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Review Article

The Emerging Role of *Saccharomyces cerevisiae* in Polyhydroxyalkanoate Biosynthesis: A Review

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ABSTRACT
Increasing environmental concerns over plastic pollution and the depletion of fossil resources have driven global interest in biodegradable polymers, such as polyhydroxyalkanoates (PHAs). While bacterial systems have traditionally dominated PHA production, *Saccharomyces cerevisiae* has recently emerged as a promising alternative due to its Generally Recognized as Safe (GRAS) status, industrial robustness, and compatibility with advanced genetic engineering tools. This review explores the current state and future potential of *S. cerevisiae* as a cell factory for PHA biosynthesis. It examines metabolic engineering strategies to enhance precursor availability, the expression of heterologous PHA biosynthetic genes, and integrating dynamic regulatory elements to optimise production. Recent advancements in feedstock utilisation, particularly lignocellulosic and industrial wastes bioreactor design, and downstream processing, are highlighted, along with challenges such as low yield, enzyme misfolding, and extraction inefficiencies. The review also emphasises the importance of omics technologies, synthetic biology, and machine learning in accelerating strain development and scalability. Overall, the article underscores the transformative potential of *S. cerevisiae* in enabling sustainable bioplastic production and contributing to circular bioeconomy goals.

Keywords: Bioplastics; Metabolic engineering; Polyhydroxyalkanoates; *Saccharomyces cerevisiae;* Sustainable biotechnology

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INTRODUCTION

Due to escalating environmental concerns and global regulatory shifts towards sustainability, polyhydroxyalkanoates (PHAs) have emerged as a prominent candidate to replace petroleum-based plastics based on their biodegradability and biocompatibility (Jimoh *et al*., 2018). These microbial polyesters are synthesized and stored intracellularly by various bacteria under excess carbon and nutrient limitation conditions, forming granules that serve as carbon and energy reserves (Jendrossek, 2009; Jimoh *et al*., 2018). Traditionally, production of PHA's has relied on well-studied bacteria such as *Cupriavidus necator* and *Pseudomonas putida*, which can accumulate up to 90% and 60% of cell dry weight as PHA under optimized fermentation conditions, respectively (Jendrossek, 2009; Zytner *et al*., 2023). However, the commercialisation of PHAs has been hindered by factors including high feedstock costs, complex downstream processing, and limited yields (Zytner *et al*., 2023; Gautam *et al*., 2024). Considering these limitations, researchers have explored alternative microbial platforms, such as archaea, microalgae, and yeasts, with renewed interest in *Saccharomyces cerevisiae*, a eukaryotic organism renowned for its industrial versatility. While yeast does not naturally synthesize PHA, recent advances

in synthetic biology have enabled the functional expression of bacterial PHA biosynthetic gene clusters in yeast, initiating intracellular production (Kocharin *et al*., 2012; Chen *et al*., 2016; Kalia *et al*., 2025). Compared to bacterial hosts, the unique advantages of *S. cerevisiae* include:

i. Robust industrial performance: It tolerates fermentation stresses such as osmotic pressure, low pH environments, ethanol, and inhibitory by-products; traits well-suited for high-cell-density and continuous cultivation systems (Jimoh *et al*., 2009a; Jimoh *et al*., 2009b; Jimoh *et al*., 2012a; Jimoh *et al*., 2013a; Kim *et al*., 2022; Elhalis, 2024);
ii. Genetic and metabolic flexibility: Varieties of genetic tools, including CRISPR-Cas9, dynamically regulated promoters, and organelle-targeting strategies have enabled precise metabolic engineering and pathway rewiring in yeast for diverse bioproducts (Jimoh *et al*., 2013b; Jakočiūnas *et al*., 2015; Jimoh *et al*., 2025; Yook and Alper, 2025; Yang *et al*., 2025) and
iii. Substrate scope: Naturally, yeast can metabolise glucose and sucrose; through genetic engineering, it can also utilise xylose, glycerol, oil-based substrates, and agricultural and industrial residues (Jimoh *et al*.; 2012b; Maicas, 2020; Asafa-Adedimeji and Jimoh, 2022; Zytner *et al*., 2023; Wives *et al*., 2024).

Based on sustainability, incorporating yeast into PHA production pipelines enables benefits such as reduced contamination risk, GRAS status for food and pharmaceutical applications, and compatibility with existing fermentation infrastructure (Kusuma *et al*., 2024; Jimoh *et al*., 2025). Despite these advances, yeast-derived PHAs currently lag bacterial yields. For instance, engineered yeast typically achieves only 3–5% cell dry weight accumulation over extended cultivations, compared to over 50% in optimised bacterial systems (Kocharin *et al*., 2012; Baek *et al*., 2024). Overcoming this gap requires integrated strategies combining metabolic modelling, organelle engineering, stress response adaptations, and process optimisation, efforts well-supported by yeast's rich biological tools and industrial track record (Xu *et al*., 2011; Jimoh *et al*., 2012a; Jimoh *et al*., 2013a; Jakočiūnas *et al*., 2015; Zytner *et al*., 2023; Kalia *et al*., 2025). Consequently, *S. cerevisiae* is a promising framework for sustainable, scalable PHA production. This review explores recent developments in pathway design, metabolic engineering, substrate utilisation, bioprocess strategies, and organism-level optimisation to close the productivity gap and unlock the full potential of yeast-based bioplastics.

Polyhydroxyalkanoate Biosynthesis: Pathways and Enzymatic Mechanisms

Polyhydroxyalkanoates (PHAs) represent a diverse family of microbial polyesters synthesised and stored intracellularly as energy and carbon reserves (Jimoh *et al*., 2018). They are classified into short-chain-length PHAs (scl-PHAs) and medium-chain-length PHAs (mcl-PHAs), depending on the number of carbon atoms in of the monomer units (Samrot *et al*., 2024). The biosynthesis of PHAs involves three core enzymes: β-ketothiolase (PhaA), acetoacetyl-CoA reductase (PhaB), and PHA synthase (PhaC). These enzymes catalyse the sequential conversion of acetyl-CoA to PHA through the intermediates acetoacetyl-CoA and 3-hydroxy butyryl-CoA (Sagong *et al*., 2018;). The biosynthetic pathway begins with β-ketothiolase condensing two molecules of acetyl-CoA to form acetoacetyl-CoA and further reduced by acetoacetyl-CoA reductase to (R)-3-hydroxybutyryl-CoA, which serves as the monomer substrate for PHA synthase (PhaC) to polymerise into PHA granules (Grage *et al*., 2009; Kudo *et al*., 2023). The specificity and activity of PHA synthase determine the type and properties of the PHA produced, making PhaC a central target for metabolic engineering (Neoh *et al*., 2022). In natural PHA-producing bacteria such as *Cupriavidus necator*, these enzymes are encoded in the phaCAB operon, allowing efficient regulation and expression (Kim *et al*., 2022; Tang *et al*., 2022). However, *Saccharomyces cerevisiae* lacks these distinctive genes and thus requires heterologous expression of bacterial PHA biosynthetic genes. Recent research focused on optimising codon usage and expression levels and localising PHA enzymes in yeast to improve PHA production efficiency (Kocharin *et al*., 2012; Sandström *et al*., 2015; Jia *et al*., 2025).

The main challenge in yeast-based PHA biosynthesis is the availability of precursors such as acetyl-CoA and NADPH. Since *S. cerevisiae* tightly regulates cytosolic acetyl-CoA pools for lipid and sterol biosynthesis, rerouting this metabolite toward PHA production demands comprehensive metabolic engineering. The regulations include overexpressing acetyl-CoA-generating pathways (e.g., pyruvate dehydrogenase bypass), minimising competitive pathways, and implementing synthetic organelles to compartmentalize PHA biosynthesis (Xu *et al*., 2016; Wives *et al*., 2024). Furthermore, cofactor imbalance can limit flux through the PHA pathway, particularly in NADPH-dependent steps. Strategies such as the co-expression of NADPH-regenerating enzymes or dynamic control systems have been proposed to address this problem (Zhao *et al*., 2017; Kalia *et al*., 2025). Despite these hurdles, engineered *S. cerevisiae* strains expressing

phaCAB genes have accumulated PHAs intracellularly, although at lower yields than bacteria. However, yeast's GRAS status, fermentation robustness, and tolerance to industrial substrates, even moderate production levels, present a compelling case for yeast-based biopolymer production in applications where food safety and biocompatibility are critical (Asafa-Adedimeji and Jimoh 2022; Gundlapalli and Ganesan 2025; Yao *et al*., 2025).

Engineering *Saccharomyces cerevisiae* for PHA Production

The engineering of *Saccharomyces cerevisiae* for polyhydroxyalkanoate (PHA) production represents a promising shift from traditional bacterial hosts to robust, industrially scalable eukaryotic systems. Although *S. cerevisiae* does not naturally produce PHAs, its genetic tractability, established fermentation technology, and GRAS status make it a compelling framework for sustainable biopolymer production (Kocharin *et al*., 2012; Sandström *et al*., 2015). Initial efforts in engineering yeast for PHA production focused on the heterologous expression of the phaCAB gene cluster, encoding the key enzymes β-ketothiolase (PhaA), acetoacetyl-CoA reductase (PhaB), and PHA synthase (PhaC), typically sourced from *Cupriavidus necator* (Zhang *et al*., 2022). Kocharin *et al*. (2012) and Ylinen *et al*. 2022) successfully integrated these genes into *S. cerevisiae*, leading to detectable PHA granule accumulation. However, PHA yields were limited due to metabolic competition and poor precursor availability. Subsequent metabolic engineering efforts have addressed crucial challenges, including improving the supply of acetyl-CoA, enhancing NADPH availability, and minimising competing fluxes. Strategies include overexpressing acetyl-CoA synthetase, introducing ATP-citrate lyase, and activating the pyruvate dehydrogenase bypass to enhance cytosolic acetyl-CoA levels (Kocharin *et al*., 2012; Rueda *et al*., 2024; Gundlapalli and Ganesan, 2025; Yao *et al*., 2025. To boost NADPH regeneration, researchers have employed overexpression of enzymes such as glucose-6-phosphate dehydrogenase or malic enzyme (Stanton *et al*., 2012; Adusumilli *et al*., 2024).

Moreover, recent advances in organelle engineering have enabled the compartmentalisation of PHA biosynthesis within peroxisomes or mitochondria (Huttanus and Senger, 2020; Gu and Oliferenko, 2023; Yin *et al*., 2024). This spatial separation reduces competition for resources and enhances precursor channelling toward PHA formation (Walker and Pretorius, 2018; Burgos-Morales *et al*., 2021; Choi *et al*., 2022). Furthermore, synthetic scaffolding and dynamic metabolic control, such as inducible promoters and riboswitches, have enhanced the regulation of pathway flux (Lee *et al*., 2018; Kent and Dixon, 2020; Li *et al*., 2022). Through CRISPR/Cas9 genome editing and precise integration, regulating PHA biosynthetic pathways in *S. cerevisiae* has become more feasible. Wives *et al*. (2024) introduced advanced promoter engineering and multiplexed gene insertions to enhance PHA titers significantly. Despite these developments, PHA productivity in yeast remains lower compared to bacterial systems. However, yeast physiological advantages, such as tolerance to lignocellulosic hydrolysates, high osmotic pressure, and low pH conditions, benefit industrial-scale fermentation (Jimoh *et al*., 2018; Jimoh *et al*., 2022). Engineering strategies aim to balance yield improvements with the inherent robustness and safety of *S. cerevisiae*, thus establishing a competitive host for PHA bioproduction.

Advances in Metabolic Engineering Strategies

Recent advances in metabolic engineering have significantly improved the potential of *Saccharomyces cerevisiae* as a microbial cell factory for polyhydroxyalkanoate (PHA) biosynthesis. These developments target several challenges in precursor supply, redox balance, pathway regulation, and metabolic burden. Since *S. cerevisiae* does not naturally synthesise PHAs, optimising endogenous metabolic pathways and introducing heterologous genes are critical strategies to enable and enhance PHA production (Koller, 2022; Paduvari and Somashekara, 2025). Natural acetyl-CoA synthesis is tightly regulated and mostly occurs in mitochondria, limiting its accessibility for cytosolic PHA synthesis. To address this, metabolic engineers have rerouted carbon flux toward acetyl-CoA through the pyruvate dehydrogenase bypass and overexpression of ATP-citrate lyase (ACL) and acetyl-CoA synthetase (ACS) variants optimised for yeast (Kocharin *et al*., 2012; Gundlapalli and Ganesan, 2025; Yao *et al*., 2025). Recent studies have also demonstrated synthetic acetyl-CoA pathways incorporating phosphoketolases and phosphotransacetylases to bypass regulatory constraints and improve flux (Yang *et al.,* 2023).

Furthermore, the redox cofactor balance, mainly the NADPH/$NADP^+$ ratio, is crucial for acetoacetyl-CoA reductase (PhaB) activity. Enhancing NADPH regeneration through overexpression of enzymes such as glucose-6-phosphate dehydrogenase or $NADP^+$-dependent malic enzyme has also improved PHA yields (Koller 2022; Li *et al*., 2020; Paduvari and Somashekara 2025). Cofactor engineering and developing NADH-preferring variants of PhaB provide alternative routes to improve pathway efficiency. Compartmentalisation strategies

targeting PHA biosynthesis to peroxisomes or mitochondria assist in separating metabolic flux and minimising competition with natural pathways. This spatial engineering increases local precursor concentration and reduces metabolic interference and toxicity from PHA intermediates (Huttanus and Senger, 2020; Gu and Oliferenko, 2023; Yin *et al*., 2024). Dynamic pathway control using inducible promoters, biosensors, and feedback regulation enables fine-tuning gene expression in response to internal or environmental signals (Andres *et al*., 2019; Li *et al*., 2022). Recent studies have incorporated synthetic biology tools such as optogenetics and metabolite-responsive transcription factors to regulate PHA pathway genes dynamically, ensuring balanced growth and production phases (Naseri and Koffas 2020; Del Valle *et al*., 2021; Rojas and Larrondo, 2023); Benisch *et al*., 2024). Also, genome-scale metabolic models (GEMs) and computational tools have been used to identify important gene targets, simulate flux distributions, and guide rational strain design. Integrated omics approaches, including proteomics, transcriptomics, and metabolomics, have further accelerated the discovery of limiting factors and regulatory nodes in PHA-producing yeast strains (Weimer *et al*., 2020; Jain *et al*., 2024; Fan *et al*., 2025). These advanced metabolic engineering strategies continue to unlock the biosynthetic potential of *S. cerevisiae*, bringing yeast-based PHA production closer to commercial viability.

Substrate Utilisation and Fermentation Optimisation

The cost-effectiveness and sustainability of PHA's production using *S. cerevisiae* are highly dependent on the type of substrates used and the efficiency of the fermentation process. Substrate utilisation in engineered yeast is critical, particularly when considering renewable, inexpensive carbon sources. Optimising fermentation conditions is vital to maximise PHA yields, minimise by-product formation, and reduce downstream processing costs (Maicas, 2020; Asafa-Adedimeji and Jimoh, 2022; Kalia *et al*., 2025). The main advantage of *S. cerevisiae* over traditional bacterial PHA producers is its ability to tolerate harsh fermentation conditions, such as low pH and high osmolarity, making it suitable for large-scale bioreactor operations (Asafa-Adedimeji and Jimoh 2022; Li *et al*., 2022). Moreover, after genetic modification, *S. cerevisiae* can metabolise various substrates, including glucose, sucrose, galactose, and lignocellulosic hydrolysates (Tsegaye *et al*., 2024).

Recent studies have focused on engineering *S. cerevisiae* strains to utilise pentose sugars (xylose and arabinose), abundant in lignocellulosic biomass. The integration of heterologous xylose isomerase and xylose reductase/xylitol dehydrogenase pathways has enabled yeast to grow on xylose and produce PHAs from non-food substrates (Lee *et al*., 2018; Wives *et al*., 2024). Co-fermentation of glucose and xylose remains challenging due to carbon catabolite repression, but synthetic regulation circuits and transporter engineering have alleviated this issue (Andres *et al.,* 2019; Yan *et al*., 2023). Fermentation optimisation involves controlling environmental factors such as pH, temperature, oxygen availability, and feeding strategies. Fed-batch and continuous fermentation modes increase PHA titers by avoiding substrate inhibition and prolonging the productive phase of the cells (Rajpurohit and Eiteman, 2022; Du *et al*., 2022). Oxygen limitation also favours acetyl-CoA accumulation by reducing respiration and diverting flux toward PHA precursors; thus, agro-industrial residues, such as molasses, glycerol, and food waste hydrolysates, serve as a cost-effective carbon source for PHA production. Recent bioprocess development studies have successfully coupled hydrolysate detoxification with yeast fermentation to produce PHAs under non-sterile and scalable conditions (Sun *et al*., 2022; Atarés *et al*., 2024). These advances in substrate utilisation and fermentation process engineering contribute significantly to making *S. cerevisiae* a practical host for PHA production in industrial settings. Future efforts will focus on integrating real-time monitoring, adaptive control, and artificial intelligence (AI) -assisted optimisation to improve yields further and reduce costs.

Genetic and Synthetic Biology Tools

The advancement of genetic and synthetic biology tools has revolutionised the ability to reprogram *Saccharomyces cerevisiae* for heterologous production of complex metabolites, including polyhydroxyalkanoates (PHAs). As *S. cerevisiae* lacks natural PHA biosynthetic pathways, synthetic biology enables the systematic integration of functional genes, dynamic control systems, and modular expression platforms to build optimised strains for PHA production (Chen *et al*., 2016; Chang *et al*., 2024). The initial step involves the introduction of PHA biosynthesis genes from natural producers such as *Cupriavidus necator* and *Ralstonia eutropha*, including *phaA* (β-ketothiolase), *phaB* (acetoacetyl-CoA reductase), and *phaC* (PHA synthase). Codon optimisation and promoter selection are essential for their functional expression in yeast. Strong constitutive or inducible promoters are commonly used to drive expression, while synthetic promoter libraries have been developed to fine-tune gene expression (Feng and

Marchisio, 2021; Orita *et al*., 2022; Ishihara *et al*., 2023).

CRISPR/Cas9 and CRISPR interference (CRISPRi) technologies greatly facilitated genome editing in *S. cerevisiae*. These tools knock out competing pathways, integrate heterologous genes at safe harbour loci (e.g., HO, delta sites), and modulate gene expression precisely (Meng *et al*., 2020; Baek *et al*., 2024; Ansori *et al*., 2024). Multiplexed genome editing strategies allow simultaneous modifications of multiple genes to accelerate strain engineering for complex phenotypes, analogue to PHA production (Kent and Dixon, 2020). Synthetic biology has also introduced dynamic control elements such as riboswitches, quorum-sensing circuits, and optogenetic systems that regulate gene expression in response to metabolic or environmental signals (Chang *et al*., 2024; Müller *et al*., 2025). These tools assist balance cell growth with product synthesis, reducing metabolic burden and improving yields (Naseri and Koffas, 2020; Müller *et al*., 2025). For example, metabolite-responsive promoters can activate PHA pathway genes only when precursors such as acetyl-CoA accumulate beyond a threshold.

Modular cloning systems such as Golden Gate, MoClo, and YeastFab allow rapid and standardised assembly of biosynthetic pathways (Shaw *et al*., 2023; Marillonnet and Werner, 2025). These platforms enable plug-and-play construction of multigene pathways with defined regulatory elements, streamlining the design-build-test-learn (DBTL) cycle in yeast metabolic engineering (Cho *et al*., 2022; Jeon *et al*., 2025). Furthermore, machine learning-assisted synthetic biology is now being explored to predict optimal gene combinations and regulatory networks for improved biosynthesis. Deep learning tools can analyse large datasets from transcriptomics or proteomics to guide rational engineering strategies (van Lent *et al*., 2023; Jeon *et al*., 2025). Together, these powerful genetic and synthetic biology tools provide a robust framework to convert *S. cerevisiae* into an efficient PHA-producing platform, pushing the boundaries of yeast biotechnology and sustainable bioplastic development.

Biopolymer Recovery and Characterization

Efficient recovery and characterisation of polyhydroxyalkanoates (PHAs) are critical for evaluating production efficiency and determining the quality and applicability of the biopolymer (Getino *et al*., 2024). In engineered *Saccharomyces cerevisiae*, downstream processing must overcome specific challenges, such as the robustness of the yeast cell wall and the typically lower intracellular PHA concentrations compared to bacterial systems (Chen *et al*., 2016; de Melo *et al*., 2023). Nevertheless, advances in biopolymer extraction and analytical techniques have significantly contributed to assessing and improving PHA production in yeast-based systems. Traditionally, PHA extraction involves solvent-based methods using chloroform or dichloromethane to dissolve PHAs, followed by precipitation with alcohol. However, these methods are not environmentally friendly or scalable. Newer approaches have explored enzymatic digestion of non-PHA cell mass, mechanical disruption (e.g., bead milling, sonication), and aqueous two-phase extraction (Cháirez-Ramírez *et al*., 2024; Zhao *et al*., 2024). Enzymatic hydrolysis and surfactant-assisted extraction have been optimised in *S. cerevisiae* to break down cell walls while maintaining the integrity of the polymer.

Characterisation of the recovered PHAs is essential to determine their molecular weight, monomer composition, crystallinity, and thermal/mechanical properties. Techniques such as Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC-MS), and gel permeation chromatography (GPC) are widely employed (Jimoh *et al*., 2018; Volova *et al*., 2021; Lorini *et al*., 2021; Baidurah, 2022; Ibrahim *et al*., 2025). For instance, ^{1}H NMR provides insight into the type of monomers (e.g., 3-hydroxybutyrate, 3-hydroxyvalerate), while differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) help evaluate polymer thermal stability and melting points. Studies using *S. cerevisiae* engineered with bacterial *phaCAB* genes produced poly (3-hydroxybutyrate) [P(3HB)] with molecular weights in the range of 100–500 kDa, depending on expression levels and fermentation conditions (Boontip *et al*., 2021; Ylinen *et al*., 2022).

Moreover, co-feeding strategies with valeric acid precursors yielded copolymers such as poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(3HB-co-3HV)], offering improved material properties such as increased flexibility and reduced brittleness (Bossu *et al*., 2020; Zhila *et al*., 2022; Jo *et al*., 2024). Biosensors and in situ, monitoring systems have also been explored to track intracellular PHA accumulation in real-time. These include fluorescence-based detection systems and Raman spectroscopy probes, which offer non-destructive alternatives for live-cell polymer quantification (Chandra *et al*., 2024). Improving the recovery yield and understanding the structure-function relationship of PHAs synthesised in *S. cerevisiae* are essential steps toward developing yeast-based platforms for commercial bioplastics. Ultimately, scalable, and sustainable purification methods

integrated with precise analytical tools will drive industrial adoption.

Challenges and Future Perspectives

Despite the promising progress in engineering *Saccharomyces cerevisiae* for PHA's biosynthesis, significant challenges that limit commercial viability and large-scale implementation persist. These challenges include metabolic challenges, low yields, high production costs, and limited polymer diversity (Gautam *et al*., 2024; Gundlapalli and Ganesan, 2025). The most significant limitation is the inherently low flux of precursor metabolites such as acetyl-CoA, malonyl-CoA, and NADPH in *S. cerevisiae*, essential for effective PHA synthesis. Redirecting metabolic fluxes toward PHA biosynthesis often compromises essential pathways, leading to growth inhibition and metabolic burden. Although dynamic regulation tools such as feedback-controlled promoters and biosensors have shown promise in balancing growth and production, their integration into robust industrial strains is complex.

Moreover, the expression of heterologous bacterial genes encoding PHA synthases in yeast often results in misfolding or improper assembly, particularly for medium-chain-length PHAs requiring more complex enzyme systems (Rigouin *et al*., 2019; Kastberg *et al*., 2022; Samrot *et al*., 2024). Protein engineering approaches and chaperones have been proposed to improve functional expression and enzymatic efficiency, but further optimisation is required. Another critical issue is the cost and sustainability of fermentation feedstocks; while glucose remains the dominant carbon source, its high rate and competition with food sources limit its usefulness. Therefore, the future depends on engineering *S. cerevisiae* to efficiently utilise lignocellulosic hydrolysates, industrial waste streams, or carbondioxide (CO_2)-derived substrates through synthetic carbon fixation pathways (Zhang *et al*., 2022).

From a downstream processing perspective, scalable and non-toxic PHA recovery from yeast cells remains a hurdle. Innovative, eco-friendly extraction technologies such as supercritical CO_2 extraction, ionic liquids, and aqueous-based systems are under investigation, but their economic feasibility needs thorough assessment. Integrating systems biology, artificial intelligence, and machine learning could accelerate the design-build-test-learn cycle for PHA-producing yeast strains. Predictive models and genome-scale metabolic reconstructions may guide rational engineering strategies, while adaptive laboratory evolution could be employed to enhance tolerance and yield. Although *S. cerevisiae* offers substantial advantages as a GRAS host, achieving economically viable PHA production will require coordinated advances in synthetic biology, metabolic engineering, bioprocess development, and sustainable feedstock integration. The promising future depends on interdisciplinary innovation and collaboration between the academia, industry, and policy frameworks.

CONCLUSION

The emerging role of *Saccharomyces cerevisiae* in PHA biosynthesis represents a promising frontier in sustainable biotechnology and industrial microbiology. As environmental concerns mount over petrochemical plastics and carbon footprints, the demand for eco-friendly bioplastics, such as PHAs, continues to grow. Traditionally dominated by bacterial systems such as *Cupriavidus necator*, this field has shifted to exploring robust, genetically tractable, and industrially proven eukaryotic hosts, such as *S. cerevisiae*, for PHA production. This review outlined the molecular and metabolic engineering strategies employed to construct PHA biosynthetic pathways in *S. cerevisiae*, including the incorporation of bacterial *phaCAB* operons, optimisation of precursor supply (e.g., acetyl-CoA and NADPH), and dynamic regulation systems to balance growth and production. Coupled with metabolic rewiring and synthetic biology tools; yeast offers advantages in industrial scalability, tolerance to harsh fermentation conditions, and GRAS status, thus making it suitable for pharmaceutical and food applications.

Furthermore, innovations in bioreactor design, fermentation optimisation, and substrate diversification from glucose and glycerol to lignocellulosic materials and agro-wastes enhance the feasibility of yeast-based PHA production. Biopolymer extraction and high-resolution analytical techniques have also enabled precise characterisation of PHA types, including P(3HB) and P(3HB-co-3HV), thus expanding their potential applications in packaging, biomedical devices, and agriculture. Nonetheless, significant challenges, such as low yields, high production costs, suboptimal enzyme activity, and difficulties in large-scale downstream processing, continue to hinder commercialisation. Future research must prioritise the development of modular and adaptive framework strains, high-throughput screening platforms, and cost-effective bioprocessing strategies. Furthermore, integrating AI and systems biology tools could significantly enhance predictive metabolic engineering and strain performance. Although *S. cerevisiae* is less productive than traditional bacteria in terms of volume, its flexibility, safety, and compatibility with synthetic biology make it a crucial option for producing new

bioplastics. Continued interdisciplinary innovation, strategic partnerships between the academia and industry, supportive bioeconomy policies, and yeast-derived PHAs transitioning from laboratory-scale prototypes to commercial reality would contribute meaningfully to a circular and sustainable economy.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

Adusumilli, S. H., Alikkam Veetil, A., Choudhury, C., Chattopadhyaya, B., Behera, D. and Bachhawat, A. K. (2024). Glucose 6-phosphate dehydrogenase variants increase NADPH pools for yeast isoprenoid production. *FEBS Open Bio,* 14(3):410–425. https://doi.org/10.1002/2211-5463.13755

Andres, J., Blomeier, T. and Zurbriggen, M. D. (2019). Synthetic switches and regulatory circuits in plants. *Plant Physiology,* 179(3):862–884. https://doi.org/10.1104/pp.18.01362

Ansori, A. N., Antonius, Y., Susilo, R. J., Hayaza, S., Kharisma, V. D., Parikesit, A. A., Zainul, R., Jakhmola, V., Saklani, T., Rebezov, M., Ullah, M. E., Maksimiuk, N., Derkho, M. and Burkov, P. (2023). Application of CRISPR-Cas9 genome editing technology in various fields: A review. *Narra Journal, 3*(2):e184. https://doi.org/10.52225/narra.v3i2.184

Asafa-Adedimeji, K. O., Jimoh, S. O., Badmos-Oladapo, R. B. and Anjous-Alao, R. M. (2022). Substrate concentration and thermal effects during polyhydroxyalkanoate bioproduction. *Fountain Journal of Natural and Applied Sciences,* 11(1):1-6. https://doi.org/10.53704/fujnas.v11i1.415

Atarés, L., Chiralt, A., González-Martínez, C. and Vargas, M. (2024). Production of polyhydroxyalkanoates for biodegradable food packaging applications using *Haloferax mediterranei* and agrifood wastes. *Foods,* 13(6):950. https://doi.org/10.3390/foods13060950

Baek, K.R., Ramakrishnan, S.R., Kim, S.J. and Seo, S.O. (2024). Yeast cell wall mannan structural features, biological activities, and production strategies. *Heliyon,* 10(6),:e27896. https://doi.org/10.1016/j.heliyon.2024.e27896

Baidurah, S. (2022). Methods of analyses for biodegradable polymers: A review. *Polymers,* 14(22):4928. https://doi.org/10.3390/polym14224928

Benisch, M., Aoki, S. K. and Khammash, M. (2024). Unlocking the potential of optogenetics in microbial applications. *Current Opinion in Microbiology,* 77102404. https://doi.org/10.1016/j.mib.2023.102404

Boontip, T., Waditee-Sirisattha, R., Honda, K. and Napathorn, S. C. (2021). Strategies for poly(3-hydroxybutyrate) production using a cold-shock promoter in *Escherichia coli*. *Frontiers in Bioengineering and Biotechnology,* 9:666036. https://doi.org/10.3389/fbioe.2021.666036

Bossu, J., Angellier-Coussy, H., Totee, C., Matos, M., Reis, M. and Guillard, V. (2020). Effect of the molecular structure of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (P(3HB-3HV)) produced from mixed bacterial cultures on its crystallisation and mechanical properties. *Biomacromolecules,* 21(12):5108-5118. https://doi.org/10.1021/acs.biomac.0c00826

Burgos-Morales, O., Gueye, M., Lacombe, L., Nowak, C., Schmachtenberg, R., Hörner, M., Jerez-Longres, C., Mohsenin, H., Wagner, H. J. and Weber, W. (2021). Synthetic biology as driver for the biologization of materials sciences. *Materials Today Bio,* 11: 100115. https://doi.org/10.1016/j.mtbio.2021.100115

Cháirez-Ramírez, M. H., Díaz-Rivas, J. O., Contreras-Ramírez, J. I., González-Laredo, R. F. and Gallegos-Infante, J. A. (2024). Green polymer-based materials as promising therapeutical agents for non-communicable diseases. *Polymer International,* 74(6):483-493. https://doi.org/10.1002/pi.6708

Chandra, A., Kumar, V., Garnaik, U. C., Dada, R., Qamar, I., Goel, V. K. and Agarwal, S. (2024). Unveiling the molecular secrets: A comprehensive review of Raman spectroscopy in biological research. *ACS Omega,* 9(51). https://doi.org/10.1021/acsomega.4c00591

Chang, M., Ahn, S. J., Han, T. and Yang, D. (2024). Gene expression modulation tools for bacterial synthetic biology. *Biotechnology for Sustainable Materials,* 1:6. https://doi.org/10.1186/s44316-024-00005-

Chen, G. Q., Jiang, X. R. and Guo, Y. (2016). Synthetic biology of microbes synthesising polyhydroxyalkanoates (PHA). *Synthetic and Systems Biotechnology,* 1(4): 236-242. https://doi.org/10.1016/j.synbio.2016.09.006

Cho, J. S., Kim, G. B., Eun, H., Moon, C. W. and Lee, S. Y. (2022). Designing microbial cell factories for the production of chemicals. *JACS Au,* 2(8):1872–1888. https://doi.org/10.1021/jacsau.2c00344

Choi, B. H., Kang, H. J., Kim, S. C. and Lee, P. C. (2022). Organelle engineering in yeast: Enhanced production of protopanaxadiol through manipulation of peroxisome proliferation in *Saccharomyces cerevisiae*. *Microorganisms,* 10(3):650. https://doi.org/10.3390/microorganisms10030650

de Melo, R. N., de Souza Hassemer, G., Steffens, J., Junges, A. and Valduga, E. (2023). Recent updates to microbial production and recovery of

polyhydroxyalkanoates. *3 Biotech,* 13(6):204. https://doi.org/10.1007/s13205-023-03633-9

Del Valle, I., Fulk, E. M., Kalvapalle, P., Silberg, J. J., Masiello, C. A. and Stadler, L. B. (2021). Translating new synthetic biology advances for biosensing into the earth and environmental sciences. *Frontiers in Microbiology,* 11:618373. https://doi.org/10.3389/fmicb.2020.618373

Du, Y. H., Wang, M. Y., Yang, L. H., Tong, L. L., Guo, D. S. and Ji, X. J. (2022). Optimisation and scale-up of fermentation processes driven by models. *Bioengineering,* 9(9):473. https://doi.org/10.3390/bioengineering9090473

Elhalis, H. (2024). Expanding the horizons of *Saccharomyces cerevisiae*: Nutrition, oenology, and bioethanol production. *Sustainability,* 16(24):11151. https://doi.org/10.3390/su162411151

Fan, B.L., Chen, L.H., Chen, L.L. and Guo, H. (2025). Integrative multi-omics approaches for identifying and characterising biological elements in crop traits: Current progress and future prospects. *International Journal of Molecular Sciences,* 26(4):1466. https://doi.org/10.3390/ijms26041466

Feng, X. and Marchisio, M. A. (2021). *Saccharomyces cerevisiae* promoter engineering before and during the synthetic biology era. *Biology,* 10(6):504. https://doi.org/10.3390/biology10060504

Gautam, S., Gautam, A., Pawaday, J., Kanzariya, R. K. and Yao, Z. (2024). Current status and challenges in the commercial production of polyhydroxyalkanoate-based bioplastic: A review. *Processes,* 12(8):1720. https://doi.org/10.3390/pr12081720

Getino, L., Martín, J. L. and Chamizo-Ampudia, A. (2024). A review of polyhydroxyalkanoates: Characterisation, production, and application from waste. *Microorganisms,* 12(10):2028. https://doi.org/10.3390/microorganisms12102028

Grage, K., Jahns, A. C., Parlane, N., Palanisamy, R., Rasiah, I. A., Atwood, J. A. and Rehm, B. H. A. (2009). Bacterial polyhydroxyalkanoate granules: Biogenesis, structure, and potential use as nano-/micro-beads in biotechnological and biomedical applications. In *International Symposium on Biological Polyesters*, Auckland, New Zealand, 23-26 November 2008. Washington, DC: American Chemical Society. https://doi.org/10.1021/bm801394s

Gu, Y., Alam, S. and Oliferenko, S. (2023). Peroxisomal compartmentalisation of amino acid biosynthesis reactions imposes an upper limit on compartment size. *Nature Communications,* 14(1):5544. https://doi.org/10.1038/s41467-023-41347-x

Gundlapalli, M. and Ganesan, S. (2025). Polyhydroxyalkanoates (PHAs): Key challenges in production and sustainable strategies for cost reduction within a circular economy framework. *Results in Engineering,* 26:105345. https://doi.org/10.1016/j.rineng.2025.105345

Hoang Nguyen Tran, P., Ko, J. K., Gong, G., *et al*. (2020). Improved simultaneous co-fermentation of glucose and xylose by *Saccharomyces cerevisiae* for efficient lignocellulosic biorefinery. *Biotechnology for Biofuels,* 13:12. https://doi.org/10.1186/s13068-019-1641-2

Huttanus, H. M. and Senger, R. S. (2020). A synthetic biosensor to detect peroxisomal acetyl-CoA concentration for compartmentalised metabolic engineering. *Peer Journal of Life and Environment,* 8: e9805. https://doi.org/10.7717/peerj.9805

Ibrahim, R., Aranjani, J., Prasanna, N., *et al*. (2025). Production, isolation, optimisation, and characterisation of microbial PHA from *Bacillus australimaris*. *Scientific Reports,* 15:8395. https://doi.org/10.1038/s41598-025-92146-x

Ishihara, S., Orita, I., Matsumoto, K. and Fukui, T. (2023). (R/S)-lactate/2-hydroxybutyrate dehydrogenases in and biosynthesis of block copolyesters by *Ralstonia eutropha*. *Applied Microbiology and Biotechnology,* 107(24):7557-7569. https://doi.org/10.1007/s00253-023-12797-6

Jain, A., Sarsaiya, S., Singh, R., Gong, Q., Wu, Q. and Shi, J. (2024). Omics approaches in understanding the benefits of plant-microbe interactions. *Frontiers in Microbiology,* 15:1391059. https://doi.org/10.3389/fmicb.2024.1391059

Jakočiūnas, T., Bonde, I., Herrgård, M., Harrison, S. J., Kristensen, M., Pedersen, L. E., Jensen, M. K. and Keasling, J. D. (2015). Multiplex metabolic pathway engineering using CRISPR/Cas9 in Saccharomyces cerevisiae. *Metabolic Engineering*, 28:213-222. https://doi.org/10.1016/j.ymben.2015.01.008

Jendrossek, D. (2009). Polyhydroxyalkanoate granules are complex subcellular organelles (carbonosomes). *Journal of Bacteriology,* 191*(*10):3195-3202.

Jeon, S., Sohn, Y. J., Lee, H., Park, J. Y., Kim, D., Lee, E. S. and Park, S.J. (2025). Recent advances in the Design-Build-Test-Learn (DBTL) cycle for systems metabolic engineering of *Corynebacterium glutamicum*. *Journal of Microbiology (Seoul, Korea),* 63(3): e2501021. https://doi.org/10.71150/jm.2501021

Jia, H.Y., Xu, T., Wang, C., Zhu, H.W., Li, B.Z., Yuan, Y. J. and Liu, Z.H. (2025). Emerging biotechnological strategies advancing biological lignin valorisation towards polyhydroxyalkanoates. *Bioresource Technology,* 424:132278. https://doi.org/10.1016/j.biortech.2025.132278

Jimoh, S. O., Ado, S. A., and Ameh, J. B. (2009a). Effect of substrate concentration and agitation rate on ethanol production using sorghum bran as substrate. *Biological and Environmental Sciences Journal for the Tropics,* 6(1):130-133.

Jimoh, S. O., Ado, S. A., Ameh, J. B. and Whong, C. M. Z. (2009b). Simultaneous saccharification and fermentation of yam peel to ethanol by co-culture of *Aspergillus niger* and *Saccharomyces cerevisiae*. *Biological and Environmental Sciences Journal for the Tropics,* 6(1):96-100.

Jimoh, S. O., Ado, S. A., Ameh, J. B. and Whong, C. M. Z. (2012a). Osmotolerance and fermentative pattern of brewer's yeast. *World Journal of Life Science and Medical Research,* 2(2):11-16.

Jimoh, S. O., Ado, S. A., Ameh, J. B. and Whong, C. M. Z. (2012b). Characteristics and diversity of yeast in locally fermented beverages sold in Nigeria. *World Journal of Engineering and Pure and Applied Science,* 2(2):40–44.

Jimoh, S. O., Ado, S. A., Ameh, J. B. and Whong, C. M. Z. (2013a). Heat-shock and ethanol-osmotic effect on fermentable yeast cells. *International Journal of Biology and Biological Sciences,* 2(6):91-96.

Jimoh, S. O., Ado, S. A., Ameh, J. B. and Whong, C. M. Z. (2013b). Expression of *ADH1* and *ADH2* genes by ethanol-producing *Saccharomyces cerevisiae* strains using PCR analysis. *International Journal of Research and Advancement in Bioscience,* 3(1):15-23.

Jimoh, S. O., Akinwande, F. O., Ayinde, O. A. and Senbadejo, T. Y. (2018). Polyhydroxyalkanoate producing potential of *Saccharomyces cerevisiae*. *Fountain Journal of Natural and Applied Sciences, 7*(1), 42-47. https://doi.org/10.53704/fujnas.v7i1.248

Jimoh, S.O., Issa, S.B., Fadiji, O.S. and Ebere, C.U. (2025). Metabolic Engineering of Saccharomyces cerevisiae for Enhanced Steroid Biosynthesis: Advances, Strategies, and Industrial Implications. *Sahel Journal of Life Sciences FUDMA,* 3(1): 538-545. https://doi.org/10.33003/sajols-2025-0301-65

Jo, S. Y., Lim, S. H., Lee, J. Y., Son, J., Choi, J. I., and Park, S. J. (2024). Microbial production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate), from lab to the shelf: A review. *International Journal of Biological Macromolecules, 274*(Part 1), 133157. https://doi.org/10.1016/j.ijbiomac.2024.133157

Kalia, V. C., Singh, R. V., Gong, C. and Lee, J. K. (2025). Toward sustainable polyhydroxyalkanoates: A next-gen biotechnology approach. *Polymers,* 17(7):853. https://doi.org/10.3390/polym17070853

Kastberg, L. L. B., Ard, R., Jensen, M. K., and Workman, C. T. (2022). Burden imposed by heterologous protein production in two major industrial yeast cell factories: Identifying sources and mitigation strategies. *Frontiers in Fungal Biology,* 3:827704. https://doi.org/10.3389/ffunb.2022.827704

Kent, R. and Dixon, N. (2020). Contemporary tools for regulating gene expression in bacteria. *Trends in Biotechnology,* 38(3):316-327. https://doi.org/10.1016/j.tibtech.2019.09.007

Kim, S., Jang, Y.J., Gong, G. Lee, S.M., Um, Y., KIM, K.H. and Ja, K.K. (2022). Engineering *Cupriavidus necator* H16 for enhanced lithoautotrophic poly(3-hydroxybutyrate) production from CO_2. *Microbial Cell Factories*, 21:231. https://doi.org/10.1186/s12934-022-01962-7

Kocharin, K., Chen, Y., Siewers, V. and Nielsen, J. (2012). Engineering of acetyl-CoA metabolism for the improved production of polyhydroxybutyrate in *Saccharomyces cerevisiae*. *AMB Expr* 2:52. https://doi.org/10.1186/2191-0855-2-52

Koller M. (2022). Advances in Polyhydroxyalkanoate (PHA) Production. *Bioengineering (Basel, Switzerland)*: 9(7):328. https://doi.org/10.3390/bioengineering9070328

Kudo, H., Ono, S., Abe, K., Matsuda, M., Hasunuma, T., Nishizawa, T., Asayama, M., Nishihara, H., and Chohnan, S. (2023). Enhanced supply of acetyl-CoA by exogenous pantothenate kinase promotes synthesis of poly(3-hydroxybutyrate). *Microbial Cell Factories*, 22(1):75. https://doi.org/10.1186/s12934-023-02083-5

Kusuma, H. S., Sabita, A., Putri, N. A., Azliza, N., Illiyanasafa, N., Darmokoesoemo, H., Amenaghawon, A. N., and Kurniawan, T. A. (2024). Waste to wealth: Polyhydroxyalkanoates (PHA) production from food waste for a sustainable packaging paradigm. *Food Chemistry. Molecular Sciences*, 9:100225. https://doi.org/10.1016/j.fochms.2024.100225

Lee, H.M., Vo, P. N. L., and Na, D. (2018). Advancement of Metabolic Engineering Assisted by Synthetic Biology. *Catalysts*, 8(12):619. https://doi.org/10.3390/catal8120619

Li, C., Jiang, T., Li, M., Zou, Y., and Yan, Y. (2022). Fine-tuning gene expression for improved biosynthesis of natural products: From transcriptional to post-translational regulation. *Biotechnology Advances*, 54:107853. https://doi.org/10.1016/j.biotechadv.2021.107853

Lorini, L., Martinelli, A., Capuani, G., Frison, N., Reis, M., Ferreira, B.S., Villano, M., Majone, M. and Valentino, F. (2021). Characterisation of Polyhydroxyalkanoates Produced at Pilot Scale from Different Organic Wastes.*Frontier in Bioengineering and Biotechnology,* 9:628719. https://doi.org/10.3389/fbioe.2021.628719

Maicas, S. (2020). The Role of Yeasts in Fermentation Processes. *Microorganisms*, 8(8): 1142. https://doi.org/10.3390/microorganisms8081142

Marillonnet, S. and Werner, S. (2025). Golden Gate Cloning of Multigene Constructs Using the Modular Cloning System MoClo. *Methods in Molecular Biology*, 2850:21-39. https://doi.org/10.1007/978-1-0716-4220-7_2

Meng, J., Qiu, Y. and Shi, S. (2020). CRISPR/Cas9 Systems for the Development of *Saccharomyces cerevisiae* Cell Factories. *Frontiers in Bioengineering and Biotechnology*, 8:594347. https://doi.org/10.3389/fbioe.2020.594347

Müller, M.M., Arndt, K.M. and Hoffmann S.A (2025). Genetic circuits in synthetic biology: broadening the toolbox of regulatory devices. Front. Synth. Biol. 3:1548572. https://doi.org/10.3389/fsybi.2025.1548572

Naseri, G. and Koffas, M.A.G. (2020). Application of combinatorial optimisation strategies in synthetic biology. *Nature Communications*, 11:2446. https://doi.org/10.1038/s41467-020-16175-y

Neoh, S. Z., Chek, M. F., Tan, H. T., Linares-Pastén, J. A., Nandakumar, A., Hakoshima, T. and Sudesh, K. (2022). Polyhydroxyalkanoate synthase (PhaC): The key enzyme for biopolyester synthesis. *Current Research in Biotechnology,* 4:87-101. https://doi.org/10.1016/j.crbiot.2022.01.002

Orita, I., Unno, G., Kato, R. and Fukui, T. (2022). Biosynthesis of Polyhydroxyalkanoate Terpolymer from Methanol via the Reverse β-Oxidation Pathway in the Presence of Lanthanide. *Microorganisms*, 10(1):184. https://doi.org/10.3390/microorganisms10010184

Paduvari, R. and Somashekara, D. M. (2025). Advancements in genetic engineering for enhanced Polyhydroxyalkanoates (PHA) production: a comprehensive review of metabolic pathway manipulation and gene deletion strategies. *Bioengineered*, 16(1): 2458363. https://doi.org/10.1080/21655979.2025.2458363

Rajpurohit, H. and Eiteman, M. A. (2022). Nutrient-Limited Operational Strategies for the Microbial Production of Biochemicals. *Microorganisms*, 10(11):2226. https://doi.org/10.3390/microorganisms10112226

Rigouin, C., Lajus, S., Ocando, C., Borsenberger, V., Nicaud, J. M., Marty, A., Avérous, L. and Bordes, F. (2019). Production and characterization of two medium-chain-length polydroxyalkanoates by engineered strains of Yarrowia lipolytica. *Microbial Cell Factories*, 18(1):99. https://doi.org/10.1186/s12934-019-1140-y

Rojas, V. and Larrondo, L. F. (2023). Coupling cell communication and optogenetics: Implementation of a light-inducible intercellular system in yeast. *ACS Synthetic Biology,* 12(1):71-82. https://doi.org/10.1021/acssynbio.2c00338

Rueda, E., Gonzalez-Flo, E., Mondal, S., Forchhammer, K., Arias, D. M., Ludwig, K., Drosg, B., Fritz, I., Gonzalez-Esquer, C. R., Pacheco, S. and García, J. (2024). Challenges, progress, and future perspectives for cyanobacterial polyhydroxyalkanoate production. *Reviews in Environmental Science and Bio/Technology,* 23:321-350. https://doi.org/10.1007/s11157-024-09689-0

Sagong, H.Y., Son, H. F., Choi, S. Y., Lee, S. Y. and Kim, K.J. (2018). Structural insights into polyhydroxyalkanoates biosynthesis. *Trends in Biochemical Sciences,* 43(10)-790-805. https://doi.org/10.1016/j.tibs.2018.08.005

Samrot, A. V., Samanvitha, S. K., Shobana, N., Renitta, E. R., Senthilkumar, P., Kumar, S. S., Abirami, S., Dhiva, S., Bavanilatha, M., Prakash, P., Saigeetha, S., Shree, K. S. and Thirumurugan, R. (2021). The Synthesis, Characterisation and Applications of Polyhydroxyalkanoates (PHAs) and PHA-Based Nanoparticles. *Polymers*, 13(19):3302. https://doi.org/10.3390/polym13193302

Sandström, A. G., Muñoz de Las Heras, A., Portugal-Nunes, D. and Gorwa-Grauslund, M. F. (2015). Engineering of *Saccharomyces cerevisiae* for the production of poly-3-d-hydroxybutyrate from xylose. *AMB Express*, 5:14. https://doi.org/10.1186/s13568-015-0100-0

Shaw, W. M., Khalil, A. S. and Ellis, T. (2023). A multiplex MoClo toolkit for extensive and flexible engineering of *Saccharomyces cerevisiae*. *ACS Synthetic Biology,* 12(11): 2752-2764. https://doi.org/10.1021/acssynbio.3c00423

Stanton, R. C. (2012). Glucose-6-phosphate dehydrogenase, NADPH, and cell survival. *International Union of Biochemistry and Molecular Biology* (*IUBMB*) *Life*, 64(5):362–369. https://doi.org/10.1002/iub.1017

Sun, W., Shahrajabian, M. H. and Lin, M. (2022). Research Progress of Fermented Functional Foods and Protein Factory-Microbial Fermentation Technology. *Fermentation*, 8(12):688. https://doi.org/10.3390/fermentation8120688

Tang, R., Peng, X., Weng, C. and Han, Y. (2022). The overexpression of phasin and regulator genes promoting the synthesis of polyhydroxybutyrate in *Cupriavidus necator* H16 under nonstress conditions. *Applied and Environmental Microbiology,* 88(2). https://doi.org/10.1128/AEM.01458-21

Tsegaye, K. N., Alemnew, M., and Berhane, N. (2024). *Saccharomyces cerevisiae* for lignocellulosic ethanol production: a look at key attributes and genome shuffling. *Frontiers in Bioengineering and Biotechnology*, 12:1466644. https://doi.org/10.3389/fbioe.2024.1466644

van Lent, P., Schmitz, J., and Abeel, T. (2023). Simulated Design-Build-Test-Learn Cycles for Consistent Comparison of Machine Learning Methods in Metabolic Engineering. *ACS Synthetic Biology*, 12(9):2588-2599. https://doi.org/10.1021/acssynbio.3c00186

Volova, T. G., Golubev, A. I., Nemtsev, I. V., Lukyanenko, A. V., Dudaev, A. E. and Shishatskaya, E. I. (2021). Laser Processing of Polymer Films Fabricated from PHAs Differing in Their Monomer Composition. *Polymers*, 13(10):1553. https://doi.org/10.3390/polym13101553

Walker, R. S. K. and Pretorius, I. S. (2018). Applications of Yeast Synthetic Biology Geared towards the Production of Biopharmaceuticals. *Genes*, 9(7):340. https://doi.org/10.3390/genes9070340

Weimer, A., Kohlstedt, M., Volke, D. C., Nikel, P. I. and Wittmann, C. (2020). Industrial biotechnology of Pseudomonas putida: advances and prospects. *Applied Microbiology and Biotechnology*, *104*(18), 7745–7766. https://doi.org/10.1007/s00253-020-10811-9

Wives, A. P., Mendes, I. S. de M., dos Santos, S. T. and Bonatto, D. (2024). Molecular techniques employed in CTG(Ser1) and CTG(Ala) D-xylose metabolising yeast clades for strain design and industrial applications. *arXiv*. 1-31. https://arxiv.org/abs/2405.11010

Xu, P., Ranganathan, S., Fowler, Z. L., Maranas, C. D. and Koffas, M. A. (2011). Genome-scale metabolic network modeling results in minimal interventions that cooperatively force carbon flux towards malonyl-CoA. *Metabolic Engineering*, 13(5), 578-587. https://doi.org/10.1016/j.ymben.2011.06.008

Yan, N., Luan, T., Yin, M., Niu, Y., Wu, L., Yang, S., Li, Z., Li, H., Zhao, J. and Bao, X. (2023). Co-Fermentation of Glucose–Xylose–Cellobiose–XOS Mixtures Using a Synthetic Consortium of Recombinant *Saccharomyces cerevisiae* Strains. *Fermentation*, 9(8):775. https://doi.org/10.3390/fermentation9080775

Yang, Y., Liu, Y., Zhao, H., Liu, D., Zhang, J., Cheng, J., Yang, Q., Chu, H., Lu, X., Luo, M., Sheng, X., Zhang, Y. P. J., Jiang, H. and Ma, Y. (2023). Construction of an artificial phosphoketolase pathway that efficiently catabolizes multiple carbon sources to acetyl-CoA. *PLoS Biology*, *21*(9), e3002285. https://doi.org/10.1371/journal.pbio.3002285

Yang, J., Song, J., Feng, Z and Ma., Y. (2025). Application of CRISPR-Cas9 in microbial cell factories. *Biotechnology Letters* , 47:46. https://doi.org/10.1007/s10529-025-03592-6

Yao, J., Xiao, X.Y., Wang, M., Zhang, Q., Chen, Y., Gou, M., Xia, Z.Y., and Tang, Y.Q. (2025). A review of low-cost production of polyhydroxyalkanoates: Strategies, challenges, and perspectives. *Bioresource Technology,* 433:132745. https://doi.org/10.1016/j.biortech.2025.132745

Yin, M.Q., Xu, K., Luan, T., Kang, X.L., Yang, X.Y., Li, H.X., Hou, Y.H., Zhao, J.Z. and Bao, X.M. (2024). Metabolic engineering for compartmentalised biosynthesis of valuable compounds in *Saccharomyces cerevisiae*. *Microbiological Research,* 286: 127815. https://doi.org/10.1016/j.micres.2024.127815

Ylinen, A., de Ruijter, J. C., Jouhten, P. and Penttilä, M. (2022). PHB production from cellobiose with Saccharomyces cerevisiae. *Microbial cell factories*, *21*(1), 124. https://doi.org/10.1186/s12934-022-01845-x

Yook, S. and Alper, H. S. (2025). Recent advances in genetic engineering and chemical production in yeast species. *FEMS Yeast Research*, 25, foaf009. https://doi.org/10.1093/femsyr/foaf009

Zhang, L., Jiang, Z., Tsui, T. H., Loh, K. C., Dai, Y. and Tong, Y. W. (2022). A Review on Enhancing *Cupriavidus necator* Fermentation for Poly(3-hydroxybutyrate) (PHB) Production from Low-Cost Carbon Sources. *Frontiers in Bioengineering and Biotechnology*, 10:946085. https://doi.org/10.3389/fbioe.2022.946085

Zhao, F., Wang, Z. and Huang, H. (2024). Physical Cell Disruption Technologies for Intracellular Compound Extraction from Microorganisms. *Processes*, 12(10):2059. https://doi.org/10.3390/pr12102059

Zhila, N. O., Sapozhnikova, K. Y., Kiselev, E. G., Nemtsev, I. V., Lukyanenko, A. V., Shishatskaya, E. I. and Volova, T. G. (2022). Biosynthesis and Properties of a P(3HB-*co*-3HV-*co*-4HV) Produced by *Cupriavidus necator* B-10646. *Polymers*, 14(19):4226. https://doi.org/10.3390/polym14194226

Zytner, P., Kumar, D., Elsayed, A., Mohanty, A., Ramarao, B. V. and Misra, M. (2023). A review on polyhydroxyalkanoate (PHA) production through the use of lignocellulosic biomass. *RSC Sustainability,* 1(9), 2120-2134. https://doi.org/10.1039/D3SU00126A.