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Advances in Biological Funneling of Lignin: Chassis Domestication, Multi-input Autonomous Dynamic Regulation, and Integrated Bioplastic Manufacturing

Weichen Zhu

Academy of Life and Natural Sciences, Xi'an Jiaotong-Liverpool University, Suzhou, China
weichen.zhu24@student.xjtlu.edu.cn

Abstract. Lignin is the most abundant renewable aromatic polymer in nature. Agro-forestry residues and pulp industries generate over 300 million tons of industrial lignin annually, yet more than 95 % is merely incinerated for low-grade heat recovery, wasting high-value aromatic carbon resources. Biological funneling addresses the structural heterogeneity of lignin monomers by converting diverse H-, G- and S-type hydroxycinnamic acids into a single central intermediate, protocatechuic acid (PCA). Traditional static constitutive metabolic engineering suffers from aromatic cytotoxicity, imbalanced upstream-downstream flux, massive intermediate accumulation and poor adaptability to fluctuating industrial hydrolysates. In 2026, a Shandong University team reported in Nature Communications a layered multi-input autonomous biological-funneling system in Corynebacterium glutamicum, integrating ALE-driven chassis domestication, six endogenous aromatic-responsive transcription-factor biosensors and a two-tier cascade positive-negative feedback circuit, achieving 99.89 % molar conversion of mixed lignin-derived aromatics. Fed-batch fermentation produced 53.40 g L^{-1} PCA, 51.90 g L^{-1} cis,cis-muconic acid (CCM) and 38.33 g L^{-1} β-ketoadipic acid, and the strain directly utilized non-detoxified corn-stover alkaline pretreatment liquor. This review systematically compares lignin pretreatment-fractionation technologies and four major microbial chassis, reviews the three regulatory generations, and dissects the molecular mechanisms and fermentation performance of this landmark work. Synthetic routes to high-value lignin-derived chemicals (PCA, CCM, β-KA, PDC) and full-chain biorefinery bottlenecks with corresponding optimization strategies are summarized, providing theoretical references for green lignin biorefinery.

Keywords: lignin, biological funneling, Corynebacterium glutamicum, dynamic metabolic regulation

1. Introduction

1.1. Resource potential and bottlenecks of lignin valorization

Lignin is one of three major structural components in plant cell walls, accounting for 15 %-40 % of biomass dry weight. It is the only large-scale naturally-renewable aromatic carbon scaffold on earth.

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Distinct from the fully aliphatic carbon skeletons of cellulose and hemicellulose, lignin is rich in benzene rings and methoxy groups, making it a promising petroleum-replacement feedstock for producing fragrances, nylon monomers and high-performance polymers. Based on botanical origin, lignin can be categorized as woody or herbaceous lignin. Softwood lignin consists exclusively of guaiacyl (G) units; hardwood lignin contains both guaiacyl (G) and syringyl (S) moieties. Herbaceous lignin from corn stover, rice straw and other grasses simultaneously harbours p-hydroxyphenyl (H), G and S units. A large number of ester- and ether-linked phenylpropane derivatives such as p-coumaric acid (p-CA), ferulic acid (FA) and caffeic acid (CaA) can be readily released via mild alkaline hydrolysis, providing favourable aromatic substrates for bioconversion.

Annually, agro-forestry waste and pulp industries produce more than 300 million tons of industrial lignin. Nevertheless, most lignin is combusted for heat recovery, and less than 10 % of its carbon value is recycled. As reported by Ragauskas et al. [1], incineration only brings economic benefits of 60-150 USD ton^{-1} biomass. By contrast, conversion of lignin into bulk aromatic chemicals can yield profits up to 1000 USD ton^{-1} biomass, far exceeding power generation, feed utilization and landfill disposal (landfill incurs an extra disposal cost of 400 USD ton^{-1}). Therefore, high-value valorization of lignin is an essential requirement for biomass-based bioeconomy.

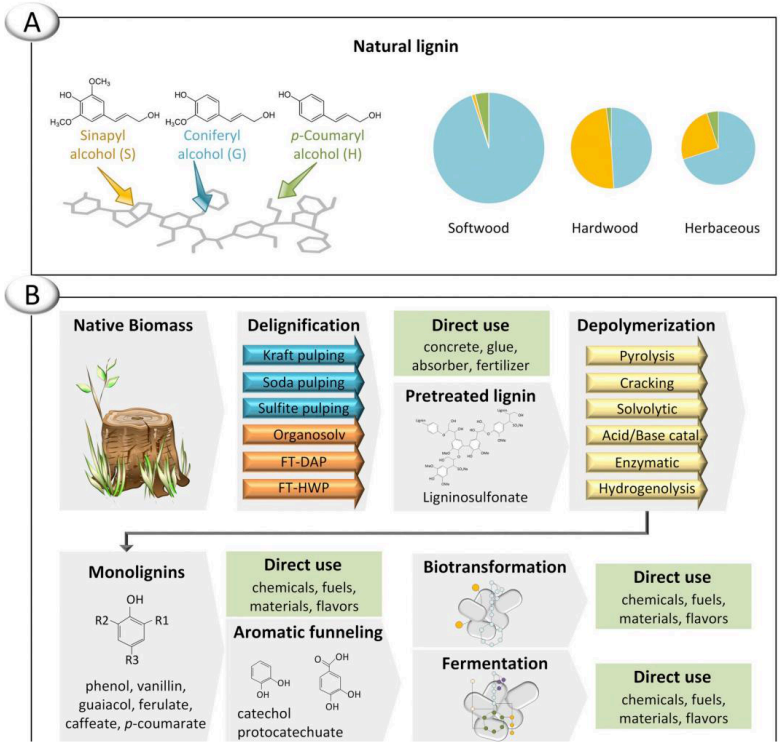

Figure 1. Structural units (H/G/S) of lignin from different feedstocks and two biorefinery workflows for lignin recovery [1]

Four inherent challenges hinder large-scale lignin valorization: (1) Dense cross-linked macromolecular structures impede efficient cleavage of degradable β-O-4 ether bonds, and repolymerization readily occurs; (2) Depolymerization releases highly-mixed monomers including p-CA, FA and sinapic acid (SA), making targeted synthesis of single products difficult; (3) Aromatic phenols exert strong cytotoxicity against microbial cell membranes and genomes and inhibit cell growth and metabolism; (4) Chemical catalytic depolymerization requires high-temperature and

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high-pressure conditions with high energy consumption, generating complex by-products and high downstream separation costs [2].

Softwood lignin is essentially G-only, hardwood mixes G and S, and herbaceous grasses add p-hydroxyphenyl (H) on top (Figure 1). This threeunit heterogeneity—rather than any single aromatic monomer—defines the substrate range a microbial chassis must accept, and it is the problem biological funneling was built to solve.

1.2. Three major technical routes for lignin valorization

Currently, lignin valorization falls into three mainstream categories: thermochemical conversion, chemical catalytic depolymerization and microbial biotransformation, which exhibit distinct characteristics and industrialization potential.

1. Thermochemical conversion (pyrolysis, hydro-liquefaction): Chemical bonds of lignin are cleaved under high-temperature conditions, yet products represent complex mixtures of phenols, aldehydes and alkanes. Subsequent purification costs are prohibitively high for selective synthesis of single high-value aromatic chemicals.

2. Acid-base catalytic depolymerization: Macromolecular lignin can be degraded, but aromatic-radical-mediated re-condensation frequently yields recalcitrant higher-molecular-weight lignin, accompanied by strong microbial inhibitors such as furans and phenolics.

3. Microbial biotransformation: Reactions proceed under mild aqueous conditions at ambient temperature and pressure. Enzymatic catalysis exhibits high substrate selectivity. Relying on biological funneling, heterogeneous aromatic monomers can be consolidated toward a single central intermediate, representing a low-carbon and cost-sustainable route.

Among the three routes, microbial biotransformation is the only one that collapses this monomeric diversity rather than sorting it: heterogeneous p-CA, FA and sinapate are funnelled through shared upper and lower pathways into a single central intermediate (Figure 2), from which downstream products branch outward.

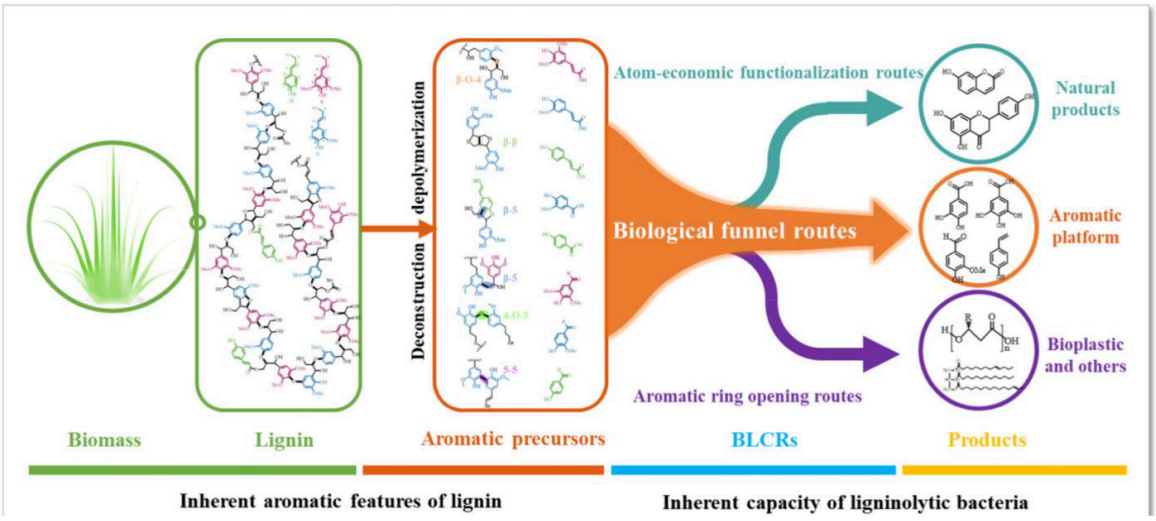

Figure 2. Schematic diagram of biological-funnel-based lignin conversion (BLCR) [3]

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1.3. Core concept and generational division of biological funneling

Natural wood-rot fungi and actinobacteria have evolved hierarchical aromatic-degradation pathways. The upstream Layer 1 pathway converts diverse H/G/S-type phenylpropane derivatives into two intermediates: 4-hydroxybenzoic acid (4-HBA) and vanillic acid (VA). The downstream Layer 2 pathway further transforms these two intermediates into protocatechuic acid (PCA) via hydroxylation and demethylation. This sequential consolidation process is defined as biological funneling, which fundamentally addresses the problem of lignin-monomer heterogeneity and serves as the theoretical cornerstone for lignin bioconversion.

According to metabolic-engineering design philosophy, biological-funneling technologies are divided into three generations:

1.First-generation (2018-2022): static constitutive metabolic engineering; strong promoters continuously overexpress rate-limiting enzymes without substrate-responsive regulation.

2.Second-generation (2022-2025): single-input aromatic-responsive transcription-factor (aTF) auto-regulatory systems; only one lignin-derived substrate can be sensed, failing to balance flux between upstream- and downstream-layer pathways.

3.Third-generation (2026, Nature Communications landmark work): layered multi-input autonomous dynamic regulatory systems. Multiple signals from upstream substrates and downstream intermediates are simultaneously perceived to establish automatic feedback loops, resolving major drawbacks of previous generations.

1.4. Scope and logic of this review

Taking the 2026 Corynebacterium glutamicum layered autonomous biological-funneling system reported by Xu and co-workers from Shandong University [4] as the core subject, this review systematically summarizes lignin pretreatment technologies and advances in four major industrial microbial chassis. Iterative improvements across three regulatory generations are compared, and three innovative modules (evolved chassis, sensory biosensors and cascade regulatory circuits) of this landmark study are dissected in detail. Downstream synthetic routes for high-value products are compiled. Industrial bottlenecks are analysed from strain, element, raw-material and process dimensions, and corresponding synthetic-biology optimization strategies are proposed, establishing a systematic theoretical framework for lignin biological-funneling research.

2. Lignin pretreatment for releasing aromatic monomers

Successful lignin bioconversion requires depolymerization of compact macromolecular lignin into free phenylpropane monomers accessible to microbes. Pretreatment directly determines aromatic-monomer yields and inhibitor loads and thus governs downstream fermentation performance. This chapter compares conventional industrial pretreatment and novel green fractionation workflows and evaluates hydrolysate composition and fermentation compatibility.

2.1. Conventional industrial lignin-fractionation workflows

Kraft/sulfite pulping represents mainstream alkaline pulping in paper manufacturing. Strong alkali at high temperature cleaves partial ether bonds of lignin, while extensive C-C condensation produces high-molecular-weight black-liquor lignin. Hydrolysates contain very low concentrations of free p-

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CA and FA, alongside large amounts of sulfide and phenolic inhibitors. Multi-step detoxification is mandatory before microbial fermentation and raises overall processing costs.

Organosolv pretreatment uses organic solvents such as ethanol and γ-valerolactone to solubilize lignin with limited re-condensation. Nevertheless, high solvent-recovery energy consumption and corrosion-resistant equipment requirements restrict its application to laboratory-scale trials rather than large-scale deployment.

Dilute-acid pretreatment preferentially degrades hemicellulose, whereas lignin undergoes severe condensation with marginal aromatic-monomer release. Meanwhile, massive furfural and hydroxymethylfurfural are generated as potent microbial toxins, making this approach unsuitable for bioconversion feedstock preparation.

2.2. Novel green lignin depolymerization-fractionation strategies

High-solid ethylenediamine (EDA) pretreatment was developed by Xu et al. [5]. The system operates at solid content up to 58 wt %. Physical confinement restricts migration of lignin-degradation intermediates and suppresses re-condensation. Ethylenediamine introduces hydrophilic amino groups onto lignin via α-amination and Schiff-base reactions. The modified lignin exhibits water solubility >86 % within pH 2-8, with molecular weight of 1200-1400 Da and abundant degradable β-O-4 ether bonds. Large quantities of p-CA and FA can be released under mild alkaline conditions, while cellulose fractions remain intact, enabling "lignin-first" biorefinery.

Following the chemistry above, the EDA process (Figure 3) cooks raw corn stover at up to 58 wt% solids, then washes out the aminomodified lignin while leaving cellulose largely intact—exactly the"lignin-first" split that the downstream fermentation demands.

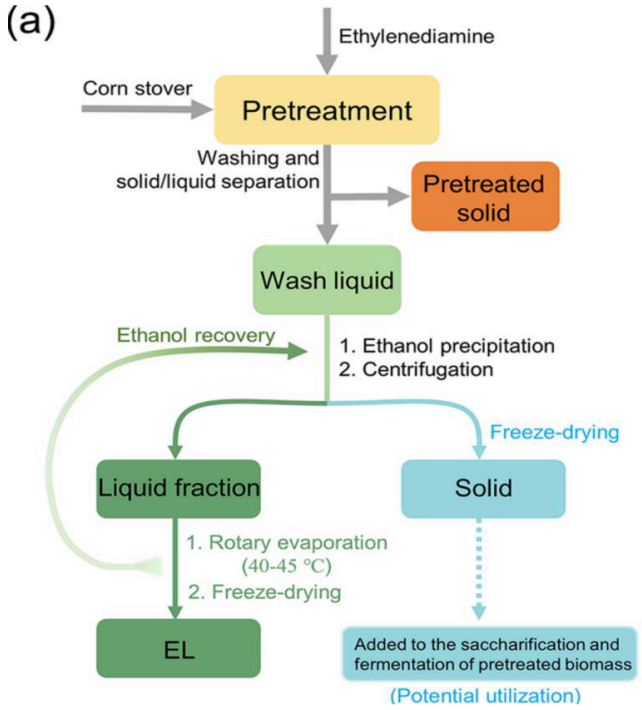

Figure 3. Process flowchart for corn-stover pretreatment by high-solid ethylenediamine for preparation of amino-modified lignin (EL) [5]

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Tandem Fractionation Technology (TAFT), described by Chen et al. [6], employs two-step acid-base fractionation to separate lignin into low-condensation liquid-phase lignin (suitable for microbial fermentation) and high-molecular-weight solid-phase lignin (for activated porous-carbon adsorbents). Hierarchical high-value utilization of lignin can therefore be realized.

TAFT pushes this logic further by splitting lignin at the fractionation stage itself (Figure 4): the low-condensation liquid phase feeds fermentation, while the high-molecularweight solid phase is redirected to porous carbon—so no stream is relegated to boiler fuel.

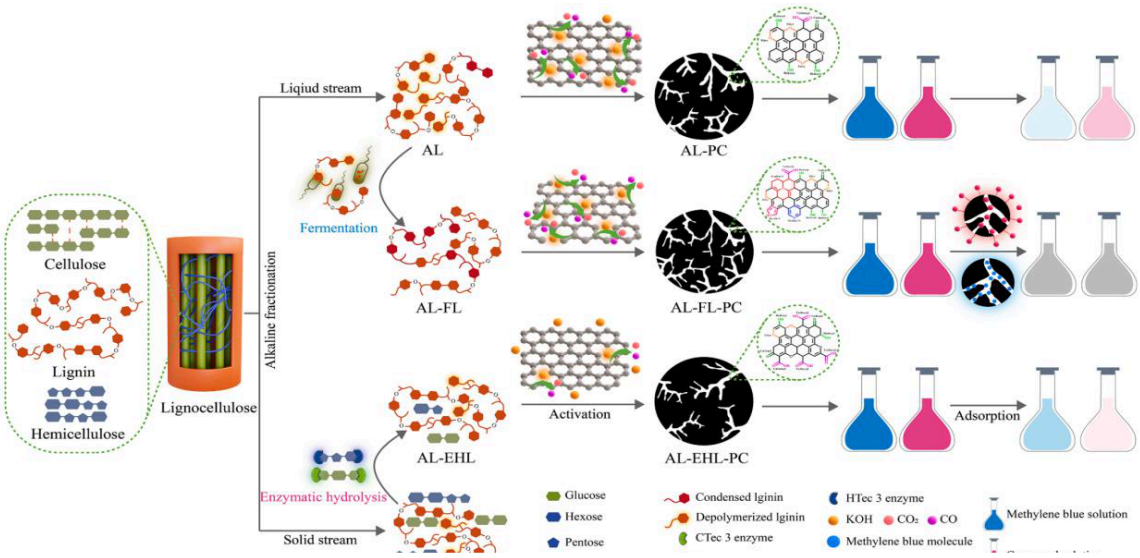

Figure 4. Schematic of TAFT tandem fractionation for preparation of porous adsorptive carbon from lignin [6]

Chemomechanical coupled pretreatment integrates ball-milling mechanical disruption and EDA chemical treatment [7]. Under mild 120 °C conditions, lignin-removal efficiency reaches 85 %, with energy consumption reduced to 41 % relative to standalone EDA treatment. Mechanical forces disrupt compact plant-cell-wall structures to enhance reagent penetration. The resultant lignin retains abundant β-O-4 linkages and serves as promising bioconversion feedstock.

2.3. Impacts of hydrolysate composition on bioconversion

The molar ratio p-CA:FA:CaA ≈4:1:0.5 is observed for corn-stover alkaline pretreatment liquor (APL), which served as standard feedstock in the 2026 Nature Communications study. EDA-pretreated hydrolysates show elevated ferulic-acid fractions, whereas pulp-mill black liquor is dominated by G-unit derivatives and inhibitory impurities. Most conventionally-engineered strains only tolerate fixed substrate ratios. Minor shifts in feedstock composition trigger massive intermediate accumulation and reduced conversion efficiency. Only the SASR7 strain constructed in 2026 exhibits robustness against broad substrate-ratio fluctuations, demonstrating industrial feedstock compatibility.

2.4. Existing challenges of coupled pretreatment-fermentation

Inhibitors (furans, phenolics) generated from conventional pretreatment suppress microbial growth. Most engineered strains require detoxification steps using activated carbon or ion-exchange resins,

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increasing process complexity and operating costs. By contrast, the ALE-domesticated C. glutamicum chassis described in the 2026 study acquires high inhibitor tolerance, allowing direct fermentation of non-detoxified corn-stover alkaline liquor and simplifying upstream workflows.

3. Microbial chassis for lignin bioconversion

Four major chassis categories are available for lignin-oriented biological funneling: Gram-negative bacteria, actinobacteria, fungi and yeast. They differ substantially in native aromatic-metabolic capacity, genetic manipulability and industrial-fermentation suitability. Advantages and limitations of each chassis are discussed below, supported by quantitative performance-comparison tables.

3.1. Gram-negative bacterium: Pseudomonas putida

Pseudomonas putida natively harbours a complete β-ketoadipate degradation pathway and exhibits moderate intrinsic aromatic tolerance, making it an early popular chassis for lignin bioconversion. Li et al. [8] constructed a p-CA- and FA-responsive auto-regulatory circuit based on the PadR transcription factor, achieving a PCA titer of 12.7 g L^{-1} in batch fermentation. Liu et al. [9] heterologously expressed VanAB demethylase to realize sinapic-acid catabolism, yielding 46.5 mM muconic acid from mixed substrates. Nevertheless, major intrinsic drawbacks persist: (1) Difficulties in high-cell-density industrial fermentation and low OD_{600} ceiling; (2) Poor co-metabolism of glucose and aromatic substrates; (3) Restrictions to single-sensor circuits; multi-layer cascade feedback circuits cannot be assembled. The maximum molar yield for mixed-aromatic-substrate conversion is below 70 %, accompanied by severe intermediate accumulation.

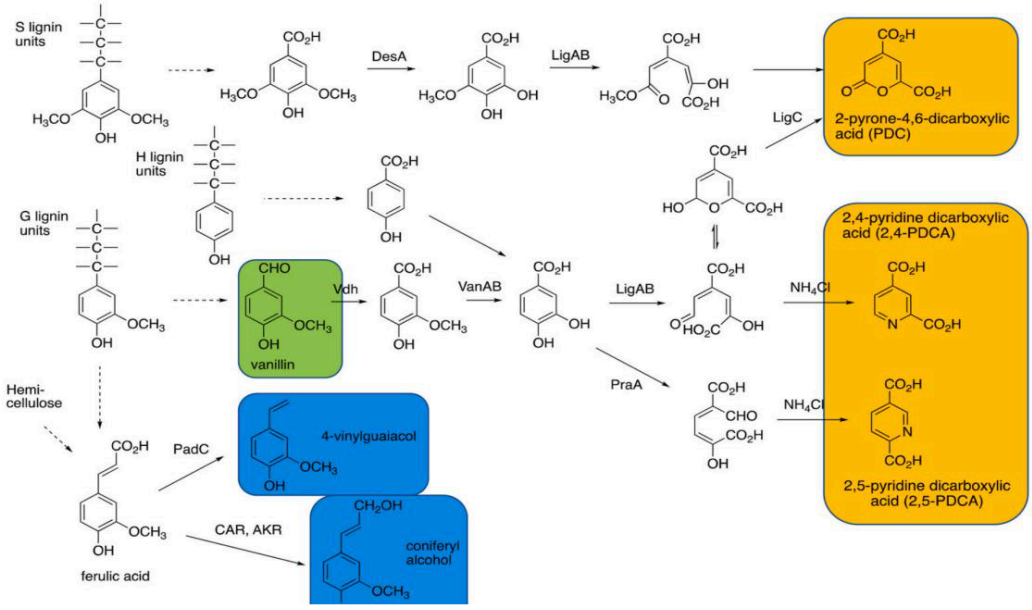

Figure 5. Metabolic pathways for synthesis of PDC and pyridinedicarboxylic acids from lignin-derived aromatic monomers in Pseudomonas putida [10]

Even with its native βketoadipate pathway, P. putida routes ligninderived aromatics primarily toward PDC and pyridinedicarboxylates (Figure 5) rather than the nylonprecursor dicarboxylic acids targeted here, and its singlesensor circuits leave mixedaromatic conversion capped near 70%.

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3.2. Actinobacterium: Rhodococcus jostii RHA1

Rhodococcus jostii RHA1 secretes DyP-type lignin peroxidase and can directly depolymerize macromolecular lignin polymers without complete pretreatment. However, severe industrial-application obstacles remain: extremely slow cell-growth rates with doubling times of tens of hours, immature genetic-editing toolboxes, and incapability of assembling multi-gene cascade regulatory circuits. It is only suitable for auxiliary in-vitro enzymatic catalysis instead of large-scale cell-factory fermentation.

3.3. Industrial model actinobacterium: Corynebacterium glutamicum

Corynebacterium glutamicum is a GRAS-classified industrial workhorse. Large-scale amino-acid-fermentation infrastructure worldwide is fully compatible with this species, rendering it the optimal chassis for third-generation dynamic-regulatory systems. Three intrinsic strengths stand out:

1.Endogenous intact phenylpropane-degradation ph operon and downstream β-ketoadipate pathways; minimal heterologous-enzyme expression is required.

2.Efficient co-utilization of glucose and aromatic substrates. Glucose supplies cellular growth energy, while aromatic carbon is channelled toward target products for high carbon-utilization efficiency.

3.Genomically encoded multiple native aromatic-responsive transcription factors including PhdR, PcaO and VanR, forming a natural element repository for multi-input biosensor construction. Earlier engineering of C. glutamicum relied on static strategies. The Weiland group [11, 12] knocked-out repressors phdR and vanR and constitutively overexpressed rate-limiting enzymes. The best strain MA-9 achieved muconic-acid titres below 30 g L^{-1}. Constitutive strong overexpression imposed heavy metabolic burden, and cell-growth was fully arrested under high aromatic-substrate concentrations. Flux-imbalance bottlenecks persisted until the breakthrough of the 2026 Nature Communications layered-regulatory system.

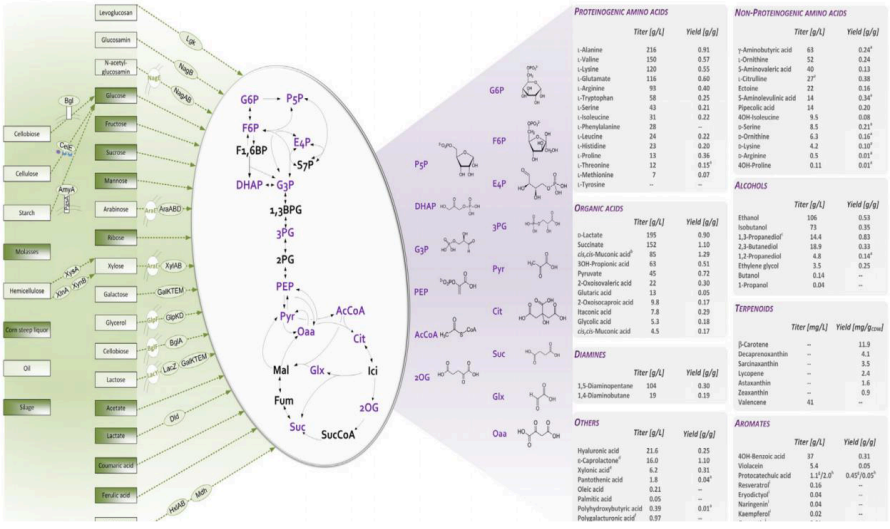

Figure 6. Central-carbon and aromatic-conversion metabolic network of Corynebacterium glutamicum [13]

The reason C. glutamicum needs so little heterologous engineering is visible in Figure 6: its native network already connects the aromatic upper-pathway ph operon to the β-ketoadipate funnel,

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so glucose feeds growth while phenylpropanes feed product flux through the same chassis.

3.4. Fungi and engineered Saccharomyces cerevisiae

Saccharomyces cerevisiae can simultaneously consume lignocellulosic hydrolysate sugars and aromatic monomers. Xin et al. [14] constructed an artificial biological-funneling pathway for vanillin biosynthesis directly from straw hydrolysate. Still, yeast lacks complete native aromatic-degradation pathways; all catabolic enzymes must be heterologously expressed with low catalytic activity. Product titres are generally lower than 2 mM. Yeast is only applicable for high-value niche flavour compounds such as vanillin rather than bulk nylon-monomer manufacturing.

3.5. Performance comparison of four microbial chassis

Benchmarked across five axes in Table 1, no wild chassis is uniformly strong. P. putida and R. jostii tolerate aromatics well but cannot carry multilayer circuits; S. cerevisiae coutilises sugars yet lacks any native aromatic funnel; classically engineered C. glutamicum is genetically tractable and fermentationmature but was held back by poor native tolerance. The decisive row in Table 1 is SASR7: the only entry combining "very high tolerance" with "excellent circuit assembly" and "very high industrial suitability"—because ALE domestication cured the single weakness of an otherwise optimal chassis, rather than starting from a lessmature host.

Early static designs were constrained by low native aromatic tolerance and constitutive overexpression. R. jostii RHA1 tolerates phenolics well yet grows extremely slowly and lacks a mature editing toolkit, restricting it to auxiliary in-vitro catalysis. Engineered S. cerevisiae accepts mixed sugar–aromatic feedstocks but carries no native aromatic-degradation routes and reaches only millimolar titres. P. putida is an early-popular chassis with an intact β-ketoadipate pathway, but its single-sensor auto-regulation and limited glucose co-utilisation cap mixed-aromatic conversion below 70 %. By contrast, the ALE-domesticated SASR7 strain combines very high aromatic tolerance with excellent circuit assembly and reaches a maximum CCM titre of 51.90 g L^{-1} under layered multi-input feedback.

Table 1. Summary of key performance metrics for microbial chassis for lignin-derived aromatic bioconversion; data compiled from references [8-11, 15]

Microbial chassis	Native aromatic tolerance	Complex genetic-circuit assembly capacity	Maximum CCM titer	Major regulatory mode	Industrial fermentation suitability
Pseudomonas putida	Moderate	Moderate	46.5 mM	Static / single-sensor auto-regulation	Low (limited cell-density)
Rhodococcus jostii RHA1	High	Poor	<10 mM	No engineered dynamic regulation	Very poor
Classically-engineered Corynebacterium glutamicum	Low	Excellent	≈30 g L^{-1}	Constitutive static overexpression	Very high
2026 NC-engineered SASR7	Very high (ALE-domesticated)	Excellent	51.90 g L^{-1}	Layered multi-input autonomous feedback	Very high
Engineered Saccharomyces cerevisiae	Very low	Moderate	<2 mM	Static heterologous pathways	Moderate

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3.6. Trends for chassis development

Considering fermentation maturity, endogenous regulatory-element repertoires and product-synthesis potential, Corynebacterium glutamicum represents the preferred chassis for third-generation multi-input dynamic-regulatory platforms. The 2026 study overcomes its intrinsic aromatic-susceptibility limitation by ALE domestication and establishes an integrated paradigm of "tolerant chassis + multi-node biosensing + closed-loop regulation", providing a standard engineering template for future lignin biorefinery.

4. Iterative developments of regulatory strategies for biological funneling

This chapter reviews three generations of biological-funneling regulatory strategies, compares their engineering logics, merits and intrinsic limitations, and dissects modules, molecular mechanisms and fermentation performance of the landmark 2026 Nature Communications layered multi-input autonomous biological-funneling system. Quantitative comparison tables illustrate technological leaps across generations.

4.1. First-generation regulation: static constitutive metabolic engineering

First-generation engineering lacked substrate-responsive regulatory logic. Strong constitutive promoters (Ptrc, Ptuf) continuously overexpressed rate-limiting enzymes of both biological-funneling layers: upstream phdAEBC cluster (Layer 1, phenylpropane β-oxidation) and downstream pobA, vanAB (Layer 2, intermediate hydroxylation / demethylation). Native transcriptional repressors phdR and vanR were deleted to relieve basal-transcription repression. Competitive bypass genes such as pcaIJ and fudC were knocked-out to divert carbon flux.

Spca1-Spca8 static-engineered reference strains were constructed in the original publication. Shake-flask experiments revealed irreversible drawbacks for static regulation. Constitutive gene transcription-translation consumes abundant amino acids and ATP even in the absence of aromatic substrates, imposing high basal metabolic burden and inhibiting cell proliferation. Severe permanent flux imbalance exists between upstream and downstream pathways. Continuous overexpression of Phd enzymes generates massive 4-HBA and VA intermediates, yet fixed expression levels of downstream PobA and VanAB cannot accelerate intermediate consumption. For strain Spca4, accumulated 4-HBA reaches 30.5 mM and VA reaches 21.0 mM under fermentation conditions. Furthermore, toxicity cannot be mitigated without tolerance engineering of the host chassis. 20 mM p-CA completely suppresses cellular respiration of wild-type strains. The highest PCA molar yield of static strains only reaches 52.83 % with product concentration of merely 25.19 mM, incompatible with industrial high-load lignin hydrolysates. Static engineering can only optimize individual pathway layers and fails to dynamically coordinate upstream-downstream reaction rates, creating major bottlenecks that drove the emergence of second-generation biosensor-based strategies.

4.2. Second-generation regulation: single-input aTF auto-regulatory systems

Single-input auto-regulatory circuits were built using repressor-type transcription factors such as Pad from P. putida and PhdR from C. glutamicum. In the absence of aromatic substrates, aTF binds promoter operators and blocks gene transcription. Upon substrate addition, ligand-bound aTF dissociates from DNA, turning on pathway expression automatically; expression is switched off

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when substrates are depleted, substantially decreasing basal metabolic burden. A representative example is the p-CA/FA regulatory circuit in Pseudomonas putida reported by Li et al. [8].

Nevertheless, this design bears fundamental limitations. Only one upstream-substrate signal can be detected; intermediates 4-HBA and VA cannot be sensed, and Layer 2 pathway expression cannot be up-regulated in response to intermediate buildup. No hierarchical synergistic feedback is implemented. Even after upstream intermediate generation, downstream-enzyme expression cannot be synchronously boosted. Intermediate accumulation can reach up to 15 mM for mixed-lignin-substrate fermentation, with molar conversion hovering around 70 %. Second-generation approaches only resolve wasteful metabolism under substrate-free conditions but do not tackle core upstream-downstream flux mismatch. Conversion efficiency deteriorates sharply when industrial hydrolysate compositions fluctuate, necessitating third-generation multi-layer cascade regulation.

4.3. Third-generation landmark: 2026 Nat Commun layered multi-input autonomous biological-funneling system

This work establishes a three-in-one engineering platform: ALE-evolved high-tolerance chassis, modular aromatic aTF biosensor toolkit, and two-tier cascade positive-negative feedback regulatory circuits. The SAS strain series was generated, and SASR7 delivered near-complete substrate conversion.

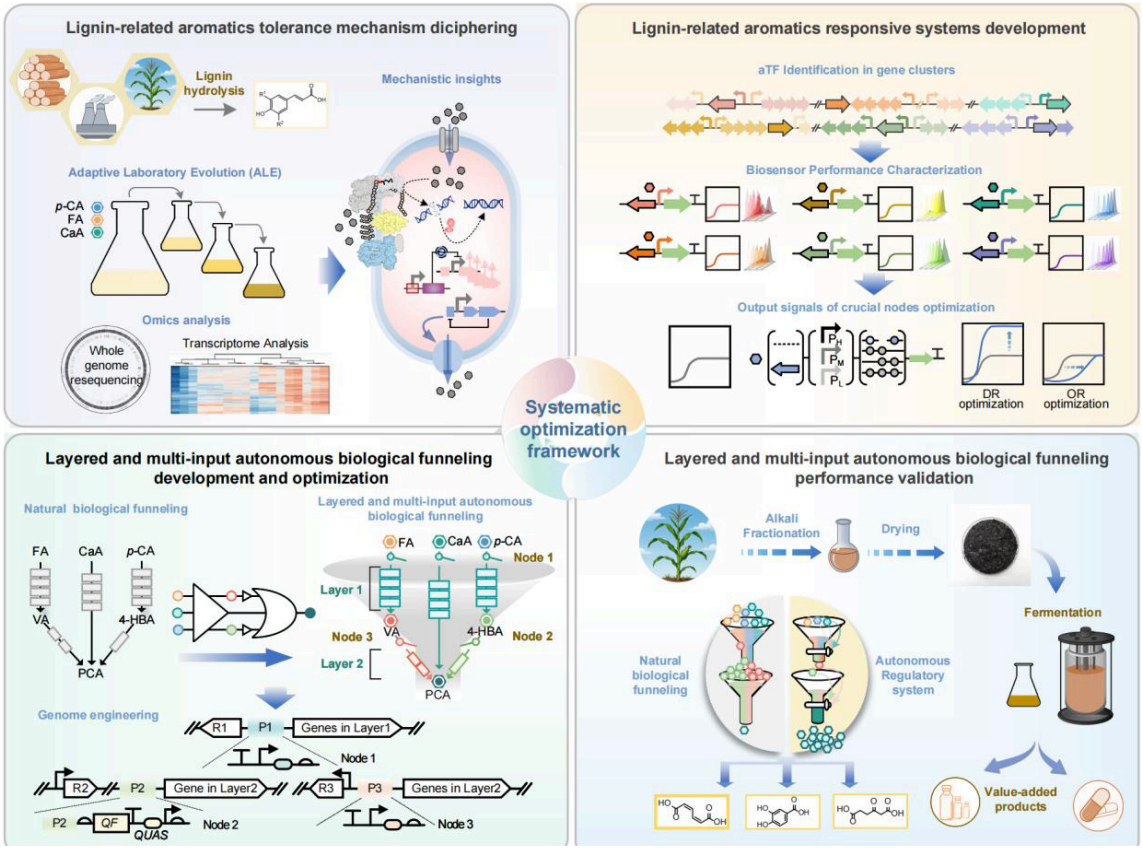

Figure 7. Overall optimization framework for the layered multi-input autonomous biological-funneling system from the 2026 Nature Communications study [4]

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Figure 7 lays out why the SASR7 result is a design logic rather than a lucky mutant: tolerance is first installed by ALE, six biosensors then instrument the substratetointermediate space, and only then is the two-tier feedback circuit wired on top. Each module depends on the one before it.

To obtain stress-resistant host chassis, ALE adaptive laboratory evolution was implemented to construct a triple-synergy aromatic-tolerant strain. Wild-type C. glutamicum ATCC 13032 cannot grow with 20 mM p-CA. Serial passaging under stepwise-increased substrate concentrations yielded the highly-tolerant clone STALE9, capable of surviving 70 mM p-CA/FA and 80 mM CaA. Whole-genome re-sequencing, multi-omics and gene-complementation experiments uncovered three synergistic anti-toxicity mechanisms. A point mutation in wzz enhances exopolysaccharide (EPS) secretion, thickening the cell-wall barrier to limit aromatic-substrate permeation and reduce intracellular toxin loads. A promoter mutation in lexA down-regulates repressor abundance and constitutively activates SOS DNA-repair responses to alleviate aromatic-agent-triggered DNA damage. Combined mutations in the AtsR repressor-binding site and its promoter derepress MMPL-family aromatic efflux pumps, accelerating expulsion of intracellular phenolic toxins to the extracellular milieu. The triple-mutant chassis designated SHT1 (STALE9 with pcaHG deletion to block PCA downstream degradation) exhibits >2-fold higher substrate-degradation rates relative to wild-type cells, resolving aromatic-toxicity bottlenecks and furnishing robust hosts for dynamic-circuit integration.

That tolerance does not come from a single largeeffect mutation: Figure 8 shows the resequencing and multiomics evidence converging on three independent mechanisms—wzzmediated EPS thickening, lexA-driven SOS upregulation, and AtsRderepressed efflux—that have to act together before the strain survives 70 mM p-CA.

Building on the robust chassis, a toolkit of six endogenous aromatic-responsive-transcription-factor biosensors was established. Six native C. glutamicum regulators (PhdR, PcaO, GenR, BenR, RolR, IhtR) covering upstream-substrate and intermediate nodes were mined. Via hybrid-promoter engineering and fungal QF transcriptional-amplifier circuits, dynamic ranges of three core regulatory nodes (PhdR, PcaO, VanR) were elevated from 2-fold ~4-fold up to 10.00-fold ~17.36-fold, with effective response windows expanded to 0.1-42 mM, enabling precise signal perception across broad concentration ranges. Node 1 (PhdR) senses upstream phenylpropanes (p-CA/FA/CaA) and controls Layer 1 phdAEBC. Node 2 (PcaO) detects intermediate 4-HBA and governs pobA with QF amplification. Node 3 (VanR) responds to intermediate VA and regulates demethylase-encoding vanAB.

Their expanded dynamic ranges—up to 17.36-fold across a 0.1–42 mM window (Figure 9)—are what make multi-input control feasible: without each sensor covering a distinct concentration band, the upstream and downstream layers could not be independently tuned.

The two-tier cascade autonomous feedback regulatory circuit in SASR7 was assembled. When lignin-derived aromatic monomers are present, PhdR dissociates from operator sites and triggers transcription of phdAEBC, catalysing conversion of p-CA/FA/CaA to 4-HBA and VA. As 4-HBA and VA accumulate, PcaO and VanR respectively activate transcription of pobA and vanAB and accelerate intermediate consumption. A positive closed-loop logic is formed: higher substrate loads drive higher upstream flux; rising intermediate concentrations further up-regulate downstream enzyme expression, automatically balancing pathway-layer fluxes and intrinsically suppressing intermediate accumulation. In shake-flask experiments simulating mixed lignin substrates (p-CA:FA:CaA = 4:1:0.5; total 65.93 mM), 65.86 mM PCA was obtained after 30 h fermentation with 99.89 % molar conversion; intermediate concentrations remained consistently below 5 mM. By contrast, the static-control strain SHT1 only reached 62.04 % conversion.

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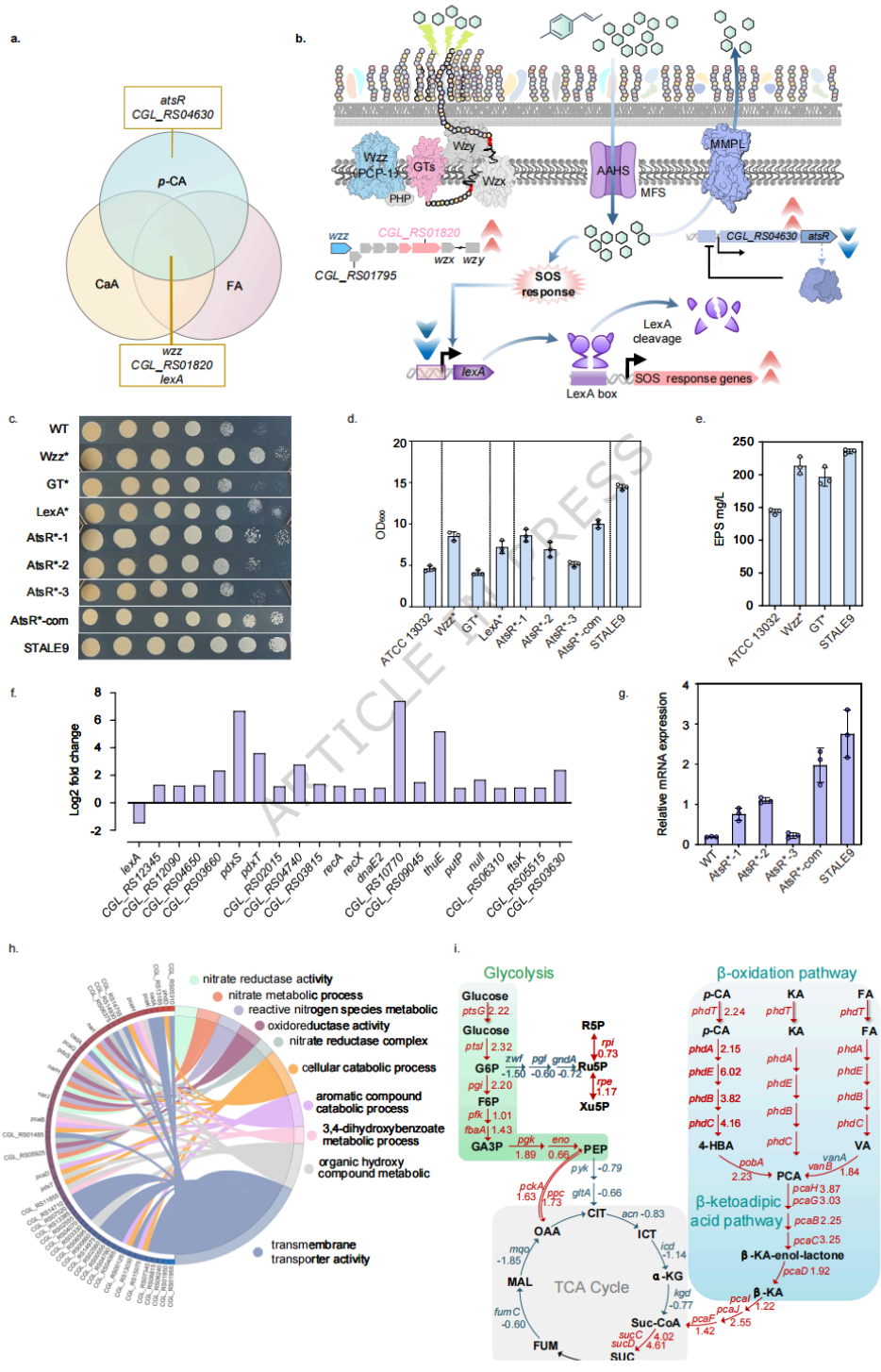

Figure 8. Multi-omics validation of triple aromatic-tolerance mechanisms for ALE-evolved strains [4]

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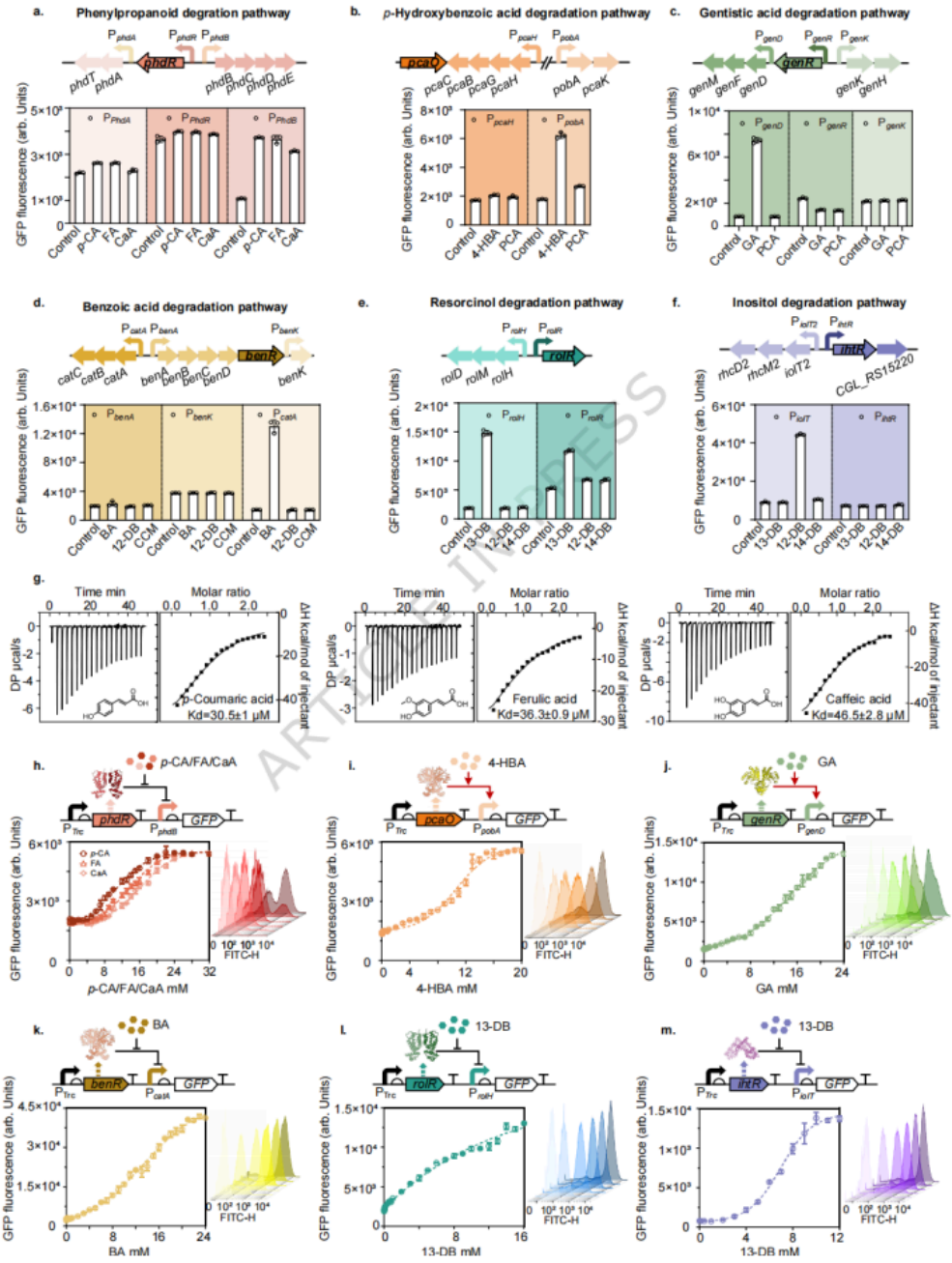

Figure 9. Full characterization of fluorescence-readout responses for six endogenous aTF aromatic biosensors [4]

The payoff of wiring these sensors into a closed loop is visible in Figure 10: where the static SHT1 control stalls at 62.04% conversion, SASR7 reaches 99.89% on the same 65.93 mM mixed substrate while holding intermediates below 5 mM—because rising 4HBA and VA automatically accelerate the downstream enzymes rather than overwhelming them.

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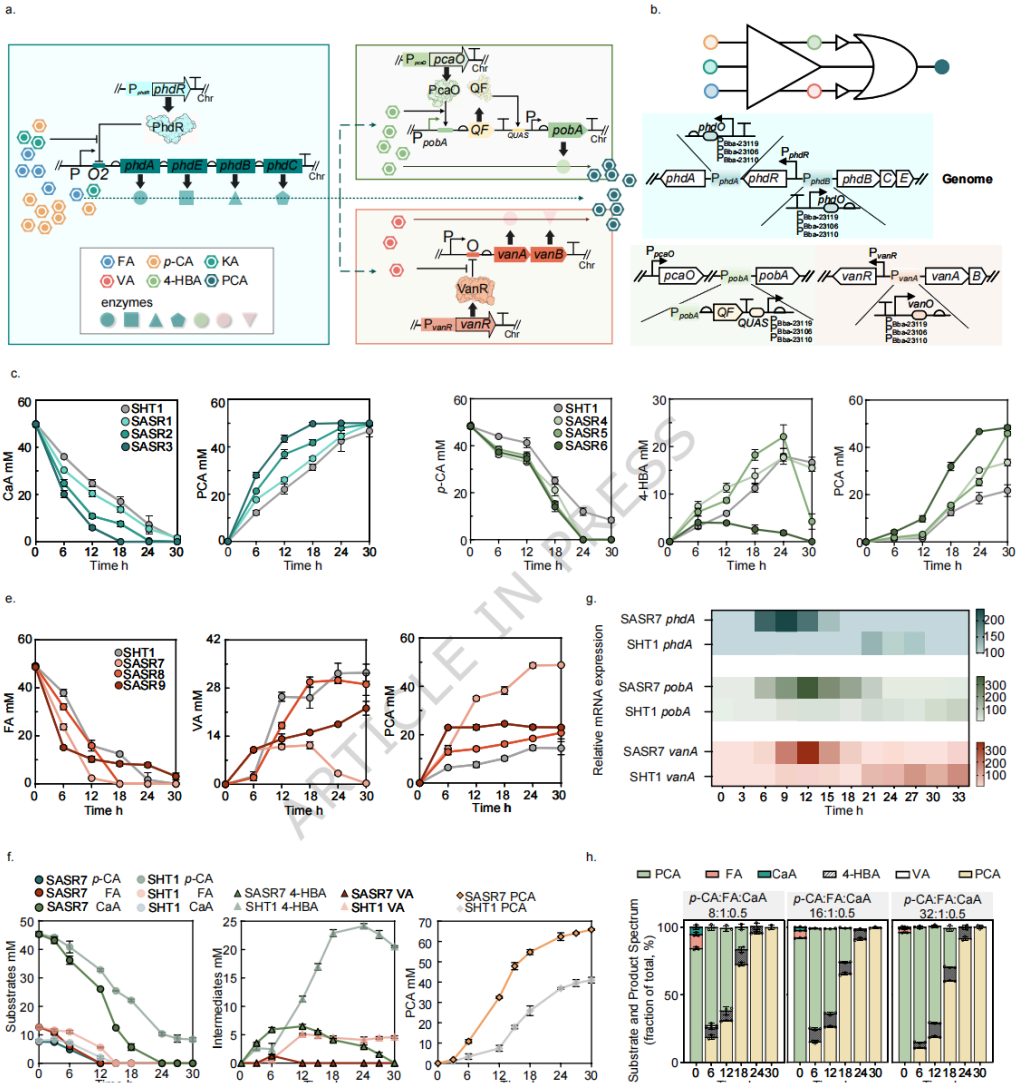

Figure 10. Genetic-circuit design and shake-flask time-course profiles of SAS-series strains [4]

Subsequently, 5 L bioreactor scale-up and real-lignin-hydrolysate validation were performed. In fed-batch fermentation with six successive substrate feedings, 388.80 mM total mixed phenylpropane substrates were consumed. The final PCA titre reached 53.40 g L^{-1} with volumetric productivity of 1.27 g L^{-1} h^{-1}. Two derivative engineered strains were generated via downstream-pathway remodelling. SASR7-C1 was constructed by introducing the aroY catechol-synthesis module and deleting competitive catB, yielding 51.90 g L^{-1} CCM in 5 L fermentation. SASR7-K19 carries a pcaIJ deletion blocking β-KA catabolism and produced 38.33 g L^{-1} β-KA. Direct fermentation of non-detoxified corn-stover alkaline pretreatment liquor achieved 99.44 % molar PCA yield, demonstrating compatibility with industrial-relevant feedstock.

Crucially, Figure 11 shows that this closedloop behaviour survives scaleup: across six fed-batch additions, 388.80 mM of mixed phenylpropane was consumed, yet the same <5 mM intermediate ceiling held throughout—so the 53.40 g/L PCA titre reflects balanced flux, not accumulated dead--end intermediates.

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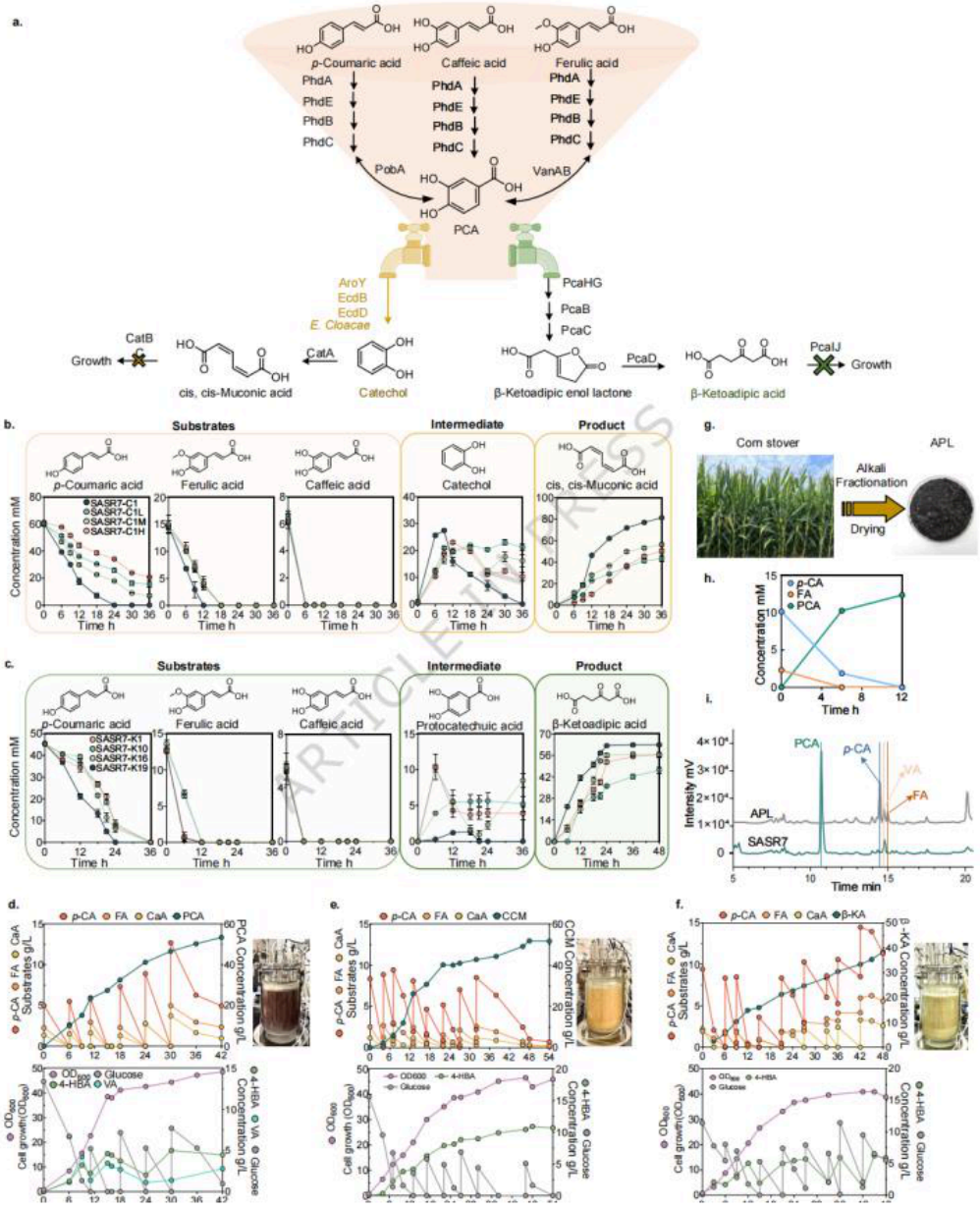

Figure 11. Time-course profiles of substrate, intermediate and product concentrations in 5 L bioreactor fermentations using SASR7 derivatives [4]

This landmark work achieves the three-pronged integration of "ALE-tolerant chassis + multi-node biosensing + layered closed-loop regulation". Three major industrial bottlenecks including aromatic cytotoxicity, upstream-downstream flux imbalance and massive intermediate accumulation are simultaneously overcome. A modular, general-purpose biological-funneling engineering paradigm is established, where diverse high-value chemicals can be synthesized merely by minor modification of downstream branch pathways.

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4.4. Quantitative cross-comparison of three regulatory generations

Table 2 quantifies why each earlier generation was a dead end. Static firstgeneration circuits run blind—no substrate sensing, one independent layer, <60% conversion with 30.5 mM intermediates piling up. Singlesensor secondgeneration loops cut that to ~70% and 15 mM, but they watch only the upstream substrate and cannot see 4HBA or VA building up. The 2026 NC design closes both intermediate loops at once: three signals, two cascade layers, 99.89% conversion, <5 mM residual, and direct use of nondetoxified hydrolysate.

Table 2. Key-performance-parameter comparison across three generations of biological-funneling regulatory strategies. Core data on mixed-substrate conversion and intermediate accumulation originate from benchmark reference [4]; remaining metrics are adapted from reviews [12, 15]

Regulatory generation	Types of input signals	Pathway-synergy layers	Molar conversion for mixed substrates	Maximum intermediate accumulation	Direct utilization of non-detoxified lignin hydrolysate
1st-generation static regulation	0 (constitutive continuous expression)	Single-layer independent	<60 %	30.5 mM	No
2nd-generation single-sensor regulation	1 upstream-substrate signal	Single-layer	≈70 %	15.0 mM	Limited compatibility
3rd-generation multi-layer autonomous regulation (2026 NC)	3 signal types (substrate + two intermediates)	Two-tier cascade closed-loop	99.89 %	<5.0 mM	Fully compatible

4.5. Inherent limitations of third-generation systems

Signal amplification depends on heterologous fungal-derived QF activator protein, imposing additional metabolic burden upon C. glutamicum. Validation was restricted to herbaceous corn-stover lignin liquor; compatibility with woody lignin and pulp-mill black liquor has not been systematically evaluated. So far, only fed-batch fermentations have been carried out; coupled continuous-fermentation and in-situ-product-removal workflows remain unexplored.

5. Downstream high-value-chemical synthesis and industrial-chain applications

As the unified central intermediate of biological funneling, PCA can be redirected via distinct downstream branches toward aromatic platform intermediates, nylon-forming dicarboxylic acids, biodegradable-plastic monomers, flavouring agents and energy-storage substrates. The 2026 layered-autonomous-funneling platform serves as a universal upstream backbone; target-product profiles can be switched simply by replacing downstream metabolic modules. This chapter summarizes synthetic pathways and industrial value of representative products.

5.1. Aromatic-platform intermediate: protocatechuic acid (PCA)

PCA acts as an antioxidant pharmaceutical intermediate and can be deployed as safe liquid fuel for microbial fuel cells. Homma et al. [16] constructed PCA-utilizing microbial fuel cells exhibiting comparable power-generation performance and superior storage stability relative to conventional ascorbate-based systems. SASR7 reached 53.40 g L^{-1} PCA in 5 L fermentation, representing the highest reported titre for lignin-derived PCA and laying a foundation for industrial isolation.

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5.2. Bulk industrial chemicals: nylon-precursor dicarboxylic acids

cis, cis-muconic acid (CCM) can be hydrogenated to adipic acid, the critical petroleum-derived monomer for nylon 6,6. In the NC-reported SASR7-C1 strain, PCA is decarboxylated by AroY to catechol, and CatA-mediated ring-opening yields CCM, with 51.90 g L^{-1} titre in 5 L bioreactors.

Kohlstedt et al. [17] validated the full cascade workflow "lignin → CCM → adipic acid → nylon 6,6" at kilogram-scale pilot conditions.

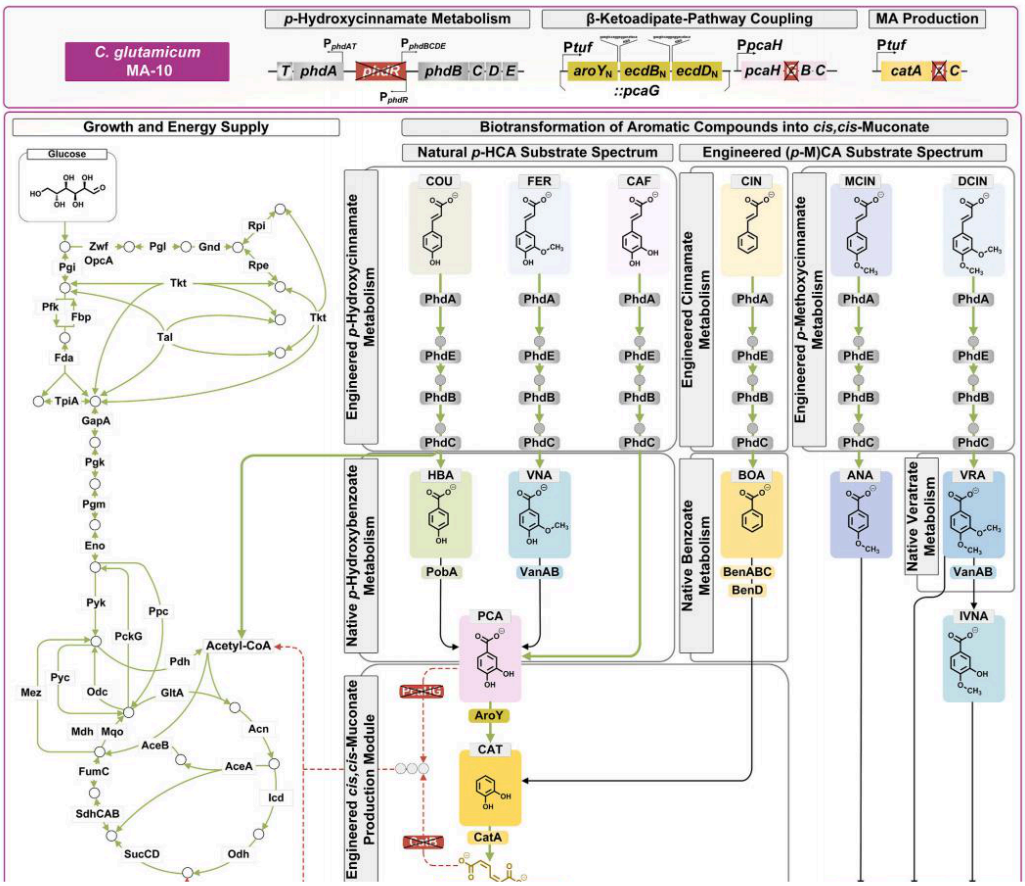

Figure 12. Complete metabolic pathway for cis,cis-muconic-acid synthesis by engineered Corynebacterium glutamicum MA-10 [12]

The route from PCA to CCM is short but enzymatically specific (Figure 12): AroY decarboxylates PCA to catechol, CatA opens the ring to cis,cis-muconate, and deleting catB blocks the competing branch—this is the minimal downstream rewiring that turns the SASR7 platform from a PCA producer into a CCM producer.

β-ketoadipic acid (β-KA) is used for high-barrier specialty-nylon films with improved moisture-blocking performance compared with conventional nylon. Deletion of pcaIJ blocks β-KA entry into the TCA cycle. SASR7-K19 produces 38.33 g L^{-1} β-KA in 5 L fermentation, delivering promising precursors for specialty bio-nylon.

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5.3. Novel biodegradable-polyester monomer: 2-pyrone-4, 6-dicarboxylic acid (PDC)

Heterologous expression of the ligABC pathway converts PCA into PDC. PDC can undergo polycondensation with 1,4-butanediol to form thermostable bio-polyester as a petroleum-polyester replacement. The highest reported PDC titre for engineered Pseudomonas putida reaches 22.7 g L^{-1}. The layered-regulatory C. glutamicum platform offers opportunities to further boost conversion performance.

PDC is unusual among ligninderived products in that it is not a dead-end chemical but a monomer (Figure 13): after ligABC converts PCA to PDC, copolycondensation with 1,4-butanediol yields a thermostable polyester—so the upstream funnel feeds directly into material manufacturing rather than stopping at a platform intermediate.

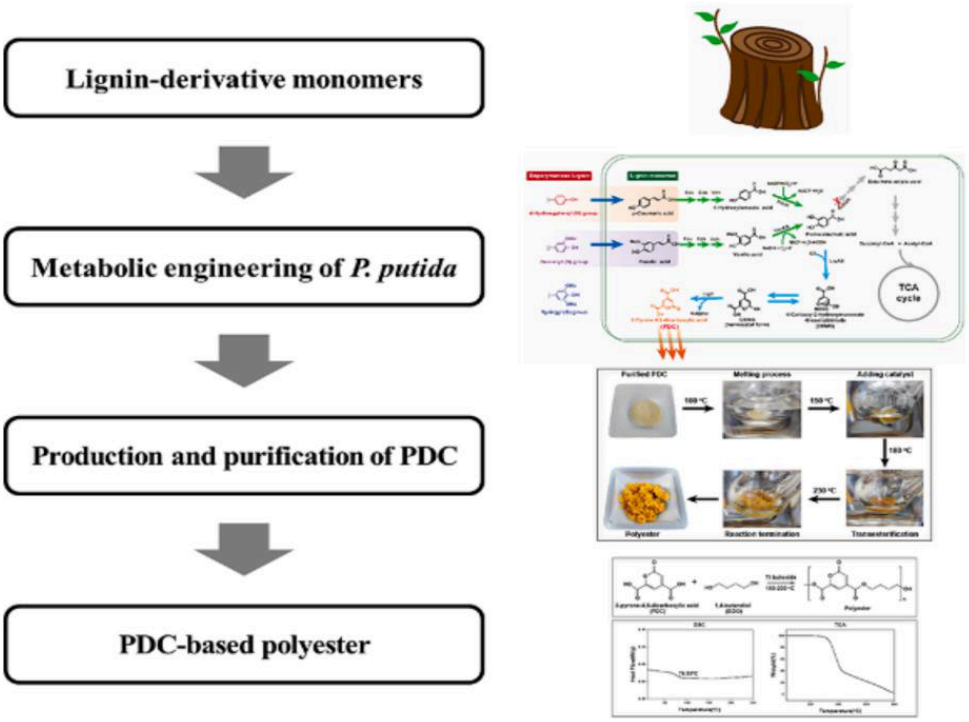

Figure 13. Workflow of PDC biosynthesis, purification and thermal-processing for bio-polyester manufacturing [10]

5.4. Niche high-value-added products

Vanillin serves as food- and cosmetic-grade fragrance, which can be biosynthesized from FA via step-wise catabolism in engineered yeast or bacteria. Polyhydroxyalkanoate (PHA) biodegradable plastics can be produced by co-assimilating lignin-derived aromatics and ethanol carbon sources. 4-vinylguaiacol is widely-used food-flavour additive.

The layered multi-input autonomous biological-funneling platform represents a versatile upstream conversion backbone. With fixed ALE-domesticated chassis and multi-sensor regulatory circuits, different target chemicals can be generated merely by swapping downstream metabolic modules. One chassis supports multiple product lines, reducing strain-development and fermentation-equipment-retrofit costs and satisfying flexible-production demands for biorefinery plants.

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6. Full-chain bottlenecks and optimization strategies for lignin biorefinery

Combining intrinsic drawbacks of the 2026 NC system and common challenges within the field, bottlenecks are analysed across chassis strain, regulatory element, raw-material compatibility and fermentation-process dimensions, and corresponding synthetic-biology and fermentation-engineering countermeasures are proposed.

6.1. Bottlenecks and optimizations for microbial chassis

Mutations conferring ALE-acquired aromatic tolerance are prone to gene loss during long-term serial passaging, compromising strain genetic stability. Targeted genomic integration of key tolerance mutations (wzz, lexA, AtsR) into stable chromosomal loci can generate robust tolerant chassis without repeated adaptive evolution. Heterologous QF transcriptional-amplifier protein imposes metabolic burden and slows cell proliferation. Endogenous orthogonal transcriptional-activator elements mined from C. glutamicum can replace eukaryotic QF and eliminate heterologous-protein-associated load. Current strains are mainly optimized for herbaceous straw hydrolysates, and conversion efficiency drops for woody lignin and pulp-mill black liquor. Composite-substrate adaptive-laboratory-evolution using mixed H/G/S aromatic feedstocks can broaden substrate-recognition and tolerance spectra.

6.2. Bottlenecks and optimizations for regulatory elements

Existing PhdR, PcaO, VanR transcription factors possess narrow ligand-binding pockets. Low-abundance intermediates fail to trigger sufficient transcriptional responses. Site-directed mutagenesis of transcription-factor ligand-binding domains can enlarge substrate-recognition spectra and improve sensing sensitivity towards dilute intermediates, adapting to industrial hydrolysates with highly variable component profiles.

6.3. Bottlenecks and optimizations for coupled pretreatment-fermentation

Although EDA-produced low-condensation lignin delivers excellent bioconversion performance, pretreatment-related costs remain substantial. Integrated "pretreatment-fermentation" workflows should be developed, in which pretreated liquor is directly transferred into bioreactors without concentration and purification steps to cut energy-intensive concentration operations and overall operating expenditure.

6.4. Bottlenecks and optimizations for industrial fermentation

Current experiments are limited to fed-batch fermentation, with volumetric productivity ranging from 1.0-1.3 g L^{-1} h^{-1}, still lagging behind requirements for continuous industrial production. Macroporous-resin-based in-situ product-removal technology can be introduced. PCA / CCM products are adsorbed and removed during fermentation to relieve product-feedback inhibition, enhancing volumetric productivity and total batch product yields. According to techno-economic estimates by Tuck et al. [18], in-situ separation can reduce downstream-purification costs by more than 30 %.

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7. Conclusions and future perspectives

Biological funneling represents the optimal microbial-conversion strategy addressing lignin-monomer heterogeneity. Regulatory technologies have evolved from static engineering, through single-sensor regulation, to multi-layer autonomous control. The layered multi-input autonomous system in Corynebacterium glutamicum reported by Shandong University in 2026 achieves substantial performance improvement. Enabled by ALE-driven triple aromatic-stress tolerance and six endogenous aTF-based two-tier cascade feedback circuits, mixed-aromatic-substrate conversion reaches 99.89 %. Non-detoxified corn-stover alkaline lignin liquor can be directly fermented, overcoming core industrial obstacles of aromatic cytotoxicity and metabolic-flux imbalance. Benefiting from high modularity, the platform can synthesize PCA, CCM, β-KA and other bio-based chemicals with minor downstream-pathway adjustments, establishing complete green industrial-chain routes converting agro-forestry waste to bioplastics.

Looking forward to future research directions: mining endogenous low-burden transcriptional amplifiers in C. glutamicum to replace heterologous QF proteins; performing composite-aromatic-substrate ALE domestication of chassis to expand compatibility toward woody lignin and pulp-mill black liquor; developing in-situ-product-separation coupled continuous-fermentation processes for improved productivity and industrial continuity; designing integrated workflows combining EDA / TAFT green pretreatment with engineered biological-funneling strains; constructing multi-input AND / OR logic-gate genetic circuits to stabilize metabolic-flux balance under extreme feedstock-composition fluctuations.

The layered multi-input autonomous biological-funneling paradigm provides standardized synthetic-biology solutions for low-cost, large-scale lignin valorization. It facilitates the transition of agro-forestry and pulp-mill waste lignin from low-value fuel feedstock toward petroleum-substituting aromatic chemical feedstock, supporting full-component green biorefinery industrial-chain implementation under carbon-neutrality goals.

References

[1] Ragauskas, A. J., Beckham, G. T., Biddy, M. J., et al. (2014). Lignin valorization: Improving lignin processing in the biorefinery. *Science, 344*(6185), 1246843.

[2] Beckham, G. T., Johnson, C. W., Karp, E. M., et al. (2016). Opportunities and challenges in biological lignin valorization. *Current Opinion in Biotechnology, 42*, 40-53.

[3] Liu, Z.-H., Li, B.-Z., Yuan, J. S., & Yuan, Y.-J. (2022). Creative biological lignin conversion routes toward lignin valorization. *Trends in Biotechnology, 40*(12), 1550–1566.

[4] Xu, J., Zhang, J., Wang, X., et al. (2026). Engineering a robust and autonomous biological funneling for efficient valorization of lignin-related aromatics. *Nature Communications*. https://doi.org/10.1038/s41467-026-74424-y [5] Xu, L., et al. (2022). High-solid ethylenediamine pretreatment to fractionate new lignin streams from lignocellulosic biomass. *Chemical Engineering Journal, 427*, 130962.

[6] Chen, K., et al. (2023). Tandem fractionation technologies (TAFT) benefit multi-functional porous carbon toward lignin valorization. *Chemical Engineering Journal, 474*, 145815.

[7] Ntakirutimana, S., et al. (2023). Chemomechanical pretreatment for efficient delignification and saccharification of corn stover biomass. *Chemical Engineering Journal, 471*, 144588.

[8] Li, J., et al. (2022). Construction of a p-coumaric and ferulic acid auto-regulatory system in Pseudomonas putida KT2440 for protocatechuate production from lignin-derived aromatics. *Bioresource Technology, 344*, 126786. [9] Liu, H., et al. (2024). Engineering Pseudomonas putida for lignin bioconversion into cis,cis-muconic acid. *Chemical Engineering Journal, 495*, 153375.

[10] Bugg, T. D. H., Williamson, J. J., & Alberti, F. (2021). Microbial hosts for metabolic engineering of lignin bioconversion to renewable chemicals. *Renewable and Sustainable Energy Reviews, 152*, 111674.

DOI: 10.54254/2753-8818/2026.39500

[11] Weiland, F., Barton, N., Kohlstedt, M., et al. (2023). Systems metabolic engineering upgrades Corynebacterium glutamicum to high-efficiency cis,cis-muconic acid production from lignin-based aromatics. *Metabolic Engineering, 75*, 153-169.
[12] Weiland, F., et al. (2025). Metabolic engineering of Corynebacterium glutamicum for increased cis,cis-muconate production from plant-derived p-hydroxycinnamates via deregulated pathway flux and increased CoA availability. *Metabolic Engineering, 92*, 262-283.
[13] Becker, J., Kuhl, M., Kohlstedt, M., Starck, S., & Wittmann, C. (2018). Metabolic engineering of Corynebacterium glutamicum for the production of cis,cis-muconic acid from lignin. *Microbial Cell Factories, 17*, 115.
[14] Xin, X., et al. (2024). Engineering yeast to convert lignocellulose into vanillin. *Chemical Engineering Journal, 485*. [15] Weiland, F., Kohlstedt, M., & Wittmann, C. (2022). Guiding stars to the field of dreams: Metabolically engineered pathways and microbial platforms for a sustainable lignin-based industry. *Metabolic Engineering, 71*, 13-41. [16] Homma, T., Tsurusaki, Y., Kamimura, N., et al. (2021). Protocatechuic acid fuel: A sustainable energy generation system based on lignin-derived aromatic compounds. *Biomass and Bioenergy, 154*, 106254.
[17] Kohlstedt, M., et al. (2018). From lignin to nylon: Cascaded chemical and biochemical conversion using metabolically engineered Pseudomonas putida. *Metabolic Engineering, 47*, 279-293.
[18] Tuck, C. O., Pérez, E., Horváth, I. T., et al. (2012). Valorization of biomass: Deriving more value from waste. *Science, 337*(6101), 695-699.