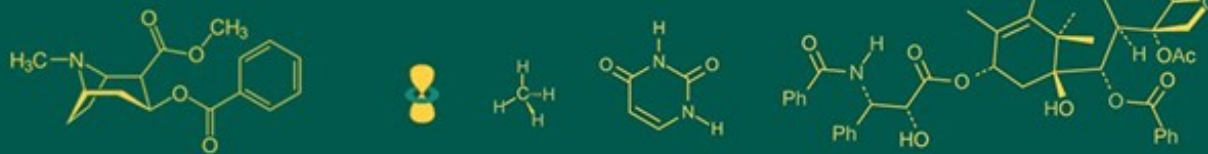


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating: (2026): 5.29
 Indexing: Embase
 IJABR 2026; SP-10(9): 2240-2244
www.biochemjournal.com
 Received: 05-07-2026
 Accepted: 30-08-2026
 Published: 26-09-2026

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Metabolic engineering of *Saccharomyces cerevisiae* for dual biofuel production from lignocellulosic biomass: Advances in pathway optimization and process integration

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i9S.ab.10154>

ABSTRACT

The increasing demand for sustainable energy has intensified research on lignocellulosic biomass as a renewable feedstock for second-generation biofuel production. Among the available microbial hosts, *Saccharomyces cerevisiae* remains the preferred industrial organism because of its robust fermentation characteristics, well-characterized genetics, and ease of genetic manipulation. However, its inability to efficiently utilize pentose sugars and cellulose-derived oligosaccharides limits its application in lignocellulosic biorefineries. Recent advances in metabolic engineering have addressed these limitations through the introduction of heterologous sugar transporters, cellulolytic enzymes, pentose assimilation pathways, and carbon flux redistribution strategies, enabling efficient utilization of mixed sugars and enhanced production of ethanol together with higher-value biofuels and biochemicals. In addition, genome-editing technologies, adaptive laboratory evolution, and systems metabolic engineering have accelerated strain improvement by enhancing substrate utilization, inhibitor tolerance, and fermentation performance. Process integration strategies, including simultaneous saccharification and fermentation, consolidated bioprocessing, and synthetic microbial consortia, have further improved biomass conversion efficiency while reducing production costs. Despite these advances, challenges related to glucose repression, metabolic burden, inhibitor toxicity, and industrial scalability continue to hinder commercial implementation. This review summarizes recent developments in the metabolic engineering of *S. cerevisiae* for dual biofuel production from lignocellulosic biomass, with emphasis on pathway optimization, mixed-sugar fermentation, carbon flux engineering, and integrated bioprocessing strategies. It also discusses current challenges and future research directions for developing economically viable and environmentally sustainable lignocellulosic biorefineries.

Keywords: Adaptive laboratory evolution, Consolidated bioprocessing, Dual biofuel production, Metabolic engineering, *Saccharomyces cerevisiae*, Synthetic biology

1. Introduction

The increasing global demand for renewable energy, coupled with concerns over climate change, depletion of fossil fuel reserves, and environmental pollution, has accelerated research into sustainable biofuel production. Among various renewable resources, lignocellulosic biomass has emerged as an attractive feedstock because of its abundance, low cost, renewability, and non-competition with food crops. Agricultural residues, forestry wastes, and dedicated energy crops represent valuable lignocellulosic resources that can be converted into biofuels while simultaneously promoting waste valorization and reducing greenhouse gas emissions (Sun *et al.*, 2021; Xu *et al.*, 2024) [1, 2].

Lignocellulosic biomass is primarily composed of cellulose, hemicellulose, and lignin. Following suitable pretreatment and enzymatic hydrolysis, cellulose and hemicellulose release fermentable sugars, mainly glucose, cellobiose, and xylose. However, efficient conversion of these mixed sugars remains a major bottleneck because conventional fermentative microorganisms preferentially utilize glucose, resulting in incomplete pentose fermentation and reduced biofuel productivity. In addition, inhibitory compounds generated during biomass pretreatment adversely affect microbial growth and fermentation efficiency, thereby limiting large-scale commercialization (Ha *et al.*, 2011; Ko *et al.*, 2019) [3, 4].

Among industrial microorganisms, *Saccharomyces cerevisiae* has long been recognized as the preferred host for bioethanol production owing to its well-characterized genetics, rapid growth, high ethanol productivity, and tolerance to industrial fermentation conditions. Nevertheless, its inability to naturally metabolize cellobiose and xylose restricts its application in lignocellulosic biorefineries. To overcome these limitations, extensive metabolic engineering efforts have focused on introducing heterologous sugar transporters, pentose

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assimilation pathways, cellulolytic enzymes, and regulatory modifications that enable efficient mixed-sugar utilization and improve fermentation performance (Jeffries *et al.*, 2011; Yamada *et al.*, 2013) [5, 6].

Recent advances in synthetic biology, genome editing, and systems metabolic engineering have significantly expanded the metabolic capabilities of *S. cerevisiae*. CRISPR/Cas9-mediated genome engineering, adaptive laboratory evolution, and pathway optimization have improved sugar uptake, carbon flux distribution, inhibitor tolerance, and product yield. Furthermore, engineering strategies now extend beyond ethanol production to enable the co-production of higher alcohols, xylitol, microbial lipids, squalene, and other value-added biochemicals, thereby enhancing the economic feasibility of integrated lignocellulosic biorefineries (Lee *et al.*, 2022; Dixit *et al.*, 2023) [8, 7]. In parallel, process integration approaches such as simultaneous saccharification and fermentation (SSF), consolidated bioprocessing (CBP), and synthetic microbial consortia have further improved lignocellulosic bioconversion by enhancing substrate utilization, reducing enzyme requirements, and increasing overall process efficiency (Jayakody *et al.*, 2018; Yan *et al.*, 2023) [9, 10]. Despite these advances, challenges related to carbon catabolite repression, metabolic burden, genetic stability, inhibitor tolerance, and industrial scalability continue to limit commercial implementation (Wagner *et al.*, 2023) [11].

This review summarizes recent advances in the metabolic engineering of *Saccharomyces cerevisiae* for dual biofuel production from lignocellulosic biomass. Particular emphasis is placed on sugar transporter engineering, pentose assimilation, carbon flux redistribution, synthetic biology tools, process integration strategies, and the major challenges and future prospects for developing efficient and economically sustainable lignocellulosic biorefineries.

2. Lignocellulosic biomass as a sustainable feedstock

Lignocellulosic biomass is considered one of the most sustainable feedstocks for second-generation biofuel production because of its abundance, renewability, and non-competitive relationship with food crops. Agricultural residues such as rice straw, wheat straw, corn stover, and sugarcane bagasse, together with forestry residues and dedicated energy crops, represent inexpensive carbon sources that can be converted into biofuels and value-added biochemicals. Their utilization not only reduces dependence on fossil fuels but also promotes waste valorization and supports the development of a circular bioeconomy (Sun *et al.*, 2021; Ahuja *et al.*, 2024) [1, 12].

Structurally, lignocellulosic biomass consists of cellulose (35–50%), hemicellulose (20–35%), and lignin (10–25%), with composition varying among feedstocks. Cellulose is the principal source of glucose, whereas hemicellulose releases pentose sugars, mainly xylose and arabinose, following hydrolysis. Lignin forms a complex aromatic network that protects structural carbohydrates from enzymatic degradation, making biomass inherently recalcitrant to microbial conversion (Yamada *et al.*, 2013) [6].

Conversion of lignocellulosic biomass into biofuels generally involves pretreatment, enzymatic saccharification, microbial fermentation, and product recovery. Pretreatment disrupts the lignin-carbohydrate matrix, improving enzyme accessibility and increasing sugar release. However, harsh pretreatment conditions often generate inhibitory compounds such as furfural, 5-hydroxymethylfurfural (HMF), acetic acid, and phenolic derivatives that impair microbial growth and reduce fermentation efficiency (Ko *et al.*, 2019) [4]. Furthermore, lignocellulosic hydrolysates contain mixed sugars, predominantly glucose and xylose, whose simultaneous utilization remains one of the major challenges for industrial biofuel production because native *Saccharomyces cerevisiae* preferentially metabolizes glucose (Ha *et al.*, 2011) [3].

Recent advances in metabolic engineering have substantially improved the utilization of lignocellulosic hydrolysates by enabling efficient uptake of mixed sugars, enhancing inhibitor tolerance, and redirecting carbon flux toward multiple biofuels. These developments

have strengthened the role of *S. cerevisiae* as an industrial cell factory and accelerated the transition from single-product ethanol fermentation to integrated biorefineries producing ethanol together with other value-added biofuels and biochemicals (Lee *et al.*, 2022; Zhao *et al.*, 2024) [8, 13].

3. Metabolic engineering strategies in *Saccharomyces cerevisiae*

Metabolic engineering has revolutionized the use of *Saccharomyces cerevisiae* as a microbial cell factory for lignocellulosic biofuel production. Although wild-type yeast efficiently ferments glucose into ethanol, it lacks the ability to directly utilize cellobiose and pentose sugars, thereby limiting its application in second-generation biorefineries. Advances in metabolic engineering have addressed these limitations by introducing heterologous pathways, optimizing native metabolic networks, and improving substrate utilization, enabling *S. cerevisiae* to convert a broader range of lignocellulosic sugars into biofuels and value-added products (Jeffries *et al.*, 2011; Sun *et al.*, 2021) [5, 1].

Current engineering strategies focus on improving sugar uptake, enhancing pentose assimilation, redirecting intracellular carbon flux, increasing tolerance to lignocellulosic inhibitors, and expanding the spectrum of biofuel products. Heterologous expression of sugar transporters and cellulolytic enzymes has enabled efficient utilization of cellobiose and cellulose-derived oligosaccharides, while engineering of xylose assimilation pathways has facilitated simultaneous fermentation of glucose and pentose sugars (Ha *et al.*, 2011; Yamada *et al.*, 2013) [3, 6]. In parallel, modifications in central carbon metabolism have improved precursor availability for the synthesis of higher alcohols, microbial lipids, and other valuable biochemicals (Lee *et al.*, 2022) [8].

The emergence of CRISPR/Cas9 genome editing, multiplex gene integration, and adaptive laboratory evolution has further accelerated strain development by enabling precise modification of multiple metabolic pathways. These technologies have improved fermentation efficiency, inhibitor tolerance, and genetic stability under industrial conditions, thereby enhancing the commercial potential of engineered yeast strains (Dixit *et al.*, 2023; Wei *et al.*, 2020) [7, 14]. In addition, synthetic microbial consortia have been explored to distribute metabolic functions among specialized strains, improving mixed-sugar utilization and reducing metabolic burden associated with single-strain engineering (Chen *et al.*, 2018; Shin *et al.*, 2024) [15, 16].

The following sections discuss the major metabolic engineering strategies employed to improve lignocellulosic biofuel production, including transporter and cellulase engineering, pentose assimilation pathways, carbon flux redistribution, genome engineering, and process integration approaches.

3.1 Engineering of sugar transporters and cellulolytic enzymes

Efficient transport and hydrolysis of lignocellulose-derived sugars are fundamental prerequisites for improving biofuel production in *Saccharomyces cerevisiae*. Native yeast primarily transports glucose through the hexose transporter (HXT) family and lacks the ability to efficiently utilize cellobiose and other cellodextrins released during cellulose hydrolysis. Consequently, engineering sugar transporters and cellulolytic enzymes has become a key strategy for expanding substrate utilization and improving lignocellulosic bioconversion (Jeffries *et al.*, 2011) [5].

A major breakthrough was the heterologous expression of the cellodextrin transporter (CDT-1) and intracellular β -glucosidase (GH1-1) from *Neurospora crassa*, which enabled direct uptake and intracellular hydrolysis of cellobiose. This strategy eliminated extracellular glucose accumulation, reduced carbon catabolite repression, and facilitated simultaneous utilization of cellobiose and xylose, thereby improving ethanol productivity from lignocellulosic hydrolysates (Ha *et al.*, 2011) [3]. Subsequent optimization of CDT-1 and CDT-2 transporters further enhanced cellodextrin uptake, transporter stability, and

fermentation efficiency under industrial conditions (Kretzer *et al.*, 2024) [17].

Besides transporter engineering, considerable efforts have focused on introducing cellulolytic enzymes, including endoglucanases, cellobiohydrolases, and β -glucosidases, into *S. cerevisiae*. Co-expression of these enzymes enables partial hydrolysis of cellulose into fermentable sugars, thereby reducing dependence on externally added cellulases. Improvements in codon optimization, promoter selection, and secretion signals have significantly enhanced enzyme expression and activity, supporting the development of consolidated bioprocessing systems (Yamada *et al.*, 2013) [6].

Cell-surface display technology has further improved cellulose degradation by anchoring cellulases directly onto the yeast cell wall, increasing enzyme proximity to insoluble cellulose and minimizing enzyme loss into the fermentation medium. This strategy improves substrate accessibility, enhances hydrolysis efficiency, and reduces enzyme costs, making it an attractive approach for industrial lignocellulosic biorefineries (Liu *et al.*, 2016) [18].

Recent advances in multiplex genome editing have enabled simultaneous integration of transporter genes and cellulase expression cassettes into the yeast genome, resulting in strains with improved cellulose utilization, enhanced genetic stability, and superior fermentation performance. Such engineered strains represent an important step toward efficient consolidated bioprocessing, where enzyme production, biomass hydrolysis, and fermentation occur within a single microorganism (Dixit *et al.*, 2023) [7]. Nevertheless, balanced expression of transporters and cellulases, efficient protein secretion, and minimization of metabolic burden remain critical challenges requiring further optimization before large-scale industrial application.

3.2 Engineering of pentose assimilation pathways

Efficient utilization of xylose is essential for maximizing lignocellulosic biofuel production because hemicellulose contributes a substantial proportion of fermentable sugars released during biomass hydrolysis. However, *Saccharomyces cerevisiae* lacks a native xylose assimilation pathway, making metabolic engineering necessary for efficient pentose fermentation (Jeffries *et al.*, 2011) [5]. Consequently, extensive efforts have focused on introducing heterologous pathways and optimizing endogenous metabolism to improve xylose conversion and overall fermentation efficiency.

Two major pathways have been engineered for xylose metabolism in *S. cerevisiae*: the xylose reductase–xylitol dehydrogenase (XR–XDH) pathway and the xylose isomerase (XI) pathway. The XR–XDH pathway, derived from *Scheffersomyces stipitis*, converts xylose into xylulose through sequential reduction and oxidation reactions before entry into the pentose phosphate pathway. Although this pathway supports rapid xylose assimilation, differences in cofactor requirements between XR and XDH often result in intracellular redox imbalance and xylitol accumulation (Zha *et al.*, 2013) [19]. Alternatively, the XI pathway directly converts xylose into xylulose without requiring redox cofactors, thereby reducing by-product formation, although further optimization is required to improve enzyme activity in yeast (Sun *et al.*, 2021) [1].

Engineering the pentose phosphate pathway (PPP) has further enhanced xylose utilization. Overexpression of XKS1, TKL1, TAL1, RPE1, and RKI1 increases carbon flux through the PPP, improving ethanol yield and reducing carbon loss during pentose metabolism. In addition, adaptive laboratory evolution has generated strains with enhanced xylose uptake, improved mixed-sugar co-consumption, increased inhibitor tolerance, and higher fermentation efficiency under industrial conditions (Papapetridis *et al.*, 2018; Wei *et al.*, 2020) [20, 14].

Another important challenge is glucose-mediated carbon catabolite repression, which delays xylose utilization until glucose is depleted. Engineering sugar transporters, modifying regulatory networks, and optimizing promoter activity have enabled simultaneous fermentation of glucose and xylose, thereby increasing substrate utilization efficiency and shortening fermentation time (Wei *et al.*, 2015). Recent

studies have also combined CRISPR/Cas9 genome editing with pathway engineering to precisely regulate genes involved in pentose metabolism, leading to improved carbon conversion efficiency and greater genetic stability (Dixit *et al.*, 2023) [7].

Despite considerable progress, complete and rapid utilization of mixed sugars remains a major challenge because of redox imbalance, transporter limitations, and metabolic burden associated with heterologous pathway expression. Future engineering strategies should integrate systems biology, dynamic pathway regulation, and computational metabolic modelling to achieve efficient co-fermentation of lignocellulosic sugars while supporting simultaneous production of multiple biofuels and value-added biochemicals.

3.3 Carbon flux redistribution for dual biofuel production

Efficient production of multiple biofuels from lignocellulosic biomass requires redistribution of intracellular carbon flux from native ethanol fermentation toward alternative biosynthetic pathways. In wild-type *Saccharomyces cerevisiae*, pyruvate generated through glycolysis is predominantly converted to ethanol, limiting the synthesis of other valuable biofuels. Metabolic engineering strategies therefore focus on redirecting metabolic intermediates such as pyruvate and acetyl-CoA towards higher alcohols, lipids, and other value-added compounds while maintaining efficient carbon utilization (Lee *et al.*, 2022) [8].

Acetyl-CoA is a central metabolic intermediate linking glycolysis with numerous biosynthetic pathways. Engineering enzymes involved in acetyl-CoA generation and reducing carbon loss through competing pathways has significantly enhanced the production of advanced biofuels, including *n*-butanol, isobutanol, and lipid-derived biofuels. Simultaneous optimization of precursor availability and cofactor balance has further improved carbon conversion efficiency and product yields (Khan *et al.*, 2022; Promdonkoy *et al.*, 2020) [21, 22].

Besides higher alcohols, metabolic pathway engineering has enabled the co-production of ethanol with industrially important compounds such as xylitol, 2,3-butanediol, and squalene. Balanced expression of xylose reductase (XR) and xylitol dehydrogenase (XDH) facilitates controlled partitioning of carbon towards both ethanol and xylitol, whereas engineering the acetolactate pathway promotes simultaneous synthesis of ethanol and 2,3-butanediol from lignocellulosic sugars. Likewise, partial suppression of ERG1 redirects carbon through the mevalonate pathway, increasing intracellular squalene accumulation without severely affecting ethanol production (Zha *et al.*, 2013; Nan *et al.*, 2014; Hull *et al.*, 2014) [19, 24, 23].

Recent advances in systems metabolic engineering have enabled dynamic regulation of carbon flux through multiplex genome editing, synthetic promoters, and computational metabolic modelling. These approaches facilitate coordinated expression of multiple biosynthetic pathways, reducing metabolic burden while improving precursor availability and product formation. Such integrated engineering strategies are expected to play a crucial role in developing economically viable biorefineries capable of simultaneously producing bioethanol and high-value biofuels from lignocellulosic biomass (Dixit *et al.*, 2023; Ravn *et al.*, 2024) [7, 25].

3.4 Process integration strategies for enhanced dual biofuel production

The successful commercialization of lignocellulosic biofuels depends not only on strain engineering but also on the integration of efficient bioprocessing strategies. Conventional biofuel production involves separate pretreatment, enzymatic hydrolysis, and fermentation steps, which increase operational complexity and production costs. Consequently, integrated process configurations have been developed to improve sugar conversion efficiency, reduce enzyme consumption, and enhance overall process economics (Jayakody *et al.*, 2018) [9].

Simultaneous saccharification and fermentation (SSF) is one of the most widely adopted strategies, in which enzymatic hydrolysis and microbial fermentation occur in a single reactor. This approach minimizes end-product inhibition of cellulases by continuously converting released glucose into ethanol, thereby improving substrate conversion

and reducing contamination risks. However, differences in the optimal temperatures for cellulase activity and yeast fermentation remain a major limitation (Song *et al.*, 2018) [26].

Another promising strategy is consolidated bioprocessing (CBP), where enzyme production, cellulose hydrolysis, and fermentation are integrated into a single microbial system. Engineering *Saccharomyces cerevisiae* to express cellulolytic enzymes and efficient sugar transporters has significantly improved cellulose utilization while reducing the requirement for externally added enzymes. Although cellulase secretion efficiency and enzyme balance remain challenging, CBP offers considerable potential for lowering production costs and simplifying industrial biofuel production (Yamada *et al.*, 2013; Minnaar *et al.*, 2023) [6, 27].

Recent studies have also explored synthetic microbial consortia to improve lignocellulosic bioconversion. By distributing metabolic functions among specialized microorganisms, co-culture systems enhance mixed-sugar utilization, reduce metabolic burden, and improve tolerance to inhibitory compounds. Such division of labour has demonstrated superior substrate conversion efficiency compared with monoculture fermentations, particularly when processing complex lignocellulosic hydrolysates (Chen *et al.*, 2018; Yan *et al.*, 2023) [15, 10].

Integration of advanced pretreatment technologies with engineered yeast strains has further improved biofuel productivity. Pretreatment methods that maximize sugar recovery while minimizing inhibitor formation enhance downstream fermentation performance and increase overall process efficiency. Coupled with advances in metabolic engineering and process optimization, these integrated strategies are expected to play a crucial role in establishing economically viable lignocellulosic biorefineries for simultaneous production of multiple biofuels and value-added biochemicals (Prado *et al.*, 2025; Pereira *et al.*, 2024) [29, 28].

4. Challenges and future perspectives

Despite remarkable progress in the metabolic engineering of *Saccharomyces cerevisiae*, several biological and technological challenges continue to limit its industrial application for dual biofuel production. One of the primary constraints is the incomplete and asynchronous utilization of lignocellulose-derived mixed sugars, particularly glucose and xylose, due to carbon catabolite repression and inefficient pentose metabolism. In addition, heterologous expression of transporters and biosynthetic enzymes often imposes a significant metabolic burden, reducing cell growth, fermentation efficiency, and overall product yield (Wei *et al.*, 2020; Wagner *et al.*, 2023) [14, 11].

Another major challenge is the presence of fermentation inhibitors, including furfural, 5-hydroxymethylfurfural (HMF), acetic acid, and phenolic compounds, which are generated during biomass pretreatment. These compounds impair cell viability, alter redox balance, and reduce biofuel productivity under industrial conditions. Furthermore, achieving stable expression of cellulolytic enzymes, maintaining genetic stability of engineered strains, and balancing multiple biosynthetic pathways remain critical obstacles for large-scale commercialization (Ko *et al.*, 2019; Zhao *et al.*, 2024) [4, 13].

Future research should focus on integrating systems biology, synthetic biology, and artificial intelligence-assisted metabolic modelling to design robust yeast strains with improved substrate utilization, stress tolerance, and carbon conversion efficiency. Advanced genome-editing technologies, dynamic metabolic regulation, and machine learning-guided pathway optimization are expected to accelerate strain development while minimizing metabolic burden. In parallel, combining engineered yeast with efficient pretreatment technologies, consolidated bioprocessing, and synthetic microbial consortia will further improve lignocellulosic biomass conversion and support economically viable biorefineries capable of producing multiple biofuels and value-added biochemicals (Dixit *et al.*, 2023; Shin *et al.*, 2024) [7, 16].

5. Conclusion

Metabolic engineering has transformed *Saccharomyces cerevisiae* into a promising microbial platform for the sustainable production of dual biofuels from lignocellulosic biomass. Advances in sugar transporter engineering, cellulolytic enzyme expression, pentose assimilation, and carbon flux redistribution have substantially improved the utilization of mixed sugars and expanded the production of ethanol alongside higher-value biofuels and biochemicals. Furthermore, the integration of genome-editing technologies, adaptive laboratory evolution, and process optimization has enhanced fermentation efficiency and strain robustness, bringing lignocellulosic biorefineries closer to industrial implementation.

Despite these advances, challenges associated with mixed-sugar utilization, inhibitor tolerance, metabolic burden, and process scalability continue to limit commercial application. Addressing these limitations will require integrated approaches combining systems metabolic engineering, synthetic biology, computational modelling, and advanced bioprocess design.

Overall, continued innovations in metabolic engineering and process integration are expected to accelerate the development of economically viable and environmentally sustainable lignocellulosic biorefineries. Such advances will not only improve renewable biofuel production but also support the broader transition towards a circular bioeconomy and reduced dependence on fossil-based energy resources.

Acknowledgements

The authors also thank the AICRP on EAAI (Bioconversion Technology), University of Agricultural Sciences, Dharwad for providing institutional support and encouragement during the preparation of this review manuscript.

Conflict of interest

The authors declare that they have no conflict of interests to declare.

Funding

The authors received no specific financial support from any funding agency in the public, commercial, or not-for-profit sectors for the preparation of this review.

References

1. Sun L, Jin YS. Xylose assimilation for the efficient production of biofuels and chemicals by engineered *Saccharomyces cerevisiae*. *Biotechnol J*. 2021;16(4):2000142.
2. Xu F, Sun D, Wang Z, Li M, Yin X, Li H, *et al.* Highly efficient production of cellulosic ethanol from poplar using an optimal C6/C5 co-fermentation strain of *Saccharomyces cerevisiae*. *Microorganisms*. 2024;12(6):1174.
3. Ha SJ, Galazka JM, Kim SR, Choi JH, Yang X, Seo J, *et al.* Engineered *Saccharomyces cerevisiae* capable of simultaneous cellobiose and xylose fermentation. *Proc Natl Acad Sci U S A*. 2011;108(2):504-509.
4. Ko JK, Enkh-Amgalan T, Gong G, Um Y, Lee SM. Improved bioconversion of lignocellulosic biomass by *Saccharomyces cerevisiae* engineered for tolerance to acetic acid. *GCB Bioenergy*. 2019;12(1):90-100.
5. Jeffries TW, Nelson SS, Mahan SD. Metabolically engineered yeasts for the production of ethanol and other products from xylose and cellobiose. *Appl Microbiol Biotechnol*. 2011;89:1097-1108.
6. Yamada R, Nakatani Y, Ogino C, Kondo A. Efficient direct ethanol production from cellulose by cellulase- and celldextrin transporter-co-expressing *Saccharomyces cerevisiae*. *AMB Express*. 2013;3:34.

7. Dixit Y, Yadav P, Sharma AK, Pandey P, Kuila A. Multiplex genome editing to construct cellulase-engineered *Saccharomyces cerevisiae* for ethanol production from cellulosic biomass. *Renew Sustain Energy Rev.* 2023;180:113310.
8. Lee Y, Tran PHN, Ko JK, Gong G, Um Y, Han SO, *et al.* Glucose/xylose co-fermenting *Saccharomyces cerevisiae* increases the production of acetyl-CoA-derived n-butanol from lignocellulosic biomass. *Front Bioeng Biotechnol.* 2022;10:942919.
9. Jayakody LN, Liu J, Yun EJ, Turner TL, Oh EJ, Jin YS. Direct conversion of cellulose into ethanol and ethyl- β -D-glucoside via engineered *Saccharomyces cerevisiae*. *Biotechnol Bioeng.* 2018;115(12):2859-2868.
10. Yan N, Luan T, Yin M, Niu Y, Wu L, Yang S, *et al.* Co-fermentation of glucose-xylose-cellobiose-XOS mixtures using a synthetic consortium of recombinant *Saccharomyces cerevisiae* strains. *Fermentation.* 2023;9:1035.
11. Wagner ER, Gasch AP. Advances in *Saccharomyces cerevisiae* engineering for xylose fermentation and biofuel production: balancing growth, metabolism, and defense. *J Fungi.* 2023;9(11):1088.
12. Ahuja V, Chinnam S, Bhatt AK. Yeast-based biorefinery for xylitol and ethanol production from sugarcane bagasse. *Chem Eng Res Des.* 2024;191:676-684.
13. Zhao J, Zhao Y, Wu L, Yan N, Yang S, Xu L, *et al.* Development of a robust *Saccharomyces cerevisiae* strain for efficient co-fermentation of mixed sugars and enhanced inhibitor tolerance through protoplast fusion. *Microorganisms.* 2024;12(8):1526.
14. Wei F, Li M, Wang M, Li H, Li Z, Qin W, *et al.* A C6/C5 co-fermenting *Saccharomyces cerevisiae* strain with alleviation of antagonism between xylose utilization and robustness. *GCB Bioenergy.* 2020;13(1):83-97.
15. Chen Y, Wu Y, Zhu B, Zhang G, Wei N. Co-fermentation of cellobiose and xylose by mixed culture of recombinant *Saccharomyces cerevisiae* and kinetic modeling. *PLoS One.* 2018;13(6):e0198984.
16. Shin J, Liao S, Kuanyshv N, Xin Y, Kim C, Lu T, *et al.* Compositional and temporal division of labor modulates mixed sugar fermentation by an engineered yeast consortium. *Nat Commun.* 2024;15:1764.
17. Kretzer LG, Knychala MM, Silva LCD, Fontoura ICC, Leandro M, Fonseca C, *et al.* Screening and engineering yeast transporters to improve cellobiose fermentation by recombinant *Saccharomyces cerevisiae*. *Fermentation.* 2024;10(9):490.
18. Liu Z, Ho SH, Sasaki K, de Haan R, Inokuma K, Ogino C, *et al.* Engineering of a novel cellulose-adherent cellulolytic *Saccharomyces cerevisiae* for cellulosic biofuel production. *Sci Rep.* 2016;6:24550.
19. Zha J, Li B, Shen MH, Hu ML, Song H, Yuan Y. Optimization of CDT1 and XYL1 expression for balanced co-production of ethanol and xylitol from cellobiose and xylose by engineered *Saccharomyces cerevisiae*. *PLoS One.* 2013;8(7):e68844.
20. Papapetridis I, Verhoeven MD, Wiersma SJ, Goudriaan M, van Maris AJA, Pronk JT. Laboratory evolution for forced glucose-xylose co-consumption enables identification of mutations that improve mixed-sugar fermentation by xylose-fermenting *Saccharomyces cerevisiae*. *FEMS Yeast Res.* 2018;18(6):foy056.
21. Khan SM, Hussain A, Hussain MI, Aqeel SM, Basharat S, Al-Ansi W, *et al.* Metabolic engineering of *Saccharomyces cerevisiae* for ethanol and butanol biofuel production. *Int J Environ Agric Biotechnol.* 2022;8(2):47-56.
22. Promdonkoy P, Mhuanong W, Champreda V, Tanapongpipat S, Rungphan W. Improvement in D-xylose utilization and isobutanol production in *Saccharomyces cerevisiae* by adaptive laboratory evolution and rational engineering. *J Ind Microbiol Biotechnol.* 2020;47(6):497-510.
23. Hull CM, Loveridge EJ, Rolley NJ, Donnison IS, Kelly SL, Kelly DE. Co-production of ethanol and squalene using a *Saccharomyces cerevisiae* *erg1* mutant and agro-industrial feedstock. *Biotechnol Biofuels.* 2014;7:133.
24. Nan H, Seo SO, Oh EJ, Seo J, Cate JHD, Jin YS. 2,3-Butanediol production from cellobiose by engineered *Saccharomyces cerevisiae*. *Appl Microbiol Biotechnol.* 2014;98(12):5757-5764.
25. Ravn JL, Manfrão-Netto JHC, Schaubeder JB, Pianale LT, Spirk S, Ciklic I, *et al.* Engineering *Saccharomyces cerevisiae* for targeted hydrolysis and fermentation of glucuronoxylan through CRISPR/Cas9 genome editing. *Microb Cell Fact.* 2024;23:152.
26. Song X, Li Y, Wu Y, Cai M, Liu Q, Gao K, *et al.* Metabolic engineering strategies for improvement of ethanol production in cellulolytic *Saccharomyces cerevisiae*. *FEMS Yeast Res.* 2018;18(8):foy079.
27. Minnaar L, de Haan R. Engineering natural isolates of *Saccharomyces cerevisiae* for consolidated bioprocessing of cellulosic feedstocks. *Appl Microbiol Biotechnol.* 2023;107:541-556.
28. Pereira IDO, Santos AA, Guimarães NC, Lima CS, Zanella E, Matsushika A, *et al.* First- and second-generation integrated process for bioethanol production: fermentation of molasses diluted with hemicellulose hydrolysate by recombinant *Saccharomyces cerevisiae*. *Biotechnol Bioeng.* 2024;121:1126-1140.
29. Prado CA, Silva A, Fernandes PA, Shibukawa VP, Jofre FM, Rodrigues BG, *et al.* Hydrodynamic cavitation-assisted photo-Fenton pretreatment and yeast co-culture as strategies to produce ethanol and xylitol from sugarcane bagasse. *Catalysts.* 2025;15(5):418.
30. Wei N, Oh EJ, Million G, Cate JHD, Jin YS. Simultaneous utilization of cellobiose, xylose and acetic acid from lignocellulosic biomass for biofuel production by an engineered yeast platform. *ACS Synth Biol.* 2015;4(6):707-713.

How to cite this article: Santosh G, Shubha S, Chetankumar R, Naveen V, Patil C. Metabolic engineering of *Saccharomyces cerevisiae* for dual biofuel production from lignocellulosic biomass: Advances in pathway optimization and process integration. *International Journal of Advanced Biochemistry Research.* 2026;SP-10(9):2240-2244. doi: <https://www.doi.org/10.33545/26174693.2026.v10.i9S.ab.10154>