


Mini review: Yeast Genes Expression Affecting Lignocellulosic Biomass Fermentation

Naveen Kumar^{1,*}, Ajay Singh², Subodh Kumar³, Himanshu Saini⁴, Hritik Srivastava⁵, Nisha Sharma⁴, Shivani Sharma⁴, Himanshu Mehta⁴, Priya Chugh⁴, Vinay Chauhan⁴

¹ Department of Microbiology, School of Agriculture and Technology, Maya Devi University, Dehradun-248011, Uttarakhand, India

² Department of Biotechnology, Deenbandhu Chhotu Ram University of Science and Technology (DCRUST), Murthal, Sonapat (Haryana) – 131039

³ School of Bioscience and Technology, Galgotias University, Greater Noida, Uttar Pradesh

⁴ School of Agriculture and Technology, Maya Devi University, Dehradun-248011, Uttarakhand, India

⁵ Department of Agriculture, Integral Institute of Agricultural Science and Technology (IIAST), Integral University, Lucknow, 226026, India

* Correspondence: naveen.kumar@mdu.edu.in;

Received: 2.03.2025; Accepted: 17.07.2025; Published: 30.09.2025

Abstract: Efficient lignocellulosic biomass fermentation by yeast is crucial for maximizing sustainable biofuel production. This abundant resource, derived from sources like forestry residues (wood chips), agricultural wastes (corn stover, wheat straw), and dedicated energy crops (switch grass), is primarily composed of cellulose, hemicellulose, and lignin. The success of turning lignocellulosic biomass into biofuels depends on yeast being able to break down complex sugars into simpler sugars, which can then be turned into useful products like ethanol and other biofuels. Consequently, genetic engineering strategies to enhance both the rate of alcoholic fermentation and yeast tolerance to ethanol have shown considerable promise. This review focuses on the key yeast genes that significantly influence lignocellulosic biomass fermentation and the overall efficiency of alcoholic fermentation.

Keywords: *Saccharomyces cerevisiae*; yeasts; gene expression; fermentation.

© 2025 by the authors. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. The authors retain copyright of their work, and no permission is required from the authors or the publisher to reuse or distribute this article, as long as proper attribution is given to the original source.

1. Introduction

Cellulose, hemicellulose, and lignin make up the bulk of lignocellulosic biomass. The primary component of lignocellulose is cellulose, which is the β -1,4 polymer of glucose, and the most common sugar in lignocellulose hydrolysates is glucose. Hemicellulose is a heteropolymer made up of galactose, xylose, and arabinose, among other substances. The second-highest sugar in lignocellulose hydrolysates is xylose, which is generated from hemicellulose. Therefore, the quick and effective fermentation of lignocellulosic sugars by microbes is critical to the financial viability of industrial bioconversion [1]. A natural method for dissolving more complex organic compounds into simpler ones is microbial fermentation. Before alcoholic fermentation, pretreatment procedures may be necessary to prepare the biomass for extraction and fermentation. Pretreatment is an important stage in the conversion of lignocellulosic materials into ethanol, as it is required for the enzymatic saccharification process. Brewer's yeast, or *Saccharomyces cerevisiae*, is the most widely used and conventional

cell factory for the commercial synthesis of bioethanol. It can ferment hexose well and make a lot of ethanol because it ferments quickly and doesn't react badly with ethanol [2]. Fermentable mono- and disaccharide sugars can be released through enzymatic hydrolysis subsequent to preparation. These sugars (such as glucose, galactose, and fructose) are then converted by yeast in metabolic processes that can take place in both aerobic and anaerobic environments to ethanol, carbon dioxide, and other byproducts. For instance, during glycolysis, glucose molecules yield two pyruvate molecules. Following this, two molecules of ethanol and carbon dioxide are produced from the two pyruvic acid molecules [3]. Pyruvate can be converted to acetaldehyde in anaerobic conditions by releasing carbon dioxide. Alcohol dehydrogenase can subsequently convert acetaldehyde to ethanol [4]. The efficient fermentation of lignocellulosic biomass by yeast involves the expression of specific genes that enable the breakdown of complex plant cell wall components into fermentable sugars and the utilization of these sugars for growth and product formation. Several key yeast genes play critical roles in lignocellulosic biomass fermentation.

2. Cellulase Genes

Genetically modified yeast strains containing cellulase genes have the ability to synthesize cellulolytic enzymes that degrade cellulose, the primary constituent of plant cell walls, into glucose and other oligosaccharides. Illustrative instances comprise genetic sequences encoding endoglucanases, exoglucanases, and β -glucosidases [5]. Together, the enzymes accelerate the hydrolysis of cellulose polymers, converting them into soluble sugars that yeast can metabolize, including glucose. By efficiently expressing cellulase genes, yeast can effectively use lignocellulosic biomass as a carbon source for growth and product synthesis. Elevated expression of cellulase genes yields heightened cellulase enzyme activity, hence enhancing the efficiency of cellulose degradation and the liberation of fermentable sugars from lignocellulosic biomass. This increases the availability of carbohydrates for yeast fermentation and improves overall process efficiency. The efficient expression of cellulase genes helps overcome the challenge provided by the resistant nature of cellulose in lignocellulosic biomass. Yeast strains that have been genetically modified to express cellulase genes at a higher level demonstrate better fermentation performance when using lignocellulosic biomass substrates. These organisms have the ability to digest released sugars effectively, resulting in higher ethanol production, faster fermentation rates, and increased productivity. High expression of cellulase genes lowers the lag period associated with lignocellulosic biomass fermentation. Yeast cells may swiftly adapt to the presence of complex substrates and commence growth and fermentation activities early, leading to shorter overall fermentation periods. Genetic engineering approaches can be utilized to optimize the expression of cellulase genes in yeast strains for certain biomass feedstocks and process conditions. This enables the production of tailor-made yeast strains with enhanced cellulolytic capabilities for a variety of lignocellulosic biomass sources. The development of a CBP host strain has been significantly assisted by cellulase engineering in *S. cerevisiae*. This has resulted in the successful secretion of the three main types of cellulase activities, namely β -glucosidase (BGL), endoglucanase (EG), and cellobiohydrolase (CBH), as well as partial ethanol conversion [6,7] (Figure 1). The processive mechanism of action of cellobiohydrolase, also known as exoglucanase (CBH, 1,4- β -D-glucan cellobiohydrolase, E.C.3.2.1.91) on crystalline cellulose substrates has drawn special attention. The majority of cellulolytic fungi generate the most protein, called cellobiohydrolases, which liberate cellobiose by acting on the reducing (CBHI) or non-

reducing (CBHII) ends of cellulose chains. These enzymes are essential to synergy [8]. Davison et al. [9] finally increased hydrolysing and fermenting ability by screening cellulosic *S. cerevisiae* transformants generated using either cocktail delta (δ)-integration and/or plasmid-borne methods. Davison et al. [9] hypothesized that the optimum recombinant expression ratio of cellulases required may also depend on the proportion of substrate employed. In comparison to co-expressing *Talaromyces emersonii* CBHI and *S. fibuligera* BGLI (with 7.08 g/L glucose and 37.1% cellulose conversion yields), a *S. cerevisiae* transformant that co-expressed the genes *Trichoderma reesei* EGII and *Saccharomycopsis fibuligera* BGLI from a single strain produced higher glucose yields (10.8 g/L) and consequently higher cellulose conversion yields (56.5%) in corn residues [9].

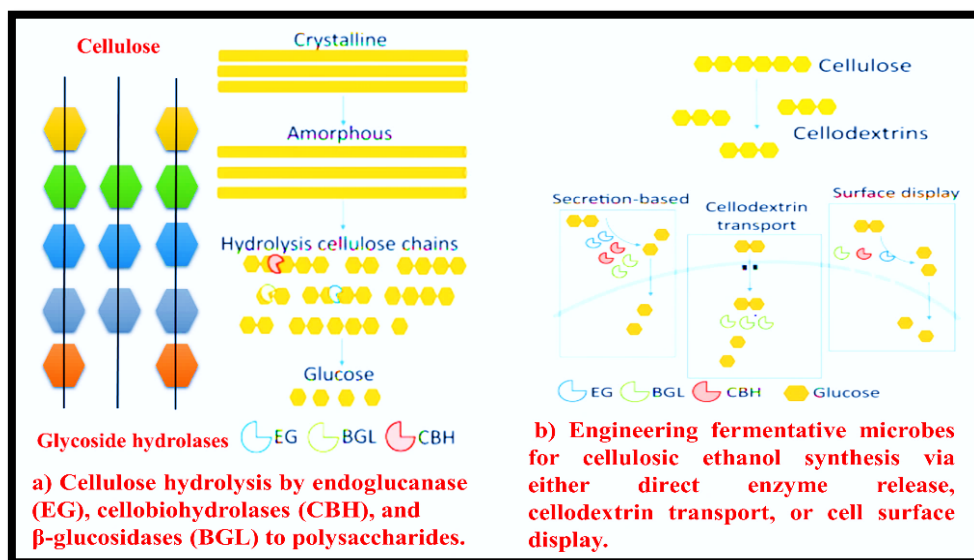


Figure 1. Lignocellulosic sugar utilization pathways using recombinant *S. cerevisiae* strains.

3. Hemicellulase Genes

Hemicellulases are enzymes that hydrolyze hemicellulose, a heterogeneous polymer found in plant cell walls. Yeast strains designed to express hemicellulase genes produce enzymes such as xylanases, arabinofuranosidase, and xyloglucanases. These enzymes target various components of hemicellulose, breaking them down into monomeric sugars such as xylose, arabinose, and galactose, which are subsequently available for fermentation by yeast [10]. Increased expression of hemicellulase genes leads to greater enzymatic activity, resulting in more effective breakdown of hemicellulose and the release of fermentable sugars. This boosts the availability of extra sugars for yeast fermentation, hence enhancing overall process efficiency and increasing ethanol production. Hemicellulase enzymes can efficiently break down hemicellulose into fermentable sugars, reducing the accumulation of hemicellulose-derived inhibitors during biomass pretreatment and hydrolysis operations. This contributes to increased yeast viability, fermentation kinetics, and overall process robustness. Two primary xylose-using routes have been discovered. Two oxidoreductases, xylose reductase (XR) and xylitol dehydrogenase (XDH), are involved in xylose consumption in fungi and yeasts that ferment xylose. These enzymes require the cofactors NADPH and NAD⁺, respectively, for their forward processes (Figure 2, pathway 1). The majority of bacterial species use a different approach that requires only one enzyme, xylose isomerase (Figure 2, Pathway 2). Both activities produce xylulose, which is phosphorylated by xylulose kinase. This enzyme can then enter the Pentose Phosphate Pathway (PPP) and produce glycolytic pathway intermediates.

Pentose sugars can enter *S. cerevisiae* spontaneously by entangling themselves with different hexose sugar transporters (Hxt7, Hxt5, Hxt4, Hxt2, and Gal2). The main issue here is that these transporters have a higher affinity for glucose, which is their native substrate. Therefore, glucose is preferentially transported while xylose uptake is hindered in media containing both hexose and pentose sugars, such as those produced by pretreating lignocellulose biomass. Two different approaches have been studied: the overexpression of genes encoding native *S. cerevisiae* transporters and the heterologous expression of genes encoding transporters from xylose-using species. Different studies have shown different growth rates and xylose intake in response to the overexpression of the hexose transporter genes Hxt1, Hxt7, Hxt13, and Gal2. Overexpressing HXT1 and GAL2 boosted xylose transport, whereas overexpressing HXT7 did not [11].

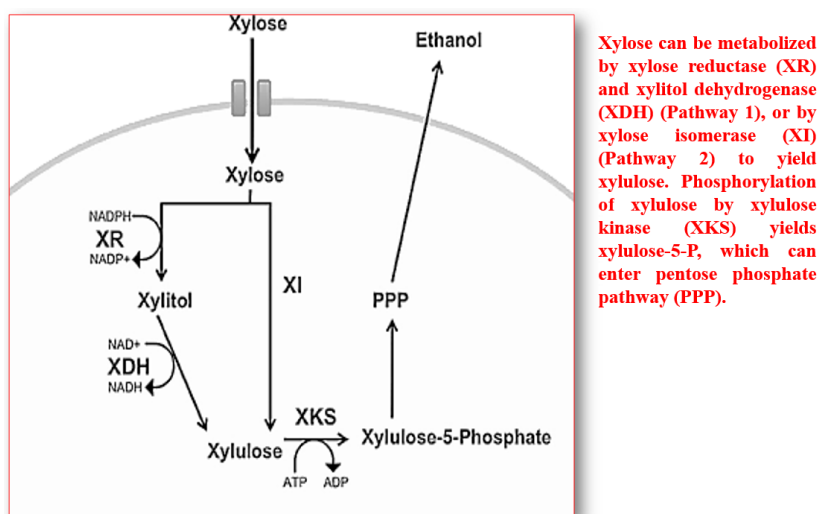


Figure 2. Xylose metabolism routes expressed heterologously in yeast.

4. Pectinase Genes

Pectinases are enzymes that decompose pectin, a complex polysaccharide found in plants' intermediate lamella and main cell walls. Yeast strains harboring pectinase genes may hydrolyze pectin into monomeric sugars like galacturonic acid, which can be fermented. Lignocellulosic biomass, such as wood, agricultural leftovers, and certain types of grass, contains not only cellulose and hemicellulose but also pectin. By breaking down pectin more efficiently, yeast can access a greater range of carbohydrates, including those produced from pectin. This can lead to higher total sugar usage during fermentation, potentially enhancing fermentation efficiency and the output of biofuels or other bioproducts. If yeast can create sufficient levels of pectinase enzymes internally, it may eliminate the need for external enzyme supplementation during lignocellulosic biomass fermentation. This can result in cost savings and simplify the fermentation process. Lignocellulosic biomass often contains inhibitors, such as organic acids and phenolic compounds, which can be created during pretreatment operations. Potential growth inhibitors that are probably present include methanol and acetic acid [12]. These substances may have an impact on the yields of metabolite production, fermentation kinetics, and yeast growth [13]. Pectinase enzymes can help in the detoxification of these inhibitors by breaking down complex compounds, hence boosting yeast tolerance to fermentation inhibitors. Furthermore, D-galacturonic acid, which is not natively utilized by *S. cerevisiae* or other important yeast species like *Kluyveromyces marxianus*, *Yarrowia lipolytica*, or *Pichia stipitis*, is present in substantial amounts in pectin-rich hydrolysates. There have been

recent reports of attempts to genetically modify *S. cerevisiae* to effectively express the catabolic pathway for D-galacturonic acid [14]. Furthermore, studies have focused on developing this pentose-fermentative route in *S. cerevisiae* strains because pectin-rich hydrolysates contain notable concentrations of L-arabinose [15]. Pectinase enzymes can function synergistically with cellulases and hemicellulases to efficiently break down the various components of lignocellulosic biomass. Coordinated expression of genes encoding these enzymes can lead to optimum biomass breakdown and fermentation performance.

5. Transporter Genes

Yeast strains may need specific transporters to efficiently absorb a variety of sugars generated from lignocellulosic biomass breakdown. Lignocellulosic biomass contains numerous sugars, including glucose, xylose, and arabinose. Genes encoding sugar transporters, such as hexose transporters (e.g., HXT genes) and pentose transporters (e.g., XUT genes), are necessary for sugar utilization during fermentation [16]. Higher expression levels of these transporter genes can accelerate the uptake of sugars, resulting in higher fermentation performance. Lignocellulosic biomass fermentation can create organic acids, such as acetic acid and formic acid, which can limit yeast growth and fermentation. Yeast transporter genes involved in organic acid transport, such as PDR12 and TPO1, are required to export these inhibitors out of the cell, boosting yeast tolerance to acidic environments and improving fermentation efficiency [17]. Lignocellulosic biomass also contains aromatic chemicals generated from lignin breakdown, such as phenolic compounds and aromatic aldehydes, which can be harmful to yeast. Transporter genes involved in the uptake or efflux of these aromatic chemicals (e.g., ATR1, FLR1) can modify yeast tolerance to lignin-derived inhibitors and affect fermentation performance. Some yeast transporter genes are involved in the uptake of cofactors or co-substrates essential for specific metabolic pathways involved in lignocellulosic biomass fermentation. For example, transporters involved in the intake of vitamins, minerals, or co-enzymes may alter the activity of important enzymes engaged in sugar metabolism or detoxification pathways, thereby influencing overall fermentation efficiency. Transporter genes can also play a role in nutrient recycling by enhancing the uptake of compounds produced during biomass decomposition. For instance, transporters involved in the uptake of short-chain fatty acids or amino acids generated during lignocellulose degradation can supply additional nutrients to assist yeast growth and metabolism during fermentation.

6. Fermentation Pathway Genes

The expression of yeast fermentation pathway genes can have a substantial impact on lignocellulosic biomass fermentation. Yeast, such as *S. cerevisiae*, utilizes several metabolic pathways to ferment sugars obtained from lignocellulosic biomass into biofuels (e.g., ethanol) or biochemicals (e.g., organic acids). Genes involved in central carbon metabolism, such as those encoding enzymes of glycolysis, the pentose phosphate route, and the ethanol fermentation pathway, are vital for converting fermentable sugars produced from lignocellulosic biomass into ethanol or other desirable products [18]. Glycolysis is the key metabolic mechanism responsible for the conversion of glucose to pyruvate. Key enzymes involved in glycolysis, such as hexokinase, phosphofructokinase, and pyruvate kinase, catalyze successive events leading to the synthesis of ATP and pyruvate. Higher expression levels of glycolytic genes can increase the rate of glucose utilization and pyruvate generation, thereby

enhancing fermentation performance. The pentose phosphate pathway is critical for the metabolism of pentose sugars such as xylose and arabinose, which are common in lignocellulosic biomass. Enzymes involved in PPP, such as transketolase and transaldolase, create NADPH and ribose-5-phosphate, which are critical for cellular redox equilibrium and nucleotide biosynthesis, respectively. Modulating PPP gene expression can boost pentose sugar consumption and improve overall fermentation efficiency. Ethanol fermentation is the conversion of pyruvate to ethanol and carbon dioxide through anaerobic metabolism. This route involves enzymes such as pyruvate decarboxylase and alcohol dehydrogenase [19]. Higher expression levels of ethanol fermentation genes can boost ethanol production rates and yields, which are critical for biofuel generation from lignocellulosic biomass. In addition to ethanol, yeast may create organic acids such as acetic acid and lactic acid under certain conditions. These organic acids can be undesirable byproducts in ethanol production as they diminish ethanol yield and productivity. Modulating the expression of genes involved in alternate fermentation pathways, such as the pyruvate decarboxylase bypass pathway, can limit the accumulation of organic acids and increase ethanol fermentation performance.

7. Stress Response Genes

Lignocellulosic biomass hydrolysates commonly contain inhibitory chemicals (e.g., furans, phenolics) that might stress yeast cells. Genes implicated in stress response pathways, such as those regulating oxidative stress (e.g., YAP1, SKN7) and osmotic stress (e.g., HOG1), play essential roles in improving yeast tolerance to biomass-derived inhibitors. Lignocellulosic biomass fermentation presents various obstacles to yeast, including high osmolarity, acidic pH, the presence of fermentation inhibitors, and changes in temperature and oxygen levels. Lignocellulosic hydrolysates frequently include high quantities of sugars and salts, resulting in osmotic stress on yeast cells. Genes involved in the osmotic stress response, such as HOG1 and its downstream targets, regulate the production of compatible solutes as well as the activation of osmoprotective systems. Modulating the expression of osmotic stress response genes can promote yeast tolerance to high osmolarity conditions and improve fermentation performance in lignocellulosic hydrolysates. Lignocellulosic hydrolysates often have an acidic pH due to the presence of organic acids created during biomass preparation. Yeast acid stress response genes, including TPS1, TPS2, and RIM15, are involved in controlling intracellular pH homeostasis, detoxification of organic acids, and maintenance of cell viability under acidic circumstances. In lignocellulosic hydrolysates, optimizing the expression of acid stress response genes can increase yeast tolerance to acidic pH and improve fermentation efficiency. During lignocellulosic biomass fermentation, yeast cells may suffer temperature variations and heat stress, especially in industrial-scale fermentations. Heat shock response genes, such as HSP70 and HSP90, play a vital role in protein folding, stability, and refolding under heat stress conditions. Modulating the expression of heat shock response genes can promote yeast thermotolerance and improve fermentation performance at elevated temperatures. Lignocellulosic hydrolysates contain fermentation inhibitors such as furfural, hydroxymethylfurfural (HMF), and phenolic chemicals, which can hinder yeast development and fermentation [20]. Genes implicated in detoxification pathways, including ADHs (alcohol dehydrogenases), ALDHs (aldehyde dehydrogenases), and other oxidoreductases, mediate the conversion of hazardous chemicals into less damaging metabolites. Optimizing the expression of detoxifying genes can boost yeast tolerance to fermentation inhibitors and improve fermentation performance in lignocellulosic hydrolysates. Wei et al. [21] effectively developed

a method to convert the harmful acetic acid into ethanol in genetically modified *S. cerevisiae* in the absence of oxygen. Furfural and HMF can be used as the sole carbon source for cell development by *Amorphotheca resinae* ZN1 and transformed into low-toxic chemicals for *S. cerevisiae* [22]. Overexpression of dehydrogenases (ADH6/7) and pentose phosphate pathway (ZWF1) genes can improve the reduction ability of *S. cerevisiae* to furfural and 5-HMF [23]. The researchers thought that the poor performance of *S. cerevisiae* strains grown in both acetic acid and furfural might be because there wasn't enough ATP and NADPH available, as shown by the results of ¹³C metabolic flux analyses. One possible way to make yeast more resistant to fermentation inhibitors in lignocellulosic materials is to change *S. cerevisiae* strains so that they make more energy and cofactors. Because fermentation inhibitors change many biological processes, it has been suggested that they change gene expression by changing the expression of a transcriptional factor. The first time that bioinformatic research found that two transcription factors, Sfp1p and Ace2p, make yeast more resistant to mixed fermentation inhibitors was when they were studied [24].

8. Regulatory Genes

Lately, nanomaterials derived from lignin have gained extensive utilization as biomaterials across a wide spectrum of uses, including antimicrobial effects, food and cosmetics, UV absorption, tissue engineering, and antioxidant functionalities. Transcription factors and regulatory proteins influence gene expression in lignocellulosic biomass fermentation. Engineering regulatory genes, such as those involved in carbon catabolite repression (e.g., MIG1) or stress response control (e.g., MSN2/MSN4), can optimize yeast performance under biomass fermentation conditions [25]. Transcription factors regulate gene expression by binding to certain DNA sequences and activating or suppressing target genes. In lignocellulosic biomass fermentation, TFs such as XlnR (XYL1 regulator) and AraR (ARA1 regulator) regulate the expression of genes involved in pentose sugar utilization pathways, facilitating the effective metabolism of xylose and arabinose. Modulating TF expression can increase consumption of alternative sugars and improve fermentation performance [26]. Regulatory genes involved in stress response pathways have a vital role in yeast's ability to cope with environmental challenges experienced during lignocellulosic biomass fermentation. For example, the transcription factor Msn2/4 modulates the expression of stress-responsive genes under diverse stress conditions, including osmotic stress, thermal shock, and oxidative damage. Modulating the expression or activity of stress response regulators can boost yeast tolerance to fermentation inhibitors and improve fermentation efficiency. Yeast regulatory genes involved in carbon catabolite suppression govern the use of different carbon sources based on their availability and preference. When glucose is present, regulatory genes such as Mig1 and Mig2 suppress the expression of genes involved in alternate carbon source utilization during lignocellulosic biomass fermentation, ensuring effective glucose consumption [27]. Modulating CCR regulators can optimize carbon source usage and increase fermentation performance in lignocellulosic hydrolysates. Regulatory genes involved in nutrition sensing and signaling pathways monitor extracellular nutrient availability and regulate cellular responses accordingly. For example, the TOR (target of rapamycin) signaling system governs cell growth and metabolism in response to nutritional availability. Modulating the activity of nutrient-sensing pathways can optimize yeast metabolism and improve fermentation efficiency in lignocellulosic biomass. Regulatory genes involved in the cell cycle control the timing of cell division and coordinate cellular processes during lignocellulosic biomass fermentation. For

instance, the cyclin-dependent kinase (CDK) pathway governs cell cycle progression and cellular responses to environmental signals. Modulating cell cycle regulators can maximize cell growth and biomass generation during fermentation, thereby enhancing overall fermentation performance.

9. Industrial-derived Tolerant Strains

Scientists are becoming more and more interested in using *S. cerevisiae* strains from tough industrial environments that have lots of sugar and ethanol, high temperatures, changing pH levels, and harmful chemicals to make 2G bioethanol. The isolated strains have demonstrated greater capabilities compared to laboratory strains, with variations in fermentation performance being attributed to metabolic activity. This includes not only sugar consumption and ethanol production, but also furan conversion. The *S. cerevisiae* ATCC96581 strain, which was found in used sulfite liquor at a Swedish pulp plant, was able to convert almost all of the furfural in spruce hydrolysate when it was put through second-generation inhibitory conditions. In contrast, the laboratory strain CBS 8066 only detoxified 25% of the furfural [28]. This phenomenon can be attributed to the increased activity of alcohol dehydrogenase, which is responsible for converting furfural into less harmful alcohols. Pereira and co-workers [29] also observed a quicker bioconversion and detoxification of furfural and HMF in eucalyptus hydrolysate by two industrial strains, PE-2 and flocculating CCUG53310 obtained from first- and second-generation bioethanol companies, respectively. The authors discovered that the ability to detoxify furan compounds is dependent on strain background, which is critical for effective ethanol production [29]. The mechanism and resilience of the flocculating CCUG53310 strain have been examined and compared with the laboratory *S. cerevisiae* CBS 8066 [30]. Despite having lower expression levels of the genes YAP1, ATR1, and FLR1 (which confer resistance to lignocellulose-derived inhibitors) than the laboratory strain, the flocculant strain demonstrated higher tolerance to the inhibitors present in a spruce hydrolysate, highlighting flocculation as a physiological trait determinant of yeast tolerance. The authors further postulated that flocculation may prevent ROS accumulation through mechanisms that are still unknown but are probably connected to a decrease in toxic concentrations outside and inside the cell, as evidenced by the lower expression of YAP1, which is normally activated in response to oxidative stress, in the CCUG53310 strain. Thus, using a strong yeast chassis for metabolic engineering applications (such as xylose consumption) provides an additional advantage for the fermentation of lignocellulose into ethanol [31]. In fact, a xylose consumption pathway was expressed in three distinct *S. cerevisiae* strains: the industrial isolates PE-2 and CAT-1 from 1G bioethanol plants, and the laboratory strain CEN.PK113-5D. It was discovered that the two industrial strains produced more ethanol and consumed more xylose than the laboratory strain in both synthetic media and a corn cob hydrolysate. By genetically modifying the industrial strain *S. cerevisiae* ATCC 4124 and the laboratory strain D452-2 for xylose intake, Kim et al. [32] also assessed the host strain background of these strains. The significance of choosing a naturally robust host strain was highlighted when they found that the industrially derived strain outperformed the laboratory strain with the same genetic modification in a Miscanthus hydrolysate, demonstrating better efficiency of xylose fermentation and ethanol production. Furthermore, Costa et al. [31] demonstrated that the type of hemicellulosic hydrolysate employed could affect the amount of xylose consumed by industrial strains that had undergone metabolic engineering. It is also

possible to enhance these desired characteristics of the industrial isolates' inhibitor tolerance via mutagenesis, metabolic engineering, genome rearranging, or evolutionary engineering.

10. CRISPR/Cas9 System for Yeast Genome Engineering

In recent years, yeast genome engineering has made use of CRISPR-Cas9 genome editing technology. The CRISPR/Cas9 system's exceptional advantages, such as its flexibility, efficiency in retargeting, capacity to introduce site-specific mutation efficiently, and multiplexing capabilities, have contributed to its appeal when compared to other conventional approaches. *S. cerevisiae* has been the focus of the majority of work describing CRISPR/Cas9 genome editing in yeasts, which has focused on *S. cerevisiae* [33]. Recent reports have shown the use of installed synthetic Cas9 target sequences in the yeast genome, which enables fine control over integration of the sequence of interest into specific sections of the genome, reduction of off-target effects, and multiplexing with a single sgRNA [34]. The CRISPR/Cas9 system can be used in many ways in *Y. lipolytica*, and studies on gene deletion have shown that it works well enough. Furthermore, a number of vectors for targeting the *Y. lipolytica* multiplex gene have been developed [35]. Recently, the expression of several Cas9 genes and gRNA molecules in *P. pastoris* has been thoroughly examined. Recently, Weninger et al. established a CRISPR/Cas9 approach for *P. pastoris*, demonstrating nearly 100% targeting effectiveness. They looked into the integration of site-specific homologous DNA, deleted a few genes, and used this better technology to do tests on gene disruption. The CRISPR/Cas9 method lets you change the genome of *P. pastoris* quickly and without using markers. This opens up new metabolic and strain engineering possibilities [36]. Single-gene nonsense mutations with up to 90% effectiveness can result from the production of the Cas9 gene and the HH/HDV-ribozyme-flanked gRNA transcript, according to reports. Despite targeting two genes, 69% of the time, nonsense mutations in both ORFs were found. The donor template had 1-kbp homologous arms; however, the integration efficiency was only 2%. This implied that the primary technique for repairing double-strand breaks was NHEJ [37]. In a separate study, the CRISPR-Cas9 system was created using the tRNA^{Leu} promoter, specifically for use in *H. polymorpha*. This system uses the endogenous tRNA processing mechanism to ensure proper sgRNA maturation. Up to 71% of the disruption was efficient, which made metabolic engineering techniques easier to use in the future [38].

11. Conclusions

Efficient utilization of lignocellulosic hydrolysates for 2G biofuel production necessitates addressing the inhibitory levels of acetic acid generated during pretreatment. A practical route to industrial-scale bioethanol synthesis lies in the creation of robust yeast strains that can effectively combat this organic acid stress, potentially by harnessing the inherent resistance mechanisms found in wild yeasts. Metabolic and genetic engineering have emerged as powerful technologies for endowing yeast with enhanced acetic acid tolerance and improved bioethanol production. By precisely modulating the expression of key genes through these engineering strategies and optimizing fermentation processes, significant advancements can be made in the efficiency of lignocellulosic biomass fermentation, driving down costs and bolstering the sustainability of biofuel production.

Author Contributions

Conceptualization, N.K., N.S; methodology, N.K.; software, N.K., H.S.; validation, H.M., S.S, N.K.; formal analysis, N.K., H.S., V.C.; investigation, H.M., S.S.; resources, N.K.; data curation, H.S., N.S.; writing—original draft preparation, N.K.; writing—review and editing, N.K., H.S., H.S., N.S., S.S., H.M., P.C., A.S. V.C.; visualization, N.K., H.S., P.C., A.S.; supervision, N.K.; project administration, N.K.. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

No new data were created or analyzed in this study. Data sharing is not applicable.

Funding

This research received no external funding.

Acknowledgments

The authors would like to express their gratitude to the management of Maya Devi University, Dehradun, for their assistance in completing this project.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Oh, E.J.; Jin, Y.S. Engineering of *Saccharomyces cerevisiae* for efficient fermentation of cellulose. *FEMS yeast research* **2020**, *20*, foz089, <https://doi.org/10.1093/femsyr/foz089>.
2. Kumar, N.; Aggarwal, N.K. Process development for fungal cellulase production and enzymatic saccharification of *Parthenium hysterophorus* for bioethanol production. *Waste Management Bulletin* **2024**, *2*, 1-8, <https://doi.org/10.1016/j.wmb.2023.11.004>.
3. Milanesi, R.; Porro, D.; Branduardi, P.; Serra, I. Production of Industrially Relevant Organic Acids by Yeasts and Filamentous Fungi. In *Biotechnology of Yeasts and Filamentous Fungi*, Sibirny, A.A., Ed.; Springer Nature Switzerland: Cham, **2025**; pp. 123-140, https://doi.org/10.1007/978-3-031-74726-7_5.
4. Malakar, S.; Paul, S.K.; Jolvis Pou, K.R. 1 - Biotechnological Interventions in Beverage Production. In *Biotechnological Progress and Beverage Consumption*, Grumezescu, A.M., Holban, A.M., Eds.; Academic Press: **2020**; pp. 1-37, <https://doi.org/10.1016/B978-0-12-816678-9.00001-1>.
5. Siqueira, J.G.; Rodrigues, C.; de Souza Vandenberghe, L.P.; Woiciechowski, A.L.; Soccol, C.R. Current advances in on-site cellulase production and application on lignocellulosic biomass conversion to biofuels: a review. *Biomass and Bioenergy* **2020**, *132*, 105419, <https://doi.org/10.1016/j.biombioe.2019.105419>.
6. Raheja, Y.; Gaur, P.; Islam, T.; Chaurasia, A.K.; Gaur, V.K.; Chadha, B.S. Advancement in lignocellulolytic enzyme production: tailored strategies to overcome challenges in biomass hydrolysis. *Systems Microbiology and Biomanufacturing* **2025**, *5*, 948-968, <https://doi.org/10.1007/s43393-025-00342-7>.

7. Kumar, N.; Sharma, R.; Aggarwal, N.K.; Yadav, A. Parthenium hysterophorus weed as a novel substrate for β -glucosidase production by *Penicillium citrinum* NAF5: application of the crude extract to biomass saccharification. *Lett Appl NanoBioSci*. **2023**, *12*, 13, <https://doi.org/10.33263/LIANBS121.013>.
8. Sukumaran, R.K.; Sankar, M.; Adarsh, V.P.; Mathew, R.M.; Sreeja-Raju, A.; Athulya; Neetha, P.S.; Raphy, B.; Gnanaraj, V.R. Technological Advancements in Enzyme Production for 2G Ethanol. In Value Addition and Product Diversification in Sugarcane, Suresha, G.S., Krishnappa, G., Palanichamy, M., Mahadeva Swamy, H.K., Kuppasamy, H., Govindakurup, H., Eds.; Springer Nature Singapore: Singapore, **2024**; pp. 337-362, https://doi.org/10.1007/978-981-97-7228-5_18.
9. Davison, S.A.; Keller, N.T.; van Zyl, W.H.; den Haan, R. Improved cellulase expression in diploid yeast strains enhanced consolidated bioprocessing of pretreated corn residues. *Enzyme and Microbial Technology* **2019**, *131*, 109382, <https://doi.org/10.1016/j.enzmictec.2019.109382>.
10. Ravn, J.L.; Engqvist, M.K.; Larsbrink, J.; Geijer, C. CAZyme prediction in ascomycetous yeast genomes guides discovery of novel xylanolytic species with diverse capacities for hemicellulose hydrolysis. *Biotechnology for Biofuels* **2021**, *14*, 150, <https://doi.org/10.1186/s13068-021-01995-x>.
11. Vasylyshyn, R.; Kurylenko, O.; Ruchala, J.; Shevchuk, N.; Kuliesiene, N.; Khroustalyova, G.; Rapoport, A.; Daugelavicius, R.; Dmytruk, K.; Sibirny, A. Engineering of sugar transporters for improvement of xylose utilization during high-temperature alcoholic fermentation in *Ogataea polymorpha* yeast. *Microbial Cell Factories* **2020**, *19*, 96, <https://doi.org/10.1186/s12934-020-01354-9>.
12. Joanna, B.; Michal, B.; Piotr, D.; Agnieszka, W.; Dorota, K.; Izabela, W. Chapter 13 - Sugar Beet Pulp as a Source of Valuable Biotechnological Products. In Advances in Biotechnology for Food Industry, Holban, A.M., Grumezescu, A.M., Eds.; Academic Press: **2018**; pp. 359-392, <https://doi.org/10.1016/B978-0-12-811443-8.00013-X>.
13. Cunha, J.T.; Romani, A.; Costa, C.E.; Sá-Correia, I.; Domingues, L. Molecular and physiological basis of *Saccharomyces cerevisiae* tolerance to adverse lignocellulose-based process conditions. *Applied microbiology and biotechnology* **2019**, *103*, 159-175, <https://doi.org/10.1007/s00253-018-9478-3>.
14. Jeong, D.; Ye, S.; Park, H.; Kim, S.R. Simultaneous fermentation of galacturonic acid and five-carbon sugars by engineered *Saccharomyces cerevisiae*. *Bioresource Technology* **2020**, *295*, 122259, <https://doi.org/10.1016/j.biortech.2019.122259>.
15. Ye, S.; Jeong, D.; Shon, J.C.; Liu, K.H.; Kim, K.H.; Shin, M.; Kim, S.R. Deletion of PHO13 improves aerobic l-arabinose fermentation in engineered *Saccharomyces cerevisiae*. *Journal of Industrial Microbiology and Biotechnology* **2019**, *46*, 1725-1731, <https://doi.org/10.1007/s10295-019-02233-y>.
16. Knychala, M.M.; Dos Santos, A.A.; Kretzer, L.G.; Gelsleichter, F.; Leandro, M.J.; Fonseca, C.; Stambuk, B.U. Strategies for efficient expression of heterologous monosaccharide transporters in *Saccharomyces cerevisiae*. *Journal of Fungi* **2022**, *8*, 84, <https://doi.org/10.3390/jof8010084>.
17. Ndukwe, J.K.; Aliyu, G.O.; Onwosi, C.O.; Chukwu, K.O.; Ezugworie, F.N. Mechanisms of weak acid-induced stress tolerance in yeasts: Prospects for improved bioethanol production from lignocellulosic biomass. *Process Biochemistry* **2020**, *90*, 118-130, <https://doi.org/10.1016/j.procbio.2019.11.009>.
18. Avanthi, A.; Kumar, S.; Sherpa, K.C.; Banerjee, R. Bioconversion of hemicelluloses of lignocellulosic biomass to ethanol: an attempt to utilize pentose sugars. *Biofuels* **2017**, *8*, 431-444, <https://doi.org/10.1080/17597269.2016.1249738>.
19. Woo, J.E.; Jang, Y.S. Metabolic engineering of microorganisms for the production of ethanol and butanol from oxides of carbon. *Applied microbiology and biotechnology* **2019**, *103*, 8283-8292, <https://doi.org/10.1007/s00253-019-10072-1>.
20. Li, B.; Liu, N.; Zhao, X. Response mechanisms of *Saccharomyces cerevisiae* to the stress factors present in lignocellulose hydrolysate and strategies for constructing robust strains. *Biotechnology for Biofuels and Bioproducts* **2022**, *15*, 28, <https://doi.org/10.1186/s13068-022-02127-9>.
21. Wei, N.; Quarterman, J.; Kim, S.R.; Cate, J.H.; Jin, Y.S. Enhanced biofuel production through coupled acetic acid and xylose consumption by engineered yeast. *Nature communications* **2013**, *4*, 2580, <https://doi.org/10.1038/ncomms3580>.
22. Wang, X.; Gao, Q.; Bao, J. Transcriptional analysis of *Amorphotheca resinae* ZN1 on biological degradation of furfural and 5-hydroxymethylfurfural derived from lignocellulose pretreatment. *Biotechnology for biofuels* **2015**, *8*, 1-3, <https://doi.org/10.1186/s13068-015-0323-y>.
23. Liu, C.G.; Li, K.; Li, K.Y.; Sakdaronnarong, C.; Mehmood, M.A.; Zhao, X.Q.; Bai, F.W. Intracellular redox perturbation in *Saccharomyces cerevisiae* improved furfural tolerance and enhanced cellulosic bioethanol

- production. *Frontiers in Bioengineering and Biotechnology* **2020**, *8*, 615, <https://doi.org/10.3389/fbioe.2020.00615>.
24. Jin, Y.S.; Cate, J.H. Metabolic engineering of yeast for lignocellulosic biofuel production. *Current opinion in chemical biology* **2017**, *41*, 99-106, <http://dx.doi.org/10.1016/j.cbpa.2017.10.025>.
 25. Minden, S.; Aniolek, M.; Noorman, H.; Takors, R. Performing in spite of starvation: How *Saccharomyces cerevisiae* maintains robust growth when facing famine zones in industrial bioreactors. *Microbial Biotechnology* **2023**, *16*, 148-168, <https://doi.org/10.1111/1751-7915.14188>.
 26. Benocci, T.; Aguilar-Pontes, M.V.; Kun, R.S.; Lubbers, R.J.; Lail, K.; Wang, M.; Lipzen, A.; Ng, V.; Grigoriev, IV.; Seiboth, B.; Daly, P. Deletion of either the regulatory gene *ara1* or metabolic gene *xkil* in *Trichoderma reesei* leads to increased CAZyme gene expression on crude plant biomass. *Biotechnology for biofuels* **2019**, *12*, 81, <https://doi.org/10.1186/s13068-019-1422-y>.
 27. Wang, L.; Wang, A.; Wang, D.; Hong, J. The novel properties of *Kluyveromyces marxianus* glucose sensor/receptor repressor pathway and the construction of glucose repression-released strains. *Microbial Cell Factories* **2023**, *22*, 123, <https://doi.org/10.1186/s12934-023-02138-7>.
 28. Becerra, M.L.; Prieto, G.A.; Rendueles, M.; Diaz, M. Biological transformations of furanic platform molecules to obtain biomass-derived furans: a review. *Biomass Conversion and Biorefinery* **2024**, *14*, 26611-26629, <https://doi.org/10.1007/s13399-022-03535-5>.
 29. Pereira, F.B.; Teixeira, M.C.; Mira, N.P.; Sá-Correia, I.; Domingues, L. Genome-wide screening of *Saccharomyces cerevisiae* genes required to foster tolerance towards industrial wheat straw hydrolysates. *Journal of Industrial Microbiology and Biotechnology* **2014**, *41*, 1753-1761, <https://doi.org/10.1007/s10295-014-1519-z>.
 30. Westman, J.O.; Taherzadeh, M.J.; Franzén, C.J. Inhibitor tolerance and flocculation of a yeast strain suitable for second generation bioethanol production. *Electronic Journal of Biotechnology* **2012**, *15*, <https://doi.org/10.2225/vol15-issue3-fulltext-8>.
 31. Costa, C.E.; Romani, A.; Cunha, J.T.; Johansson, B.; Domingues, L. Integrated approach for selecting efficient *Saccharomyces cerevisiae* for industrial lignocellulosic fermentations: importance of yeast chassis linked to process conditions. *Bioresource technology* **2017**, *227*, 24-34, <https://doi.org/10.1016/j.biortech.2016.12.016>.
 32. Kim, S.R.; Skerker, J.M.; Kong, I.I.; Kim, H.; Maurer, M.J.; Zhang, G.C.; Peng, D.; Wei, N.; Arkin, A.P.; Jin, Y.S. Metabolic engineering of a haploid strain derived from a triploid industrial yeast for producing cellulosic ethanol. *Metabolic engineering* **2017**, *40*, 176-185, <https://doi.org/10.1016/j.ymben.2017.02.006>.
 33. Wang, Z.; Qi, X.; Ren, X.; Lin, Y.; Zeng, F.; Wang, Q. Synthetic evolution of *Saccharomyces cerevisiae* for biomanufacturing: Approaches and applications. *mLife* **2025**, *4*, 1-16, <https://doi.org/10.1002/mlf2.12167>.
 34. Giersch, R.M.; Finnigan, G.C. Method for multiplexing CRISPR/Cas9 in *Saccharomyces cerevisiae* using artificial target DNA sequences. *Bio-protocol* **2017**, *7*, e2557, <https://doi.org/10.21769/BioProtoc.2557>.
 35. Shi, T.Q.; Huang, H.; Kerkhoven, E.J.; Ji, X.J. Advancing metabolic engineering of *Yarrowia lipolytica* using the CRISPR/Cas system. *Applied microbiology and biotechnology* **2018**, *102*, 9541-9548, <https://doi.org/10.1007/s00253-018-9366-x>.
 36. García-Calvo, L.; Kummén, C.; Rustad, S.; Rønning, S.B.; Fagerlund, A. A toolkit for facilitating markerless integration of expression cassettes in *Komagataella phaffii* via CRISPR/Cas9. *Microbial Cell Factories* **2025**, *24*, 97, <https://doi.org/10.1186/s12934-025-02716-x>.
 37. Stovicek, V.; Holkenbrink, C.; Borodina, I. CRISPR/Cas system for yeast genome engineering: advances and applications. *FEMS yeast research* **2017**, *17*, fox030, <https://doi.org/10.1093/femsyr/fox030>.
 38. Löbs, A.K.; Schwartz, C.; Wheeldon, I. Genome and metabolic engineering in non-conventional yeasts: current advances and applications. *Synthetic and systems biotechnology* **2017**, *2*, 198-207, <https://doi.org/10.1016/j.synbio.2017.08.002>.

Publisher's Note & Disclaimer

The statements, opinions, and data presented in this publication are solely those of the individual author(s) and contributor(s) and do not necessarily reflect the views of the publisher and/or the editor(s). The publisher and/or the editor(s) disclaim any responsibility for the accuracy, completeness, or reliability of the content. Neither the publisher nor the editor(s) assume any legal liability for any errors, omissions, or consequences arising from the use of the information presented in this publication. Furthermore, the publisher and/or the editor(s) disclaim any liability for any injury, damage, or loss to persons or property that may result from the use of any ideas, methods,

instructions, or products mentioned in the content. Readers are encouraged to independently verify any information before relying on it, and the publisher assumes no responsibility for any consequences arising from the use of materials contained in this publication.