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Advancing lignocellulosic conversion though biosensor-enabled metabolic engineering

Qi Gan, Jianli Zhang, Xinyu Gong, Yusong Zou and Yajun Yan *

Lignocellulosic biomass holds great potential to produce a wide range of chemicals, including biofuels, biomaterials, and bioactive compounds. Effective utilization of these biomass feedstocks can significantly benefit human well-being while helping to mitigate climate change and reduce the environmental damage associated with fossil fuel use. Microbial synthesis plays a key role in converting biomass into valuable products. However, further optimization of these metabolic pathways is required to improve productivity. The design and optimization of these pathways remain major bottlenecks due to the complexity of biological systems and our limited understanding of them. Biosensors hold significant potential in advancing microbial metabolic engineering and enhancing substrate-to-product bioconversion. In this review, we discuss the major microbial conversion pathways for lignocellulosic biomass, the development and optimization of biosensors, and their applications in efficient biocatalytic processes for lignocellulosic conversion.

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1. This review highlights recent progress in integrating biosensors with metabolic engineering to enhance the biological conversion of lignocellulosic biomass – a key strategy for sustainable biomanufacturing.
2. Biosensor-enabled metabolic engineering offers precise, real-time control over microbial pathways, allowing systems to adapt dynamically to changing substrates or stress conditions. This makes the approach widely relevant to biotechnology and sustainable industrial bioprocessing.
3. The convergence of biosensor technology, systems biology, and machine learning will drive the next generation of smart, adaptive microbial platforms for biomass valorization. The insights presented in this review provide a foundational framework for designing such systems and will help shape the future of green chemistry by advancing cleaner, more efficient, and more sustainable conversion technologies.

Introduction

Lignocellulosic biomass, one of the most abundant renewable resources on earth, is sourced from plant materials such as agricultural residues, forestry by-products, and energy crops.[1] Composed primarily of cellulose, hemicellulose, and lignin, its depolymerization yields hexoses, pentoses, and aromatic compounds that serve as crucial substrates for biorefineries.[2] The conversion of these compounds supports the development of sustainable alternatives to fossil-based resources.[3] Lignocellulose holds significant value for producing an extensive array of valuable products, including biofuels, biomaterials, and bioactive compounds.[1,4] For instance, lignocellulosic biomass has been utilized to produce bioethanol, biodiesel, and other valuable products, such as butanol, isoamyl alcohol, coumarin and terpenes.[5,6] Despite this immense potential, the inherent recalcitrance of lignocellulosic biomass presents a substantial barrier to its efficient bioconversion, ultimately limiting overall productivity.[7,8]

Microbial conversion plays critical role in transforming lignocellulosic biomass into valuable products, but it faces significant challenges, particularly in the development of lignocellulosic biomass biodegradation and conversion techniques for platform chemicals that can contribute to increased biofuel production while lowering the cost.[9,10] While certain microorganisms can degrade lignin, limitations such as low enzyme activity, slow growth rates, and cultivation difficulties limit their effectiveness. Robust engineered strains with high depolymerization efficiency are therefore essential to overcoming these challenges and maximizing lignin breakdown. For industrial applications, optimal strains must achieve high lignin conversion efficiency, elevated product titers, and consistent performance stability. Recent advancements in synthetic biology have expanded opportunities to produce a

School of Chemical, Materials, and Biomedical Engineering, College of Engineering, The University of Georgia, Athens, GA 30602, USA. E-mail: yajunyan@uga.edu

variety of high-value compounds using microbial cell factories.[11] However, introducing heterologous or novel biosynthetic pathways into microbial hosts often leads to metabolic stress and imbalances in metabolic flux.[12] Therefore, precise pathway design and optimization are crucial to balance achieve metabolic balance, thereby improving both product yield and titer.[6]

To achieve pathway optimization and protein engineering in biocatalysts often requires extensive screening of mutation libraries to identify high-yielding strains and efficient biocatalysts. Biosensors are biological components that detect and respond to specific molecules or conditions in a cell or environment, often producing a measurable output. And they play a vital role in this process by detecting specific metabolites, environmental conditions, or cellular signals, thus enabling precise monitoring of cellular responses.[13,14] This capability facilitates high-throughput phenotypic screening, allowing rapid identification of desired traits. Furthermore, the integration of biosensors with metabolic engineering enables fine-tuning of metabolic activities based on real-time data, enhancing microbial adaptability to fluctuations in fermentation conditions and improving both the efficiency and stability of product formation.

Previous reviews have comprehensively summarized recent advancements, advantages, and limitations of both thermochemical depolymerization and biological conversion processes for the valorization of lignocellulosic biomass into valuable products.[15–17] They have also explored the potential for sustainable industrial application of waste lignin through integrated valorization strategies that combine with artificial intelligence. Here, our review primarily focuses on the application of biosensors in lignocellulosic biomass conversion, with particular emphasis on biological conversion processes. This review is intended for early-stage researchers, graduate students, and interdisciplinary scientists who are entering the fields of synthetic biology, metabolic engineering, and biomass valorization. We first summarized the basic advances in biomass conversion, followed by an overview of the discovery and optimization of biosensors that respond specially to the key intermediates in the lignocellulosic biomass conversion process. We then explore major applications of these biosensors: visualization of metabolite dynamics, dynamic metabolic regulation, and high-throughput screening and evolution. Finally, we provide conclusions and perspectives on the future development of small molecule biosensors and their application in enhancing lignocellulosic biomass conversion.

Lignocellulosic biomass and its bioconversion pathways

Lignocellulose is mainly composed of three key structural components: lignin, cellulose and hemicellulose, each of which can be converted into valuable products through distinct metabolic pathways.[1] Cellulose is a linear polymer of glucose, hemicellulose is a heteropolysaccharide with various sugar monomers, and lignin is a complex phenolic compound. Due to its high carbon-to-oxygen ratio and aromatic content, lignin holds significant potential as a feedstock for biofuels and high-value chemicals.[18,19] However, the complex structure and recalcitrance of lignocellulosic biomass present considerable challenges for efficient conversion into bioproducts.

The typical process for converting lignocellulose into valuable products involves pretreatment, enzymatic degradation, and fermentation. During pretreatment, lignocellulosic biomass is broken down to increase the accessibility of cellulose and hemicellulose for enzymatic or microbial action, although the intricate structure of lignin can complicate this step.[2,20,21] Following pretreatment, enzymes such as cellulases and hemicelluloses are added to degrade cellulose and hemicellulose into fermentable sugars like glucose and xylose. This enzymatic hydrolysis is critical, as the released sugars are required for microbial fermentation. However, enzyme activity must be optimized, as residual lignin and inhibitors generated during pretreatment can impede efficiency. In the fermentation step, specialized microorganisms, including yeasts and bacteria, are used to convert these sugars into desired bioproducts. For instance, *Saccharomyces cerevisiae* is commonly used for ethanol production, while other engineered microbes are tailored for the synthesis of biofuels, bioplastics, and other valuable chemicals.[22]

Microbial deconstruction of lignocellulose depends on enzymatic processes that break down cellulose and hemicellulose, with cellulases converting cellulose into cellobiose and glucose and hemicelluloses releasing pentoses (*e.g.*, xylose) and hexoses. These sugars enter central metabolic pathways such as glycolysis and the pentose phosphate pathway, leading to biofuel and chemical production (Fig. 1). However, the breakdown of lignin into aromatic compounds, like vanillin or ferulic acid, requires specialized enzymes (*e.g.*, peroxidases, laccases) and is less efficient, as lignin-derived intermediates can inhibit microbial growth. Additional bottlenecks include substrate inhibition, where the accumulation of sugars or intermediates impedes enzyme function, and metabolic pathway inefficiencies, caused by imbalanced fluxes and slow reaction rates at key steps.[20] To overcome these challenges, innovative approaches in metabolic engineering, enzyme optimization, and dynamic regulation strategies is essential, enabling efficient and sustainable lignocellulosic bioconversion.[18]

Biosensors in microbial metabolic engineering

Biosensors, such as transcription factor-based, whole cell based, nucleic acid-based, and protein-level biosensors, play a critical role in simplifying the engineering process by providing precise and efficient ways to monitor and control metabolic activities,[23,24] Each type has distinct mechanisms and applications that contribute to advancements in biotechnology and synthetic biology (Fig. 2). Among them, transcription

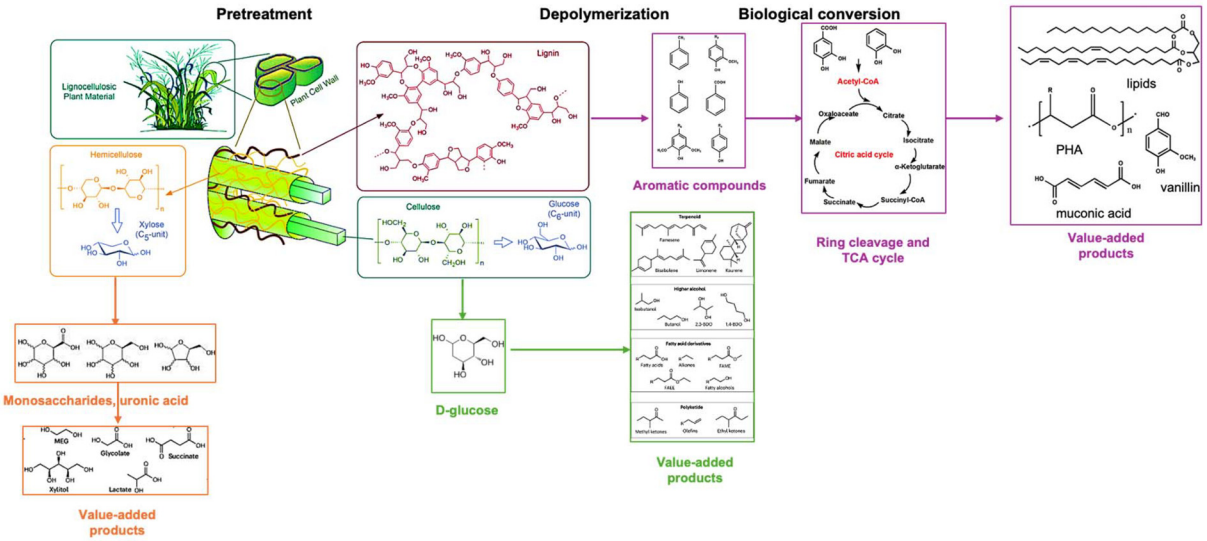

Fig. 1 Key steps in the conversion of lignocellulosic biomass to value-added products.

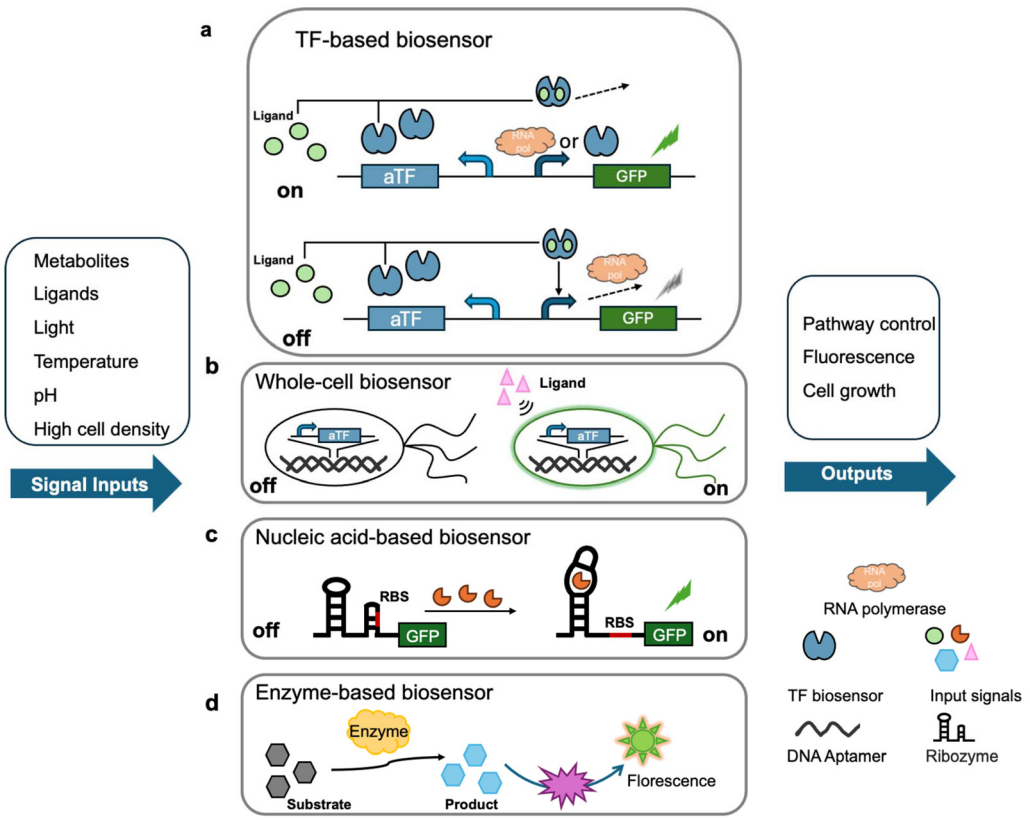

Fig. 2 The major categories of genetically encoded biosensors. (a) Transcriptional Activation *via* TF-Based Biosensor. Upon signal input, the TF undergoes a conformational change, dissociates from the promoter region, and allows RNA polymerase to initiate transcription. Transcriptional Repression *via* TF-Based Biosensor, in the presence of the signal, the transcription factor (TF) binds to its target promoter, blocking RNA polymerase binding and repressing gene expression. (b) Whole-cell biosensors are engineered microorganisms that respond to specific environmental or intracellular signals by producing a measurable output, such as fluorescence, colour change, or electrical signal. (c) Nucleic acid-based biosensors, utilize nucleic acids (DNA or RNA) as recognition elements to detect specific biomolecules, pathogens, or other analytes. (d) Enzyme-based biosensors, utilize enzymes as biological recognition elements to detect specific substrates or other analytes. These biosensors capitalize on the catalytic activity of enzymes, which can produce a measurable signal in response to the presence of a target substance.

factor-based biosensor is most widely used in metabolic engineering.[25] These biosensors utilize transcription factors that respond to specific molecules by activating or repressing gene expression. Transcription factor repressor is a molecule that inhibits gene expression by preventing transcription, such as the tetracycline repressor tetR and the tryptophan repressor TrpR. The repressor binds to specific DNA sequences or interacts with transcription factors to block RNA polymerase from transcribing the target genes. In the absence of the target compound, repressor can be used to "switch off" gene expression. When the target metabolite is present, it may interact with the repressor and then change its conformation, thereby relieving repression and allowing gene expression.[26] In contrast, a transcription factor activator is a molecule that binds to a transcription factor or directly to DNA, enhancing gene expression. Activators increase the transcription of specific genes, often in response to the presence of a target metabolite or environmental signal. For instance, in a biosensor designed to detect a particular metabolite, the metabolite itself can act as an activator, binding to the transcription factor and inducing the expression of a reporter gene, such as green fluorescent protein (GFP). To optimize the biosensor performance, various engineering approaches, including modifications to promoters, ribosome binding sites (RBS), and operators, have been employed to fine-tune the expression of biosensor components.[25] Additionally, structural domain exchanges of RNA and proteins have proven effective in altering specificity. Whole-cell biosensors are genetically engineered microbial systems that integrate sensing, signal transduction, and reporting functions within a living cell to detect specific target molecules. The mechanism typically involves three key components: a sensing module, a regulatory circuit, and a reporter output. The sensing module consists of a transcription factor or receptor that selectively recognizes the target analyte, often leading to a conformational change that modulates gene expression. This molecular interaction regulates a synthetic or native promoter that controls the downstream expression of a reporter gene, such as those encoding fluorescent proteins, enzymes, or luminescent markers. Upon activation, the reporter gene generates a quantifiable signal that correlates with the concentration of the target molecule. This design enables dynamic and real-time monitoring of environmental or intracellular conditions, making whole-cell biosensors powerful tools for applications in environmental monitoring, metabolic engineering, and synthetic biology (Fig. 2). Nucleic acid-based biosensors use engineered DNA or RNA elements to detect specific targets with high sensitivity and selectivity. Common designs include aptamers, DNAzymes, and toehold switches. Aptamers fold into defined structures that bind target molecules, triggering measurable outputs such as fluorescence or electrochemical signals. DNAzymes catalyse substrate cleavage in the presence of specific analytes, generating a detectable response. Toehold switches are synthetic RNA structures that block translation until a trigger RNA binds, activating reporter gene expression. Enzyme-based biosensors utilize the catalytic activity of enzymes to detect specific analytes through biochemical reactions that generate a measurable signal. These biosensors typically consist of an enzyme immobilized on a transducer surface, where the enzyme selectively reacts with the target molecule, producing a product that induces a detectable change—commonly in fluorescence, colour, pH, or electrochemical properties. The signal intensity correlates with analyte concentration, enabling quantitative analysis. Enzyme-based biosensors offer high specificity, fast response times, and sensitivity under mild conditions. They are widely used in clinical diagnostics, environmental monitoring, and food safety applications.

In microbial metabolic engineering, biosensors are employed to monitor and regulate the behavior of engineered microbes by providing real-time feedback on key metabolites or environmental conditions. The biological component, such as enzymes, nucleic acids, or receptors, interacts with the target molecule, and this interaction is converted into a measurable signal by the transducer, which can be optical, electrochemical, or acoustic.[13] Various types of biosensors are used in microbial systems, with optical, electrochemical, and fluorescence-based sensors being the most common. Biosensors have become indispensable tools in microbial metabolic engineering due to their ability to monitor critical parameters in real time. One of their primary applications is tracking metabolite concentrations within microbial cells, providing crucial insights into metabolic flux and the effectiveness of engineered pathways. They are also used to detect environmental changes, such as fluctuations in pH, light, or temperature, which can significantly impact microbial performance and product yields (Fig. 2).[13,27,28] Additionally, biosensors enable real-time feedback for optimizing metabolic pathways, allowing for dynamic adjustments based on immediate cellular responses. This real-time data is vital for fine-tuning metabolic activities, improving overall efficiency in fermentation processes, and reducing waste or by-product accumulation.[29] By continuously monitoring and responding to the internal and external states of microbial systems, biosensors help streamline and enhance the bioconversion of lignocellulosic biomass.

Recent advancements in biosensor technology have significantly expanded their capabilities and applications in microbial metabolic engineering (Fig. 3). Emerging biosensor technologies, such as synthetic RNA-based biosensors and CRISPR-based sensing systems, offer higher sensitivity, specificity, and the ability to respond to a wider range of metabolites and environmental signals.[30] Integrating these biosensors with synthetic biology tools, allowing for the creation of more sophisticated feedback loops and control systems. For example, Jiang *et al.* developed an autonomous cascaded artificial dynamic (AutoCAD) regulation circuit based on the PadR and FdeR biosensor systems to maintain pathway balance, resulting in a 16.5-fold increase in naringenin titer. In fed-batch fermentation, this system achieved a naringenin titer of 277.2 mg L^{-1}.[31] Biosensors can be coupled with genetic circuits to trigger the expression of specific genes in response to detected metabolites, providing a means for autonomous regu-

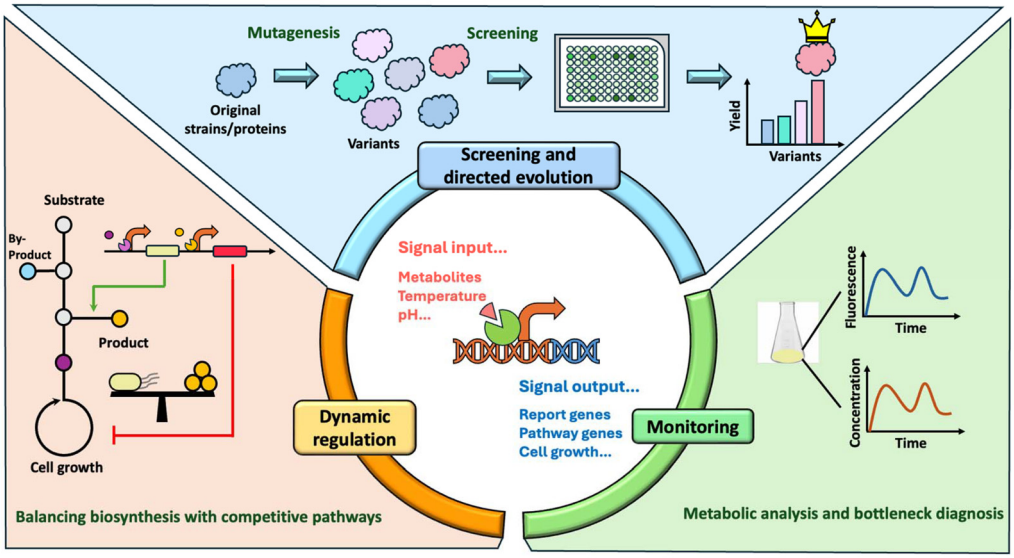

Fig. 3 Versatile applications of TF-based biosensors. (a) In real time monitoring the products or intermediates. (b) Dynamic pathway regulation system for improved production. Biosensors were utilized to activate the synthetic pathway and repress the TCA cycle, to direct the carbon flux to synthesis. (c) Transcriptional biosensors as screening methods to allow enzymes or strains characterization and evolution.

lation of metabolic pathways. This integration of biosensors with synthetic biology enables the construction of self-regulating microbial systems that can adapt to changing conditions, optimize resource utilization, and enhance overall productivity. These innovations are pushing the boundaries of microbial metabolic engineering, making it possible to create more robust and efficient microbial cell factories for sustainable biomanufacturing.

Biosensor-enabled optimization of lignocellulosic conversion

Real-time monitoring of metabolic intermediates

To improve the lignocellulosic conversion, it first requires the efficient depolymerization of lignocellulose into bioavailable substrates. Then achieve more efficiently break down complex sugars derived from cellulose and hemicellulose or to direct metabolic intermediates to desired end products. And it's also critical to increase the overall yield and efficiency of the bioconversion process and overcome challenges such as metabolic bottlenecks and by-product formation.[55] The ligninolytic activity of bacteria can release unmodified low-molecular-weight aromatics from lignin-rich waste streams. Several bacterial enzymes are capable of degrading lignocellulosic biomass and converting it into monomers. However, due to the heterogeneous and highly crosslinked structure of lignin, an efficient enzymatic process for this conversion is not yet available. Therefore, advancing the development and discovery of new enzymes is a critical step toward realizing an integrated lignocellulosic biorefinery.[56]

As summarized in Table 1, numerous biosensors that respond to sugars or key intermediates have been identified and applied to monitor product or substrate concentrations. For instance, the XylR–PxylF system responds to xylose by using GFP as a reporter to monitor xylose concentration, with GFP intensity indicating the amount of xylose present. Tang *et al.* employed a range of biosensors containing a mutant XylR, which expanded the detection range nearly 10-fold compared to the control, enabling the detection of varying xylose concentrations (Table 2).[33] Also, biosensors can be used to detect compounds that are difficult to monitor using conventional methods. For example, CouR is a MarR family regulator, that negatively regulates the transcription of genes involved in the catabolism of *p*-coumarate in bacteria. *p*-coumaroyl-CoA cannot be detected using standard analytical methods, Liu *et al.* developed a novel biosensor, CouR, in *Saccharomyces cerevisiae* that responds to *p*-coumaroyl-CoA. Although it is challenging to quantify the sensor's concentration threshold and operating range, Liu successfully devised a dynamic regulatory circuit that enables real-time adjustment of *p*-coumaroyl-CoA production.[57] The correlation between fluorescence output and enzyme or pathway efficiency enables the monitoring of substrate utilization and final product production, facilitating optimization. These biosensors play a crucial role in enhancing substrate-to-product bioconversion in lignocellulosic biomass processing by enabling real-time tracking of essential metabolic intermediates. This capability enables researchers to identify bottlenecks within the metabolic network and screen and optimize key enzymes.

Biosensors in dynamic regulation

Biosensors can respond to a broad array of metabolites across diverse living microorganisms to maintain balanced cellular metabolism, extensive exploration of biosensor discovery and optimization techniques has led to the identification of an increasing number of metabolites as signaling molecules,

Table 1 Summary of notable examples of biosensors applied for lignocellulose valorization

Biosensors	Sensor type	Sensing molecular	Reporter	Host	Purpose	Ref.
XylR	TF-based, repressor	Xylose	*yEGFP*	*S. cerevisiae*	Dynamic regulation of gene expression	32
XylR	TF-based, repressor	Xylose	*β-Galactosidase gene*	*Escherichia coli*	Monitoring production	33
araF	FERT nano sensor	L-Arabinose	*eYFP*	*Escherichia coli*	Monitoring production	34
AraC	TF-based, repressor	Arabinose	*GFP*	*Escherichia coli*	Change the inducer scope	35
FLIPmal	FERT	Maltose	*eYFP*	*Escherichia coli*	Quantify production	34
RhaS	TF based, activator	L-Rhamnose, L-mannose	*sfgfp*	*Escherichia coli*	Change the inducer scope and sensitivity	36
CelR	TF-based, repressor	Cellulase	*GFP*	*Escherichia coli*	Monitoring gene expression	37
MglB	FERT	Glucose	*GFP*	*Escherichia coli*	Analyse the designed FERT biosensor structure	38
YbeJ	FERT	Glutamate	*Fluorescence*	*Escherichia coli*	Screening mutants	39
PcaU	TF-based, repressor and an activator	Protocatechuic acid	*GFP*	*Escherichia coli*	Screening enzyme activity	40
VanR	TF-based, repressor	Vanillic acid	*YFP*	*Escherichia coli*	Optimize biosensor dynamic range	41
ADH7	Inducible promoter	Vanillin	*3-O-Methylgallate-O-demethylase (LigM), vanillin dehydrogenase (LigV) and protocatechuate decarboxylase (AroY)*	*Escherichia coli*	Dynamic regulation of gene expression	42
MuYqhC	TF-based, repressor	Vanillin	RFP	*B. subtilis*	Screening enzyme activity	43
PadR	TF-based, repressor	*p*-Coumaric acid	*YFP*	*Escherichia coli and Corynebacterium glutamicum*	Screening and selection for optimal chassis	44
FerC	TF-based, repressor	Ferulic acid	*eGFP*	*Escherichia coli*	Screening biosensor substrate	45
BenR	TF-based, activator	Benzoic acid	*gfp*	*Escherichia coli*	Screening novel enzyme	46
NahR	TF-based, activator	2-Hydroxybenzoic acid	*lacZα and tetA*	*Escherichia coli*	Screening and selection active strains	47
BldR	TF-based, activator	Benzaldehyde	*gfp*	*Escherichia coli*	Optimize biosensor specify and sensitivity	48
EmrR	TF-based, repressor	Phenol	*gfp*	*Escherichia coli*	Screening target genes	49
LvaR	TF-based, repressor	Levulinic acid	*sfgfp*	*P. putida KT2440*	Screening optimal chassis	50
ArgP	TF-based, repressor	L-Arginine	*gfp*	*Escherichia coli*	Screening enzyme mutant with better activity	51
BenM	TF-based, repressor	Adipic acid	*sfgfp*	*Escherichia coli*	Screening optimal production strains	*52*
FadR	TF-based, repressor	Acyl-CoA	*gfp*	*Saccharomyces cerevisiae*	Screening gene targets	53
FapR	TF-based, repressor	Malonyl-CoA	*RFP*	*Escherichia coli*	Dynamic regulation of metabolic flux	54

FERT (ferulic acid-responsive transcriptional regulator), TF (ranscription Factor), COMT (caffeic acid *O*-methyltransferase). eYFP: enhanced yellow fluorescent protein; Sfgfp: Superfolder green fluorescent protein; YFP: enhanced yellow fluorescent protein; rfp, mCherry.

which can be integrated into biosensor applications within dynamic regulatory circuits.[63] These biosensors are essential for monitoring and controlling metabolic flux in engineered microbial systems, adjusting pathways in real time based on environmental and intracellular cues.

Carbon flux optimization is essential for ensuring that the carbon atoms are efficiently channeled into desired metabolic products rather than being diverted into by-products or lost through inefficient pathways. By detecting key metabolites involved in central carbon metabolism, biosensors can inform

Table 2 Typical applications of biosensors in lignocellulose valorization

Applications	Products	Biosensors	Mechanisms	Effectors	Achievements	Ref.
Monitoring	Xylose	XylR	In the absence of xylose, XylR does not bind to the operator xylO, repressing *reporter gene* expression. When xylose is present, XylR binds xylose, enabling its binding to xylO and activating transcription of *reporter*.	Xylose	Screening the XylR mutants, the detecting range was increased by nearly 10-fold	33
	Salicylic acid	MarR	MarR protein binds on PmarO to inhibit promoter expression and SA binds with MarR protein to release the repression.	Salicylic acid	Two promoter variants I12AII4T and I12AII14T that exhibited improved responsive strengths and shifted dynamic ranges were obtained	58
	Levulinic acid	LvaR	The LvaR regulator senses LA as a cognate ligand	Levulinic acid	Screening optimal chassis, like *P. putida* KT2440, the maximal fluorescence increase was 12.3-fold	50
Screening enzymes or genes	Lignin transformation products	EmrR	EmrR is necessary and sufficient for the compound-dependent activation of the emrRAB operon.	Phenol	Identified genes encoding six functional classes mediating lignin transformation phenotypes	49
	Vanillin	MuYqhC	In the presence of an aldehyde, the transcriptional regulator YqhC activates the promoter of the *yqhD* gene, which drives the expression of a reporter gene (*RFP*), resulting in red fluorescence.	Vanillin	Identified a COMT variant, Mu176, that displayed a 7-fold increase in the conversion rate	43
Dynamic regulation	Protocatechuic acid	Transcriptional repressor PadR	An auto-regulatory system was established, by coexpressing key rate-limiting enzymes, 4-hydroxybenzoate hydroxylase, vanillate-*O*-demethylase, and transporter HcnK under the biosensor element	*p*-Coumaric acid (*p*-CA)	A titer of 12.7 g L^{-1} of protocatechuic acid	59
	Vanillic acid	Transcriptional repressor PP3359	Two-layer genetic controller that interconnects two independent genetic sensors to decouple growth and production	Feruloyl-CoA	Reduced metabolic stress by two-fold, while also increasing the growth rate and productivity by two-fold and five-fold, produced vanillic acid at titers up to 880 mg/L	60
	Itaconic acid	Nitrogen starvation-detecting biosensor	Limiting the expression of the apparently toxic CadA protein to the production phase *via* dynamic regulation	Nitrogen starvation	Maximum yield of 56% (mol mol^{-1}) and titer of 1.3 g L^{-1} from *p*-coumarate, and 1.4 g L^{-1} titer from monomeric aromatic compounds produced from alkali-treated lignin	61
	Catechol	Vanillin-inducible promoter ADH7	This system is composed of a catechol biosynthesis pathway coexpressed with an active aromatic transporter CouP under induction by a vanillin self-inducible promoter, ADH7, to effectively convert the lignin-derived aromatics into value-added chemicals	Vanillin	Improved the catechol yields about 30% and 40% under promoter pTrc and ADH7, respectively.	42
	Polyhydroxybutyrate	PcaQ	Control the functional genes expression in response to heterologous lignin-derived aromatics	Various lignin-derived monomers	Polyhydroxybutyrate titer of 2.38 g L^{-1} from the alkaline pretreatment liquor	62

real-time adjustments to metabolic pathways, such as upregulating or downregulating specific enzymes to maintain balanced flux. This precision improves the overall efficiency of microbial conversion processes, ensuring that more of the lignocellulosic substrate is converted into valuable products while minimizing waste.

To alleviate resource competition between cell growth and product synthesis, a dynamic regulation strategy was implemented by decoupling the two processes. Lo *et al.* has developed two versions of the controller, each equipped with glucose nutrient sensors but differing in their substrate sensing modules.[60]One controller specifically targets hydroxycinnamic acid, while the other targets oleic acid. The substrate-sensing module was placed under the direct control of the nutrient-sensing module, enabling decoupling of growth and metabolite production in the two-layer genetic circuit. To be specifically, they used the lignin-responsive transcriptional repressor PP3359, which inhibits the *ech* promoter and is deactivated by hydroxycinnamic CoA. The *fcs* gene was placed under the glucose-responsive *csiD* promoter, while *ech* and *vdh* were controlled by the *ech* promoter. PP3359 was expressed constitutively. This two-layer genetic circuit enabled prioritized biomass accumulation, followed by the induction of *fcs* expression to convert lignin-derived aromatics into thioester intermediates, thereby relieving repression of *ech* and *vdh*. The engineered ciircuits exhibited a 2-fold increase in growth rate and produced vanillic acid at titers up to 880 mg L^{-1}.[60]

In the process of lignocellulosic conversion, engineered microbes often experience metabolic stress due to the demands placed on their metabolic machinery. This metabolic burden can lead to reduced growth rates, lower production efficiency, and instability in engineered strains. Biosensors offer a solution by allowing for the identification and mitigation of metabolic stress in real time. For example, biosensors that detect stress-related metabolites or intracellular signals can trigger adaptive responses in microbial systems, such as the downregulation of stress-inducing pathways or the activation of stress-relief mechanisms. Lignin-derived vanillin has been utilized as an inducer to develop an autoregulatory system for lignin valorization to catechol. The vanillin-inducible promoter *ADH7* was employed to dynamically regulate both the catechol biosynthetic and aromatic transport modules. Wu *et al.* constructed autoregulatory system, efficiently transports vanillin across the cell membrane and converts it to catechol, resulting in a yield improvement of approximately 30% and 40% under the control of the pTrc and ADH7 promoters, respectively.[42] This approach addresses challenges related to the toxicity of lignin-derived aromatics and the high cost of exogenous inducers. Also, the biosensor responsive to lignin-derived *p*-coumaric acid was developed to intelligently sense lignin substrates and dynamically regulate the lignin metabolism network. In this system, *p*-coumaric acid binds to the PadR protein, releasing it from the *PpadC* promoter and activating the protocatechuic acid biosynthesis pathway. This dynamic regulation achieved a protocatechuic acid titer of 12.7 g L^{-1}.[59]

A pivotal discovery is the regulatory element in *Ralstonia eutropha* H16. The regulatory protein PcaQ and its activated promoter *Ppca* were used to construct a self-induction system responsive to various lignin-derived monomers, enabling dynamic control of rate-limiting enzyme expression. Protein engineering of PcaQ, guided by hotspot prediction and molecular docking, yielded a high-sensitivity mutant, PcaQ-R145K. The engineered strain, equipped with multifunctional modules, achieved a record polyhydroxybutyrate titer of 2.38 g L^{-1} from the alkaline pretreatment liquor of *Pinus massoniana.*[62]

Additionally, Restricting the expression of toxic pathway enzymes to the product synthesis phase can effectively balance cell growth and bioproduction. During nitrogen limitation, the PHA pathway competes with the citric acid (TCA) cycle for acetyl-CoA *via* fatty acid biosynthesis. Producing itaconic acid during growth is challenging due to its inhibitory effects on enzymes in the glyoxylate shunt and citramalate cycle. A two-stage process that decouples growth from product synthesis can overcome issues such as product toxicity and slow catalyst growth. These strategies often leverage natural responses to nutrient or environmental stresses (*e.g.*, nitrogen, sulfur, phosphate limitation; oxygen or temperature shifts) that halt growth but preserve metabolic activity. Transcriptomic analysis identified the nitrogen starvation-responsive promoter *PurtA*, which was combined with T7 RNA polymerase and T7 lysozyme to construct a nitrogen-limitation biosensor. Dynamic control of the cytotoxic enzyme CadA (enzyme lysine decarboxylase 1) using this system significantly enhanced cell growth and offered a promising strategy for lignin-to-itaconic acid conversion.[61]

The development of aromatic-sensitive intelligent regulatory systems offers a powerful strategy for time-sequential regulation of multi-substrate transformations, a key requirement for efficient lignin valorization. By leveraging the responsiveness of engineered biosensors and transcriptional regulators to lignin-derived monomers, these systems enable precise, real-time control over metabolic flux. The continued refinement of these systems-through advances in synthetic biology, protein engineering, and systems metabolic modeling-will be crucial for optimizing pathway performance and scalability. Coupling such regulatory frameworks with robust microbial chassis and sustainable feedstock processing can pave the way toward economically viable and environmentally friendly lignin biorefineries.

High-throughput screening and strain improvement

In addition to enabling dynamic regulation, biosensors also support high-throughput screening and strain optimization.[64] By linking biosensor outputs to key metabolites or target products, researchers can rapidly identify strains with improved metabolic performance or higher product yields. This automated screening approach greatly accelerates the discovery of promising enzymes, saving both time and labor.[65] Additionally, biosensors enable the rapid evaluation of thousands of genetic variants, facilitating the identification of novel metabolic configurations that conventional methods might overlook. For instance, the biosynthesis of vanillin is constrained by the conversion of protocatechuate to vanillate, a reaction catalyzed by catechol *O*-methyltransferase. Kunjapur *et al.* optimized the VanR–VanO system. The best rationally constructed sensor achieved 14-fold dynamic range. They

employed the evolved biosensor for rapid bioprospecting of natural catechol *O*-methyltransferases, leading to the identification of three previously uncharacterized active *O*-methyltransferases.[41] Dong *et al.* successfully applied a MuYqhC-based vanillin biosensor to identify a COMT variant, Mu176. This variant exhibited a 7-fold increase in conversion rate compared to the wild-type COMT. When large gene libraries are generated *via* mutagenesis or rational design, biosensors enable rapid screening of these libraries to identify efficient microbial strains. Siedler *et al.* applied padR to quickly sort droplets containing yeast cells that produced significant amounts of extracellular *p*-coumaric acid by utilizing the fluorescent signal from an *E. coli* biosensor.[44]

In strain improvement programs, biosensors not only aid in the initial screening process but also enable automated selection of high-performing strains throughout the development pipeline. Linking sensing and cellular adaptation specifically through the regulation of antibiotic resistance genes can enhance the evolution of biosensors, allowing for the enrichment of mutant libraries with improved phenotypes and productivity *via* adaptive laboratory evolution (ALE). This method has proven effective in increasing enzyme activity and metabolic productivity under selective conditions, boosting the production of metabolites such as tyrosine, L-valine, and muconic acid,[52,66] Overall, directed evolution using genetically encoded biosensors has facilitated the rapid and efficient engineering of proteins, metabolic pathways, and global metabolic networks.[67,68]

Artificial intelligence and machine learning in biosensor development

Recently, the integration of artificial intelligence (AI) and machine learning (ML) has opened new frontiers in biosensor development, enabling smarter, faster, and more precise sensing platforms.[69,70] They are applied across various stages of biosensor development, from the initial design of recognition elements to advanced data analysis and real-time decision-making. These technologies facilitate the handling of large and complex biological datasets, allowing for real-time data analysis, predictive modelling, and adaptive system optimization. For example, Ding *et al.* trained a convolutional neural network (CNN) on large datasets linking ribosome binding sites (RBS) with transcription factor binding (TFB) dynamic ranges, developing a classification model (CLM-RDR) to enable intelligent regulation of TFB dynamic range.[71] And Zhou *et al.* generated a gradient promoter dataset using DNA barcoding to build a TFB library and characterized response curves *via* FACS-seq. Using an XGBoost model, they accurately predicted genotype-to-phenotype relationships and validated high-performing sequences, ultimately developing a malonyl-CoA biosensor with a dynamic range.[72]

Also, advancements in artificial intelligence (AI) have empowered machine learning and deep learning algorithms to effectively identify patterns in biological data and accurately predict the performance of bio-elements, offering a promising solution to overcome key technical barriers in regulating lignin biological metabolism. It can predict optimal synthetic pathways and regulatory elements by analysing genomic, transcriptomic, and metabolic data. This reduces the trial-and-error typically associated with biosensor tuning and accelerates the design-build-test cycle. Moreover, AI-driven pattern recognition enhances signal interpretation in various biosensing modalities, including optical, fluorometric, and electrochemical sensors. Looking ahead, the field of AI-assisted design and activity prediction of bio-elements will be shaped by advances in deep reinforcement learning, unsupervised learning, and neural network architectures. Integrating AI with disciplines like systems biology and bioinformatics will deepen our understanding of complex biological systems and enhance the precision of bio-element design.[70] Continued interdisciplinary collaboration will be key to translating these tools from proof-of-concept to industrial implementation.

Conclusion and outlook

The future economic viability of lignocellulosic biorefineries depends heavily on effective lignin valorization, as lignin serves as a valuable feedstock to produce a wide range of high value bioproducts. However, biological lignin valorization has not yet reached industrial or commercial scale. Recently, several reviews provided a comprehensive overview of lignin valorization, emphasizing multiscale metabolic regulation, covering key aspects such as critical enzymes involved in lignin biotransformation, metabolic pathway networks, genome-to-phenotype relationships, and predictive learning strategies.[15,73] In this review, we mainly focused on Biosensor-enabled metabolic engineering for lignocellulosic conversion. The biosensor-based technologies enable real-time detection, dynamic regulation, and the identification of potential enzyme catalysts, as well as efficient strain screening for targeted evolution. These capabilities could greatly accelerate the engineering of proteins, metabolic pathways, and global metabolic networks, which are crucial for facilitating lignocellulosic conversion. However, several technical and economic challenges must be addressed before full commercialization is feasible. A primary concern is the long-term stability and sensitivity of biosensors in industrial environments. Developing robust biosensors is critical to ensure their effectiveness in large-scale operations.

Additionally, further research is needed to explore biocatalytic enzymes and biotransformation pathways, enhance substrate fermentability and biotransformation efficiency, design high-performance microbial strains, and optimize fermentation conditions for the efficient production of a wide range of chemicals. Establishing biosensors that meet specific criteria, such as specificity, dynamic range, and detection range is essential for practical applications. The design of ideal biosensors remains a significant challenge, yet artificial intelligence techniques, particularly deep learning and machine learning, are beginning to be integrated into biosensor design.

These advances allow for the development of sophisticated biosensors with greater accuracy and efficiency.

When combined with machine learning algorithms that can analyze the large datasets generated by biosensors, these tools enable the screening and enrichment of positive mutations in proteins, metabolic pathways, and genome-wide networks from extensive libraries. Such innovations will not only increase the efficiency and resilience of biosensor-enabled biorefineries, but they will also transform the way lignocellulosic biomass converted into biofuels and biochemicals, paving the way for more sustainable and efficient industrial processes. As researchers work to overcome challenges in scalability and robustness, biosensor-driven systems will play a vital role in advancing next-generation biorefineries, contributing to a cleaner and more sustainable bioeconomy.

Author contributions

Conceptualization, Y. Y. and Q. G.; writing – original draft preparation, Q. G.; visualization, Q. G., J. Z. and X. G; writing – review and editing, Q. G. and Y. Y.; All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

No primary research results, software, or code have been included, and no new data were generated or analyzed as part of this review.

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