REVIEW

Lignin valorization meets synthetic biology

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Lignin, an abundant renewable resource in nature, is a highly heterogeneous biopolymer consisting of phenylpropanoid units. It is essential for sustainable utilization of biomass to convert lignin to value-added products. However, there are technical obstacles for lignin valorization due to intrinsic heterogeneity. The emerging of synthetic biology technologies brings new opportunities for lignin breakdown and utilization. In this review, we discussed the applications of synthetic biology on lignin conversion, especially the production of value-added products, such as aromatic chemicals, ring-cleaved chemicals from lignin-derived aromatics and bio-active substances. Synthetic biology will offer new potential strategies for lignin valorization by optimizing lignin degradation enzymes, building novel artificial converting pathways, and improving the chassis of model microorganisms.

KEYWORDS

artificial pathway, bio-degradation, lignin valorization, synthetic biology, value-added products

1 | INTRODUCTION

The content of lignin is 15–40% of dry weight in most terrestrial plants, which provides the strength and rigidity, facilitates water and nutrient transport, and forms heterogeneous, recalcitrant barriers to microbial attack [1,2]. Lignin is the most abundant renewable feedstock for aromatics [3,4]. Cellulosic biofuel production will generate about 62 million tons, in addition, pulping and paper industries also generate approximately 50 million tons of lignin annually (e.g., kraft lignin, lignosulfonate, soda lignin) [5,6]. Lignin valorization will produce large quantity of value-added chemicals and fuels, which could be considered as potential lignin valorization platform in the future [7]. Lignin is a highly heterogeneous biopolymer consisting of phenylpropanoid units, including three monolignols, e.g., *p*-coumaryl alcohol (H), coniferyl alcohol (G), and sinapyl alcohol (S)

(Supporting Information Figure 1) [8]. The percentage of each unit depends on the plant species and tissue [8]. The phenylpropanoid units basically consist of several types of ether bonds (e.g., β -O-4, α -O-4, 4-O-5) and carbon–carbon linkages (e.g., 5-5, β - β , β -1, and β -5), whose formation is mediated by laccases and peroxidases (Supporting Information Figure 2) [9]. The lignin random structures and intrinsic heterogeneity lead to significant technical challenges for lignin valorization. Therefore, lignin breakdown requires unusual chemistry or biochemistry for efficient utilization of lignin [10]. The lignin can be degraded to soluble fragments by the aqueous ammonia pretreatment [11]. In addition, ethylenediamine pretreatment can also degrade lignin due to the break of the ether bonds linking the phenolic monomers [12–14]. And some lignin pretreatment could produce lignin-derived aromatic molecules such as ferulic acid, vanillin, and syringaldehyde for utilization of lignin (Supporting Information Figure 3) [15,16]. In addition, lignin can also be bio-degraded by lignin-degrading microorganisms. Lignin-degrading microorganisms usually secrete peroxidase enzymes for lignin degradation such as lignin

Abbreviations: MA, muconic acid; PCA, protocatechuic acid; PHA, polyhydroxyalkanoate; *p*HBA, *p*-hydroxybenzoic acid.

peroxidases (LiPs), manganese peroxidases (MnPs), versatile peroxidases (VPs), dye-decolorizing peroxidases (DyPs), and laccases [17]. The whole genomes sequencing and system biology analysis of the lignin degrading microorganisms supply abundant enzyme and lignin degradation pathway for synthetic biology [18,19]. Some specific enzymes for lignin bond cleavage and lignin-derived aromatic molecule degradation have been reported. For example, *Sphingomonas paucimobilis* SYK-6 (SYK-6) can degrade β -aryl ether, the most abundant linkages in lignin [20]. The heterologous expression of *LigDEFG* gene from SYK-6 showed the cleavage of β -aryl ether in lignin [21]. Some microorganisms can degrade parts of lignin to protocatechuic acid (PCA), breakdown the aromatic rings and utilize them as the carbon sources [22]. Therefore, based on the lignin degradation pathway, the microorganisms have been applied to produce value-added products [16]. However, due to the complex structure of lignin, these microorganisms often exhibit low efficiency on the degradation and conversion [6]. Synthetic biology, aiming to design, build, and optimize metabolic pathways, or to redesign natural pathways, will facilitate the bio-conversion of lignin by developing the designer microorganisms [23]. For synthetic biology application, the *Saccharomyces cerevisiae* genome was redesigned and synthesized, and the rearrangement of the synthetic genome was used for improving strain tolerance and product yield [24,25]. Synthetic biology would facilitate lignin valorization for producing chemicals, such as vanillin, muconic acid (MA), and polyhydroxyalkanoates (PHAs), by redesigning natural lignin biodegradation pathways or building novel artificial pathways (Figure 1).

In this review, lignin valorization through synthetic biology was discussed, especially the production of the value-added products, aromatic chemical, ring-cleaved chemicals from lignin-derived aromatics, and bio-active substances. In addition, we also discussed the potential strategies of synthetic biology on other products in lignin valorization.

2 | SYNTHETIC BIOLOGY APPLICATIONS IN LIGNIN VALORIZATION

As the polymer of aromatic molecules, lignin valorization may become the new platform for some aromatic chemicals. In nature, some microorganisms possess degrading pathways of lignin [26]. To date, more and more conversion routes of lignin-derived aromatic molecules were identified, which facilitates the building of more lignin conversion pathways in model microorganisms. The synthetic biology approach would promote lignin valorization for producing value-added products, including aromatic chemicals, ring-cleaved

PRACTICAL APPLICATION

Lignin is an abundant renewable resource in nature. In this mini-review, we summarized the recent applications of synthetic biology in lignin valorization. Synthetic biology facilitated the aromatic chemicals production from lignin degradation and conversion. In addition, the variety of cleavage products from lignin-derived aromatics and value-added bio-active substances could be produced in lignin bio-conversion with the aid of synthetic biology.

chemicals from lignin-derived aromatics and bio-active substances (Figure 2).

2.1 | Production of aromatic chemicals

Recently, it was reported that some aromatic chemicals can be produced from lignin, such as *p*-hydroxybenzoic acid (*p*HBA), vanillin and gallic acid (Figure 2). *p*HBA, an industrial platform chemical, is generally used as a monomer for liquid crystal polymers and the starting feedstock for biosynthesis of a variety of industrially pertinent compounds, such as resveratrol, MA, gastrodin, xiamenmycin, and vanillyl alcohol [27,28]. *p*HBA was produced efficiently from lignin unit *p*-coumaric acid in *Burkholderia glumae* strain BGR, by deleting two genes related with *p*HBA degradation [29]. In further, *phcs* II encoding *p*-hydroxycinnamoyl-CoA synthetase II was identified and overexpressed, which improved *p*HBA production in batch reaction [29]. In addition, *E. coli* with heterologous expression of a novel *p*HBA decarboxylase can convert *p*HBA to phenol, a representative bulk chemical in chemical industry [30].

Vanillin is one of the most important aromatic flavor compounds in novel food and fragrances in perfumes. Deletion of vanillin dehydrogenase led to accumulation of vanillin up to 96 mg/L in *Rhodococcus jostii* RHA1 (*R. jostii* RHA1) on minimal medium containing 2.5% wheat straw lignocellulose and 0.05% glucose [3]. Ferulic acid, a common lignin unit, was catalyzed to produce the vanillin using a temperature-routed system in *E. coli*, by expressing *Fcs* and *Ech* from thermophilic actinomycete *A. thermoflava* N1165. In this system, vanillyl alcohol was formed at 30°C, while vanillin was accumulated at 50°C due to high activities of functional *Fcs* and *Ech* at the high temperature and elimination of ADHs activities [31]. Nevertheless, the biosynthetic route containing *Fcs* and *Ech* has seriously hindered the effective conversion of lignin-derived aromatics due to constant requirement for ATP and CoA [32]. In further, Pad and Ado, a coenzyme-free pathway was introduced into *E. coli* to produce vanillin and 4-vinylguaiacol from ferulic acid [32].

Heterologous expression of *VpVAN* gene from *Vanilla planifolia* catalyzed lignin unit ferulic acid to vanillin in *S. cerevisiae* [33]. Vanillin can be efficiently converted to vanillyl alcohol due to the alcohol dehydrogenase 6 (ADH6) in *S. cerevisiae* [34]. In addition, the detoxification study indicated that the *S. cerevisiae* also could convert coniferyl aldehyde, ferulic acid, and *p*-coumaric acid in to less toxic metabolites [35]. Aminated compounds are useful synthons in the manufacture of a wide range of fine chemicals and pharmaceuticals. Vanillin could be aminated to vanillylamine by heterologous expressing CV2025 w-transaminase (w-Tam) from *Chromobacterium violaceum* DSM30191 in *E. coli* [36]. Except for the vanillylamine, *vdh* or *ligV* encoded vanillin dehydrogenase enable conversion of vanillin to vanillic acid that has pharmacologic activities [37–39]. Vanillic acid can be converted by aryl O-demethylase to protocatechuic acid (PCA), a building block for polymers [37,40,41]. The expression of vanillic acid O-demethylase (*ligM*) from SYK-6 and plant methionine synthase enhanced the PCA production in *E. coli* from vanillic acid [42]. *VanA* and *vanB* genes of lignin-degrading microorganisms, such as *R. jostii* RHA and *Pseudomonas putida* KT2440 (*P. putida* KT2440), also catalyze the O-demethylation of vanillic acid [37,43]. In addition, PCA can also be produced from *p*HBA in *Corynebacterium glutamicum* with expression of *p*HBA hydroxylase encoded by *pobA* [41]. PCA decarboxylation can produce catechol, a versatile building block for the synthesis of a wide range of polymeric materials [44]. A synthetic route with the genes *ligV*, *ligM*, and *aroY* (encoding PCA decarboxylase) converted vanillin to catechol in *E. coli* with expression of an aromatic transporter CouP to alleviate the toxicity [45]. In addition, a vanillin self-inducible promoter ADH7 from *S. cerevisiae* was introduced in *E. coli* to eliminate the cost of an external inducer [45]. Besides PCA decarboxylation, catechol can also be produced from guaiacol, a product of lignin depolymerization, via expressing a cytochrome P450 aromatic O-demethylase from the family CYP255A (*GcoA*) and a three-domain reductase (*GcoB*) in *P. putida* [46].

Syringic acid can be produced from syringaldehyde, one of the lignin-derived aromatic molecules, by heterologous expression of *desV* or *ligV* in *E. coli* [47]. In further, syringic acid could be converted by the demethylase (*desA* and *ligM*) to gallic acid, which is one of naturally occurring phytochemicals, and has strong antioxidant and antibacterial activities [15]. Gallic acid was also produced from *p*HBA in *E. coli* with heterologous expression of *PobA* of *p*HBA hydroxylase mutant from *Pseudomonas aeruginosa* [48]. Gallic acid can be decarboxylated encoded by *lpdc* gene to produce pyrogallol, a precursor for production of the antibiotics trimethoprim, the muscle relaxant gallamine triethiodide, and insecticide bendiocarb [15,49].

2.2 | Production of ring-cleaved chemicals from lignin-derived aromatics

PCA or catechol, central intermediates of lignin-derived catabolism, can be ring-cleaved and utilized as the carbon sources in natural lignin degrading microorganisms [16]. Some important ring-cleaved chemicals, such as MA and PHA, were produced in modifying microorganisms with building artificial pathways (Figure 2). MA is an important chemical building block and precursor of adipic and terephthalic acids in the production of nylon and PET polymers [50]. MA can be produced from catechol with the ortho-cleavage. The *catA* encoding dioxygenase for catechol ortho-cleavage and *aroY* gene encoding PCA decarboxylase were heterologously expressed in *P. putida* KT2440 as a host to achieve MA production from lignin-derived aromatics [51]. In further, the *dmpKLMNOP* gene encoding the phenol monooxygenase from *Pseudomonas* sp was integrated into *P. putida* KT2440 to achieve MA production from phenol [51]. The yield of MA was reduced due to the PCA decarboxylase as a metabolic bottleneck. Coexpression of a PCA decarboxylase associated protein reduced PCA accumulation and enhanced MA production in *P. putida* KT2440-derived strains [52]. In addition, the lignin-derived aromatics were highly toxic, which poses a challenge for the bio-production of MA [53]. Both native catechol 1,2-dioxygenases, *catA* and *catA2*, under the control of the *Pcat* promoter was introduced into *P. putida* KT2440-M6, which increased catechol tolerance, catechol 1,2-dioxygenase levels, and catechol conversion rates [53]. A 64.2 g/L MA from catechol was achieved in a fed-batch process with repeatedly regenerated the energy levels via specific feed pauses in the engineered MA-6 strain [53]. MA could be also produced from A guaiacol-rich true lignin hydrolysate from pine through hydrothermal conversion by deletion of two putative muconate cycloisomerases from the mutant *Amycolatopsis* sp. ATCC 39116 MA-2 [54]. Without using glucose, MA can be produced from vanillic acid while growing on syringic acid in engineered SYK-6 [55]. Besides, the lignin degradation microorganisms were modified. The model microorganisms, *E. coli*, was used to produced MA from vanillin by heterologously expressing a synthetic route with *ligV*, *ligM*, *aroY* gene [15]. Pyruvate, a key intermediate in central metabolism, is fundamental to metabolic engineering strategies for bio-based production of amino acids, alcohols including ethanol and isobutanol, terpenoids, and lactate [56]. Pyruvate can be achieved from catechol and protocatechuate in *P. putida* KT2440, by replacement of the catechol ortho degradation pathway with an exogenous meta-cleavage pathway from *P. putida* mt-2 or replacement of the endogenous PCA ortho pathway with a meta-cleavage pathway from SYK-6 [56]. In addition, L-lactate was produced from the pyruvate through

FIGURE 1 The framework of synthetic biology applied in lignin valorization

the incorporation of a bovine lactate dehydrogenase [56]. Aromatic dicarboxylic acids are commonly used for the synthesis of polyester thermoplastic polymers such as polyethylene terephthalate (PET) and polybutyrate adipate terephthalate (PBAT) [57]. Pyridine 2,4-dicarboxylic acid or pyridine 2,5-dicarboxylic acid was generated by insertion of recombinant genes for PCA 4,5-dioxygenase or PCA 2,3-dioxygenase into *R. jostii* RHAI, when grown on minimal media containing 1% wheat straw lignocellulose [57]. 2-Pyrone-4,6-dicarboxylate (PDC), a building block of functional polymers, was produced from PCA as a precursor in SYK-6 mutant by expressing *pcaK* to enhance PCA uptake in SYK-6, which exhibited 1.27-fold greater PCA conversion and 1.24-fold greater PDC production compared to the control strain [58]. 3-Carboxy muconate, one possible application to a crosslinking agent in polymeric materials such as unsaturated polyester resins, can be achieved from PCA by expressing PCA 3,4-dioxygenase in *E. coli* [59]. Polyhydroxyalkanoates (PHAs) comprising various hydroxycarboxylic acids are natural biodegradable

microbial polyesters, which are accumulated by numerous microorganisms under nutrient limited conditions in the presence of excess carbon sources [60]. Intracellular chain-length polyhydroxyalkanoates (mcl-PHAs) was produced from alkaline pretreated liquor (APL) without appreciable dilution in *P. putida* [16]. For improving the yield of PHA, the dyp peroxidase-based enzymatic system of *P. putida* A514 was enhanced by overexpressing a secreted multifunctional dyp, in further, a variety of peripheral and central catabolism pathways to metabolize aromatic compounds were optimized by overexpressing key enzymes [18]. As a result, PHAs content reached 73% per cell dry weight [18].

2.3 | Production of bio-active substances

As we know, flavonoids, stilbenoids, and coumarins as the phenylpropanoid-derived substances have widely used in human health care [61,62]. *p*-coumaric acid and ferulic acid, present in the lignin pretreatment liquor [16], were used to

the current lignin conversion routes. Especially, *S. cerevisiae*, as the strain for cellulosic ethanol fermentation may enable biotransformation of lignin, cellulose, and hemicellulose to high value-added products by integrating various metabolic pathways. In further, the synthetic biology platform and the gene editing tools can be used to improve the host tolerance to aromatic chemicals. Deep insights into the synthetic biology applications will provide promising and economical prospect for lignin valorization.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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