

Lignin valorization into vanillin: Paving the way for a sustainable aromatic bioeconomy

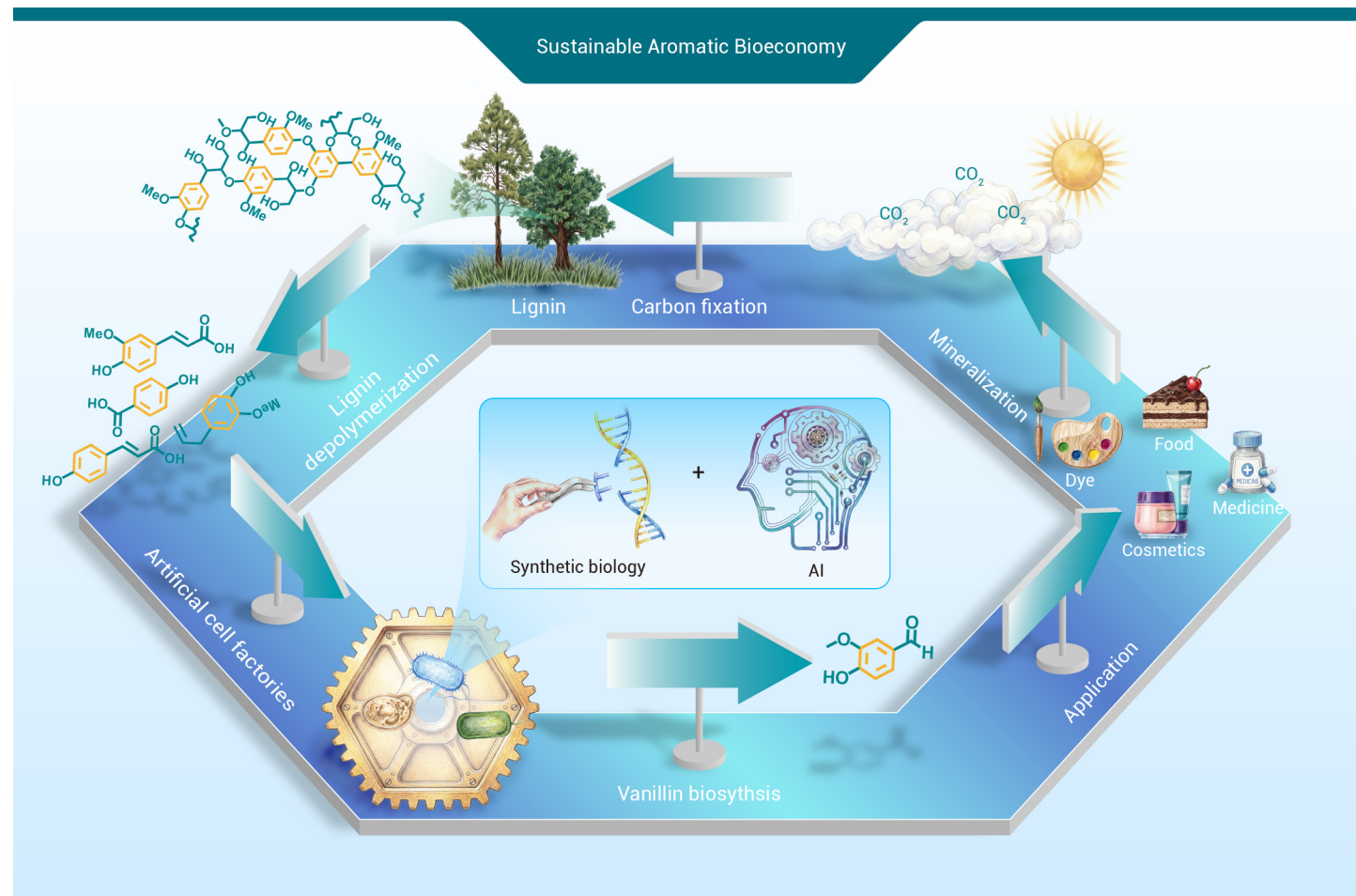
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GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- Lignin valorization into vanillin promotes a sustainable aromatic bioeconomy.
- Microbial conversion of lignin to vanillin boosts lignin valorization.
- Regulating metabolic pathways of cell factory is crucial for vanillin biosynthesis.
- Fermentation strategies could alleviate aromatic toxicity and enable high-titer production.
- Emerging technologies hold the promise for enhancing vanillin biosynthesis.

Lignin valorization into vanillin: Paving the way for a sustainable aromatic bioeconomy

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Lignin is the most abundant renewable aromatic resource on earth. The bioconversion of lignin into aromatic fine chemicals like vanillin aligns with the principles of sustainable "aromatic bioeconomy" concept. However, its recalcitrance and inherent heterogeneity pose significant challenges to efficient biological valorization. Therefore, this review provides a multidisciplinary perspective on aromatic ring-preserving, atom-economical pathways, emphasizing cutting-edge microbial strategies that bridge lignin-derived aromatics to vanillin production without sacrificing aromaticity. Depolymerization strategies are pivotal for enhancing the bioavailability of lignin derivatives, enabling their effective utilization as substrates for downstream vanillin biosynthesis. Furthermore, the design of robust microbial cell factories and the precise regulation of metabolic pathways increase the performance of vanillin biosynthesis. Additionally, advanced fermentation strategies are essential to alleviate vanillin cytotoxicity and enable high-titer production. Furthermore, techno-economic analysis was integrated to benchmark the industrial viability of lignin-based routes. Overall, continued advances in engineering strategies, from lignin depolymerization to vanillin biosynthesis, are reshaping lignin valorization paradigms and driving the development of a sustainable aromatic bioeconomy grounded in aromatic ring conservation.

INTRODUCTION

Sustainability has emerged as a central imperative in modern science, driving intensive efforts toward the development of green and renewable energy sources.^{1,2} Lignin, accounting for about 15%-30% of the total weight of dry biomass, is mainly derived from agricultural residues and forest biomass.³ Currently, the paper and pulp industry produces more than 50 million tons of lignin per year, mostly for combustion to generate electricity.⁴ As the most abundant renewable aromatic compound resource on earth, lignin is characterized by its high carbon and low oxygen content, making it a promising feedstock for energy products, chemicals, and biomaterials.^{5,6} Lignin valorization aligns with the principles of aromatic bioeconomy, which is a bio-based manufacturing paradigm aimed at selectively retaining, upgrading, and valorizing renewable aromatic carbon into high-value chemicals, thereby decoupling aromatic chemical production from fossil resources.

However, lignin is an amorphous three-dimensional reticulated polymer consisting of three structural monomers *p*-coumaryl alcohol (H), coniferyl alcohol (G), and sinapyl alcohol (S) polymerized by cross-linking of several types of ether bonds (e.g., β -O-4, α -O-4, and 4-O-5) and carbon-carbon bonds (e.g., 5-5, β - β , β -1, and β -5).⁷ The inherent heterogeneity of lignin and its diverse linkage types pose significant challenges to efficient depolymerization and subsequent conversion into high-value products.⁸ Nature provides a blueprint for lignin biotransformation. Many microorganisms have evolved sophisticated ligninolytic enzyme tools and metabolic pathways capable of depolymerizing lignin and upgrading its heterogeneous aromatics into diverse bioproducts.³ Moreover, biological funneling strategies that reconcile lignin's inherent heterogeneity into unified metabolic routes are key to improving carbon efficiency of lignin.^{9,10} Building on these biological principles, emerging technologies, particularly synthetic biology, could further reshape how aromatic carbon resources are accessed and converted, and reinforce lignin's strategic role in a sustainable future.¹¹

Vanillin, 3-methoxy-4-hydroxybenzaldehyde, is among the most widely used flavor additives, underpinning applications that span food, fragrance,

pharmaceutical, and fine chemicals. Currently, global consumption of vanillin exceeds 18,000 tons annually, reflecting its indispensable role in modern consumer and specialty products.¹² Despite this demand, the supply of natural vanillin, traditionally derived from vanilla pods, remains inherently limited. Cultivation-intensive production, susceptibility to environmental variability, and complex extraction processes constrain scalability and drive prices to \$1,250-4,400 per kilogram.¹³ As a result, most commercial vanillin on the market is produced via chemical synthesis. However, these routes require harsh reaction conditions and impose a substantial environmental burden.¹⁴ Against the backdrop of sustainability-driven innovation and rising consumer preference for naturally sourced products, bio-based vanillin production has emerged as a compelling alternative. Microbial synthesis offers an efficient and environmentally benign route while preserving the molecular identity and natural status of vanillin derived from renewable substrates.¹³

Glucose has been widely explored as a readily available substrate for microbial vanillin production.¹⁵ However, glucose-derived vanillin synthesis requires carbon flux to traverse central metabolism and the shikimate pathway to generate aromatic amino acid precursors, followed by additional downstream modules to assemble the vanillin biosynthesis. This multistep pathway not only leads to a low theoretical carbon yield (approximately 40%) but also imposes substantial metabolic burdens on the host cell.¹⁶ In contrast, lignin offers inherent green advantages at the source. A growing body of literature summarized engineered microbial platforms for producing various chemicals from lignin-derived aromatics across different host strains.^{5,17,18} Owing to its natural aromatic structure, lignin-derived aromatics can also be served as suitable precursors for vanillin through aromatic atom-economical pathways, achieving substantially higher theoretical carbon efficiencies (60%-100%).¹⁹ This aromatic ring-preserving strategy directly unlocks lignin's inherent aromatic potential, providing a more efficient and sustainable route for vanillin biosynthesis.^{1,20} Nevertheless, the valorization of lignin into vanillin remains far from trivial. During lignin depolymerization, uncontrolled bond cleavage and random condensation reactions generate structurally diverse aromatic fragments, complicating the selective production of monomers amenable to downstream bioconversion. Beyond substrate availability, intracellular metabolic networks further impose constraints. Heterologous vanillin biosynthetic pathways inevitably compete with endogenous metabolic branches, leading to imbalances in carbon flux, energy supply, and redox cofactors, all of which limit vanillin productivity. Moreover, vanillin itself is cytotoxic, representing a critical bottleneck for cell viability in industrial bioproduction.

This review aims to provide the principles of a sustainable "aromatic bioeconomy-oriented framework" for vanillin production by fully leveraging lignin's intrinsic aromaticity. Multidisciplinary strategies across the entire biomanufacturing system are introduced. Advanced lignin depolymerization approaches are discussed to address lignin heterogeneity and low bioavailability, thereby delivering suitable aromatic precursors. Microbial pathway design and metabolic regulation are presented to overcome low carbon efficiency and pathway bottlenecks, enabling high-flux vanillin biosynthesis. Fermentation engineering strategies are summarized to facilitate high-titer and industrially relevant production. Finally, economic and environmental feasibility analysis are highlighted to guide the rational design of waste-free lignin valorization. Microbial valorization of lignin into vanillin thus bridges renewable aromatic carbon with high-value flavor production.

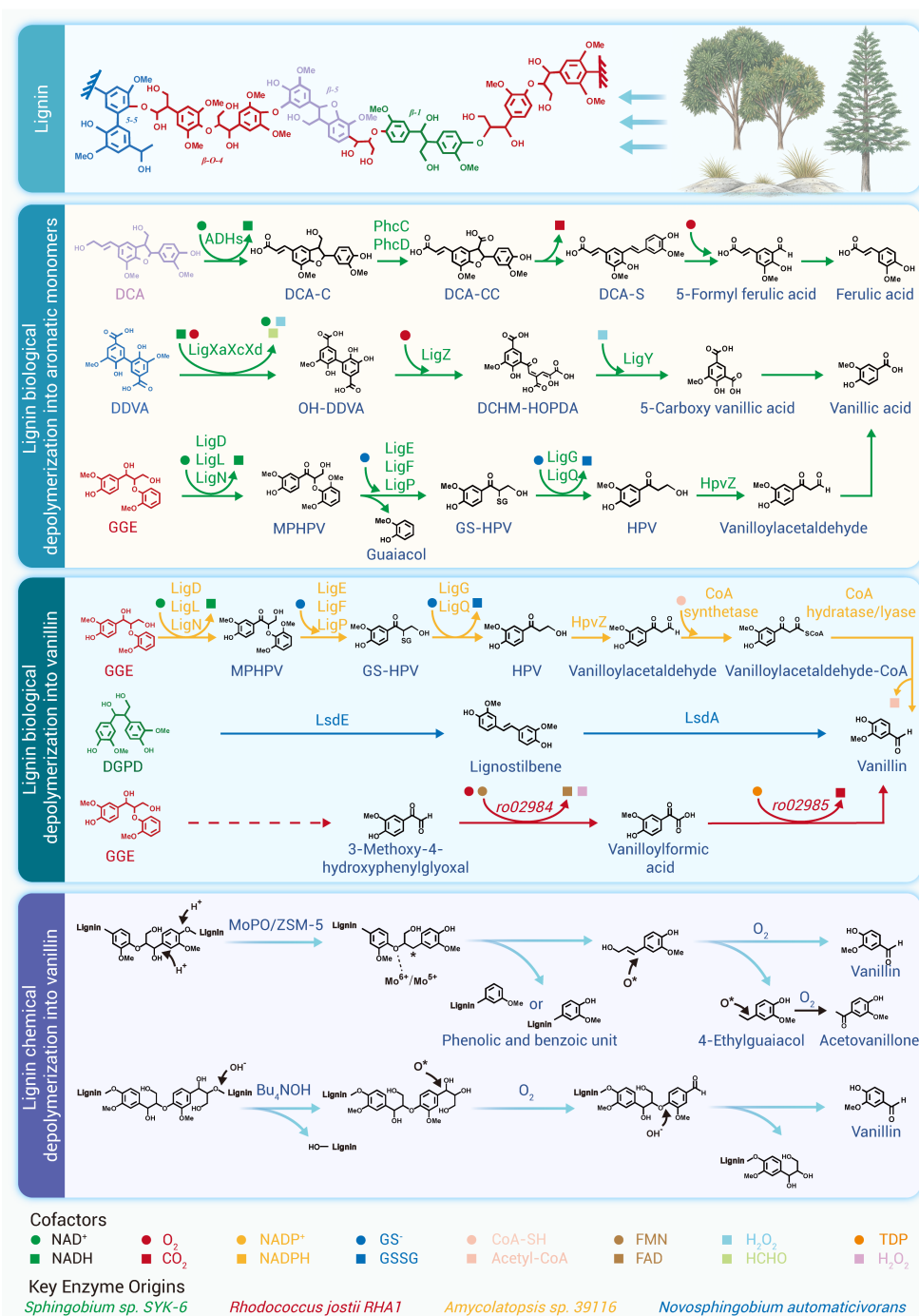


Figure 1. Depolymerization strategies producing aromatic precursors or vanillin from lignin DCA, dehydrodiconiferyl alcohol; DCA-C, 3-(2-(4-hydroxy-3-methoxyphenyl)-3-(hydroxy-methyl)-7-methoxy-2,3-dihydrobenzofuran-5-yl)acrylate; DCA-CC, 5-(2-carboxyvinyl)-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydro-benzofuran-3-carboxylate; DCA-S, 3-(4-hydroxy-3-(4-hydroxy-3-methoxystyryl)-5-methoxyphenyl)acrylate; DDVA, 5,5'-dehydrodivanillate; OH-DDVA, 2,2',3-trihydroxy-3'-methoxy-5,5'-dicarboxybiphenyl; DCHM-HOPDA, 4,11-dicarboxy-8-hydroxy-9-methoxy-2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate; GGE, guaiacylglycerol- β -guaiacyl ether; MPPHV, α -(2-methoxyphenoxy)- β -hydroxypropiovanillone; GS-HPV, α -glutathionyl- β -hydroxypropiovanillone; HPV, β -hydroxypropiovanillone; DGPD, diguaiacylpropanediol; ADH, alcohol dehydrogenase; PhcC, (+)-3-(2-(4-hydroxy-3-methoxyphenyl)-3-(hydroxymethyl)-7-methoxy-2,3-dihydrobenzofuran-5-yl)acrylate oxidase; LigXaXcXd, three-component *O*-demethylase; LigZ, 2,2',3-trihydroxy-3'-methoxy-5,5'-dicarboxybiphenyl dioxygenase; LigY, 2,2',3-trihydroxy-3'-methoxy-5,5'-dicarboxybiphenyl meta-cleavage compound hydrolase; LigD, LigL and LigN, *C α* -dehydrogenases; LigE, LigF and LigP, β -etherases; LigG and LigQ, glutathione *S*-transferases; HpvZ, β -hydroxypropiovanillone oxidase; DypB, dye decolorizing peroxidase; LsdE, erythro-1,2-diguiacylpropane-1,3-diol γ -formaldehyde lyase; LsdA, carotenoid oxygenase.

generated inhibitors such as furans and organic acids derived from carbohydrate degradation further compromise microbial growth. Overall, conventional lignin depolymerization methods typically yield lignin products that are poorly suited for subsequent bioconversion.^{7,22}

The type and yield of the resulting lignin derived aromatics are key determinants of lignin bioavailability. Some depolymerization strategies have been developed to generate aromatic precursors for vanillin, thereby supporting resource supply in the aromatic bioeconomy (Table S1 & Figure 1). For instance, alkaline depolymerization typically produces a relatively simple monomer profile; treatment of corn stover with 1% NaOH yields 8.9% *p*-coumaric acid and 3.3% ferulic acid.⁹ In contrast, advanced integrated depolymerization approaches enhance the cleavage of β - β and β -O-4 linkages, increasing hydroxycinnamate release and improving the suitability of lignin-derived streams for bioconversion.²⁵ Moreover,

DEPOLYMERIZATION STRATEGIES PROMOTED THE GENERATION OF AROMATIC PRECURSORS

Depolymerization strategies producing aromatic precursors for vanillin

Lignins generated by the traditional pulping and biorefining industries undergo significant structural alterations and exhibit broader molecular weight distributions.²¹ Moreover, recondensation reactions form recalcitrant carbon-carbon bonds that severely hinder subsequent depolymerization and bioconversion.²² Kraft pulping process cleaves most alkyl-aryl ether bonds in lignin, but sulfur incorporation and severe recondensation substantially limit downstream biological valorization.²³ Alkali pulping produces sulfur-free lignin via aqueous NaOH treatment, offering improved feedstock compatibility despite lower delignification efficiency.¹ Lignosulfonates, generated through sulfite pulping, exhibit high solubility due to sulfonate groups but contain elevated sulfur content and remain prone to recondensation.²² Although organosolv lignin, extracted in organic or biphasic solvent systems, better preserves native β -aryl ether linkages, acid-catalyzed conditions still promote condensation reactions to limit its bioconversion.²⁴ Additionally, co-

alkaline oxidative depolymerization further diversifies the product spectrum by promoting the formation of aromatic aldehydes and acids, including vanillin, vanillic acid, and *p*-hydroxybenzaldehyde.^{26,27} More recently, lignin-first biomass depolymerization technologies have emerged as promising alternatives. These approaches selectively solubilize lignin while preserving carbohydrate integrity, enabling controlled depolymerization into aromatic platform molecules.²⁸ Reductive catalytic depolymerization can achieve near-theoretical yields of phenolic monomers. For example, reductive depolymerization of methanol-soluble pine wood yielded 16.5% phenolic monomers, including 12% isoeugenol.²⁹ Oxidative catalytic depolymerization allows one-pot lignin extraction and depolymerization under relatively mild conditions, yielding aromatic monomers, such as syringaldehyde, vanillin, methylparaben, and methyl syringate.³⁰ Although chemical depolymerization strategies offer efficient routes for lignin breakdown, their environmental concerns and limited biocompatibility underscore the need for integrated depolymerization-bioconversion frameworks tailored for sustainable lignin bioconversion.

Biological depolymerization of lignin is considered to be a promising method due to its specificity and environmental friendliness.³¹ Many fungi and bacteria have evolved to naturally depolymerize lignin by coordinating various extracellular enzymes.³² Lignin peroxidase (LiP), manganese peroxidase (MnP) and multifunctional peroxidases (VP) are H₂O₂-dependent heme proteins. Some auxiliary oxidases can supply H₂O₂ to them and synergistically depolymerize lignin.³³ For example, MnP-glucose oxidase catalyzed the degradation of corn stover by 65.9% after three days.³⁴ The lytic polysaccharide monooxygenase-peroxidase efficiently degraded the β -O-4 and 5-5' lignin model dimers, with 46.5% and 37.7% degradation rates, respectively.³⁵ It is also effective in depolymerizing natural lignin from *bamboo culms* and kraft lignin.³⁵ Additionally, the combined treatment using MnP, LiP and laccase from *Phanerochaete chrysosporium* reduced the molecular weight of lignin by 29.0% more effectively than either enzyme alone.³⁶ Molecular docking and molecular dynamics simulation predicted the interaction of laccase with lignin, and experimental validation confirmed that laccase degraded 100% of syringol, 99.3% of dihydroconiferyl alcohol, and 95.7% of guaiacol.³⁷ A key advantage of bacterial laccases is their ability to minimize lignin recondensation, especially when combined with veratryl alcohol oxidase.³³ Moreover, laccase binding to various mediators selectively targets different chemical bonds of lignin and enhances its catalytic activity. Treatment of alkali lignin with laccase/2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) resulted in 39.6% vanillin among the identified aromatic compounds and organic acids.³⁸ Lignin depolymerization also produces a range of aromatic dimers, which can be funneled by ligninolytic microorganisms through specialized metabolic pathways into platform aromatic monomers, thereby facilitating downstream lignin valorization. *Sphingobium* sp. SYK-6 can convert lignin-derived dimers, such as β -aryl ether, phenylcoumaran, biphenyl and diarylpropane into aromatic monomers, such as ferulic acid, vanillic acid.³⁹ *Bacillus ligniniphilus* L1 secreted laccase and metabolized lignin dimers via its native catabolic pathway, yielding vanillic acid, benzoic acid, vanillin, and *p*-hydroxybenzoic acid.⁴⁰ Therefore, rationally designed enzyme-microbe synergistic systems represent a powerful strategy for efficient lignin depolymerization and subsequent bioconversion.

Lignin-derived aromatics constitute a versatile reservoir of precursors for the biosynthesis of high-value aromatic chemicals. However, effective depolymerization remains challenging due to the intrinsic structural heterogeneity of lignin, which varies with biomass source and extraction process, rendering universal depolymerization strategies impractical. Consequently, increasing attention has been paid to tailored depolymerization approaches that are specifically matched to lignin types. Beyond efficient bond cleavage, these strategies must also stabilize reactive intermediates to prevent recondensation, thereby generating a bioaccessible aromatic pool for downstream bioconversion. Looking ahead, the development of integrated processes that balance yield, selectivity, and bioaccessibility will be essential. Advances in both depolymerization technologies and subsequent bioconversion pathways are critical to strengthening the value chain of the aromatic bioeconomy.

Direct depolymerization route of lignin to yield vanillin

Direct depolymerization of lignin to produce vanillin represents a streamlined strategy for valorizing this abundant aromatic polymer. About 15% of global vanillin is derived from the directly oxidative depolymerization of lignin, a route that bypasses the synthesis of intermediate aromatic compounds.⁴¹ β -O-4 linkages in lignin can be cleaved to form phenolate species, which are subsequently oxidized to phenoxy radicals. The phenoxy radical forms quinone methyle, which reacts with hydroxide ions and further breaks the C α -C β bond by inverse hydroxyl aldehyde cleavage reaction, ultimately yielding vanillin.^{7,42,43} Using nitrobenzene as a strong oxidant under alkaline conditions, different technical lignins exhibited distinct vanillin yields and selectivities, with sodium lignosulfonate achieving up to 11.6% yield and 100% selectivity.⁴⁴ Despite this high efficiency, the environmental risks associated with nitrobenzene severely limit its practical application. Greener catalytic systems are thus developed to promote lignin dissolution, selective bond cleavage, and stabilization of reactive intermediates. For example, using MoPO/ZSM-5 as the catalyst, the oxidative depolymerization of alkali lignin from rice straw under alkaline conditions yielded 5.6% vanillin.⁴⁵ The ionic liquid [MIM][Me₂PO₄] system enabled 100% catalytic conversion of organosolv lignin, producing 14.7% vanillin.⁴⁶ Moreover, oxidative depolymerization of

Japanese cedar wood using tetrabutylammonium hydroxide achieved a vanillin yield of 23.2%.⁴⁷

Extracellular enzymes secreted by certain ligninolytic microorganisms, along with their intrinsic metabolic pathways, facilitate lignin depolymerization for the direct synthesis of vanillin. For example, the dye decolorizing peroxidase from *Rhodococcus jostii* RHA1 in conjunction with a glycolate oxidase catalyzed the cleavage of the C α -C β bond of lignin to yield vanillin.⁴⁸ Recombinant *Arthrobacter* sp. C2 degrades alkali lignin to produce 96 mg/L of vanillin.⁴⁹ *S. sp.* SYK-6, *Novosphingobium* sp. PP1Y, *N. aromaticivorans*, *Dichomitus squalens*, and *Amvcolatopsis* sp. 39116, can produce vanillin from β -aryl ether dimer by C α -dehydrogenase LigD and β -etherase LigE or LigF. α -Glutamate- β -hydroxypropiovanillone can be oxidatively cleaved by glutathione-S-transferase LigG to yield β -hydroxypropylvanillone and further oxidized to vanillin.²³ *N. aromaticivorans* depolymerized β -1 lignin model compounds diguaiacylpropanediol to produce vanillin via a two-step enzymatic reaction using erythro-1,2-diguiacylpropane-1,3-diol γ -formaldehyde lyase and carotenoid oxygenase.³⁹

Given the structural similarity between vanillin and lignin's native phenylpropanoid units, direct depolymerization offers clear advantages in terms of atom economy, process simplicity, and shorter production cycles. However, achieving directional and selective lignin depolymerization toward vanillin remains challenging. In particular, the heterogeneous structure of lignin, the coexistence of multiple interunit linkages, and the high reactivity of intermediates often lead to low selectivity and undesired recondensation, thereby limiting vanillin yield. Moreover, process conditions such as harsh oxidation environments and catalyst instability further complicate scalability and sustainability. To address these challenges, future efforts should focus on developing highly selective catalytic systems, integrating mild and controllable depolymerization conditions, and coupling *in situ* stabilization or extraction strategies to suppress side reactions. Additionally, combining chemical depolymerization with biological funneling or hybrid chemo-enzymatic routes may provide new opportunities to enhance vanillin yield and specificity.

VANILLIN BIOSYNTHESIS PATHWAYS FROM LIGNIN-DERIVED AROMATICS

Following depolymerization, lignin is converted into a diverse pool of aromatic monomers, such as ferulic acid, *p*-coumaric acid, vanillic acid, and *p*-hydroxybenzoic acid. These compounds serve as highly compatible substrates for microbial vanillin biosynthesis, enabling a seamless transition from depolymerization to biological upgrading. By preserving the aromatic backbone, lignin-derived aromatics support more direct and carbon-efficient conversion routes, thereby aligning with the principles of the aromatic bioeconomy (Table S2 & Figure 2).

Ferulic acid is one of the most abundant phenolic compounds among lignin from grass biomass and represents an excellent substrate for vanillin biosynthesis. Biosynthetic routes of vanillin from ferulic acid include non- β -oxidation pathway and β -oxidation pathway.⁵⁰ In the most common CoA-dependent non- β -oxidation pathway, ferulic acid is converted to feruloyl-CoA by hydroxycinnamate-CoA ligase (4CL/FCS), followed by hydration and cleavage catalyzed by 4-hydroxycinnamoyl-CoA hydratase/lyase, or enoyl-CoA hydratase/aldolase (ECH), ultimately yielding vanillin or vanillic acid intermediates.^{51,52} However, cofactor and energy limitations often restrict pathway flux, which requires engineering strategies that enhance cofactor supply or bypass CoA dependence.⁵³ An even greater advantage is the utilization of a coenzyme-independent non- β -oxidation pathway, which significantly reduces system complexity.⁵⁴ Ferulic acid is first converted to 4-vinylguaiacol by ferulic acid decarboxylase (FDC), which is subsequently converted into vanillin by 4-vinylguaiacol oxygenase (CSO2). Because these enzymes exhibit distinct optimal conditions, CSO2 frequently becomes the rate-limiting step. Process intensification strategies, including two-stage fermentation, can alleviate intermediate accumulation and increase vanillin titers by up to 6.5-fold.⁵⁵ Moreover, screening and co-expression of phenolic acid decarboxylase (Pad) and aromatic dioxygenase (Ado) with better catalytic efficiency and thermal stability in *Escherichia coli* enabled rapid conversion of ferulic acid to vanillin at high efficiency with a titer of 27.5 mM vanillin.⁵⁶

p-Coumaric acid is another most abundant monomer in herbaceous lignin

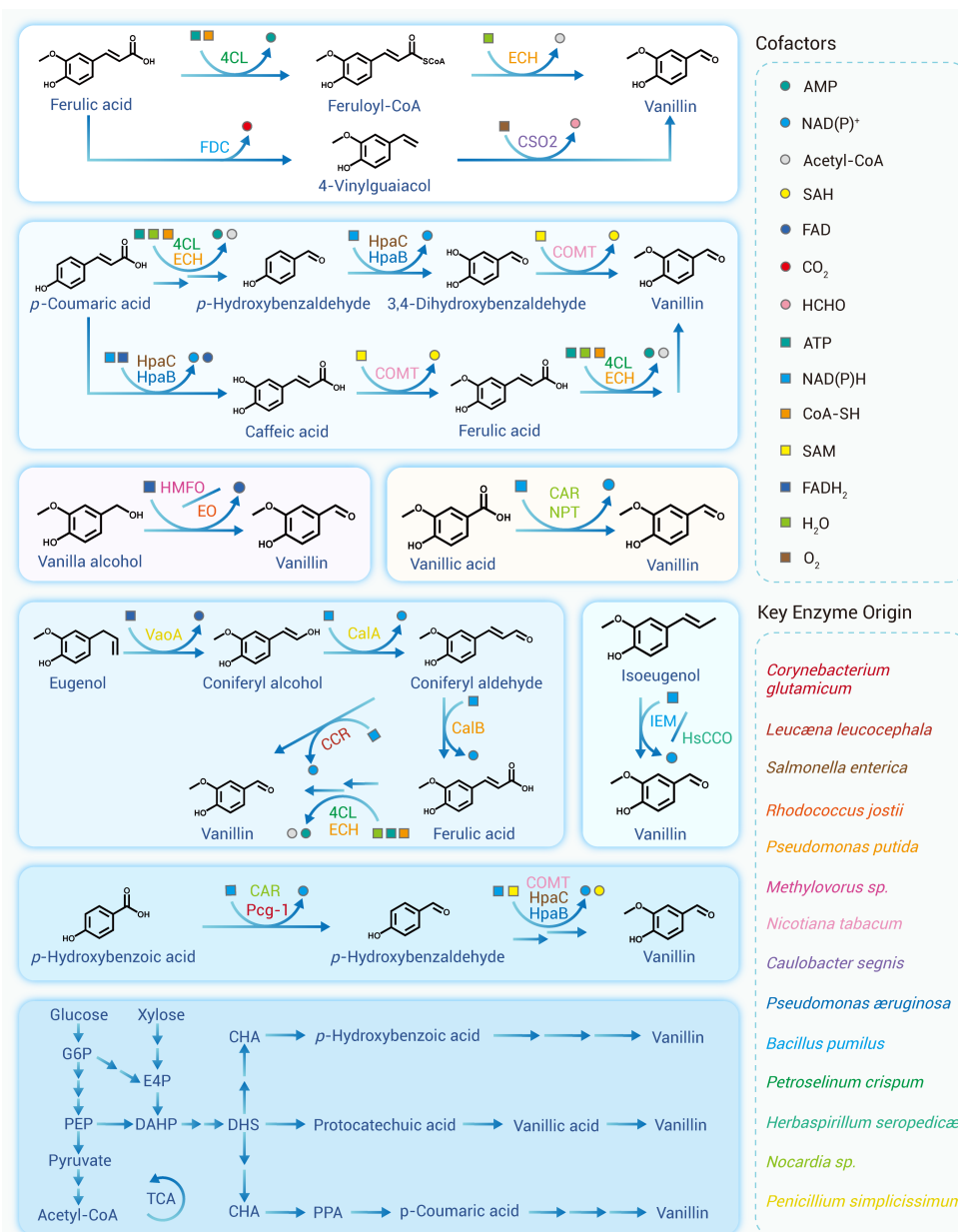


Figure 2. Pathways and key enzymes for vanillin biosynthesis from lignin-derived aromatic monomers 4CL, hydroxycinnamate-CoA ligase; ECH, enoyl-CoA hydratase/aldolase; FDC, ferulic acid decarboxylase; CSO2, 4-vinylguaicol oxygenase; HpaB, *p*-hydroxyphenylacetic acid 3-monooxygenase; HpaC, *p*-hydroxyphenylacetic acid 3-monooxygenase reductase; COMT, caffeic acid *O*-methyltransferase; HMFO, 5-hydroxymethylfurfural oxidase; EO, eugenol oxidase; CAR, carboxylic acid reductase; NPT, Pcg-1, two phosphopantetheinyl transferases; VaoA, vanillyl alcohol oxidase; CalA, coniferyl alcohol dehydrogenase; CalB, coniferyl aldehyde dehydrogenase; CCR, cinnamoyl-CoA reductase; IEM, isoeugenol monooxygenase; CCO, carotenoid cleavage oxygenase; DAHP, 3-deoxy-D-arabino-heptulosonate 7-phosphate; CHA, chorismate; DHS, 3-dehydroshikimate; PPA, prephenate.

catabolized to vanillin through coniferyl alcohol, coniferyl aldehyde, and ferulic acid intermediates. To enhance pathway efficiency, the introduction of a vanillyl alcohol oxidase VaoA and two coniferyl alcohol dehydrogenase CalB, CalA into *E. coli* XL1-Blue resulted in a molar yield of 91% and a ferulic acid concentration of 8.6 g/L.⁶⁰ Subsequently, recombinant *E. coli* harboring FCS and ECH produce 0.3 g/L of vanillin from ferulic acid.⁶⁰ However, coniferyl aldehyde inhibited vanillin synthesis, and CalB converted vanillin into vanillic acid. To overcome this limitation, cinnamoyl-CoA reductase (CCR) was introduced to catalyze the direct conversion of coniferyl aldehyde to feruloyl CoA. Recombinant *E. coli* constructed with the simplified pathway obtained 0.07 mM vanillin from 2 mM eugenol within 12 h.⁶¹ The vanillin yield from eugenol can be further enhanced through strategies such as pathway reconstruction and the optimization of key catalytic enzymes.

Isoeugenol, 4-propenyl-2-methoxyphenol, is a natural, low-cost constituent of plant essential oils used for vanillin production. Numerous microorganisms have been identified that naturally convert isoeugenol to vanillin. *P. chlororaphis* CDAE5 produced a vanillin concentration of 1.2 g/L from 10 g/L of

and serves as an important precursor substance for vanillin synthesis. Conversion of H-type lignin monomers to vanillin requires sequential hydroxylation at the 3' position and methylation of the phenolic hydroxyl group. The biosynthetic pathways from *p*-coumaric acid to vanillin include the ferulate pathway and the benzoate pathway, which differ in the sequence of catalytic steps. *Saccharomyces cerevisiae* harbored 4CL, ECH, a fusion enzyme comprising *p*-hydroxyphenylacetic acid 3-monooxygenase (HpaB) and its reductase (HpaC), along with *O*-methyltransferase (OMT) and enabled the production of 0.4 mM vanillin from 1.3 mM *p*-coumaric acid.⁹ Additionally, introducing this pathway into a tyrosine-overproducing *E. coli* strain enabled the production of 97 mg/L vanillin from simple carbon sources. Nevertheless, inefficient formation of key pathway intermediates remains a major bottleneck, highlighting rate-limiting steps as prime targets for pathway optimization.

Eugenol, 4-allyl-2-methoxyphenol, is the major constituent of clove oil and widely explored as a precursor for biobased vanillin production. Notably, *Pseudomonas fluorescens* E118 can produce 5.8 g/L ferulic acid with intermittent addition of clove oil, highlighting its potential for vanillin biosynthesis.⁵⁷ Microbial conversion of eugenol was first demonstrated in *P. resinovorans* SPR1, which naturally converted eugenol to 0.24 g/L vanillin and 1.1 g/L vanillic acid, respectively.⁵⁸ Wild strain *B. cereus* NCIM-5727 was able to synthesize 6.6 g/L vanillin from eugenol with a molar yield of 71.2% under optimized fermentation condition.⁵⁹ Across these strains, eugenol is

isoeugenol.⁶² *P. putida* IE27 cells produced 16.1 g/L vanillin from 150 mM isoeugenol with 71% molar conversion.⁶³ *B. fusiformis* SW-B9 produced 32.5 g/L vanillin in 72 h using 60% (v/v) isoeugenol as substrate.⁶⁴ With the identification of the key enzyme therein, some recombinant strains achieved the conversion of isoeugenol to vanillin. The gene encoding isoeugenol monooxygenase (IEM) from *B. pumilus* ZB1 was heterologously expressed in *E. coli* BL21 (DE3).⁶⁵ As a result, vanillin yield reached 139.5 mg/L from horse-tail pine pyrolysis bio-oil concentration of 10 g/L.⁶⁵ Beyond IEM, several carbon-carbon bond-cleaving oxygenases with good catalytic activity have been discovered, including carotenoid cleavage oxygenases (CCOs) from *Herbaspirillum seropedicæ* and *Rhodobacteraceae* and isoeugenol dioxygenase from *N. aromatoovorans*.^{66,67} These enzymes exhibit favorable properties such as cofactor independence and enhanced thermal stability. Notably, the recombinant *E. coli* heterologously expressing HsCCO produced 1.2 g/L of vanillin from isoeugenol with a molar conversion of 80%.⁶⁶ Despite these advances, the low activity of oxygenases and the inhibitory effect of the product on the oxygenases remain critical bottlenecks. Saturation mutagenesis of the novel oxidase IEM720 in *E. coli* increased vanillin concentration by 2.2-fold. The application of sol-gel chitosan membrane significantly enhanced the vanillin concentration to 4.5 g/L with a yield of 75%.⁶⁸ Additionally, an IEM mutant with enhanced thermal stability was selected through a combined strategy of surface residue substitution and consensus mutation. The triple

mutant K83R/K95R/L273F produced 240.1 mM vanillin from isoeugenol with a conversion rate of 77.6%.⁶⁹ Overall, isoeugenol stands out as a highly attractive precursor for vanillin biosynthesis due to its short conversion pathway, well-defined enzymatic machinery, and consistently high yield.

Other aromatic monomers also serve as potential precursors for vanillin synthesis. For example, vanillyl alcohol is a lignin-derived intermediate that can be converted to vanillin in a single oxidation step. Early studies employed oxidative enzymes, flavoprotein vanillin oxidase, catalyzed the oxidation of vanillyl alcohol to produce vanillin.⁷⁰ Immobilized eugenol oxidase from *R. jostii* catalyzed vanillyl alcohol to synthesize vanillin at a productivity of 9.9 g/L/h.⁷¹ Furthermore, the gene encoding 5-hydroxymethylfurfural oxidase (HMFO) was heterologously expressed in *E. coli*, oxidizing vanillyl alcohol to vanillin with high selectivity and efficiency.⁷² 250 mM vanillyl alcohol was converted to vanillin with a yield of 96% and no vanillic acid by-products were detected, reflecting the strong substrate specificity of HMFO.⁷² These results underscore the importance of highly selective biocatalysts in achieving high yield and purity in lignin-based vanillin biosynthesis. Vanillic acid, a common lignin-derived monomer, can be reduced to vanillin in single step. Carboxylic acid reductase (CAR) catalyzes this reaction in the presence of NADPH, and its activity can be enhanced by phosphopantetheinyl transferases derived from various sources, such as NPT and Pcg-1. Co-expression of CAR and NPT in *E. coli* resulted in a 1.8-fold increase in conversion rate and improved catalytic efficiency compared to expression of CAR alone.⁷³ Further introduction of glucose dehydrogenase (GDH) promoted NADPH regeneration and accelerated vanillic acid conversion.^{73,74} Protocatechuic acid also represents a central intermediate node for vanillin synthesis. Genes encoding 4-hydroxybenzoic acid hydroxylase, vanillic acid-*O*-demethylase, and the transporter protein HcnK were co-expressed in *P. putida* KT2440, which enabled the conversion of *p*-coumaric acid and ferulic acid to 12.7 g/L protocatechuic acid.⁷⁵ Subsequently, protocatechuic acid can be converted to vanillic acid by caffeic acid *O*-methyltransferase (COMT), which is then reduced to vanillin by CAR.⁷⁶ Co-expression COMT and CAR in *E. coli*, along with deletion of aldoketo reductase and alcohol dehydrogenase, yielded 481.2 mg/L vanillin.⁷⁷ Enzyme engineering of COMT and optimization of methyl-donor supply have further increased vanillin production at a titer of 1 mM in *Bacillus* hosts.⁷⁸ *p*-Hydroxybenzoic acid is also present in lignin depolymerization streams. Although fewer studies exist, CAR-mediated reduction followed by hydroxylation and methylation enables its conversion to vanillin.⁷⁹ Using this strategy, the *de novo* synthesis pathway of vanillin was constructed in engineered *S. cerevisiae* using *p*-hydroxybenzoic acid, achieving the vanillin titers exceeding 533 mg/L.⁷⁹

In summary, the biosynthesis of vanillin from lignin-derived aromatics represents a promising route for sustainable valorization of this abundant renewable resource. While native microorganisms can convert some of these compounds into vanillin, most engineered biosynthetic pathways have been established in model hosts like *E. coli* and *S. cerevisiae*. However, the efficiency of vanillin production depends not only on the type and yield of these substrates but also on their structural complexity, the presence of inhibitory byproducts, and the stability of reactive intermediates formed during depolymerization. Key challenges also include the structural heterogeneity of lignin-derived substrates, suboptimal catalytic activity of heterologous enzymes, accumulation of inhibitory intermediates, and diversion of carbon flux into competing endogenous pathways. Moreover, the toxicity of vanillin and other intermediates can restrict microbial growth and overall yield. Looking forward, overcoming these challenges will require integrated strategies combining advanced metabolic engineering, enzyme evolution, dynamic pathway regulation, and hybrid chemical-biological approaches. Expanding the accessible substrate space, stabilizing reactive intermediates, and designing host strains capable of robustly channeling diverse lignin-derived aromatics toward vanillin will be critical. Such efforts are expected to enhance process efficiency, improve selectivity, and support a scalable, sustainable aromatic bioeconomy, ultimately bridging lignin valorization with high-value aromatic chemical production.

METABOLIC REGULATION STRATEGIES FOR THE BIOSYNTHESIS OF VANILLIN

Engineered microbial cell factories offer a sustainable and versatile plat-

form for producing natural vanillin. However, the efficiency of vanillin biosynthesis is often constrained by multiple factors, including metabolic bottlenecks, cofactor imbalances, toxic intermediate accumulation, and stress responses triggered by vanillin or other aromatics. These limitations frequently result in low productivity and suboptimal carbon utilization. To overcome these challenges, a suite of metabolic regulation strategies has been developed, encompassing enzyme discovery and engineering, cofactor optimization, methylation control, carbon flux redistribution, and biosensor-mediated dynamic regulation (Table S3).

Enzyme bioprospecting and engineering for vanillin biosynthesis

The biosynthesis efficiency of vanillin is strongly constrained by the catalytic performance of key enzymes. Many native enzymes exhibit low turnover rates, poor stability under industrial conditions, or limited expression in heterologous hosts, often leading to byproduct formation and reduced vanillin yields. Consequently, systematic enzyme bioprospecting and engineering have become indispensable for advancing lignin-based vanillin biosynthesis. The emergence of metagenomics and sequence alignment tools has accelerated the discovery of novel ligninolytic enzymes.⁸⁰ Enzymes with higher homology to CSO2 were expressed in suitable host systems, where several enzymes exhibit superior catalytic activity towards 4-vinylguaiacol and enhanced stability, such as Ado from *Thermothelomyces thermophila*, and CCOs from *H. seropedicae* and *R. bacterium*.^{56,66} Such integrated strategies substantially narrow the screening space and enable rapid identification of high-potential biocatalysts.

Despite these advances, the catalytic properties of natural enzymes often remain suboptimal for targeted industrial conditions. Enzyme engineering therefore plays a central role in refining enzyme performance.⁸¹ For example, after multiple rounds of directed evolution, the enzyme variant II-16-26E of CCR produced approximately 4-fold higher vanillin yield from coniferyl aldehyde compared to the wild-type enzyme.⁶¹ Error-prone PCR combined with high-throughput screening is commonly used to explore vast mutational landscapes.⁸² Droplet microfluidics has further revolutionized this process by encapsulating single cells in picoliter droplets and linking enzyme activity to fluorescence signals, enabling the screening of more than 10⁷ variants per day with high enrichment efficiency.^{83,84} Furthermore, orthogonal DNA replication system accelerates continuous evolution and cell factory construction.⁸⁵

Semi-rational or rational enzyme design aim to improve engineering efficiency by restricting mutations to structurally or functionally relevant regions. Conservation analysis of CSO2 homologs identified both strictly conserved and distinctive residues, guiding targeted mutagenesis. The A49P variant showed 1.6-fold increase in catalytic efficiency towards 4-vinylguaiacol relative to the wild-type enzyme. Further refinement based on homology modeling and molecular docking revealed non-conserved residues near the substrate-binding pocket, while the Q390A variant showed a 1.64-fold improvement in catalytic efficiency.⁸⁶ Recently, machine learning-driven enzyme engineering accelerates the identification of high-performance variants.⁸⁷ Deep learning model such as CataPro can accurately predict enzyme kinetic parameters, and guide variant selection prior to experimental validation. Using this approach, the activity of the CSO2 mutant T216M-M351F-V384G was improved by 65.2-fold.⁸⁸ Beyond optimizing existing enzymes, *de novo* enzyme design offers a transformative route to creating biocatalysts unconstrained by natural evolution. This strategy typically involves backbone generation, sequence optimization, and structural validation. Physics-based methods (e.g., Rosetta) and deep learning frameworks (e.g., LigandMPNN) are increasingly used to navigate the vast protein design space, although challenges remain due to the immense structural diversity of possible proteins.^{89,90} Continued expansion of structural databases and machine learning capabilities is expected to accelerate progress in this area. Overall, enzyme bioprospecting identifies the available enzymatic tools in nature, while enzyme engineering refines these catalysts into precision tools suitable for industrial deployment. Together, they bridge the gap between natural enzymatic diversity and scalable vanillin production, pointing toward a future of data-driven and intelligent biocatalyst design.

Cofactor enhancement promoted the biosynthesis of vanillin

Cofactors are essential for maintaining cellular redox balance and powering

enzymatic activities in vanillin biosynthesis, such as hydroxylation and decarboxylation. However, when multiple reactions simultaneously consume large amounts of cofactors, intracellular cofactors are depleted, carbon flux stagnates, leading to slowed cell growth, accumulation of intermediates, and low vanillin yield. Therefore, rational cofactor engineering has emerged as a critical strategy for improving vanillin yield and productivity.⁹¹ In the coenzyme-dependent non- β -oxidation pathway, CoA and ATP are essential cofactors. Targeted metabolic rewiring by knocking out *ptsH* and *acs* in engineered *E. coli* resulted in a 1.9-fold increase in vanillin yield, which was further enhanced by the additional introduction of *gltA*.⁹² The gene *ptsH* encodes a key component of the phosphotransferase system. Its deletion promoted phosphoenolpyruvate accumulation, facilitating ATP regeneration and accelerating acetyl-CoA turnover. Knockout of *acs*, encoding acetyl-CoA synthetase, prevented ATP depletion caused by irreversible acetate assimilation. Additionally, deletion of *icdA* redirected carbon flux through the glyoxylate shunt, improving the efficiency of CoA generation. In contrast, the citrate synthase *GltA* catalyzes the conversion of acetyl-CoA and oxaloacetate to citric acid and CoA, directly enhancing vanillin synthesis. Using 3.0 g/L ferulic acid as substrate, *gltA*-amplified *E. coli* produced 1.98 g/L vanillin, approximately twice that of the control strain.⁹³ Amplification of *gltA* and deletion of *icdA* exerted a synergistic effect, further enhancing vanillin yield.⁹³

Additionally, sufficient NADPH supply is indispensable for redox reactions throughout vanillin biosynthesis. NADPH is primarily generated via the Pentose Phosphate Pathway (PPP). Overexpressing key genes encoding glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase boosts NADPH availability. However, modulation of downstream PPP flux often offers greater leverage.⁹⁴ *De novo* metabolic pathway for ferulic acid biosynthesis was constructed in *S. cerevisiae* by overexpressing phosphoketolase, transaldolase, transketolase and phosphotransacetylase, while deleting glyceraldehyde-3-phosphate phosphatase. This strategy increased caffeic acid production to 385.2 mg/L, representing a 45% improvement.⁹⁴ GDH has been employed as an efficient NADPH regeneration module.⁷³ Co-expression of CAR, NPT and GDH in *E. coli* greatly accelerated the reduction of vanillic acid, yielding approximately 2.2 g/L vanillin.⁷³

Flavin cofactors also play important roles in vanillin precursor synthesis. HpaB, a FAD-dependent enzyme, catalyzes the conversion of *p*-coumaric acid to caffeic acid.⁸¹ In *E. coli*, overexpression of *RibF*, a gene involved in FAD synthesis, increased caffeic acid production by 34.9%.⁸¹ In *S. cerevisiae*, systematic optimization for FAD biosynthesis and transport, including overexpression of *RIB1*, *FLX1*, riboflavin kinase/FAD synthase, and the riboflavin transporter MCH5, raised caffeic acid titers to 248.2 mg/L.⁹⁴ These results highlight the importance of tailoring flavin availability to support oxygenase-catalyzed reactions. Beyond regulating cofactor supply, shifting enzyme cofactor preference represents a powerful strategy to overcome intracellular limitations. Directed evolution of the CCR shifted its cofactor specificity from NADP⁺ to the more abundant NAD⁺ in *E. coli* cells.⁶¹ With intermittent isoeugenol feeding, the engineered system achieved 31.8 mM vanillin with yields exceeding 88.2%.⁶¹ Structure-guided semi-rational strategy enabled systematic redesign of nicotinamide cofactor specificity, exemplified by the development of the CSR-SALAD web tool.⁹⁵ Consequently, cofactor engineering strategies, including the regulation of cofactor regeneration and enzymatic cofactor rewiring, significantly facilitated vanillin synthesis. Nonetheless, regulating cofactors for specific reactions face challenges in balancing and coordinating the demands across different reactions. Future efforts will rely on more holistic designs enabled by advances in genome-scale modeling, sequencing, and precision gene editing, ultimately paving the way toward the regulation of microbial cell factories.

Methylation regulation enhanced the biosynthesis of vanillin

The bioconversion of H-type lignin-derived aromatics toward vanillin requires site-specific the methylation of phenolic hydroxyl groups. This methylation step frequently constitutes a major bottleneck in vanillin biosynthesis. In heterologous hosts, insufficient expression of OMTs results in inadequate intracellular enzyme levels, while OMTs with broad substrate promiscuity can divert carbon flux away from the desired pathway, resulting in byproduct accumulation. Furthermore, OMT-catalyzed reactions depend on S-adenosylmethionine (SAM) as the universal methyl donor. SAM biosynthesis is ATP- and methionine-intensive, and its intracellular regeneration efficiency

is inherently limited, further constraining methylation capacity.⁹⁶

To address these challenges, methylation engineering strategies have focused on both enzyme optimization and SAM cycle reinforcement. Based on the engineered *S. cerevisiae* capable of synthesizing vanillin from *p*-coumaric acid, AtCOMT from *Arabidopsis thaliana* was identified as exhibiting the highest catalytic efficiency toward both key intermediates, protocatechuic aldehyde and caffeic acid.⁹ Increasing the copy number of AtCOMT and overexpressing the SAM cycle-related genes *SAH1*, *MET6*, and *MET13atsc* enabled the engineered strain to produce 0.5 mM vanillin from 1.4 mM *p*-coumaric acid, achieving a molar yield of 38.1%.⁹ The *de novo* synthesis pathway of vanillamine was assembled into *S. cerevisiae* with a titer of 6.47 g/L.⁹⁷ The conversion of caffeoyl-CoA to feruloyl-CoA requires SAM as a methyl donor. Engineered *S. cerevisiae* co-expressing genes *SahH* and *SAM2* produced vanillamine at a concentration of 14.89 g/L, showing a 125% enhancement. Co-expressing genes *Mtn* and *LuxS* can also promote SAM cycling.⁹⁷ Furthermore, coupling OMT with halide methyltransferase serves as an effective strategy to establish an efficient SAM regeneration cycle system. Enhancing SAM biosynthesis and recycling has proven effective in bacterial systems. Deleting the gene *metJ* and overexpressing the genes *metA* and *cysE* lifted the restriction of SAM synthesis in *E. coli*, resulting in a 33% increase in the titer of vanillic acid.⁹⁸

Overall, these studies highlight methylation as a critical rate-limiting step in vanillin biosynthesis governed by both enzymatic performance and methyl-donor availability. Overcoming this bottleneck requires coordinated optimization of OMT specificity and expression, along with systematic reinforcement of SAM biosynthesis and recycling pathways. Future advances will depend on a deeper, systems-level understanding of SAM metabolism and its integration into the cellular metabolic network, enabling more precise balancing of methylation demand with overall metabolic flux.

Regulation of carbon flux toward vanillin biosynthesis

Regulating carbon flux aims to rationally allocate metabolic resources to sustain cell growth while maximizing carbon channeling toward vanillin biosynthesis. Achieving this balance requires coordinated optimization of heterologous pathway design, gene expression strategies, and competition among endogenous metabolic routes. Rational design of heterologous vanillin pathways must balance carbon efficiency and host compatibility. Current efforts focus on optimizing flux in existing routes and discovering new biosynthetic pathways. Computational pathway design tools, such as RetroPath, enable the inference of feasible biosynthetic routes based on biochemical reaction rules.⁹⁹ These pathways can be embedded into genome-scale metabolic models to simulate growth-production trade-offs, predict theoretical yields, and identify flux bottlenecks. Visualization platforms such as iPath3 further facilitated intuitive assessment of pathway integration and guide experimental implementation.¹⁰⁰

The mode of pathway expression profoundly influences carbon flux distribution. Plasmid-based expression remains a convenient strategy to accelerate the design-build-test cycle, with gene copy number serving as an effective lever for tuning enzyme levels.⁵¹ For example, FCS and ECH from *Streptomyces* sp. V-1 were introduced into *E. coli* at an optimized 1:1 ratio, and 20 mM ferulic acid was converted to 15 mM vanillin.⁵¹ For stable, long-term flux regulation, CRISPR/Cas-mediated genomic integration introduced *fcs* and *ech* into the *HO* locus of the *S. cerevisiae* genome and enabled a 70.6% molar conversion of ferulic acid to vanillin, demonstrating the advantage of chromosomal expression.¹⁰¹ Beyond expression level control, multienzyme architectural engineering has emerged as a powerful strategy to enhance cascade efficiency by shortening diffusion distances and minimizing intermediate loss. For example, fusion of HpaC and HpaB with a 3-copy GGGGS linker significantly improved hydroxylation efficiency in vanillin biosynthesis.⁹ To systematically optimize such architectures, the iMARS platform was developed to rationally design enzyme scaffolds and linkers.¹⁰² Using this approach, a customized Pad-Ado complex achieved 210.9 mg/L vanillin from ferulic acid, representing a 133% improvement over non-assembled systems.¹⁰²

Blocking or attenuating branch pathways of vanillin synthesis is also essential to minimize carbon loss and byproduct formation. Intracellular vanillin is readily converted to vanillic acid and vanillyl alcohol, which reduce synthesis efficiency and complicate downstream purification.⁹² Deleting the

vanilloid dehydrogenase gene *vdh* in the *Amycolatopsis* sp. ATCC 39116 reduced the degradation of vanillin by more than 90%.¹⁰³ In *E. coli*, systematic knockout of aldehyde reductases (*YahK*, *YjgB*, and *YqhD*) and aldo-keto reductases (*DkgB*, *YeaE*, and *DkgA*) dramatically suppressed aromatic aldehyde reduction, enabling a 55-fold increase in vanillin titer compared with the wild-type strain.^{77,104} In *S. cerevisiae*, multiple enzyme families, including medium-chain dehydrogenase/reductases, aldo-keto reductases, short-chain dehydrogenase/reductases, and aldehyde dehydrogenases, contribute to vanillin degradation and require coordinated regulation.⁹ In *P. putida* KT2440, the knockout of the molybdate ABC transporter gene cluster PP_3827-3832 resulted in deficiency of the molybdenum cofactor, thereby blocking the molybdenum cofactor-dependent vanillin degradation pathway and increasing vanillin accumulation to 1.3g/L.⁵² Process-level strategies can also shape flux distribution. For instance, temperature-directed whole-cell biocatalysis exploited the thermal robustness of FCS and ECH from *thermophilic actinomycetes* while inactivating endogenous alcohol dehydrogenases, achieving a vanillin productivity of 1.1 g/L/h at 50 °C.¹⁰⁵

Emerging systems-level strategies further refine carbon flux control. Genomic integration sites strongly influence gene expression and global metabolism.⁷⁹ Relocation of *NtCOMT* from *YPRCdelta15* to the *PDC5* locus in *S. cerevisiae* resulted in a 15.5-fold increase in vanillin titer, with transcriptomic analysis revealing broad metabolic reprogramming effects. Advances in whole-genome sequencing and genome-scale engineering now enable simultaneous modification of multiple metabolic nodes, providing a holistic framework for flux redistribution.^{79,106}

Temporal regulation of pathway expression offers an additional layer of control. Promoter engineering can decouple vanillin biosynthesis from primary growth metabolism, reducing metabolic burden. For example, *fsc* and *ech* were expressed in *A. sp.* ATCC 39116 by using a strongly constitutive promoter, *PerME**, which eliminated adaptation delays and enabled production of 19.3 g/L vanillin with a molar yield of 94.9%.¹⁰³ In *S. cerevisiae*, vanillin pathways derived from lignocellulose components were placed under a bidirectional inducible promoter, activating *fcs* and *ech* expression only after glucose depletion. This strategy minimized interference with sugar metabolism and increased vanillin titer by 1.4-fold.⁷⁹ Overall, effective regulation of carbon flux toward vanillin biosynthesis requires integrated optimization across pathway design, enzyme expression, competing reactions, and global metabolic networks. For complex aromatic products, future microbial cell factories will increasingly rely on systems biology-guided strategies, dynamic regulation, and real-time metabolic sensing to achieve precise and high-yield vanillin biomanufacturing.

Biosensor design regulated the biosynthesis of vanillin

Traditional static metabolic regulation is limited by low-throughput, costly analyses and can cause metabolic imbalances when pathways are constitutively overexpressed. Engineered strains also struggle with fluctuating aromatic concentrations that impair growth and production. Biosensors, as powerful analytical tools that convert the intracellular concentration of a metabolite into a detectable signal, enable the construction of self-adaptive and self-optimizing engineered strains.¹⁰⁷ Biosensors have been developed as high-throughput screening tools for vanillin production. For example, an *E. coli*-based biosensor was constructed to detect vanillin via fluorescent output, allowing rapid screening of enzyme libraries involved in lignin depolymerization and vanillin formation.¹⁰⁸ In this system, a vanillin-inducible promoter *yeiW* was identified to drive green fluorescent protein expression, generating easily detectable fluorescent phenotype.¹⁰⁸ Similarly, evolution of the aldehyde transcriptional regulator *YqhC* in *B. subtilis* yielded a biosensor with a positive response to vanillin, a negative response to protocatechuic acid, and no response to intermediates.⁷⁸ This high specificity enabled screening of COMT variant, leading to the identification of a superior enzyme that converted 3 mM protocatechuic acid to 2.38 mM vanillin, approximately 7-fold higher activity than the wild-type enzyme.⁷⁸

Biosensors have also proven valuable for targeted genome evolution and pathway optimization. The transcriptional regulator VanR, which naturally controls the conversion and transport of vanillic acid in *E. coli*, was engineered to respond specifically to vanillin.¹⁰⁹ This vanillin-responsive biosensor enabled high-throughput screening of randomly mutagenized genomes, revealing that inactivation of the efflux protein AcrA significantly increased

intracellular vanillin accumulation by 1.3-fold.⁹² These findings highlight the utility of biosensors in uncovering previously overlooked factors, such as transport systems, that critically influence vanillin productivity.⁹² Biosensors also enable dynamic regulation of metabolic pathways by automatically controlling metabolic flux in response to intracellular or extracellular signals. Such feedback-controlled systems help balance metabolism flux, alleviate toxicity, and improve carbon efficiency. For example, a self-inducible engineered *E. coli* was constructed using the vanillin-inducible promoter ADH7 coupled with the aromatic compound transporter protein CouP.¹¹⁰ In the presence of vanillin, this system automatically activated downstream pathways to convert vanillin into catechol, demonstrating responsive control over aromatic metabolism.¹¹¹ Similarly, a transcriptional repressor PadR-based biosensor responsive to *p*-coumaric and ferulic acids was designed in *P. putida* KT2440.⁷⁶ Through promoter engineering including -10 box mutation, UP-element insertion, PadR mutagenesis, and addition of binding sites the dynamic range and sensitivity of the biosensor was significantly enhanced. The engineered bacteria rapidly co-expressed genes encoding the key enzymes 4-hydroxybenzoic acid hydroxylase, vanillic acid-*O*-demethylase, and the transporter protein HcnK in the presence of this biosensor.⁷⁵

Overall, the biosynthesis of vanillin from lignin derivatives involves reactions such as hydroxylation, methylation, decarboxylation, and redox transformations, which impose high demands on the performance of key enzymes and the supply of cofactors, including CoA, NADPH, FAD, and SAM (Figures 3A-D). Biosensors enable the real-time coupling of cofactor regeneration and pathway activation with intracellular metabolic states. When integrated with enzyme engineering, metabolic engineering, systems biology, and computational modeling, biosensors provide a transformative platform for the precise and efficient development of robust cell factories for sustainable vanillin production (Figures 4A-E). These efforts in cellular metabolic regulation are expected to serve as a key bridge linking pathway potential with actual aromatic production performance.

FERMENTATION STRATEGIES AND TECHNO-ECONOMIC ANALYSES FOR VANILLIN BIOSYNTHESIS

Vanillin biosynthesis at scale remains constrained by toxicity and process inefficiencies, posing significant challenges to industrial implementation. Optimizing fermentation strategies is therefore critical to translate upstream innovations into economically viable and sustainable production systems. To address these limitations, this section focuses on integrated approaches that couple intracellular robustness engineering with fermentation process intensification and techno-economic considerations. Strategies such as toxicity mitigation, *in situ* product removal, process optimization, and scale-up design are essential to enhance productivity. Through multi-level coordination, these efforts aim to bridge laboratory-scale performance with the industrial feasibility of vanillin biosynthesis (Figures 5A-E).

Glycosidation-assisted vanillin production

Introducing or strengthening vanillin detoxification pathways improve chassis strain tolerance by converting vanillin into less toxic derivatives, thereby indirectly increasing vanillin yield. Vanillin β -D-glucoside (glucosylvanillin) is the natural storage form of vanillin. In contrast to free vanillin, glucosylvanillin displays low cellular toxicity, making glucosylation an effective strategy to improve yield and product value. Plant-derived glycosyltransferases efficiently catalyze the glycosylation of natural products. For example, 98 natural uridine diphosphate-glucuronosyltransferases (UGTs) were heterologously expressed, and several UGTs catalyzed the glycosylation of vanillin *in vitro*.⁷⁶ Among them, UGT72B1, UGT71C2, and UGT72E2 exhibited superior catalytic activity and affinity toward vanillin. Additionally, upon knockout of the endogenous glycoside hydrolase gene *BGL1* in *S. cerevisiae*, the hydrolysis of glucosylvanillin was less than 5%.⁷⁶ A novel *de novo* synthesis pathway of vanillin was further constructed in *S. cerevisiae* using *p*-hydroxybenzoic acid as a key node.⁷⁹ Introduction of *UGT72B1*, deletion of the 1,3- β -glucan exonuclease gene *EXG1*, and reinforcement of uridine diphosphate-glucose supply via *PGM1* overexpression enabled production of 1339.5 mg/L vanillin β -D-glucoside. Subsequent fermentation optimization resulted in an increased titer of vanillin β -D-glucoside, reaching 7476.5 mg/L, without detectable by-product accumulation (Table S4). Overall, glucosylation repre-

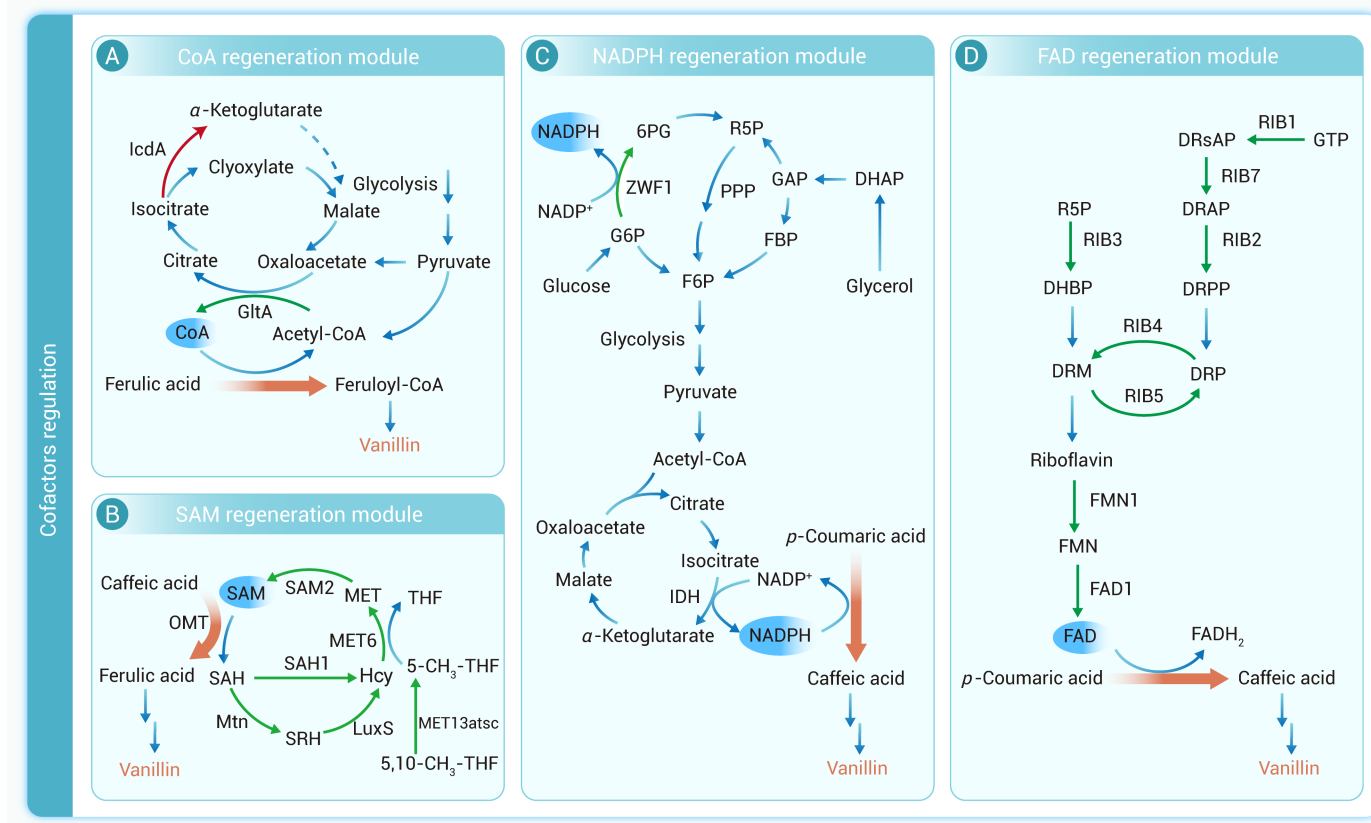


Figure 3. Cofactors regulation strategies for the biosynthesis of vanillin (A) CoA regulation module. (B) SAM regulation module. (C) NADPH regulation module. (D) FAD regulation module. 6PG, 6-phosphogluconate; R5P, ribose 5-phosphate; GAP, glyceraldehyde 3-phosphate; FBP, fructose 1,6-bisphosphate; F6P, fructose 6-phosphate; G6P, glucose 6-phosphate; DHAP, dihydroxyacetone phosphate; PPP, pentose phosphate pathway; GTP, guanosine triphosphate; DRsAP, 2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 5'-phosphate; DRAP, 2,5-diamino-6-ribitylamino-4(3H)-pyrimidinone 5'-phosphate; DRPP, 5-diamino-6-ribitylamino-2,4(1H, 3H)-pyrimidinedione 5'-phosphate; DRP, 5-diamino-6-ribitylamino-2,4(1H, 3H)-pyrimidinedione; DRM, 6,7-dimethyl-8-ribityllumazine; DHBP, 3,4-dihydroxy-2-butanone-4-phosphate; FMN, flavin mononucleotide; SAM, S-adenosylmethionine; MET, methionine; Hcy, homocysteine; SRH, S-ribosyl-L-homocysteine; SAH, S-adenosyl-L-homocysteine; THF, tetrahydrofolic acid; 5-CH₃-THF, 5-methylenetetrahydrofolic acid; 5,10-CH₃-THF, 5,10-methylenetetrahydrofolic acid; IcdA, gene encoding isocitrate dehydrogenase (IDH); GltA, citrate synthase; ZWF1, glucose-6-phosphate dehydrogenase; RIB1, guanosine triphosphate cyclohydrolase II; RIB2, 2,5-diamino-6-ribitylamino-4(3H)-pyrimidinone 5'-phosphate deaminase; RIB3, 3,4-dihydroxy-2-butanone-4-phosphate synthase; RIB4, lumazine synthase; RIB5, riboflavin synthase; RIB7, 2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 5'-phosphate reductase; FMN1, riboflavin kinase; FAD1, flavin adenine dinucleotide synthetase; SAM2, S-adenosylmethionine synthetase; MET6, homocysteine S-methyltransferase; LuxS, S-ribosylhomocysteine lyase; Mtn, adenosylhomocysteine nucleosidase; OMT, O-methoxytransferase; MET13atsc, methylenetetrahydrofolate reductase.

sents a powerful strategy to mitigate vanillin toxicity while enhancing product stability and accumulation. Coupling detoxification pathways with metabolic and process engineering can further enable high-titer production. Such approaches highlight the potential of derivative-based strategies to advance scalable and robust vanillin biosynthesis.

Enhanced tolerance of chassis strains

Vanillin exhibits strong antimicrobial properties against a broad range of fungi and bacteria.^{52,112} Exposure of *E. coli* to vanillin at 2 mg/mL disrupts membrane integrity, induces membrane depolarization, and causes leakage of nucleic acids and proteins, accompanied by ATP depletion and cell death.¹¹³ When the vanillin concentration exceeds 7.5 mM, it inhibits translation of mRNA in *S. cerevisiae* by causing the formation of messenger ribonucleoprotein granules, ultimately preventing protein synthesis.¹¹⁴ Elevated intracellular reactive oxygen species further indicate severe oxidative stress.¹¹⁵ These cytotoxic effects substantially compromise the performance of microbial cell factories. Enhancing chassis tolerance to such stresses is therefore essential for maintaining pathway stability and improving the performance of vanillin biosynthesis.¹¹⁶

Current strategies to enhance microbial tolerance to vanillin are primarily focused on optimizing the material transport system and reinforcing the stability of the cell membrane. Several efflux pumps (e.g., AcrAB/TolC, MdtEF/TolC, YohG), influx transporters (e.g., MhpT, NanT, YgcS), and porins have been reported to confer microbial resistance to a range of toxic compounds.^{81,92,112} The *acrB*-deficient *E. coli* mutant exhibited a 1000-fold decrease in colony formation under 12.5 mM vanillin relative to its wild-type.¹¹² Overexpression of *mhpT* led to more severe growth inhibition in *E. coli*

under 3 mM vanillin. In contrast, knockout of *mhpT* mitigated the inhibitory effect of vanillin. The engineered strain $\Delta\text{acs}\Delta\text{ptsH}\Delta\text{mhpT}$ achieved a vanillin titer of 55.4 mM with a yield of 90%, representing a significant increase from the original 17 mM.⁹² Additionally, ergosterol, a key component of cell membrane of certain microorganisms, was found to enhance membrane robustness and vanillin tolerance, although the underlying mechanisms remain incompletely understood.¹¹⁷

Adaptive laboratory evolution (ALE) has emerged as an effective approach to improve microbial tolerance by cultivating strains under sustained stress. A *B. subtilis* B7-S strain capable of converting ferulic acid to vanillin was subjected to domestication, resulting in a vanillin synthesis efficiency of 63.3%.¹¹⁸ However, stringent mismatch repair systems limit mutation rates and genetic diversity during ALE.¹¹⁹ To overcome this bottleneck, ethyl methanesulfonate mutagenesis of *S. cerevisiae*, followed by 113 serial passages in corn stover hydrolysate containing 0.2 g/L vanillin, yielded the evolved strain EMV-8.¹²⁰ This strain exhibited a maximum specific growth rate of 0.31 per hour in 1.0 g/L vanillin and maintained growth (0.10 per hour) even at 2.0 g/L vanillin, conditions under which the wild-type strain failed to grow.¹¹⁴ Heavy-ion beam irradiation induced broad-spectrum mutations in *S. cerevisiae*, followed by ALE under gradually increasing vanillin concentrations.¹²¹ The resulting mutant strain reached an average OD₆₀₀ of 0.95 in 1.6 g/L vanillin, 3.4-fold higher than that of wild type strain, and showed improved substrate utilization.¹²¹ Multi-omics analyses of highly tolerant strains have provided mechanistic insights and identified new engineering targets.¹²² For example, genomic analysis of vanillin-resistant *S. cerevisiae* mutants revealed that deleting transcription factor *Yrr1p* enhanced ATP-binding cassette transporters expressing, rRNA processing, and ribo-

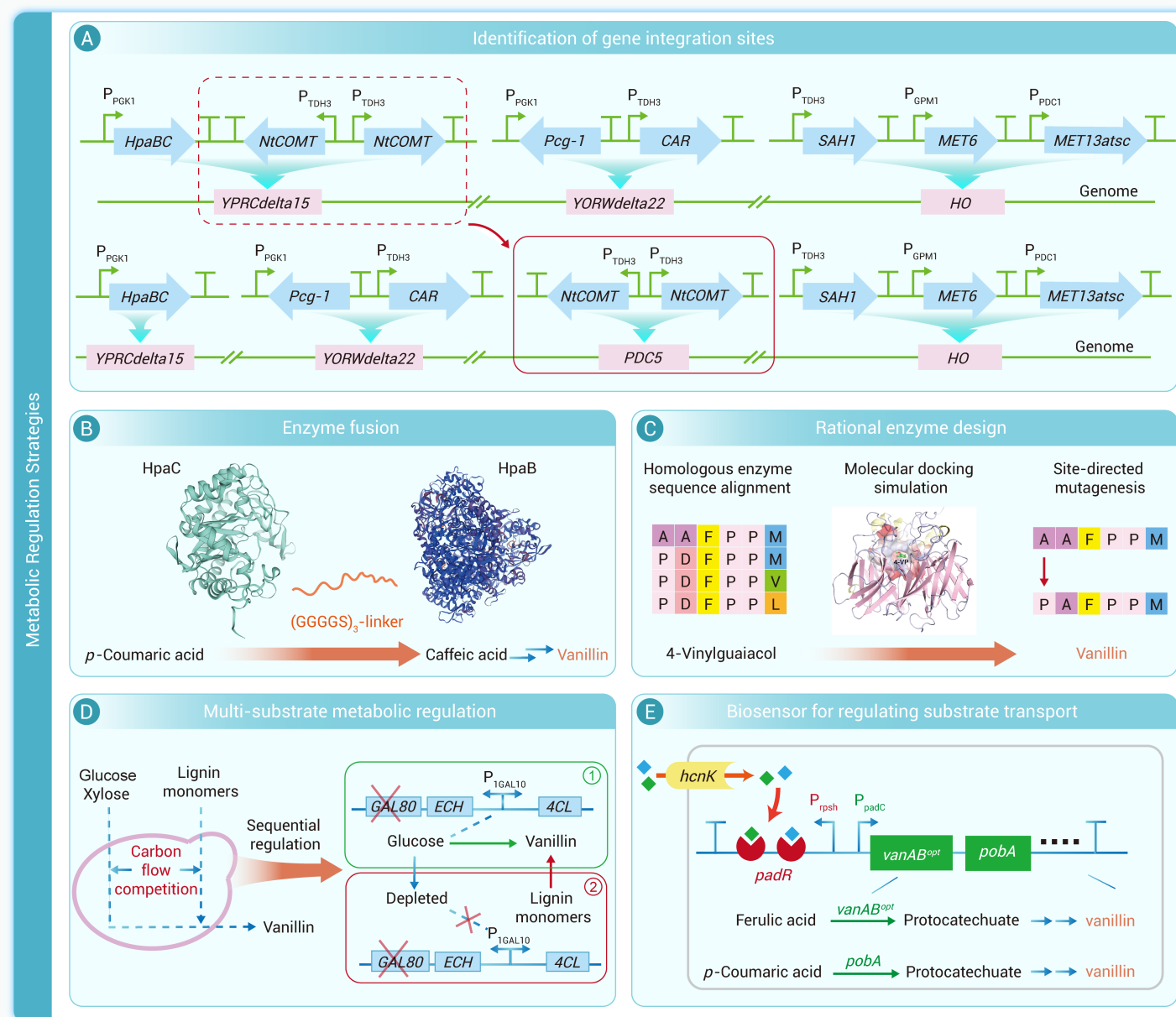


Figure 4. Frontier metabolic regulation strategies for the biosynthesis of vanillin (A) Identification of gene integration sites. (B) Enzyme fusion. (C) Rational enzyme design. (D) Multi-substrate metabolic regulation. (E) Biosensor for regulating substrate transport.

some biogenesis, thereby increasing vanillin tolerance. Targeted knockdown of *Yrr1p* in the wild strain reproduced the tolerant phenotype.¹²³ Whole genome and transcriptome sequencing analyses further indicated down-regulation of genes associated with sugar metabolism and translation (*COX9*, *SDH3*, *IDP1*, and *MRPS5*), alongside upregulation of genes involved in flocculation, rDNA transcription, DNA replication fidelity, and protein palmitoylation (*AAT1*, *FLO9*, *GRC3*, *SWF1*, and *PSP2*) collectively contributing to enhanced vanillin stress resistance.¹²¹ Collectively, these results highlight that vanillin tolerance is a multifactorial trait, while integrating rational engineering with evolutionary strategies and systems-level analyses provides a powerful framework to construct robust microbial chassis.

In-situ separation strategies improved the vanillin fermentation performance

In situ product separation has emerged as an effective strategy to mitigate vanillin toxicity and enhance fermentation performance. Biphasic fermentation systems employ water-immiscible organic solvents to selectively extract hydrophobic substances from the aqueous phase, thereby alleviating vanillin inhibition on cells and enzymes. Certain solvent-tolerant strains, such as

Brevibacillus agri and *B. subtilis*, are particularly suitable for biphasic fermentation systems.^{124,125} Using *B. agri* 13 as a whole-cell biocatalyst for the bioconversion isoeugenol, the addition of 30% (v/v) butyl acetate significantly reduced substrate toxicity and accelerated vanillin formation, achieving 1.7 g/L with a molar conversion of 27.8%.¹²⁴ Oleyl alcohol has also identified as an efficient extraction solvent. In an engineered *S. cerevisiae* for *de novo* p-coumaric acid synthesis, oleyl alcohol increased product titer and reduced by-product accumulation.^{126,127} However, when applied to vanillin biosynthesis, oleyl alcohol not only extracted vanillin efficiently but also partitioned key metabolic intermediates into the organic phase, interfered with cell growth, and impaired substrate utilization, ultimately reducing vanillin yield.⁷⁹ These observations suggest that biphasic fermentation is better suited to short, growth-independent bioconversions rather than complex, multi-step biosynthetic pathways.⁷⁹ Overall, biphasic fermentation represents a synergistic integration of phase engineering and microbial metabolism. Continuous extraction relieves vanillin-induced stress, sustains metabolic activity, and simplifies downstream purification by enriching the product in the organic phase. Nevertheless, careful selection of solvents is required to minimize adverse effects on cell physiology and intermediate retention.

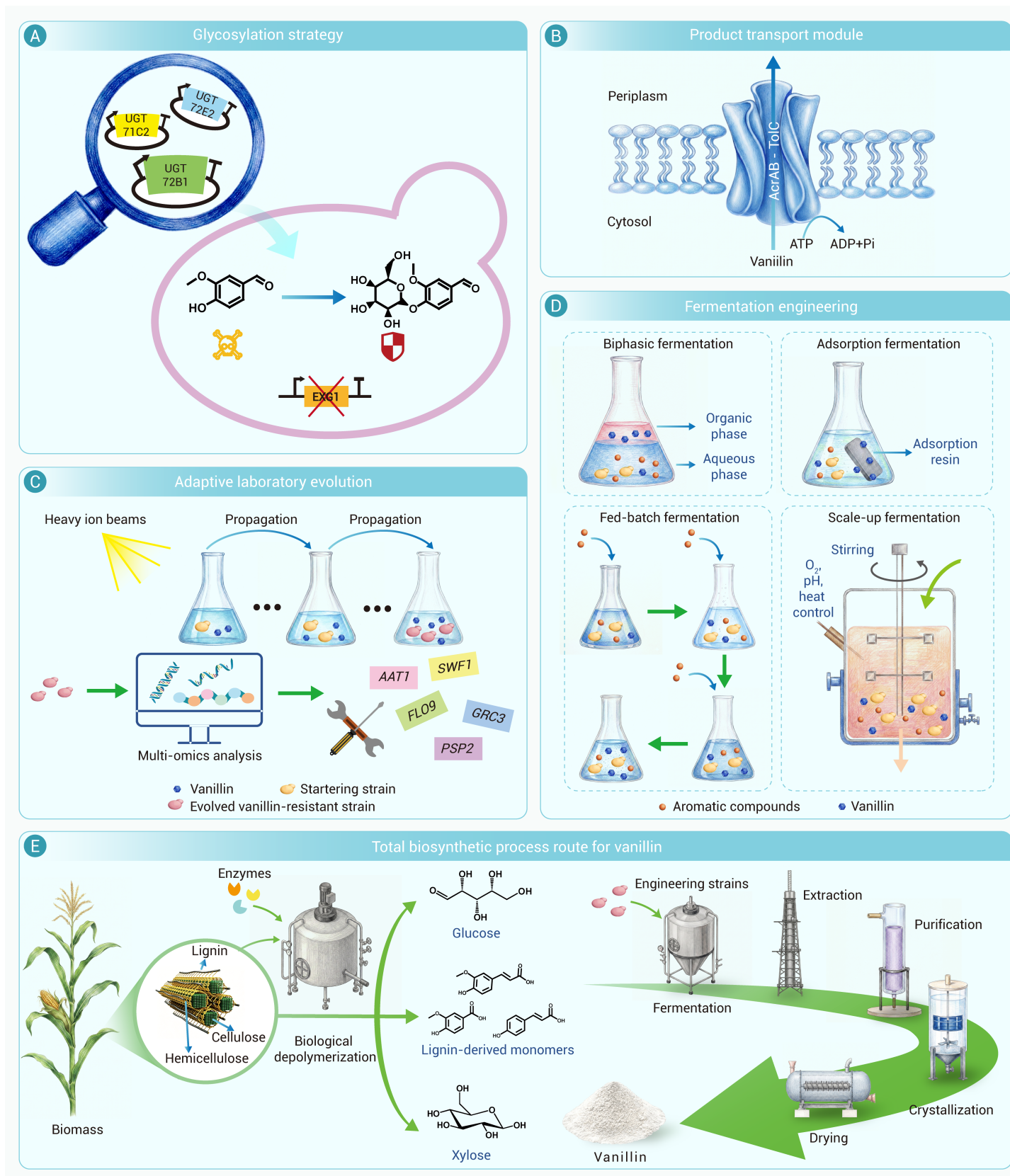


Figure 5. Fermentation strategies for enhancing vanillin yield (A) Glycosylation strategy for enhancing vanillin yield. (B) Transport process of vanillin. (C) Adaptive laboratory evolution strategy for enhancing strain tolerance to vanillin. (D) Fermentation engineering strategies for enhancing vanillin yield. (E) Bioconversion process of biomass towards vanillin.

Beyond liquid-liquid extraction, solid-phase adsorption has been widely explored as an alternative *in situ* separation strategy. Various adsorbent resins and polymers have been reported to effectively adsorb vanillin during fermentation, reducing its intracellular accumulation and toxicity while facili-

tating product recovery. For example, co-fermentation of a vanillin-resistant *E. coli* DH5a mutant with 10 g/L ferulic acid and 50% (w/v) XAD-2 resin yielded 2.9 g/L vanillin, doubling the production achieved without resin addition.⁹³ In an engineered *P. putida* system, delayed addition of 100 g/L XAD-2

resin maintained dissolved vanillin below its inhibitory threshold, resulting in a final titer of 4.47 g/L.⁵² Screening of adsorption resins using engineered *S. sp.* V-1 identified DM11 as the most effective, producing 19.2 g/L vanillin from 45 g/L ferulic acid.¹²⁸ In another example, conjugation of a sol-gel chitosan membrane with engineered *E. coli* enhanced vanillin adsorption and enabled the production of 4.5 g/L vanillin from 6 g/L isoeugenol.⁶⁸ Collectively, adsorption-assisted *in situ* separation represents a promising process-intensification strategy for vanillin fermentation, alleviating product toxicity while facilitating downstream processing. Future efforts should focus on the rational co-design of adsorbents and microbial cell factories to enhance product selectivity while maintaining nutrient availability, metabolic flux, and cell-environment interactions, enabling scalable and industrially relevant vanillin production platforms.

Fermentation optimization for vanillin production

Fermentation optimization represents a decisive step in translating vanillin biosynthesis from laboratory proof of concept to industrial implementation. Early efforts focused on empirical optimization of culture conditions. Solid-state fermentation of *P. chrysosporium* on sun-dried green coconut husk yielded 44.4 µg/g vanillin, which increased to 52.5 µg/g through the Plackett-Burman design-guided optimization of key variables.¹²⁹ More advanced statistical approaches, integrating Plackett-Burman screening with response surface methodology and central composite design, have enabled more precise and efficient identification of optimal fermentation conditions.¹³⁰ For example, *B. aryabhattai* BA03 produced 138.3 mg/L vanillin from ferulic acid under optimized temperature, pH, agitation, and nitrogen supply, demonstrating the effectiveness of multi-parameter optimization strategies.¹³¹

Beyond static optimization, dynamic control of substrate availability has emerged as an effective means to alleviate substrate inhibition and suppress by-product formation.¹³² Controlled or staged feeding strategies allow microbial metabolism to remain within favorable operating ranges. In *B. aryabhattai* BA03 strain, intermittent addition of ferulic acid significantly increased vanillin titers at a titer of 147.1 mg/L relative to batch fermentation.¹³¹ Similarly, sol-gel encapsulation enabled the controlled release of ferulic acid during fermentation by engineered *E. coli*, resulting in a vanillin titer of 20.6 mM, which is more than double the titer obtained from direct substrate addition.¹³⁰ These results highlight the importance of synchronizing substrate supply with cellular catalytic capacity.

Reactor configuration and process intensification further expand the scope of fermentation strategies. In a 2 L bioreactor, a triple-pulse feeding strategy enabled engineered *A. sp.* ATCC 39116 to reach a maximum vanillin titer of 7.76 g/L with a productivity of 0.46 g/L/h.¹³³ Immobilization-based systems also offer advantages in long-term operation. *B. subtilis* on carbon fiber textiles maintained stable activity through repeated medium exchange cycles, achieving sustained vanillin production with a molar yield exceeding 60%.^{134,135} Fermentation with *in situ* product removal represents a particularly powerful strategy for overcoming vanillin toxicity. A hybrid bioreactor combining fed-batch operation with adsorption using the thermoplastic polymer Hytrel® G4078W enabled *A. sp.* ATCC 39116 to accumulate 19.5 g/L vanillin at an overall productivity of 450 mg/L/h.¹³⁶ Overall, fermentation optimization plays a pivotal role in advancing vanillin biosynthesis toward industrial application. Statistical design methods and dynamic feeding approaches effectively enhance vanillin titers by alleviating substrate inhibition and improving metabolic efficiency. Furthermore, advanced reactor configurations, including fed-batch and pulse-feeding systems, significantly boost productivity at larger scales. These integrated fermentation strategies highlight the importance of coupling process engineering with microbial metabolism to achieve efficient vanillin production.

Techno-economic analyses and industrial feasibility of lignin valorization

Converting lignin into high-value aromatics such as vanillin offers a pathway to transform lignin from a low-value byproduct into a value-generating feedstock. While dedicated techno-economic analyses (TEA) specifically evaluating lignin-to-vanillin bioconversion are still lacking, existing TEA studies on lignin valorization provide a useful reference framework for assessing its potential feasibility and competitiveness.

A TEA model of vanillin production from Kraft lignin was designed via alkaline oxidation depolymerization by optimizing reactor conditions to achieve a

9.25% vanillin yield.¹³⁵ The results showed that separation routes using solvent extraction and distillation were identified as the most suitable, offering the lowest energy use, a payback period of 6.19 years, and an internal rate of return of 22.63%. These results effectively quantify core economic drivers, including feedstock availability, vanillin yield, separation energy and solvent choice, and capital costs. Although the lignin-based route consumes more energy and emits more CO₂ than the petrochemical route, its substantially lower raw material cost drives superior economic returns. The work demonstrates that lignin valorization can be economically viable, provided that low-cost feedstock and efficient purification are secured.¹³⁷ TEA of integrated lignin valorization within existing biorefinery infrastructures indicates that sugar-lignin co-production models can be economically viable. The process yields ~49.6 tpd of chemicals (e.g., vanillin, syringaldehyde, phenol) with the electrochemical unit consuming only 3.5% of input energy. The baseline minimum selling price (MSP) of mixed chemicals is \$16.86/kg. Key cost drivers identified include capital cost of the electrochemical reactor, lignin split fraction, and operating days. Sensitivity analysis reveals that oxidation yield, conversion, and reduction yield are the most influential parameters on MSP. It is demonstrated that lignin conversion can improve biorefinery profitability and environmental performance.¹³⁸ The plug-in processes of lignin achieved a lignin solubilization rate of 65%, a polyhydroxyalkanoate titer of 4.5 g/L, and an annual production of 3.5 MM kg, resulting in a minimum selling price of polyhydroxyalkanoate as low as \$6.18/kg.¹³⁹ Annual production and titer were identified as the most sensitive parameters affecting the minimum selling price. Lignin solubilization rate serves as a key upstream leverage point influencing bioconversion efficiency, highlighting the importance of aligning lignin depolymerization with downstream bioconversion processes. Feedstock costs, operating costs, and capital costs are also closely linked to process economics.¹³⁹ Additionally, future TEA frameworks for lignin-based vanillin biosynthesis should explicitly incorporate biological constraints. Engineered *P. putida* synthesized 25 g/L of β-ketoadipic acid from authentic lignin-derived feedstocks.¹⁴⁰ Metabolic regulation and fermentation optimization are central to improving yield and translate into quantifiable economic benefits in TEA. A 20% reduction in yield would increase the minimum selling price of β-ketoadipic acid by \$0.40/kg (approximately 20%).¹⁴⁰ These analyses highlight the critical factors determining commercial viability, including capital efficiency enabled by modular deployment, additional energy demand, effective management of lignin heterogeneity, improved utilization of aromatic carbon, product yields, and downstream separation costs. Importantly, lignin upgrading can contribute positively to overall process economics even at moderate product yields, provided that capital intensity and operational complexity are tightly controlled.

A biological funneling pathway for vanillin synthesis from lignocellulosic monomers was constructed in *S. cerevisiae*.⁹ Through a series of metabolic regulation strategies, including blocking competing pathways, enzyme fusion, and methylation regulation, the engineered *S. cerevisiae* achieved a record yield of 10.5 mg vanillin/g carbon source in real corn stover hydrolysates.⁹ However, improving vanillin biosynthesis also requires enhancing long-term cellular conversion capacity under inhibitory conditions. This necessitates complementary solutions at both the strain and process levels. Strain-level strategies include engineering vanillin-tolerant chassis and introducing detoxification or derivatization pathways. Process-level strategies, such as adsorption-assisted or biphasic fermentation for continuous product removal, as well as fed-batch operation and dynamic process control, further mitigate substrate and product toxicity during scale-up. Looking forward, advanced technologies such as synthetic biology are expected to progressively narrow the performance gap between lignin-based and sugar-based aromatic production routes (Figure 6).¹⁴¹ By enabling selective retention and efficient utilization of aromatic carbon, lignin bioconversion represents a compelling long-term pathway toward a low-carbon aromatic bioeconomy. Systematic techno-economic evaluation of lignin-to-vanillin bioprocesses will therefore be essential for defining viable operating windows, guiding research priorities, and accelerating industrial translation within a circular bio-based industry.

CONCLUSIONS AND OUTLOOKS

Lignin-to-vanillin bioconversion represents a promising and sustainable strategy to valorize renewable aromatic resources and promotes a sustain-

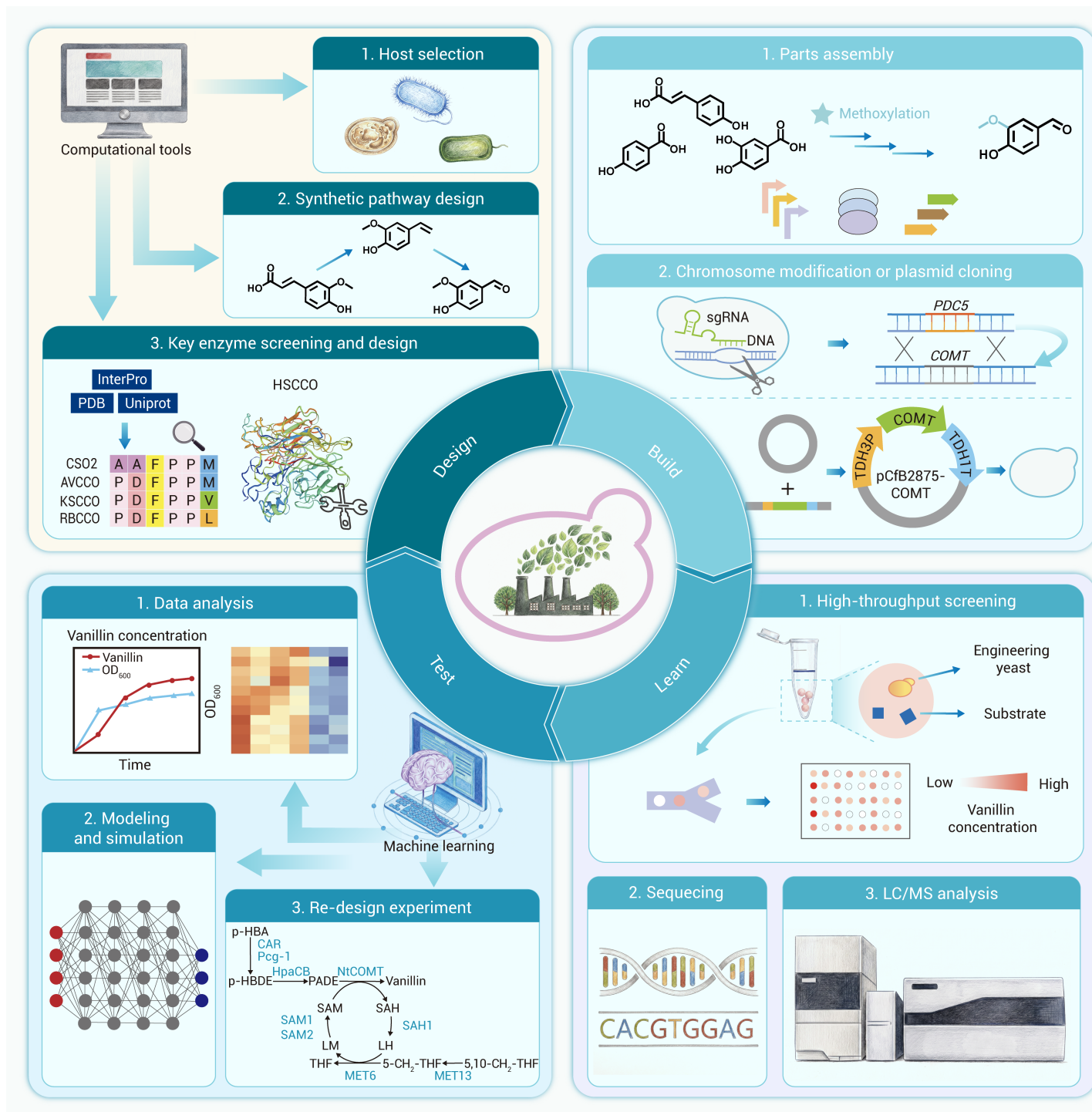


Figure 6. The application of synthetic biology technologies in vanillin biosynthesis.

able biobased economy. Advanced depolymerization methods have enhanced the bioavailability of lignin. Synthetic biology offers powerful tools for designing microbial cell factories to convert lignin derivatives into vanillin. Furthermore, advanced metabolic engineering and fermentation strategies have improved vanillin biosynthesis performance, demonstrating strong potential for industrial-scale production.

Nevertheless, several challenges remain in further optimizing the valorization of lignin into vanillin. First, the structural heterogeneity of lignin constrains both its depolymerization efficiency and subsequent bioconversion performance. Therefore, green and efficient depolymerization strategies are required to generate well-defined aromatic intermediates amenable to downstream bioconversion. Promising approaches include engineering microbes to secrete ligninolytic enzymes, employing synergistic multi-

enzyme or microbial consortia, and integrating tailored depolymerization processes with bioconversion. Second, the cytotoxicity of vanillin and other aromatics remains a major bottleneck limiting production yields. Addressing this challenge requires a combination of strategies, including metabolic detoxification, chassis engineering, and process-level interventions. For example, engineered strains can incorporate vanillin detoxification pathways to convert toxic intermediates into less harmful compounds. Concurrently, selecting naturally tolerant microorganisms or enhancing stress resistance in chassis strains can improve robustness and productivity. At the process level, strategies such as *in situ* product removal through adsorption or extraction can continuously reduce vanillin concentrations, alleviating toxicity. Third, most microorganisms inherently lack the metabolic pathways necessary to convert lignin-derived intermediates into vanillin. Introducing heterologous

biosynthetic routes often creates metabolic bottlenecks, highlighting the need for optimization of key enzyme activities and cofactor balance. Advanced strategies, such as AI-guided enzyme engineering and the use of biosensors, can help enhance metabolic flux toward vanillin production. This challenge underscores the need to move beyond single-pathway optimization toward a holistic management of aromatic carbon flux. Accordingly, selecting chassis strains with high genetic tractability and strong metabolic compatibility is critical for constructing efficient and robust vanillin-producing biocatalysts.

Collectively, these efforts will advance the valorization of lignin and facilitate the industrial-scale production of vanillin. High-yield conversion of heterogeneous lignin into aromatics aligns with atom-economy principles, and supports a circular bioeconomy. Beyond vanillin, these approaches establish a generalizable framework for upgrading lignin, improving both the economic and environmental competitiveness of biobased aromatic chemicals. Continued integration of synthetic biology, metabolic engineering, and process optimization will be critical to accelerating industrial translation and unlocking the full potential of lignin-derived aromatics.

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AUTHOR CONTRIBUTIONS

Writing - original draft, R.J.S.; conceptualization, R.J.S and Z.H.L.; writing - editing, S.R.J., D.F.L, X.X. and N.L.; funding acquisition, B.Z.L. and Z.H.L.; supervision, Z.H.L.; All authors contributed to the manuscript and approved the final version.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DATA AND CODE AVAILABILITY

No new data were created or analyzed in this study.

SUPPLEMENTAL INFORMATION

It can be found online at <https://doi.org/10.59717/j.xinn-life.2026.100229>