Lignin obtained from solid residues contains impurities in the form of residual carbohydrate, protein, and ash [42], which undergoes post-treatment processes (e.g., mild acid hydrolysis or extraction with organic solvent) to increase its purity. None of biomass pretreatment methods are individually efficient for successful extraction and separation of the three major biomass components (cellulose, hemicellulose, and lignin). Recent studies have reported the application of combined pretreatment methods to overcome these challenges [36,43,44]. Processes such as acid catalysis and mechanical milling [45,46], deacetylation and mechanical refining [47-49], inorganic salts and calcium peroxide [50], concentrated phosphoric acid with hydrogen peroxide [51], deep eutectic solvent with microwave [52], and electrolysis combined with ultrasonication [53] have been successfully established to fractionate lignocellulosic biomass.

As mentioned, structural modification of lignin occurs during

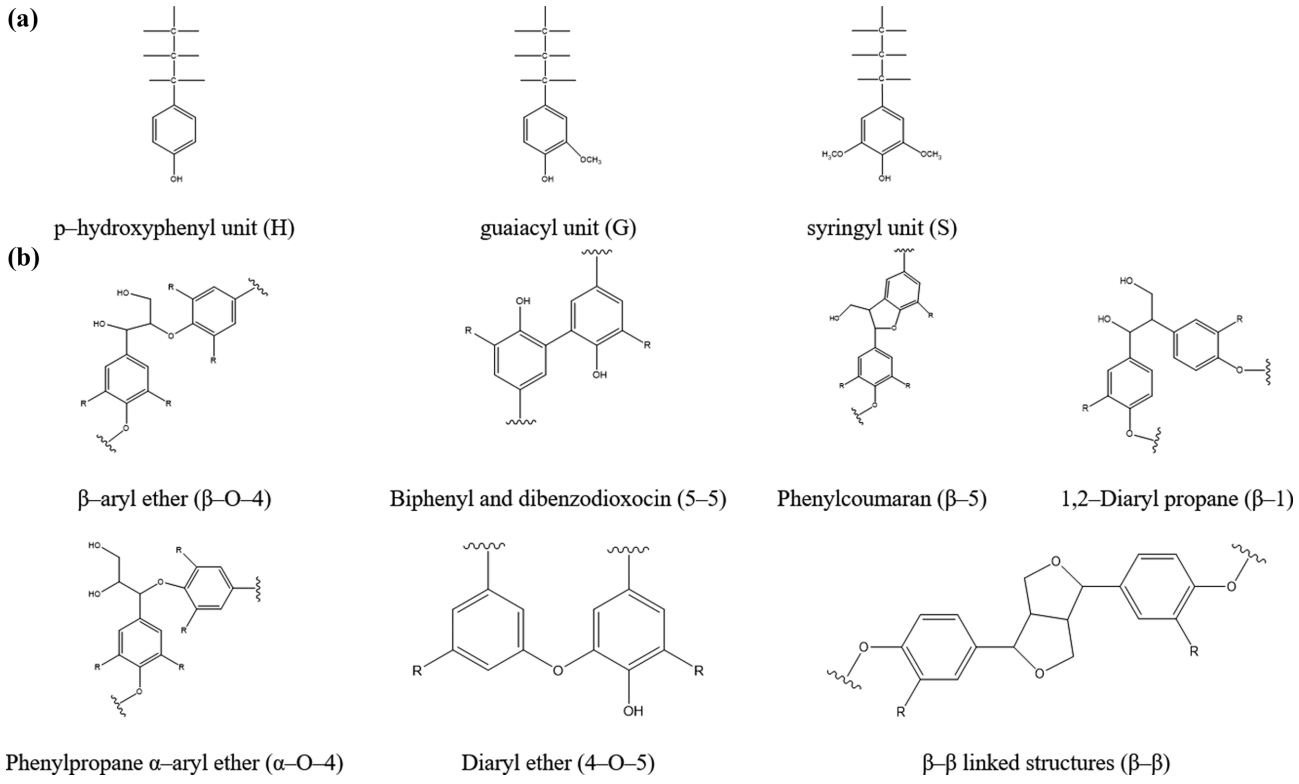


Fig. 2. Structural characteristics of lignin and its typical linkages. (a) Structure of three monomeric subunits (H, G, and S) in lignin. (b) Typical linkages found in lignin structure and their dimer structure.

the isolation and purification process, since these steps require the application of strong chemicals and harsh operating conditions. Therefore, the structural characterization of lignin is highly essential before application to various processes. The characterization of isolated lignin is carried out by the following three major protocols: (1) milled wood lignin [54], (2) cellulolytic enzyme lignin [55], and (3) enzymatic mild acidolysis lignin [42]. Molecular weight analysis (light scattering, vapor pressure osmometry, gel permeation chromatography, ultrafiltration, and mass spectrometry), nuclear magnetic resonance analysis (^{1}H, ^{13}C, ^{31}P, 2D heteronuclear single quantum coherence), and Fourier-transform infrared spectroscopy are some of the common methods adopted for the characterization of lignin [14].

STRUCTURAL CHARACTERISTICS OF LIGNIN

Phenylpropanoid monomers derived from coniferyl alcohol, sinapyl alcohol, and p-coumaryl alcohol are joined by various ether and carbon-carbon bond to form the polymeric structure of lignin (Fig. 2(a)). Radical polymerization of guaiacyl (G) units of coniferyl alcohol, syringyl (S) units of sinapyl alcohol, and p-hydroxyphenyl (H) units of p-coumaryl alcohol forms a heterogeneous structure of lignin. These alcohols (coniferyl alcohol, sinapyl alcohol, and p-coumaryl alcohol), collectively called monolignols, are derived from the aromatic amino acid, phenylalanine [56]. The ratio of these subunits (G : S : H) in lignin structure differs between plant species. G units mainly dominate softwood lignin, while the mixture of G and S type lignin is found in hardwood, and grass lignin is composed of G type lignin with a higher proportion of H units [22,28]. These monomers are joined by several bonds, and some of the typical linkages found in lignin structure include β-β, β-O-4, and β-5 (Fig. 2(b)). The formation of these interionic bonds and linkages with cell wall polysaccharides render the lignin breakdown and separation process more challenging [57]. Additionally, the recalcitrant and heterogeneous nature of lignin and the lack of suitable process technology are major hindrances in the lignin biorefinery process. Harsh operating conditions (high temperature and pressure) and massive energy requirements are other major drawbacks of lignin depolymerization during the use of chemical catalysts and thermochemical treatments for the degradation process [58]. Moreover, these methods cause environmental hazards by releasing unused catalysts into the surrounding environment. Biodegradation of lignin and its derivatives for the production of value-added products has emerged as a potential alternative to chemical and thermochemical treatments. The rigidity and high degree of polymerization nature of lignin provide resistance to various microorganisms for degradation. However, several groups of microorganisms, including bacteria and fungi, have been successfully verified for efficient degradation of lignin [59]. A detailed discussion of the various metabolic pathways and their associated microorganisms is given in the following sections.

MAJOR PATHWAYS FOR LIGNIN DEGRADATION

Numerous metabolic pathways are involved in the biological degradation of lignin using bacteria and fungi. The major catabolic pathways for lignin degradation are shown in Fig. 3. Some of the most common pathways that are involved in the degradation process have been already discussed [22,60]. Monomers of lignin are conjugated via different bonds to form the phenylpropanoid structure of lignin, which is highly resistant to chemical and biological depolymerization. Apart from other linkages, β-aryl ether (β-O-4) and biphenyl linkages are the most dominant in lignin structure. Therefore, the corresponding catabolic pathways for degradation of these linkages such as β-aryl ether and biphenyl pathways are of great importance. These two pathways are also associated with the formation of other value-added products such as vanillin or 4-hydroxybenzoic acid. While, the production of high molecular weight compounds such as lipid and polyhydroxyalkanoate (PHA) are associated with the β-ketoadipate (β-KAP) pathway.

1. β-Aryl Ether (β-O-4) Pathway

Cleavage of β-aryl ether linkage, which accounts for more than 50% of the total ether linkages, is considered one of the crucial steps in the depolymerization of lignin structure [61,62]. Previous studies have reported the identification of catabolic gene cluster *lig*DFEG from proteobacterium *Sphingobium* sp. SYK6 for the degradation of β-aryl ether linkage [61,62]. In addition to *Sphingobium* sp., *Novosphingobium aromaticivorans*, *Novosphingobium* sp. PP1Y, and *Dichomitus squalens* have also been reported to be the degrader of β-aryl ether linkage [63-65]. These genes encode alcohol dehydrogenase and three glutathione S-transferases enzymes, which are successively involved in the cleavage of β-O-4 linkage. The reaction starts with the oxidation of β-aryl ether linkage by LigD, a Cα-dehydrogenase, in the presence of NAD^{+}. This reaction is followed by the cleavage of ether linkage by glutathione *S*-transferases (LigE or LigF). Finally, glutathione lyase (LigG) oxidizes glutathione with the involvement of additional glutathione [66]. The enzymes involved in this process are highly stereospecific, and the involvement of three additional enzymes, e.g. LigL, LigN, and LigO, is also observed for the degradation of β-aryl ether stereoisomers [61,66]. Mnich et al. reported the expression of genes encoding the complete set of enzymes, i.e., LigD, F, G from *Sphingomonas paucimobilis* SYK-6 in *Arabidopsis thaliana* and post-lignification modifications were carried out to improve the digestibility of lignin by enzymes after its production [66].

2. Biphenyl Catabolic Pathway

Biphenyl linkage is another major linkage in the lignin structure, representing ~10% in softwood and ~6% in hardwood lignin total linkages [28,67]. The biphenyl catabolic pathway has been extensively studied in the degradation of polychlorinated biphenyls (PCBs). PCBs are a group of manmade chemicals that are released into the environment from several industries, adversely affecting human health and aquatic life [68]. The first step in the degradation of biphenyl is by the enzyme 2,3-dioxygenase (LigX), which adds molecular oxygen to the ring structure. A model biphenyl compound 5,5′-dehydrodivanillate (DDVA) has been studied for the degradation process. Initially, DDVA is O-demethylated resulting in the formation of 2,2′,3-trihydroxy-3′-methoxy5,5′-dicarboxybiphenyl (OH-DDVA). This is followed by ring cleavage by OH-DDVA dioxygenase (LigZ) encoded by *lig*Z gene. The hydrolase enzyme LigY further cleaves the resulting product in the previous step into 4-carboxy-2-hydroxypentadienoic acid and 5-car-

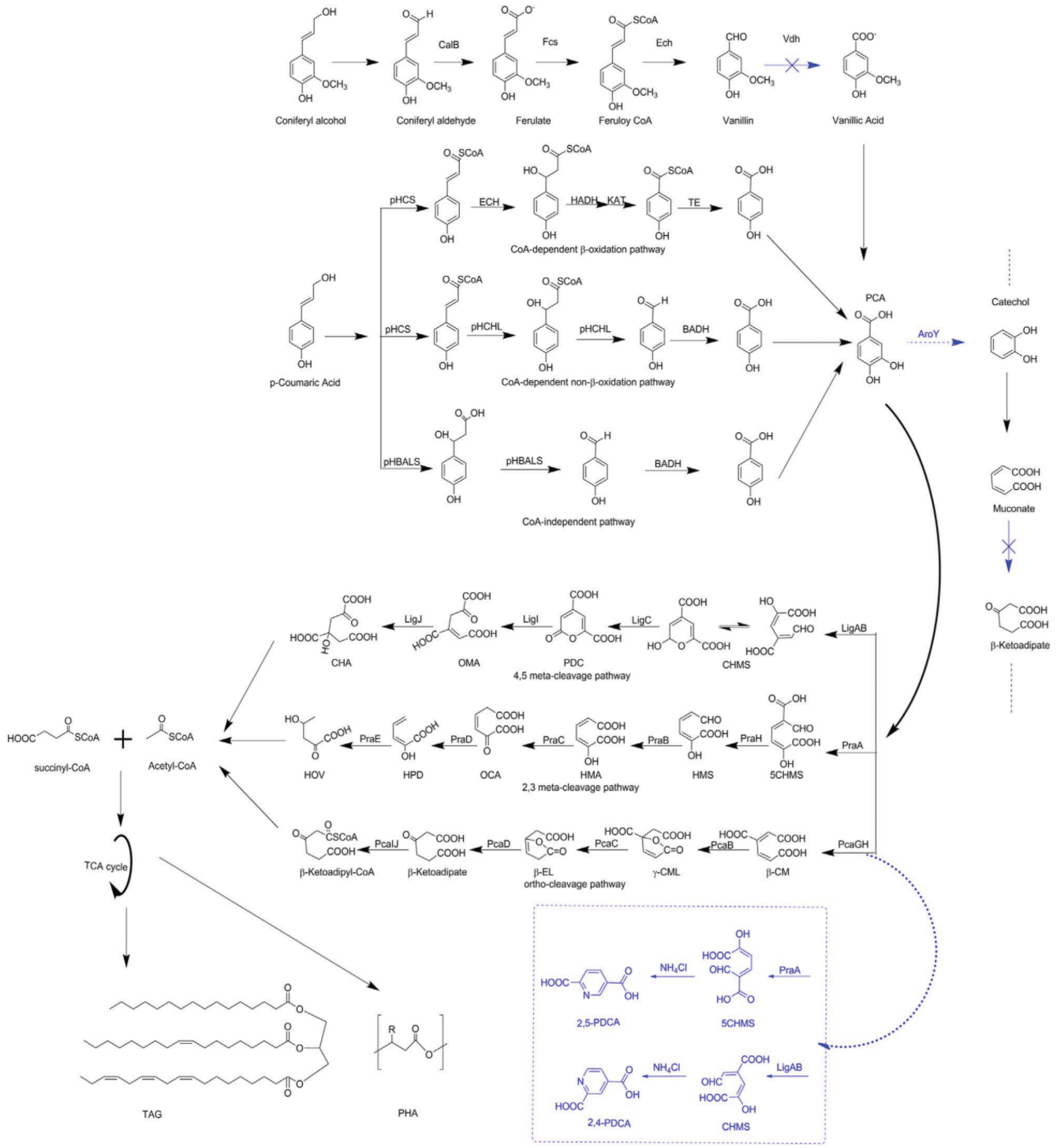


Fig. 3. Catabolic pathways for lignin degradation. Adopted from Chen and Wan [28] with kind permission from Elsevier. The pathways highlighted in blue are metabolically engineered (The readers are suggested to refer to the online version of the article for color images.).

boxyvanillic acid, which are subsequently converted to vanillic acid (VA) by LigW/LigW2 [60].

3. *β*-Ketoadipate (*β*-KAP) Pathway

The *β*-KAP pathway is most widely distributed among soil bacteria and fungi for the degradation of aromatic compounds. In 1966, this pathway was first recognized by L. Nicholas Ornston in Gram-negative *Pseudomonas putida*, with the identification of nine important enzymes and intermediate products [69]. The *β*-KAP pathway is divided into two branches concerning the end product of the pathway. In one branch, phenols (p-cresol, 4-hydroxybenzoate, etc.) and lignin-derived compounds (vanillate and coniferyl alcohol) are converted to protocatechuate, while the other branch

converts aromatic hydrocarbons, amino aromatics, and lignin monomers such as cinnamate to catechol [28,70]. The final products, protocatechuate and catechol of these branches, are further converted to β-carboxymuconate and muconate with the help of protocatechuate 3,4-dioxygenase and catechol 1,2-dioxygenase enzymes, respectively. In addition, β-carboxymuconate and muconate undergo a series of enzyme-catalyzed reactions before reaching the final products succinyl-CoA and acetyl-CoA. Subsequently, enzymes fatty acid synthase II, acetyl-CoA carboxylase, and other metabolic processes can utilize these final products for the production of various chemicals, including lipid and PHA.

VALORIZATION OF LIGNIN USING MICROORGANISMS

Due to the abundant availability of lignin in nature, microorganisms have developed metabolic systems for the effective degradation of lignin and its conversion into aromatic compounds. Several bacteria and fungi have been identified as potential degraders of lignin. Fungi are the most widely studied organisms for lignin degradation, preferentially over bacteria. A summary of the literature for the various microorganisms used in lignin degradation, lignin sources and pretreatment processes, and fermentation conditions is given in Table 3. Since anaerobic processes do not degrade the aromatic rings of lignin, biological lignin degradation is carried out by aerobic processes. Several bacteria belonging to the genus *Rhodococcus*, *Pseudomonas*, and *Streptomyces* are most widely considered for the effective degradation of lignin. Potential metabolic pathways adopted by bacteria for the valorization of lignin have been discussed in detail [71,72]. A detailed review of lignin degradation for the formation of value-added chemicals using various bacteria and fungi and the involvement of enzymes during the metabolic processes is given in the present section.

1. Polyhydroxyalkanoates (PHAs)

PHAs are groups of polyesters synthesized by various bacteria as a reserve carbon source under environmental stress conditions [73]. Recently, Kumar et al. [74] reported a detailed review of the utilization of lignin and its derivatives for PHA production. Among the various strains, *Cupriavidus necator* (previously known as *Ralstonia eutropha*) is the most commonly used strain for short-chain-length PHA (*scl*-PHA) production. *C. necator* can accumulate high PHA up to 80% w/w while growing a wide range of substrates, including waste. However, only a few strains of *C. necator* have been reported to utilize lignin and its derivatives for PHA production. Yu and Stahl reported effective utilization of metabolic inhibitors such as formic acid, acetic acid, furfural, and acid-soluble lignin by *Ralstonia eutropha* for the accumulation of PHA [75]. A tolerant *R. eutropha* strain was capable of metabolizing higher concentrations of inhibitors in the presence of diluted sugarcane hydrolysate (50 : 50) in the medium. In another study, the PHA accumulation efficiency of 11 different strains was investigated by culturing in mineral salt medium containing 18 different lignin derivatives [76]. Strain *R. eutropha* H16 showed positive growth in the presence of 3-hydroxybenzoic acid (3-HBA) and 4-hydroxybenzoic acid (4-HBA) with a final poly(3-hydroxybutyrate) (PHB) content of 65 and 63 wt%, respectively. In addition, *P. putida* JCM13063 showed positive growth in medium containing

Table 3. Summary of biological conversion of lignin for the production of value-added products

Lignin source/ derivatives used	Pretreatment techniques	Microorganism used	Fermentation type	References
Polyhydroxyalkanoates (PHAs)				
Sugarcane bagasse	Acid pretreatment	*Ralstonia eutropha*	Shake flask	[75]
3-HBA, 4-HBA	-	*R. eutropha H16*	Shake flask	[76]
VA, 4-HBA, 3,4-DHBA	-	*Pseudomonas putida* JCM13063	Shake flask	[76]
p-Coumaric acid, ferulic acid, VA, 4-HBA, 3,4-DHBA	-	*P. putida Gpo1*	Shake flask	[76]
Rice straw	Alkaline pretreatment	*Cupriavidus basilensis B-8*	Shake flask	[77]
Corn stover	Combinatorial thermochemical pretreatment	*P. putida* KT2440	Fed-batch fermentation	[79]
Water hyacinth	Grinding and autoclaving	*Bacillus cereus*	Submerge fermentation	[80]
Corn stover	Alkaline pretreatment	*P. putida* KT2440	Shake flask; Batch fermentation	[78]
Kraft lignin, 4-HBA, p-coumaric acid, VA, 2,6-imethoxyphenol	-	*Pandoraea* sp. ISTKB	Shake flask	[85]
Kraft lignin	No pretreatment	*C. basilensis* B-8	Fed-batch fermentation	[153]
Lignin	-	*Oceanimonas doudoroffii*	Shake flask	[84]
Soft wood (*Pinus radiate*) hydrolysate	High-temperature mechanical pretreatment/ steam explosion	*Novosphingobium nitrogenifigens Y88*	Shake flask	[81]
VA		*P. putida* (A514) AphaJ4C1	Shake flask	[83]

Table 3. Continued

Lignin source/ derivatives used	Pretreatment techniques	Microorganism used	Fermentation type	References
Microbial lipid/single cell oils				
4-HBA and VA	-	*Rhodococcus opacus* PD630	Shake tube and flask	[94]
Kraft lignin	-	*R. opacus* PD630	Shake flask	[98]
Softwood (*Pinus taeda*)	Ethanol organosolv (EO), Ultrasonicated EO	*R. opacus* DSM 1069 and PD630	Shake flask	[96]
Kraft lignin	Oxygen pretreatment	*R. opacus* DSM 1069	Shake flask	[154]
Softwood loblolly pine (*Pinus taeda*)	Organosolv pretreatment	*R. opacus* DSM 1069	Shake flask	[155]
4-HBA and VA	-	*R. opacus* DSM 1069 and PD630	Shake tube and flask	[94]
4-HBA, VA, and syringaldehyde	-	*Trichosporon cutaneum* ATCC 20271	Shake flask	[156]
Phenol, VA, and 4-HBA	-	*R. rhodochrous* ATCC 21198	Shake flask and bioreactor study (5 L)	[95]
Corn stover lignin	Alkaline pretreatment	*R. opacus* PD630 and *R. jostii RHA1 VanA*$^{-}$	Shake flask	[97]
Corn stover	Ammonia fiber expansion and oxygen pretreatment	*R. opacus* NRRL B-3311	Shake flask	[157]
Cis, cis-muconic acid				
Soft wood lignin derivatives (Catechol and phenol)	Hydrothermal treatment	*Corynebacterium glutamicum* MA-2	Shake flask	[99]
VA and 4-HBA	Alkaline nitrobenzene oxidation	*P. putida* strain IDPC/pTS110	Shake flask	[158]
Corn stover alkaline pretreated liquor	Alkaline pretreatment	*P. putida* KT2440-CJ242	Batch and fed-batch fermentation	[159]
VA	-	Engineered *E. coli*	Shake flask	[108]
Coniferyl alcohol	-	Recombinant *P. putida* KT2440	Shake flask and fed-batch	[106]
Corn stover	Base-catalyzed depolymerization	Recombinant *P. putida* KT2440	Bioreactor	[160]
p-Coumaric acid	-	*P. putida* KT2440-CJ184	Shake flask, batch and fed-batch fermentation	[104]
Guaiacol	Hydrothermal	Recombinant *Amycolatopsis* sp. ATCC 39116	Fed-batch fermentation	[105]
Vanillin				
Wheat straw	Alkali pretreatment	*Rhodococcus jostii* RHA045 (Δvdh) mutant	Shake flask	[111]
Ferulic acid	-	Engineered *P. putida* KT2440	Shake tube	[112]
Ferulic acid	-	*Lactobacillus plantarum* CECT 748(T)	Shake flask	[113]
Ferulic acid	-	Engineered *Pseudomonas fluorescens*	Shake flask and bioreactor	[114]

Annotations: 4-HBA: 4-Hydroxybenozoic acid, 3-HBA: 3-Hydroxybenozoic acid, VA: Vanillic acid, 3,4-DHBA: 3,4-Dihydroxybenzoic acid.

VA, 4-HBA, and 3,4-dihydroxybenzoic acid (3,4-DHBA). Strain *P. putida* Gpo1 grew in the presence of *p*-coumaric acid, ferulic acid, VA, 4-HBA, and 3,4-DHBA. However, PHA accumulation by these strains was very low (less than 1 wt%). The conversion of lignin derivatives to intermediates such as 4-HBA, 2,5-DHBA, 3,4-DHBA, and VA is a major limitation in the PHA synthesis process.

Several lignocellulosic biomass such as rice straw, corn stover, water hyacinth, and *Pinus radiate* are subjected to various pretreatment techniques such as alkaline pretreatment, combined thermochemical pretreatment, high temperature mechanical pretreatment to obtain lignin-containing hydrolysate, which is further used in PHA production process [77-81]. Various microbial strains, such as

Cupriavidus basilensis B-8, *Pseudomonas putida* KT2440, *Novosphingobium nitrogenifigens* Y88, and *Bacillus cereus*, have proven to be efficient producers of PHA by utilizing these hydrolysates. *Pseudomonas putida* KT2440 is considered the most efficient PHA producer with a wide range of substrate specificities, and it produces medium-chain-length PHAs (*mcl*-PHAs) through fatty acid biosynthesis using acetyl-CoA as a central metabolite [78,82]. Transcription level optimization of genes involved in PHA biosynthesis was carried out in *Pseudomonas putida* strain A514 grown on VA as a carbon source [83]. Enhancement in cell growth and 34 wt% PHA accumulation were achieved in *P. putida* $A_{xyl\_alkKphaGC1}$ strain regulated by an inducible strong promoter.

Apart from these strains, *Oceanimonas doudoroffii*, a newly isolated marine bacterium from allantoin polluted site, accumulated PHA by directly utilizing lignin and its derivatives [84]. The authors reported the production of PHA was mainly carried out by the formation of sinapinic acid and syringic acid intermediates instead of other lignin derivatives. Kumar et al. reported PHA production from 4-HBA, p-coumaric acid, VA, 2,6-dimethoxyphenol, and Kraft lignin by *Pandoraea* sp. ISTKB [85]. PHA production was highest on 4-HBA and lowest on Kraft lignin after 96 h of fermentation. *Burkholderia* sp. F24 isolated from soil has been reported to utilize pentose sugars to produce PHB with the concurrent utilization of organic inhibitors from the medium [86]. Microbial strains such as *Clostridium beijerinckii* and *Burkholderia cepacia* have been reported to be resistant to a wide range of inhibitors, including levulinic acid, acetic acid, ferulic acid, furfural, hydroxymethylfurfural, and syringaldehyde [74].

2. Microbial Lipids/Single Cell Oils

Recent studies have focused on the production of microbial lipids or single cell oils (SCOs), the source of third generation biofuels. Several microorganisms (microalgae, fungi, bacteria, and yeast) have been reported as producers of SCOs while growing on various carbon sources [87]. However, only a few oleaginous microbes are reported to accumulate lipids from lignin and its derivatives. Several bacteria in the Actinomycetes group have been reported to accumulate lipid in storage form by metabolizing lignin derivatives via the *β*-KAP pathway [88,89]. The Gram-positive species, *Rhodococcus* has attracted various research attention over the past few years due to its robust growth, broad substrate specificity, tolerance to various aromatics, and ability to accumulate high lipids [90]. The ability of *Rhodococcus* strain to grow on coniferyl alcohol and other lignin derivatives was observed by *R. opacus* DSM 1069 strain in 1980 [91]. Later, several *Rhodococcus* species were identified to be oleaginous, and strain *R. opacus* PD630 was most extensively studied with lipid accumulation of more than 80% [92]. Previously, this strain has been reported to accumulate lipids on aromatic compounds such as phenylacetic acid, and has been extended to other lignin model compounds [93,94]. In addition, studies have reported higher lipid accumulation (more than 40%) in *R. rhodochrous* using glucose as a supplementary carbon source along with HBA and VA [95]. In addition to the lignin model compounds, studies have reported the utilization of actual lignin from biorefinery as the sole carbon source for this process. Kosa and Ragauskas claimed that *R. opacus* can grow and accumulate lipids using ethanol organosolv lignin (EOL) and ultrasonicated EOL as the sole carbon source [96]. However, compared to the results of the lignin model compounds, lower lipid accumulation (4.08%) was observed from both EOL sources. Quantification of various lignin fractions during the fermentation process suggested that microbial strains could efficiently utilize the low molecular weight lignin fractions compared to recalcitrant polymers. Hence, depolymerization of lignin prior to fermentation will be efficient for higher biomass growth and lipid accumulation. In another study, He et al. reported higher lipid accumulation in engineered *R. jostii* RHA1 $VanA^{-}$ compared to wild type *R. opacus* PD630 strain using dilute alkali corn stover lignin [97]. In addition, lipid production was higher in co-fermentation of these strains than using a single strain in fermentation. Zhao and co-workers demonstrated a possible synergistic effect between *R. opacus* PD630 cells and laccase degradation of lignin [98]. Enhanced lipid accumulation was observed by using simultaneous depolymerization and fermentation process, where laccase mediated degradation provides sufficient monomers to *R. opacus* for fermentation.

3. Cis, Cis-muconic Acid (MA)

MA is a commercially important chemical with a wide range of applications that is being used as an intermediate for the synthesis of adipic acid, a building block for the production of nylons and polyurethanes, and terephthalic acid, a monomer for the synthesis of polyethylene terephthalate [99]. Additionally, adipic acid and terephthalic acid are also used in cosmetic, pharmaceutical, and food industries [100]. Traditionally, industrial-scale production of MA is mainly carried out from petroleum-based feedstock, which further causes environmental pollution, depletion of petroleum resources, and an increase in purification cost. Thus, the utilization of renewable feedstock such as lignin with biological conversion route provides a possible substitution to alleviate these issues. MA is an intermediate product of *β*-KAP pathway and it is not accumulated by native strains. Enzyme catechol 1,2-dioxygenase synthesizes MA using catechol as a substrate. MA was accumulated in the culture broth by blocking the MA degradation pathway in recombinant strains. To date, several aromatic compounds (benzoate, toluene, catechol, phenol, p-coumarate, and guaiacol) have been used as substrates for the production of MA [101-105]. In native strains, lignin-derived compounds (benzene, phenol, toluene benzoate, cinnamic acid, etc.) are directed towards the production of catechol, which is further converted to MA. However, other lignin-derived compounds such as VA, ferulic acid, and p-coumarate are converted using protocatechuic acid as intermediate instead of catechol. Therefore, native strains lack the ability to convert these lignin-derived compounds for MA production. Vardon and co-workers reported the linking of protocatechuic acid and catechol branches by blocking the protocatechuic acid degradation pathway in *Pseudomonas putida* KT2440 [106]. Due to this, the aromatics metabolized through the protocatechuic acid branch were directed towards the formation of catechol, and subsequently to MA. This increased the broad substrate selectivity of the strain for MA production. In another study, MA production was enhanced by deleting the catabolite repression control protein encoding genes, which regulate higher accumulation of 4-hydroxybenzoate and vanillate instead of MA [104,107]. Recently, Salvachúa et al. reported improvements in MA titer, yield, and productivity in

recombinant *P. putida* using lignin model compounds (hydroxycinnamic acids, *p*-coumaric acid, and ferulic acid). The combination of gene overexpression, removal of global catabolic regulator, and fed-batch operation increased the final MA concentration to 50 g/L with a productivity of 0.5 $g \cdot L^{-1} \cdot h^{-1}$ from p-coumaric acid. In addition to *P. putida*, other bacteria including recombinant *E. coli,* recombinant *Amycolatopsis* sp. and *C. glutamicum* have been reported as a producer of MA from lignin derivatives [99,105,108].

4. Vanillin

Vanillin is a high-value chemical used primarily in the food and flavor industry. It is mainly produced by chemical conversion of petrochemicals and thermochemical mediated degradation of lignin. Commercial production of vanillin is carried out from lignin, which contributes to 15% of the total vanillin production (~3,000 tons per year) [109]. As the demand for biological methods increases, vanillin production is moving towards the use of biological catalysts (bacteria, fungi, and plant cells) in the production process. [110]. In the past few years, some bacteria and fungi have been identified as producers of vanillin from lignin. However, a major disadvantage of industrial production of vanillin using biological methods is lower product yield. In view of this, a recent study by Sainsbury and coworkers reported higher vanillin accumulation up to 96 mg/L in recombinant *R. jostii* RHA1 strain [111]. This recombinant strain lacking the vanillin dehydrogenase gene utilized wheat straw lignocellulose with supplementation of additional glucose to the medium. Studies have also reported the conversion of ferulic acid, an abundant phenolic acid compound in plant cell wall for vanillin production using various bacteria, including recombinant *P. putida* KT2440, *L. plantarum*, and engineered *P. fluorescens* [112-114].

LIGNIN-DEGRADING ENZYMES

Apart from microbial lignin conversion, several enzymes isolated from fungi or bacteria have been reported to degrade lignin via nonspecific cleavage. These enzymes are applied to *in vitro* experiments to depolymerize or degrade lignin. Reduction of cultivation time, enhancement of substrate and enzyme interaction, and reduction of ATP/NAD(P)H imbalance are some of the additional advantages of using *in vitro* enzymatic conversion compared to the whole cell lignin conversion [115]. Most studies have reported the use of a single enzymatic system in this process. However, for the efficient conversion of lignin, a complicated enzymatic lignin digestion/depolymerization reaction involving mixed enzymes needs to be developed [116].

Most of the lignin degrading enzymes belong to the family of laccase or peroxidase. In the peroxidase family, lignin peroxidase (LiP) and manganese peroxidase (MnP) have been well-studied enzymes over the years. Recently, two additional peroxidases, such as versatile peroxidase (VP) and dye-decoloring peroxidase (DyP), have attracted research interest due to their versatile applications. Oxidation of lignin by these enzymes is carried out randomly, i.e., without any specificity of enzymes towards substrates or linkages. A detailed description of the various enzymes and their reaction mechanisms is given in the following section.

1. Laccases

Laccase is the most common lignin-degrading enzyme that can be produced by both bacteria and fungi [117]. White-rot fungus (wood-decaying basidiomycetes) is a well-known lignin degrader that is one of the major producers of laccase [118]. The degradation of both phenolic and non-phenolic lignin by laccase is carried out by accepting a molecule of oxygen as an electron acceptor [119]. The phenolic subunits of lignin can be directly oxidized by laccase, and the oxidized phenolic compounds are subsequently converted to free phenoxy radicals, which further leads to polymer cleavage reaction [120]. Additionally, this enzyme is a multi-copper containing enzyme that carries four copper atoms (Cu^{2+}) at its active sites. Copper at the active site helps in the oxidation and decarboxylation of phenolic and methoxyphenolic acids [117].

Laccase-mediated degradation of non-phenolic compounds requires the presence of additional mediators. Initially, the mediator is oxidized by laccase, which later oxidizes the non-phenolic substrates via radical hydrogen atom transfer or electron transfer mechanisms [121]. 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) and 1-hydroxybenzotriazole are reported to be the most commonly used mediators by laccase for nonphenolic degradation of lignin. These mediators aid laccase in the formation of stable intermediate with the substrate. Since oxidation of non-phenolic compounds in lignin requires additional mediator compared to the phenolic subunits, the current research is more focused on mediator-assisted oxidation of non-phenolic lignin subunits [119, 122-125]. With the help of this laccase-mediator system, 80-90% of lignin degradation can be achieved [126]. In addition, numerous studies have reported the use of molecular techniques for improving enzyme stability and performance. Apart from depolymerization, laccase is also used in several other processes, including delignification, bioremediation and biodegradation, dye decolorization, and wastewater treatment [127,128].

2. Lignin Peroxidases (LiP)

LiP has been isolated from several white-rot fungi, which follows a similar catalytic and oxidative cycle as other peroxidases. LiP is a heme-containing glycoprotein with a molecular mass in the range of 38-43 kDa [129]. It catalyzes the oxidative degradation of phenolic and non-phenolic compounds of lignin in the presence of hydrogen peroxide. Oxidation of these compounds is carried out by cleavage of C_α-C_β and aryl-C_α bonds, aromatic ring opening, demethoxylation, and phenolic oxidation [130]. Additionally, this enzyme also requires veratryl alcohol (VA), a nonphenolic metabolite, as a mediator to complete the catalytic cycle and protect the enzyme from being inactivated by H_2O_2 [131]. LiP also catalyzes the oxidation of VA to form VA radical cation ($VA^{\bullet+}$), which further oxidizes lignin and other recalcitrant molecules by indirect oxidation [131]. The high redox potential (~1.2 V at pH 3.0) allows LiP to oxidize a wide range of substrates that are not oxidized by other peroxidases [132]. Several fungal strains such as *Phanerochaete chrysosporium*, *Trametes versicolor*, *Bjerkandera* sp., and *Phlebia tremellosa*, and a few bacterial strains including *Acinetobacter calcoaceticus* and *Streptomyces viridosporus* have been identified as producers of LiP [132]. Besides depolymerization and delignification, the potential of LiPs has been explored in a variety of other fields including biorefinery, bioremediation, textiles, cosmetics, and dermatology [133].

3. Manganese Dependent Peroxidases (MnP)

MnP is another lignin-degrading enzyme similar to LiP with a

molecular weight in the range of 38 to 62.5 kDa. This enzyme was first detected in *P. chrysosporium*, and several other Basidiomycota species, such as *Lenzites betulinus*, *Panus tigrinus*, *Bjerkandera* sp., *Agaricus bisporus*, and *Nematoloma frowardii*, have been reported as producers of MnP [132]. The catalytic activity of MnP is similar to LiP, but MnP utilizes Mn^{2+} as the reducing substrate to convert it to Mn^{3+}. This Mn^{3+} is a strong oxidizing agent that diffuses into the lignocellulosic structure to oxidize the lignin phenolic compounds. Stabilization of Mn^{3+} outside the enzyme is ensured by chelation with organic acids such as fumarate, oxalate, malate, and glyoxylate [11]. Additionally, the release of Mn^{3+} from the active site of the enzyme is facilitated by these organic acids [134]. MnP oxidizes lignin phenolic compounds to phenoxyl radicals by the abstraction of a hydrogen atom, which later leads to depolymerization of lignin [135].

4. Versatile Peroxidases (VP) and Dye-decoloring Peroxidases (DyPs)

In addition to LiP and MnP, two other peroxidases, such as VP and DyPs, are used for the degradation of lignin and its model compounds. VPs have similar substrate specificities to LiP and MnP and oxidize veratryl alcohol, methoxybenzenes, non-phenolic model lignin compounds, and Mn^{2+} [132]. VPs were secreted in several isoforms with molecular mass ranging from 40 to 45 kDa [136]. Fungal species such as *Bjerkandera* and *Pleurotus* have been reported as producer of VP [11]. Due to its bifunctionality nature (similar to LiP and MnP), it has attracted several research attentions in the field of depolymerization, delignification, and industrial dye decolorization [137-139]. Over-expression of VP from *Bjerkandera adusta* in *Escherichia coli* achieved faster and large-scale production of VP for industrial applications [140].

Another peroxidase, DyP-type peroxidase (a heme-containing peroxidases), was first reported in the fungus *Bjerkandera adusta*, which was later named due to its ability to degrade a wide range of dyes [141,142]. After isolation of DyP from *B. adusta*, several fungal strains such as *Termitomyces albuminosus* [143], *Auricularia auricula-judae* [144], *Irpex lacteus* [145] have been reported to be producers of DyP with ligninolytic activity. In addition to fungal strains, DyPs have been found in a wide range of bacteria, including *Rhodococcus josti* [146], *Thermobifida fusca* [145], and *Pseudomonas fluorescens* [147]. DyP-type peroxidases are further classified into four classes (A, B, C, and D) according to their amino acid sequence [148]. DyP classes A, B, and C are mainly found in bacteria, while class D DyP is primarily produced by fungi. Types C and D DyPs show higher activity towards substrate oxidation than the other two types. DyP from *Thermobifida fusca* (TfuDyP) expressed its activity towards a wide range of substrates, including lignin-derived compounds, flavors, and various dyes [149]. Furthermore, the oxidation activity of recombinant TfuDyP towards Kraft lignin and dimeric lignin model compound (guaiacylglycerol-β-guaiacyl ether) was also demonstrated [150]. Recent studies have reported the involvement of solubilized molecular oxygen in preference to H_2O_2 during oxidation of substrates by DyP [151,152].

MAJOR CHALLENGES AND FUTURE PROSPECTS

Several efforts have been applied for successful conversion of lignin to value-added products over the past few years. However, successful extraction, purification, and valorization of lignin encounter several challenges in each step. Biological conversion of lignin is more challenging due to insufficient information on degradation pathways and availability of low yield microbial strains. The structural integrity of lignin is affected by the application of strong chemicals and harsh operating conditions during the extraction and purification process. Hence, an optimal pretreatment and isolation technique that can efficiently fractionate lignin from lignocellulosic biomass is essential for a successful valorization process. Since the biomass composition--cellulose, hemicellulose, and lignin content--varies depending on the type of biomass and its source, optimal pretreatment techniques should also focus on this aspect. The recalcitrant and heterogeneous nature of lignin prevents its dissolution in the reaction mixture, causing hindrance in the reaction process. Depolymerization of lignin to reduce molecular size is an essential step in this process. Furthermore, during the depolymerization of lignin, the highly reactive lignin intermediates are repolymerized in the reaction mixture which affects the yield of lignin derivatives. Detailed investigation of metabolic pathways and proper design of methodology can overcome these challenges. The solubility of lignin and its byproducts in the solvent can be improved by using organic solvents, and repolymerization of reactive intermediates can be obviated by chemical quenching of β-O-4 ether linkage. In addition, tolerance of enzymes and microbes towards inhibitory intermediates can be enhanced using synthetic biology and gene editing tools. The efficiency of lignin depolymerization for the production of monomers can be augmented by incorporating two or more techniques simultaneously. Although several aerobic microbial strains and their catabolic pathways have been identified, the knowledge of anaerobic lignin degradation process is still unknown. A detailed exploration of this area can provide a new dimension to lignin valorization.

CONCLUSIONS

Lignin in plant biomass is one of the promising renewable sources for the production of several high-value products, including biopolymers, microbial lipids, MA, and vanillin. In view of the advantages of biological lignin conversion over chemical conversion, a detailed discussion of biological lignin valorization was carried out. Recent studies on the biological valorization of lignin for the production of fuels and chemicals have been reviewed in the present study. This study also discusses various lignin extraction techniques, pathways adopted by microorganisms for lignin depolymerization, and enzymes involved in the degradation process. Successful extraction of lignin from lignocellulosic biomass is one of the essential steps in lignin valorization. The optimal lignin extraction and purification process can produce high purity lignin, which can further augment the conversion process. Depolymerization of lignin often leads to the formation of a wide range of lignin-derived aromatics, which further require the involvement of several enzymes and metabolic pathways for conversion. A single microbial system, often devoid of all the essential enzymes, retards the conversion process. Therefore, metabolic engineering of lignin degradation pathways by blocking unwanted side products can

further accumulate a higher concentration of the desired product. Recombinant strains can utilize a wide range of lignin derivatives with higher product titers. Isolation of novel lignin-degrading wild type strains and improvement of genetic engineering tools can further explore new dimensions in the lignin valorization process. A greater understanding of microbial system, enzyme interaction, and metabolic pathways, and the isolation of novel lignin-degrading microorganisms or enzymes can further make the biological lignin valorization process more economic and industrially more suitable.

ACKNOWLEDGEMENTS

This research was supported by the National Research Foundation of Korea (NRF-2017R1A2B4002371 and 2019R1I1A3A02058523). PKD is thankful for the financial support provided by a Brain Korea 21 plus project grant. The authors declare no conflict of interest.

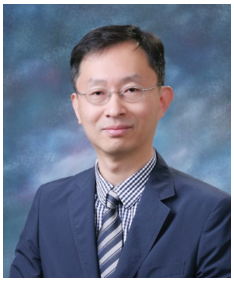
REFERENCES

1. W. J. Sagues, H. Bao, J. L. Nemenyi and Z. Tong, *ACS Sustain. Chem. Eng.*, **6**, 4958 (2018).
2. I. K. M. Yu and D. C. W. Tsang, *Bioresour. Technol.*, **238**, 716 (2017).
3. M. D. Kärkäs, *ChemSusChem*, **10**, 2111 (2017).
4. C. Li, X. Zhao, A. Wang, G. W. Huber and T. Zhang, *Chem. Rev.*, **115**, 11559 (2015).
5. G. Chatel and R. D. Rogers, *ACS Sustain. Chem. Eng.*, **2**, 322 (2014).
6. R. J. A. Gosselink, E. De Jong, B. Guran and A. Abächerli, *Ind. Crops Prod.*, **20**, 121 (2004).
7. A. Grossman and V. Wilfred, *Curr. Opin. Biotechnol.*, **56**, 112 (2019).
8. G. T. Beckham, C. W. Johnson, E. M. Karp, D. Salvachúa and D. R. Vardon, *Curr. Opin. Biotechnol.*, **42**, 40 (2016).
9. A. J. Ragauskas, G. T. Beckham, M. J. Biddy, R. Chandra, F. Chen, M. F. Davis, B. H. Davison, R. A. Dixon, P. Gilna, M. Keller, P. Langan, A. K. Naskar, J. N. Saddler, T. J. Tschaplinski, G. A. Tuskan and C. E. Wyman, *Science*, **344**, 1246843 (2014).
10. J. Zakzeski, P. C. A. Bruijnincx, A. L. Jongerius and B. M. Weckhuysen, *Chem. Rev.*, **110**, 3552 (2010).
11. Á. T. Martínez, M. Speranza, F. J. Ruiz-Dueñas, P. Ferreira, S. Camarero, F. Guillén, M. J. Martínez, A. Gutiérrez, J. C. Del Río, J. C. Río, A. T. Martínez, M. J. Martínez and J. C. Del Río, *Int. Microbiol.*, **8**, 195 (2005).
12. F. X. Collard and J. Blin, *Renew. Sustain. Energy Rev.*, **38**, 594 (2014).
13. Z. H. Liu, R. K. Le, M. Kosa, B. Yang, J. Yuan and A. J. Ragauskas, *Renew. Sustain. Energy Rev.*, **105**, 349 (2019).
14. H. Wang, Y. Pu, A. Ragauskas and B. Yang, *Bioresour. Technol.*, **271**, 449 (2019).
15. C. Liu, S. Wu, H. Zhang and R. Xiao, *Fuel Process. Technol.*, **191**, 181 (2019).
16. V. Subramani and S. K. Gangwal, *Energy Fuel*, **22**, 814 (2008).
17. S. Bahri, T. Patra, Sonal and S. Upadhyayula, *Micropor. Mesopor. Mater.*, **275**, 1 (2019).
18. S. Wang, G. Dai, H. Yang and Z. Luo, *Prog. Energy Combust. Sci.*, **62**, 33 (2017).
19. D. Shen, W. Jin, J. Hu, R. Xiao and K. Luo, *Renew. Sustain. Energy Rev.*, **51**, 761 (2015).
20. C. Liu, J. Hu, H. Zhang and R. Xiao, *Fuel*, **182**, 864 (2016).
21. C. Liu, X. Wang, F. Lin, H. Zhang and R. Xiao, *Fuel Process. Technol.*, **169**, 50 (2018).
22. T. D. H. Bugg, M. Ahmad, E. M. Hardiman and R. Rahmanpour, *Nat. Prod. Rep.*, **28**, 1883 (2011).
23. C. R. Taylor, E. M. Hardiman, M. Ahmad, P. D. Sainsbury, P. R. Norris and T. D. H. Bugg, *J. Appl. Microbiol.*, **113**, 521 (2012).
24. S. Xie, X. Qin, Y. Cheng, D. Laskar, W. Qiao, S. Sun, L. H. Reyes, X. Wang, S. Y. Dai, S. E. Sattler, K. Kao, B. Yang, X. Zhang and J. S. Yuan, *Green Chem.*, **17**, 1657 (2015).
25. S. Xie, R. Syrenne, S. Sun and J. S. Yuan, *Curr. Opin. Biotechnol.*, **27**, 195 (2014).
26. J. Ni and G. Tokuda, *Biotechnol. Adv.*, **31**, 838 (2013).
27. W. Schutyser, T. Renders, S. Van Den Bosch, S. F. Koelewijn, G. T. Beckham and B. F. Sels, *Chem. Soc. Rev.*, **47**, 852 (2018).
28. Z. Chen and C. Wan, *Renew. Sustain. Energy Rev.*, **73**, 610 (2017).
29. O. Y. Abdelaziz, D. P. Brink, J. Prothmann, K. Ravi, M. Sun, J. García-Hidalgo, M. Sandahl, C. P. Hulteberg, C. Turner, G. Lidén and M. F. Gorwa-Grauslund, *Biotechnol. Adv.*, **34**, 1318 (2016).
30. N. Mahmood, Z. Yuan, J. Schmidt and C. C. Xu, *Bioresour. Technol.*, **139**, 13 (2013).
31. A. Tribot, G. Amer, M. Abdou Alio, H. de Baynast, C. Delattre, A. Pons, J. D. Mathias, J. M. Callois, C. Vial, P. Michaud and C. G. Dussap, *Eur. Polym. J.*, **112**, 228 (2019).
32. G. E. Fredheim, S. M. Braaten and B. E. Christensen, *J. Chromatogr. A*, **942**, 191 (2002).
33. X. Zhao, K. Cheng and D. Liu, *Appl. Microbiol. Biotechnol.*, **82**, 815 (2009).
34. B. K. Avellar and W. G. Glasser, *Biomass Bioenerg.*, **14**, 205 (1998).
35. K. Rajendran, E. Drielak, V. Sudarshan Varma, S. Muthusamy and G. Kumar, *Biomass Convers. Biorefinery*, **8**, 471 (2018).
36. W. Liu, W. Chen, Q. Hou, S. Wang and F. Liu, *RSC Adv.*, **8**, 10207 (2018).
37. V. A. Thomas, B. S. Donohoe, M. Li, Y. Pu, A. J. Ragauskas, R. Kumar, T. Y. Nguyen, C. M. Cai and C. E. Wyman, *Biotechnol. Biofuels*, **10**, 252 (2017).
38. A. Shahbazi and B. Zhang, *Bioalcohol production*, Woodhead Publishing, United Kingdom (2010).
39. T. A. Lloyd and C. E. Wyman, *Bioresour. Technol.*, **96**, 1967 (2005).
40. A. B. Ibáñez and S. Bauer, *Biomass Bioenerg.*, **68**, 75 (2014).
41. J. S. Kim, Y. Y. Lee and T. H. Kim, *Bioresour. Technol.*, **199**, 42 (2016).
42. A. Tolbert, H. Akinosho, R. Khunsupat, A. K. Naskar and A. J. Ragauskas, *Biofuel. Bioprod. Bior.*, **8**, 836 (2014).
43. S. S. Hassan, G. A. Williams and A. K. Jaiswal, *Bioresour. Technol.*, **262**, 310 (2018).
44. G. Kumar, P. Sivagurunathan, G. Zhen, T. Kobayashi, S.-H. Kim and K. Xu, *Bioresour. Technol.*, **245**, 196 (2017).
45. M. Käldström, N. Meine, C. Farès, R. Rinaldi and F. Schüth, *Green Chem.*, **16**, 2454 (2014).
46. G. Calvaruso, M. T. Clough and R. Rinaldi, *Green Chem.*, **19**, 2803 (2017).
47. X. Chen, J. Shekiro, T. Pschorn, M. Sabourin, L. Tao, R. Elander, S. Park, E. Jennings, R. Nelson, O. Trass, K. Flanegan, W. Wang, M. E. Himmel, D. Johnson and M. P. Tucker, *Biotechnol. Biofuels*, **7**, 98 (2014).
48. X. Chen, W. Wang, P. Ciesielski, O. Trass, S. Park, L. Tao and M. P.

Tucker, *ACS Sustain. Chem. Eng.*, **4**, 324 (2016).
49. X. Chen, E. Kuhn, E. W. Jennings, R. Nelson, L. Tao, M. Zhang and M. P. Tucker, *Energy Environ. Sci.*, **9**, 1237 (2016).
50. R. G. Saratale, H. S. Shin, G. S. Ghodake, G. Kumar, M. K. Oh and G. D. Saratale, *Bioresour. Technol.*, **258**, 26 (2018).
51. Q. Wang, Z. Wang, F. Shen, J. Hu, F. Sun, L. Lin, G. Yang, Y. Zhang and S. Deng, *Bioresour. Technol.*, **166**, 420 (2014).
52. Z. Chen, and C. Wan, *Bioresour. Technol.*, **250**, 532 (2018).
53. G. Kumar, P. Sivagurunathan, G. Zhen, T. Kobayashi, S.-H. Kim and K. Xu, *Bioresour. Technol.*, **245**, 196 (2017).
54. A. Björkman, *Nature*, **174**, 1057 (1954).
55. B. Jiang, T. Cao, F. Gu, W. Wu and Y. Jin, *ACS Sustain. Chem. Eng.*, **5**, 342 (2017).
56. R. Whetten and R. Sederoff, *Plant Cell*, **7**, 1001 (1995).
57. E. Ten, C. Ling, Y. Wang, A. Srivastava, L. A. Dempere and W. Vermerris, *Biomacromolecules*, **15**, 327 (2014).
58. A. K. Mackenzie, A. E. Naas, S. K. Kracun, J. Schückel, J. U. Fangel, J. W. Agger, W. G. T. Willats, V. G. H. Eijsink and P. B. Pope, *Appl. Environ. Microbiol.*, **81**, 187 (2015).
59. D. W. S. Wong, *Appl. Biochem. Biotechnol.*, **157**, 174 (2009).
60. E. Masai, Y. Katayama and M. Fukuda, *Biosci. Biotechnol. Biochem.*, **71**, 1 (2007).
61. Y. Sato, H. Moriuchi, S. Hishiyama, Y. Otsuka, K. Oshima, D. Kasai, M. Nakamura, S. Ohara, Y. Katayama, M. Fukuda and E. Masai, *Appl. Environ. Microbiol.*, **75**, 5195 (2009).
62. J. Reiter, H. Strittmatter, L. O. Wiemann, D. Schieder and V. Sieber, *Green Chem.*, **15**, 1373 (2013).
63. M. Marinović, P. Nousiainen, A. Dilokpimol, J. Kontro, R. Moore, J. Sipilä, R. P. De Vries, M. R. Mäkelä and K. Hildén, *ACS Sustain. Chem. Eng.*, **6**, 2878 (2018).
64. D. L. Gall, J. Ralph, T. J. Donohue and D. R. Noguera, *Environ. Sci. Technol.*, **48**, 12454 (2014).
65. P. Picart, C. Müller, J. Mottweiler, L. Wiermans, C. Bolm, P. D. De Marfa and A. Schallmey, *ChemSusChem*, **7**, 3164 (2014).
66. E. Mnich, R. Vanholme, P. Oyarce, S. Liu, F. Lu, G. Goeminne, B. Jørgensen, M. S. Motawie, W. Boerjan, J. Ralph, P. Ulvskov, B. L. Møller, N. Bjarnholt and J. Harholt, *Plant Biotechnol. J.*, **15**, 581 (2017).
67. M. P. Pandey and C. S. Kim, *Chem. Eng. Technol.*, **34**, 29 (2011).
68. D. H. Pieper, *Appl. Microbiol. Biotechnol.*, **67**, 170 (2005).
69. L. N. Ornston, *J. Biol. Chem.*, **241**, 3787 (1966).
70. D. Zhu, P. Zhang, C. Xie, W. Zhang, J. Sun, W. J. Qian and B. Yang, *Biotechnol. Biofuels*, **10**, 44 (2017).
71. J. H. Tian, A. M. Pourcher, T. Bouchez, E. Gelhaye and P. Peu, *Appl. Microbiol. Biotechnol.*, **98**, 9527 (2014).
72. T. D. H. Bugg, M. Ahmad, E. M. Hardiman and R. Singh, *Curr. Opin. Biotechnol.*, **22**, 394 (2011).
73. S. Pradhan, P. K. Dikshit and V. S. Moholkar, *Polym. Adv. Technol.*, **29**, 2392 (2018).
74. P. Kumar, A. Maharjan, H. B. Jun and B. S. Kim, *Biotechnol. Appl. Biochem.*, **66**, 153 (2019).
75. J. Yu and H. Stahl, *Bioresour. Technol.*, **99**, 8042 (2008).
76. S. Tomizawa, J. A. Chuah, K. Matsumoto, Y. Doi and K. Numata, *ACS Sustain. Chem. Eng.*, **2**, 1106 (2014).
77. M. Si, X. Yan, M. Liu, M. Shi, Z. Wang, S. Wang, J. Zhang, C. Gao, L. Chai and Y. Shi, *ACS Sustain. Chem. Eng.*, **6**, 7969 (2018).
78. J. G. Linger, D. R. Vardon, M. T. Guarnieri, E. M. Karp, G. B. Hunsinger, M. A. Franden, C. W. Johnson, G. Chupka, T. J. Strathmann, P. T. Pienkos and G. T. Beckham, *Proc. Natl. Acad. Sci. U.S.A.*, **111**, 12013 (2014).
79. Z. H. Liu, M. L. Olson, S. Shinde, X. Wang, N. Hao, C. G. Yoo, S. Bhagia, J. R. Dunlap, Y. Pu, K. C. Kao, A. J. Ragauskas, M. Jin and J. S. Yuan, *Green Chem.*, **19**, 4939 (2017).
80. R. Mehrabi, G. Bagheri and F. Alipour, *J. Entomol. Zool. Stud.*, **3**, 33 (2015).
81. T. Bowers, A. Vaidya, D. A. Smith and G. Lloyd-Jones, *J. Chem. Technol. Biotechnol.*, **89**, 1030 (2014).
82. B. H. A. Rehm, T. A. Mitsky and A. Steinbüchel, *Appl. Environ. Microbiol.*, **67**, 3102 (2001).
83. X. Wang, L. Lin, J. Dong, J. Ling, W. Wang, H. Wang, Z. Zhang and X. Yu, *Appl. Environ. Microbiol.*, **84**, e01469 (2018).
84. K. Numata and K. Morisaki, *ACS Sustain. Chem. Eng.*, **3**, 569 (2015).
85. M. Kumar, A. Singhal, P. K. Verma and I. S. Thakur, *ACS Omega*, **2**, 9156 (2017).
86. M. S. G. Lopes, J. G. C. Gomez, M. K. Taciro, T. T. Mendonça and L. F. Silva, *J. Ind. Microbiol. Biotechnol.*, **41**, 1353 (2014).
87. S. A. Shields-Menard, M. Amirsadeghi, W. T. French and R. Boopathy, *Bioresour. Technol.*, **259**, 451 (2018).
88. C. S. Harwood and R. E. Parales, *Annu. Rev. Microbiol.*, **50**, 553 (1996).
89. A. M. Warhurst and C. A. Fewson, *Crit. Rev. Biotechnol.*, **14**, 29 (1994).
90. Z. Xu, P. Lei, R. Zhai, Z. Wen and M. Jin, *Biotechnol. Biofuels*, **12**, 1 (2019).
91. L. Eggeling and H. Sahm, *Arch. Microbiol.*, **126**, 141 (1980).
92. H. M. Alvarez, H. Luftmann, R. A. Silva, A. C. Cesari, A. Viale, M. Wältermann and A. Steinbüchel, *Microbiology*, **148**, 1407 (2002).
93. H. M. Alvarez, F. Mayer, D. Fabritius and A. Steinbüchel, *Arch. Microbiol.*, **165**, 377 (1996).
94. M. Kosa and A. J. Ragauskas, *Appl. Microbiol. Biotechnol.*, **93**, 891 (2012).
95. S. A. Shields-Menard, M. AmirSadeghi, M. Green, E. Womack, D. L. Sparks, J. Blake, M. Edelmann, X. Ding, B. Sukhbaatar, R. Hernandez, J. R. Donaldson and T. French, *Int. Biodeterior. Biodegrad.*, **121**, 79 (2017).
96. M. Kosa and A. J. Ragauskas, *Green Chem.*, **15**, 2070 (2013).
97. Y. He, X. Li, H. Ben, X. Xue and B. Yang, *ACS Sustain. Chem. Eng.*, **5**, 2302 (2017).
98. C. Zhao, S. Xie, Y. Pu, R. Zhang, F. Huang, A. J. Ragauskas and J. S. Yuan, *Green Chem.*, **18**, 1306 (2016).
99. J. Becker, M. Kuhl, M. Kohlstedt, S. Starck and C. Wittmann, *Microb. Cell Fact.*, **17**, 115 (2018).
100. N. Z. Xie, H. Liang, R. B. Huang and P. Xu, *Biotechnol. Adv.*, **32**, 615 (2014).
101. J. B. J. H. Van Duuren, D. Wijte, A. Leprince, B. Karge, J. Puchałka, J. Wery, V. A. P. M. Dos Santos, G. Eggink and A. E. Mars, *J. Biotechnol.*, **156**, 163 (2011).
102. M. Kohlstedt, S. Starck, N. Barton, J. Stolzenberger, M. Selzer, K. Mehlmann, R. Schneider, D. Pleissner, J. Rinkel, J. S. Dickschat, J. Venus, J. B. J. H. van Duuren and C. Wittmann, *Metab. Eng.*, **47**, 279 (2018).

103. J. W. Chua and J. H. Hsieh, *World J. Microbiol. Biotechnol.*, **6**, 127 (1990).
104. C. W. Johnson, D. Salvachúa, P. Khanna, H. Smith, D. J. Peterson and G. T. Beckham, *Metab. Eng. Commun.*, **3**, 111 (2016).
105. N. Barton, L. Horbal, S. Starck, M. Kohlstedt, A. Luzhetskyy and C. Wittmann, *Metab. Eng.*, **45**, 200 (2018).
106. D. R. Vardon, M. A. Franden, C. W. Johnson, E. M. Karp, M. T. Guarnieri, J. G. Linger, M. J. Salm, T. J. Strathmann and G. T. Beckham, *Energy Environ. Sci.*, **8**, 617 (2015).
107. C. W. Johnson, P. E. Abraham, J. G. Linger, P. Khanna, R. L. Hettich and G. T. Beckham, *Metab. Eng. Commun.*, **5**, 19 (2017).
108. W. Wu, T. Dutta, A. M. Varman, A. Eudes, B. Manalansan, D. Loqué and S. Singh, *Sci. Rep.*, **7**, 8420 (2017).
109. M. Fache, B. Boutevin and S. Caillol, *ACS Sustain. Chem. Eng.*, **4**, 35 (2016)
110. H. Priefert, J. Rabenhorst and A. Steinbüchel, *Appl. Microbiol. Biotechnol.*, **56**, 296 (2001).
111. P. D. Sainsbury, E. M. Hardiman, M. Ahmad, H. Otani, N. Seghezzi, L. D. Eltis and T. D. H. Bugg, *ACS Chem. Biol.*, **8**, 2151 (2013).
112. N. Graf and J. Altenbuchner, *Appl. Microbiol. Biotechnol.*, **98**, 137 (2014).
113. B. Kaur, D. Chakraborty and B. Kumar, *Biomed Res. Int.*, **2013**, 590359 (2013).
114. D. Di Gioia, F. Luziatelli, A. Negroni, A. G. Ficca, F. Fava and M. Ruzzi, *J. Biotechnol.*, **156**, 309 (2011).
115. Y. H. P. Zhang, *Biotechnol. Adv.*, **33**, 1467 (2015).
116. P. Picart, P. D. De María and A. Schallmey, *Front. Microbiol.*, **6**, 916 (2015).
117. A. Leonowicz, N. Cho, J. Luterek, A. Wilkolazka, M. Wojtas-Wasilewska, A. Matuszewska, M. Hofrichter, D. Wesenberg and J. Rogalski, *J. Basic Microbiol.*, **41**, 185 (2001).
118. A. Kinnunen, P. Maijala, P. Jarvinen and A. Hatakka, *Curr. Biotechnol.*, **6**, 105 (2016).
119. R. Bourbonnais and M. G. Paice, *FEBS Lett.*, **267**, 99 (1990).
120. L. Gianfreda, F. Xu, J. Bollag, L. Gianfreda, F. Xu and J. Bollag, *Bioremediat. J.*, **3**, 37 (2013).
121. R. Hilgers, J. P. Vincken, H. Gruppen and M. A. Kabel, *ACS Sustain. Chem. Eng.*, **6**, 2037 (2018).
122. R. Bourbonnais, M. G. Paice, B. Freiermuth, E. Bodie and S. Borneman, *Appl. Environ. Microbiol.*, **63**, 4627 (1997).
123. P. Baiocco, A. M. Barreca, M. Fabbrini, C. Galli and P. Gentili, *Org. Biomol. Chem.*, **1**, 191 (2003).
124. C. Eggert, U. Temp, J. F. D. Dean and K. E. L. Eriksson, *FEBS Lett.*, **391**, 144 (1996).
125. P. Astolti, P. Brandi, C. Galli, P. Gentili, M. F. Gerini, L. Greci and O. Lanzalunga, *New J. Chem.*, **29**, 1308 (2005).
126. S. Camarero, O. García, T. Vidal, J. Colom, J. C. del Río, A. Gutiérrez, J. M. Gras, R. Monje, M. J. Martínez and Á. T. Martínez, *Enzyme Microb. Technol.*, **35**, 113 (2004).
127. Shraddha, R. Shekher, S. Sehgal, M. Kamthania and A. Kumar, *Enzyme Res.*, **2011**, 217861 (2011).
128. P. Upadhyay, R. Shrivastava and P. K. Agrawal, *3 Biotech*, **6**, 1 (2016).
129. J. Pérez, J. Muñoz-Dorado, T. De La Rubia and J. Martínez, *Int. Microbiol.*, **5**, 53 (2002).
130. M. H. Gold, H. Wariishi and K. Valli, in *Biocatalysis in agricultural biotechnology*, J. R. Whitaker and P. E. Sonnet Eds., *ACS Symp. Ser.*, **389**, 127 (1989).
131. V. Christian, R. Shrivastava, D. Shukla, H. Modi and B. R. M. Vyas, *Enzyme Microb. Technol.*, **36**, 426 (2005).
132. G. Janusz, A. Pawlik, J. Sulej, U. Świderska-Burek, A. Jarosz-Wilkolazka and A. Paszczyński, *FEMS Microbiol. Rev.*, **41**, 941 (2017).
133. A. O. Falade, U. U. Nwodo, B. C. Iweriebor, E. Green, L. V. Mabinya and A. I. Okoh, *Microbiologyopen*, **6**, e00394 (2017).
134. K. N. Niladevi, *Biotechnology for agro-industrial residues utilisation: utilisation of agro-residues*, Springer Netherlands, 397 (2009).
135. M. Hofrichter, *Enzyme Microb. Technol.*, **30**, 454 (2002).
136. T. Mester and J. A. Field, *J. Biol. Chem.*, **273**, 15412 (1998).
137. M. C. Baratto, K. Juarez-Moreno, R. Pogni, R. Basosi and R. Vazquez-Duhalt, *Environ. Sci. Pollut. Res.*, **22**, 8683 (2015).
138. A. Ravichandran, R. G. Rao, V. Thammaiah, S. Maheswarappa Gopinath and M. Sridhar, *BioResources*, **14**, 5132 (2019).
139. J. Zeng, M. J. L. Mills, B. A. Simmons, M. S. Kent and K. L. Sale, *Green Chem.*, **19**, 2145 (2017).
140. M. Mohorčič, M. Benčina, J. Friedrich and R. Jerala, *Bioresour. Technol.*, **100**, 851 (2009).
141. E. Fernández-Fueyo, D. Linde, D. Almendral, M. F. López-Lucendo, F. J. Ruiz-Dueñas and A. T. Martínez, *Appl. Microbiol. Biotechnol.*, **99**, 8927 (2015).
142. S. J. Kim and M. Shoda, *Appl. Environ. Microbiol.*, **65**, 1029 (1999).
143. T. Johjima, M. Ohkuma and T. Kudo, *Appl. Microbiol. Biotechnol.*, **61**, 220 (2003).
144. C. Liers, C. Bobeth, M. Pecyna, R. Ullrich and M. Hofrichter, *Appl. Microbiol. Biotechnol.*, **85**, 1869 (2010).
145. D. Salvachúa, A. Prieto, Á. T. Martínez and M. J. Martínez, *Appl. Environ. Microbiol.*, **79**, 4316 (2013).
146. J. N. Roberts, R. Singh, J. C. Grigg, M. E. P. Murphy, T. D. H. Bugg and L. D. Eltis, *Biochemistry*, **50**, 5108 (2011).
147. R. Rahmanpour and T. D. H. Bugg, *Arch. Biochem. Biophys.*, **574**, 93 (2015).
148. S. Lee, M. Kang, J.-H. Bae, J.-H. Sohn and B. H. Sung, *Front. Bioeng. Biotechnol.*, **7**, 209 (2019).
149. N. Lončar, D. I. Colpa and M. W. Fraaije, *Tetrahedron*, **72**, 7276 (2016).
150. R. Rahmanpour, D. Rea, S. Jamshidi, V. Fülöp and T. D. H. Bugg, *Arch. Biochem. Biophys.*, **594**, 54 (2016).
151. A. Avram, A. Sengupta, P. H. Pfromm, H. Zorn, P. Lorenz, T. Schwarz, K. Q. Nguyen and P. Czermak, *Biocatalysis*, **4**, 1 (2018).
152. H. Zorn, S. Langhoff, M. Scheibner, M. Nimtz and R. G. Berger, *Biol. Chem.*, **384**, 1049 (2003).
153. Y. Shi, X. Yan, Q. Li, X. Wang, M. liu, S. Xie, L. Chai and J. Yuan, *Process Biochem.*, **52**, 238 (2017).
154. Z. Wei, G. Zeng, F. Huang, M. Kosa, D. Huang and A. J. Ragauskas, *Green Chem.*, **17**, 2784 (2015).
155. T. Wells, Z. Wei and A. Ragauskas, *Biomass Bioenerg.*, **72**, 200 (2015).
156. M. Hu, J. Wang, Q. Gao and J. Bao, *J. Biotechnol.*, **281**, 81 (2018).
157. Z. Wang, N. Li and X. Pan, *Fuel*, **240**, 119 (2019).
158. T. Sonoki, K. Takahashi, H. Sugita, M. Hatamura, Y. Azuma, T. Sato, S. Suzuki, N. Kamimura and E. Masai, *ACS Sustain. Chem. Eng.*, **6**, 1256 (2018).

159. D. Salvachúa, C. W. Johnson, C. A. Singer, H. Rohrer, D. J. Peterson, B. A. Black, A. Knapp and G. T. Beckham, *Green Chem.*, **20**, 5007 (2018).
160. A. Rodriguez, D. Salvachúa, R. Katahira, B. A. Black, N. S. Cleveland, M. Reed, H. Smith, E. E. K. Baidoo, J. D. Keasling, B. A. Simmons, G. T. Beckham and J. M. Gladden, *ACS Sustain. Chem. Eng.*, **5**, 8171 (2017).



Beom Soo Kim is a professor of chemical engineering at Chungbuk National University, Cheongju, Korea. He studied chemical engineering at Seoul National University (1988), obtained a PhD in biochemical engineering at KAIST (1993), completed postdoctoral work at MIT Prof. Robert Langer's lab (1998). He started his lab at Chungbuk National University (2001) and spent sabbatical research in Dr. Ching T. Hou's lab at National Center for Agricultural Utilization Research (NCAUR), United States Department of Agriculture (USDA), Peoria, Illinois (2005). He served as Editor-in-Chief, Korean Society for Biotechnology and Bioengineering Journal and is an Editorial Board member of several journals such as BioMed Research International, Biocatalysis and Agricultural Biotechnology, Korean Journal of Chemical Engineering, Biotechnology and Bioprocess Engineering, Polymers, and BMC Biotechnology. He published over 150 papers and one of his articles on the biosynthesis of silver nanoparticles has been cited more than 1200 times (Google scholar). His research interests include high cell density culture, biodegradable polymers, polyhydroxyalkanoates, biosynthesis and applications of nanomaterials, and biorefinery.