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Enzymatic and microbial valorization of lignocellulosic biomass for food applications

Mya Myintzu Hlaing,^a Naazneen Sofeo,^b David Wollborn,^a
Netsanet Shiferaw Terefe^a and Barindra Sana^{a,*b}

Meeting the increasing global food demand requires innovative production strategies using sustainable technologies, including biocatalysis and microbial technology. The long-term viability of any such strategy, however, depends on access to affordable and renewable feedstocks. The sustainable production of novel foods and food ingredients from lignocellulosic biomass (LCB), particularly from agrifood side streams, offers an attractive strategy to enhance both waste management and food security, simultaneously. LCB, the most abundant renewable material on earth, represents a promising sugar-rich feedstock. Regardless of origin, LCB are composed of three main components: cellulose, hemicellulose, and lignin, though their proportions vary depending on the source. Due to its high sugar content, LCB is widely regarded as a promising renewable carbon source for microbial fermentation, with potential applications in food industries. However, polysaccharides in LCB are not readily hydrolysable to supply fermentable sugars essential for microbial growth, as they form a complex, interconnected network with lignin. In addition to physical and chemical methods, enzymes and microorganisms are extensively used in LCB valorization processes, for biomass pre-treatment, hydrolysis, and fermentation. The production of several important food ingredients, such as single-cell proteins, microbial oils, dietary fibers, vitamins, and organic acids, has been demonstrated through the biological conversion of LCB. Isolated lignolytic and polysaccharolytic enzymes, enzyme cocktails, microbial secretomes, isolated bacteria and fungi, and natural and synthetic microbial consortia have been studied for this purpose. This review discusses recent advances in microbial and enzymatic valorization of agrifood-derived LCB for food-related applications. We specifically highlight the composition of major lignocellulosic sidestreams and biobased tools and techniques used for their conversion into food ingredients, with a special reference to enzymatic and microbial technologies. Furthermore, we discussed current challenges and prospects of LCB valorization for food applications via biological routes.

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Sustainability spotlight

Food insecurity and climate change are deeply interconnected challenges that threaten the future of humanity. The United Nations has recognized their urgency through the Sustainable Development Goals: Zero Hunger (SDG 2) and Climate Action (SDG 13). One promising approach to tackling both issues lies in converting lignocellulosic wastes into novel food ingredients. Considering the sheer abundance of lignocellulosic biomass within agrifood residues, this strategy holds immense potential to ease global food demand while lowering the environmental footprint of the agrifood system. In this review, we examine sustainable and eco-friendly pathways for breaking down complex lignocellulosic biomass and transforming it into diverse food ingredients, using nature's most powerful tools: enzymes and microorganisms.

1 Introduction

Food demand is increasing steadily with the increase in global population and changing dietary preferences. The world

population is expected to peak at 10.3 billion in 2084 from 8.2 billion in 2024.¹ Producing food for 25% higher population using existing methods could be challenging, as it would place further strain on already depleted agrifood resources, including fresh water and arable land. This will not only intensify resource depletion but also accelerate biodiversity loss, deforestation, and greenhouse gas emissions. As such, it warrants a thorough investigation into innovative food production approaches utilizing renewable resources. In recent years, significant research efforts and industrial initiatives have been directed

^aCommonwealth Scientific and Industrial Research Organisation (CSIRO), Agriculture and Food, 671 Sneydes Road, Werribee, Victoria 3030, Australia

^bSingapore Institute of Food and Biotechnology Innovation (SIFBI), Agency for Science, Technology and Research (A*STAR), 31 Biopolis Way, Nanos, Singapore 138669, Singapore. E-mail: barindra_sana@a-star.edu.sg



towards developing novel food ingredients using innovative processes and alternative resources. Plant-based and microbial proteins are being produced in large scale for human consumption.^{2,3} Emerging technologies such as engineering biology, cellular agriculture, and precision fermentation are rapidly evolving, offering complementary solutions to traditional agriculture and farm-based food production.⁴⁻⁹ Together, these technologies have the potential to reshape the global food system, making it more resilient, resource-efficient, and sustainable.

However, the long-term viability of these alternative food systems depends on a continuous supply of cheap and renewable feedstocks, at scale. Current fermentation-based food production strategies are heavily reliant on first-generation feedstocks (such as sugars derived from corn or sugarcane) that directly compete with food crops for agricultural land, water, and fertilizer. The need for alternative feedstocks has become increasingly evident as microbial and fermentation-based food production continue to advance.^{6,8} Second-generation feedstocks, particularly lignocellulosic biomass (LCB) from agricultural and forestry residues, have emerged as promising options in this respect.^{10,11} Derived from inedible parts of plants, LCB are the most abundant renewable carbon source on earth, which remain largely untapped. They are available in large quantities from forest and energy crop residues, agricultural wastes, agrifood industry side-streams, and other industrial solid wastes, that can provide substantial raw materials for the sustainable production of human food to supply a significant portion of global food demand.¹² Generally regarded as waste and often discarded or underutilized, these feedstocks do not compete with traditional agriculture for land, water, and other resources. In addition, utilizing these wastes reduces the economic and environmental burdens associated with waste management and disposal. Repurposing LCB from agricultural wastes into edible food ingredients could simultaneously reduce the global food shortage and the carbon footprint of traditional agriculture.

Despite its immense potential, the conversion of LCB into edible food ingredients is technically challenging. The primary obstacle lies in the chemical complexity and recalcitrance of lignocellulosic structures. The sugars present in agricultural wastes are not readily available for microbial metabolism. Instead, they exist in the form of complex inter-connected polysaccharides. Disintegrating these complex lignocellulosic polysaccharides into fermentable sugars is a major challenge to their valorization through microbial fermentation. Nonetheless, the field has progressed significantly with the development of innovative pre-treatment methods and sustainable saccharification strategies. In this regard, biology is playing an increasingly important role in LCB valorization. For example, biocatalysts (isolated enzymes and whole microbial cells) enable biomass pre-treatment and hydrolysis at mild conditions, and advanced fermentation techniques (like precision fermentation) are being used for microbial production of high-value food ingredients, while traditional microbial fermentation remains the key to microbial protein production from LCB hydrolysates. Also, there is an emerging trend of integrating

biological and chemical methods to overcome the recalcitrance of LCB to hydrolytic degradation.¹³

Eco-friendly extraction of fermentable sugars remains at the core of biobased conversion of LCB into food ingredients. A typical process commonly involves (1) biomass pretreatment using biological, chemical, or thermochemical methods, (2) biocatalytic or chemo-enzymatic hydrolysis to convert cellulose and hemicellulose into fermentable sugars, and (3) converting the sugars into a wide spectrum of valuable food ingredients through microbial fermentation (Fig. 1). These steps are not necessarily performed individually. Strategies have been developed for combining more than one step, and in some instances, biomass has been hydrolyzed and fermented to food and feed ingredients without any pre-treatment.¹⁴ Simultaneous saccharification and co-fermentation has been used to increase efficiency and reduce operation time and cost.¹⁵ In this approach, the enzymatic hydrolysis of lignocellulose into fermentable sugars and their fermentation are performed together in a single reactor. In another approach, whole or partially processed LCB is repurposed for producing dietary fibers. Using these strategies, various LCB have been successfully transformed into several food ingredients, including single-cell proteins, microbial oils, nutraceuticals, functional foods, and other innovative food ingredients.¹⁶⁻²⁸ However, most of these processes are yet to be scaled up and commercialized.

Being a cheap, carbon neutral, and sugar-rich feedstock, LCB presents immense potential in sustainable food production through microbial fermentation. By closing the loop between agricultural waste and food production, LCB valorization contributes directly to a circular food system. This reduces waste disposal burdens, enhances resource efficiency, and aligns the agrifood sector with the principles of food sustainability and circular economy. Thanks to latest biological tools and technologies, in recent years, there has been a significant scientific advancement in the sustainable conversion of LCB into edible nutritional ingredients. Fig. 2 presents two network maps summarizing the research trends over the past decade on 'lignocellulosic biomass' in general and 'biobased lignocellulose valorization', generated by VOSviewer 1.6.20.²⁹ Journal articles from the Europe PMC database were retrieved from 2015 to 2025 that had mentioned 'lignocellulosic biomass' or

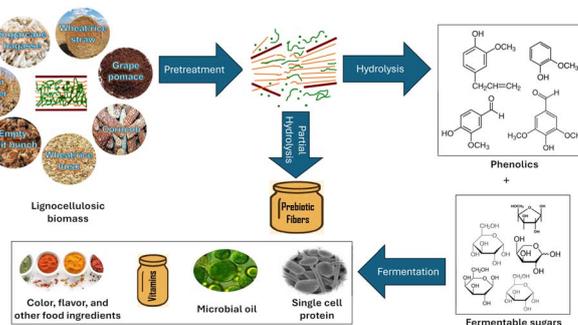


Fig. 1 Schematic diagram for lignocellulosic side-stream valorization for food applications.



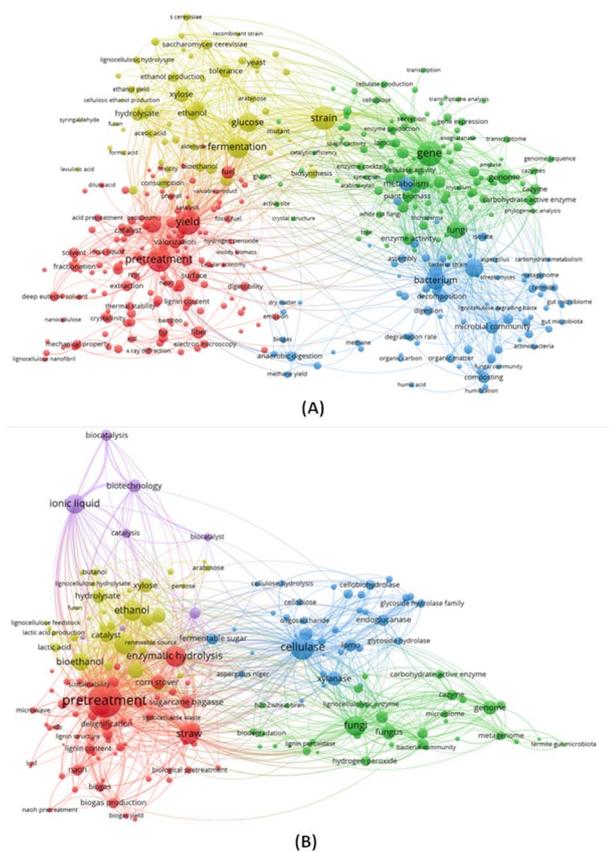


Fig. 2 Recent (2015–2025) research trend on (A) lignocellulose in general and (B) biobased lignocellulose valorization, as depicted in the map generated by VOSviewer 1.6.20 showing clusters based on terms derived from title and abstract. Different clusters are presented in various colors, while term occurrence is presented by circle size.²⁹

'biobased lignocellulose valorization' in their title or abstract. The nodes in the network represent keywords, while the edges represent co-occurrences. Overall, a substantial part of the research has focused on biomass pretreatment, a critical step in overcoming the recalcitrance of lignocellulosic materials. The maps further highlight the pivotal role of biological tools and technologies in advancing lignocellulose valorization. Fig. 2A shows importance of enzymes (green cluster), various microorganisms and microbial communities including gut microbiome (blue cluster), and microbial fermentation (yellow cluster) in LCB valorization. Cellulase is the most widely studied enzyme in this respect along with other polysaccharolytic enzymes including lytic polysaccharide monoxygenase (LPMO), cellobiohydrolase, glucoside hydrolase, and xylanase (Fig. 2B, blue cluster). These enzymes are the driving force of biomass saccharification and are widely used in various industrial processes. On the other hand, fungi and fungal ligninolytic enzymes, including laccase and lignin peroxidase, are being studied for LCB pretreatment to replace acid/alkali digestion (Fig. 2B, green cluster). Despite recent focus on food, polymers, and high-value chemicals, bioethanol remains the most sought-after products in LCB valorization (Fig. 2A and

B, yellow clusters). Glucose, xylose, arabinose, and other fermentable sugars are LCB degradation products, which are the key intermediates in biomass conversion through microbial fermentation for producing lactic acid, single-cell proteins, vitamins, and other high-value food ingredients. Another interesting group of products are oligosaccharides, which are an emerging class of dietary fibers, produced by partial hydrolysis of cellulose and hemicellulose using enzymes like endoglucanase and endoxylanase (Fig. 2B, blue cluster). Recent advancements in conversion of LCB to biofuels, value-added chemicals, and various materials have been reviewed elsewhere.^{30–37} The present article focuses specifically on microbial and enzymatic valorization of lignocellulosic agricultural wastes for producing ingredients relevant to food and nutrition. In this context, this article highlights the latest tools and technologies for disintegrating complex lignocellulosic biomass into simple sugars and their conversion into various ingredients useful in the food industry. The current challenges and possible solutions in developing scalable and economically viable technologies for LCB-derived food production are also discussed, offering fresh insights into this niche research area with the potential to immensely contribute to the sustainability of our food system.

2 Lignocellulosic side streams: source, composition and opportunities in food applications

Lignocellulosic biomass is the most abundant and economical renewable feedstock on earth.^{38,39} Agricultural residues and agri-food processing side-streams are the main sources of LCB. Typical examples include sugarcane bagasse, corn stover, rice straw, rice husks, wheat straw, shells and empty fruit bunches from oil palm. Country-specific production of some of the most abundant lignocellulosic biomass is summarized in Fig. 3. Beyond illustrating global availability, Fig. 3 also highlights the direct relevance of lignocellulosic biomass to food systems, as the dominant residue streams originate from primary food production and agri-food processing chains. Residues such as wheat straw, rice straw and husks, maize stover, and sugarcane bagasse are generated in parallel with global food supply and are therefore intrinsically linked to food-producing regions as opposed to dedicated energy or industrial crops. Their widespread geographic distribution (Fig. 3B) creates opportunities for decentralized and regionally adapted valorization strategies, particularly in areas with established agricultural infrastructure. Importantly, these residues represent non-food-competing carbon resources that can be converted into food and feed ingredients *via* fermentation-based processes, including single-cell protein and functional microbial biomass. In this context, lignocellulosic residues provide a pathway to expand sustainable protein and food ingredient production while avoiding diversion of edible crops and minimizing additional land-use pressure.

Irrespective of origin, all kinds of LCB are composed of three main components – cellulose, hemicellulose, and lignin, but



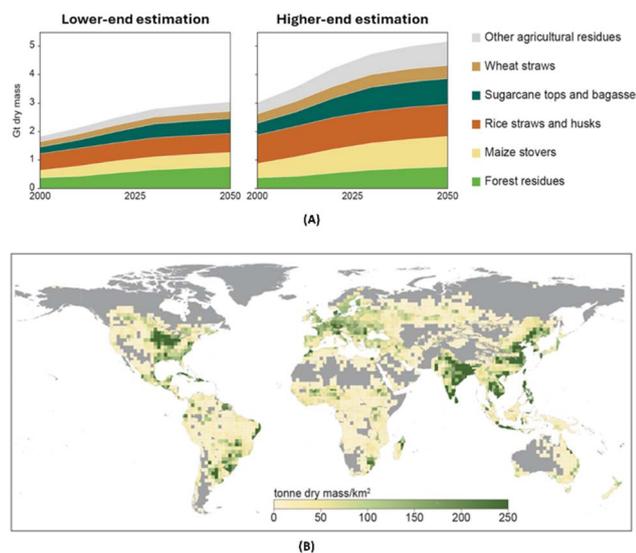


Fig. 3 (A) Lower- and higher-end estimations of the global available potential of lignocellulose residues by biomass type from 2000 to 2050. (B) Spatial distribution (higher-end estimate) of lignocellulose residues in 2050 at 200 km × 200 km resolution. The gray area reflects no cropland or managed forest in the specific region. The figure is reproduced from Huo *et al.* (2024; <https://doi.org/10.1021/acs.est.4c03005>) with permission from ACS.⁷⁷ Further permissions related to the material excerpted should be directed to the ACS.

their proportions vary extensively depending on the source (Table 1). Cellulose is the most abundant component, accounting for around 40–50% weight of lignocellulosic biomass. It is a polysaccharide, made up of long chains of glucose molecules linked by β -1,4-glycosidic bonds, forming crystalline structures that provide mechanical strength to plant cells. The other polysaccharide, hemicellulose, makes up 20–35% weight of lignocellulosic biomass; it is a heterogeneous polysaccharide made up of a variety of sugars, such as xylose, arabinose, mannose, and galactose. Lignin is a complex heteropolymer made up of mainly three phenolic building blocks called monolignols – coniferyl alcohol, sinapyl alcohol, and *p*-coumaryl alcohol; it accounts for about 15–30% weight of lignocellulosic biomass.

Recent estimations suggest only about 4.5% of globally produced LCB are utilized for various applications.^{40,41} This demonstrates an unprecedented waste management challenge, as a significant fraction of the unused LCB ends up in open burning and landfill. At the same time, this biomass present

a huge, untapped resource and a sustainable feedstock that can be potentially converted into a wide range of bioproducts. Lignin has the potential to be broken down into valuable phenolic compounds, while cellulose and hemicellulose can be hydrolyzed into fermentable sugars that serves as a carbon source for microbial growth, ultimately leading to the production of various metabolites and microbial biomass.⁴² However, in LCB, cellulose, hemicellulose and lignin are rigidly bound to each other through covalent and non-covalent linkages that form extremely complex structures. Breaking down these complex structures and separating cellulose and hemicellulose from lignin is the primary bottleneck in LCB valorization.⁴³ Nonetheless, several physical (steam explosion), chemical (acid/alkali hydrolysis and organosolv process), and biological (enzymatic hydrolysis and microbial degradation) methods have been employed to overcome this barrier. Recent advancements in enzyme and microbial technology are positioning lignocellulosic biomass as a promising resource for a green and sustainable bioeconomy.

Due to established glucose fermentation technology, out of the three main LCB components, cellulose is the most investigated for food applications. It is the primary feedstock studied for single-cell protein and microbial lipid production for food and feed industries.^{44–47} In contrast, pentose sugars, the building block of hemicellulose, are difficult to use as fermentation feedstock, since the common industrial microbes cannot naturally ferment them. Although several novel pentose-fermenting microbes have been identified and some industrial microorganisms have been engineered for hemicellulose fermentation into ethanol, the processes are often not cost-effective as the fermentation rate is significantly slower than glucose.^{48–52} Nonetheless, several food additives, such as lactic acid, acetic acid, and xylitol have been produced from hemicellulose-based sugars.^{48,53–56} In addition, oligosaccharides obtained by partial hydrolysis of cellulose and hemicellulose are useful as prebiotics, as they selectively feed beneficial gut microorganisms like *Bifidobacterium*, improving digestion, immunity, and metabolic health. One such group of oligosaccharides, xylooligosaccharides (XOS), have been recognized as safe for human consumption by regulatory bodies like the US Food and Drug Administration (FDA) and European Food Safety Authority (EFSA).⁵⁷

Lignin, the third component of LCB, has high valorization potential due to its unique aromatic building block, which is widely considered as a potential source of high-value

Table 1 Global production and composition of major lignocellulosic agricultural wastes

| Source | Estimated global annual production | Composition (wt%) | | | Reference |
|----------------------------|------------------------------------|-------------------|---------------|--------|-----------|
| | | Cellulose | Hemicellulose | Lignin | |
| Sugarcane bagasse | 700 million tons (ref. 66) | 40–50 | 25–30 | 20–25 | 67 and 68 |
| Corn stover | 1 billion tons (ref. 69) | 33–43 | 26–36 | 17–21 | 69 and 70 |
| Rice straw | 731 million tons (ref. 71) | 33–47 | 19–27 | 5–24 | 72 |
| Wheat straw | 529 million tons (ref. 73) | 34–40 | 21–26 | 11–23 | 74 |
| Oil palm empty fruit bunch | 99 million tons (ref. 75) | 42–65 | 17–34 | 13–26 | 76 |



compounds, including innovative food ingredients. Lignin-derived phenolic compounds are known to have applications as natural antioxidants, prebiotics, functional foods, natural preservatives, food additives, and flavoring agents.^{58,59} Lignin valorization received special attention due to its abundance as an industrial side-stream of cellulosic bioethanol production and paper industries. It has been regarded as one of the major renewable alternatives to fossil-fuel-based production of several high value fine chemicals including vanillin, catechol, guaiacol, syringic acid, eugenol, and *p*-coumaric acid, and bulk chemicals like phenol, benzene, and toluene.^{60,61} However, successful lignin valorization faces several challenges due to its complex heterogeneous structures, which limit extraction efficiency and scalable conversion.⁶² Despite decades-long efforts there is limited success in producing lignin-derived small molecules. To date, vanillin is the only commercially produced lignin-derived phenolic compound. Due to its characteristic 'vanilla' flavor, it is widely used as a flavoring agent in the food and beverage industries. In addition, it has antidiabetic, antioxidant, antimicrobial, and anti-inflammatory properties.⁶³ With an estimated production of over 9000 tons per year lignin-derived vanillin accounts for approximately 15% of global commercial vanillin production.⁶⁴ Although significant challenges remain for scale-up and commercialization, with advances in technology, lignin has the potential to emerge as a promising renewable source of intrinsic functionalized phenols, which are currently produced *via* multi-step conversion of fossil-fuel-derived bulk chemicals.⁶⁵

3 Biobased tools for converting LCB into food ingredients

3.1. Enzymes

3.1.1. Isolated enzymes. Enzymatic depolymerization of the polymeric building blocks is the key to biobased LCB valorization. Various hydrolase and oxidoreductase enzymes have been reported to depolymerize complex polymers.⁷⁸ Effective breakdown of cellulose, hemicellulose, and lignin requires different types of enzymes, which often need to act synergistically to achieve efficient LCB degradation. Cellulose is the glucose-rich part of LCB and is the primary target for producing fermentable sugar, which in turn could be converted to single-cell proteins, microbial oils, and various value-added food ingredients through microbial fermentation. A group of enzymes that can hydrolyze cellulose is collectively known as cellulases, consisting of three main sub-groups: endoglucanase, exoglucanase, and β -glucosidase. Members of all three subgroups work synergistically to produce glucose through a multi-step hydrolysis of cellulose. Endoglucanases break down cellulose internally by hydrolyzing β -1,4-glycosidic linkages located in the amorphous regions inside the cellulose chain, resulting in the formation of smaller oligomeric species (consisting of 2–6 glucose units) called cello-oligosaccharides. These shorter and soluble fragments are more accessible to the exoglucanase enzymes, which can hydrolyse short chain celluloses only at the reducing or non-reducing ends. This

group of enzymes breaks down the cello-oligosaccharides to a glucose disaccharide called cellobiose. Finally, β -glucosidases hydrolyse cellobiose to two molecules of glucose.^{79,80} It is worth mentioning that cello-oligosaccharides are recognized for their prebiotic properties, which promote the growth of beneficial gut bacteria.

The second group of polysaccharides in LCB is hemicellulose. While cellulose is a relatively simple linear homopolymer composed of glucose, hemicellulose consists of a group of linear and branched heteropolysaccharides made up of various pentose and hexose sugars such as arabinose, xylose, rhamnose, mannose, glucose, glucuronic acids, and galacturonic acids. Due to this structural heterogeneity, enzymatic hydrolysis of hemicellulose involves a range of enzymes collectively called hemicellulase. While *endo*- and *exo*-hemicellulase enzymes hydrolyze the polysaccharide backbones by cleaving various glycosidic linkages present in these heteropolymers, several accessory enzymes play important roles in cleaving the side-chain substituents present in various hemicellulose building blocks. Examples of hemicellulase enzymes include *endo*- β -1,4-xylanase, β -xylosidases, *endo*- β -1,4-mannase, mannosidase, *endo*- α -1,5-arabinanase, α -galactosidase, and *endo*-1,4- β -galactanase, while acetyl-xylan esterase, ferulic and *p*-coumaric acid esterases, and acetyl mannan esterase are examples of accessory enzymes involved in hemicellulose hydrolysis.

Although cellulase and hemicellulase enzymes can hydrolyze cellulose and hemicellulose individually, enzymatic saccharification of LCB is far more complex. In LCB, cellulose, hemicellulose, and lignin form intertwined cross-linked structures that need to be partially disintegrated for saccharification of the polysaccharides. Lignin, being the most heterogeneous and recalcitrant component of LCB, presents the greatest challenge to enzymatic degradation. As such, lignin degradation is often regarded as the primary bottleneck in enzymatic LCB valorization. Nonetheless, lignin has been reported to be degraded by several enzymes, such as laccase, lignin peroxidase, manganese peroxidase, versatile peroxidase, and multiple β -etherase enzymes.⁸¹ These enzymes often work together to cleave lignin synergistically.^{80,82,83} The roles of major lignocellulolytic enzymes are summarized at Table 2.

3.1.2. Enzyme cocktails. Due to the structural complexity of lignocellulosic polymers, achieving complete depolymerization to the monomeric level using a single enzyme is not feasible. Even for cellulose, a linear homopolymer, complete depolymerization requires at least three distinct cellulase enzymes, each targeting cellulose with varying degrees of polymerization. Amongst the three LCB components, enzymatic degradation of cellulose is well-understood, and a handful of commercial cellulase cocktails have been reported to be highly efficient on certain cellulosic materials. Some cellulolytic enzyme cocktails have been optimized to degrade pretreated LCB.^{84,85} However, fine-tuning the cocktails for individual cellulosic substrates remains a significant challenge. The enzymatic breakdown of heteropolymers, such as hemicellulose and lignin, is even more intricate and less understood, as it involves the coordinated action of multiple enzyme classes. Although some enzyme



Table 2 Summary of the main lignocellulolytic enzymes and their roles in lignocellulosic biomass (LCB) valorization to food ingredients

| Enzyme class | Enzyme | Reactions | Role in LCB valorization for food applications |
|--------------------------|---|---|--|
| Cellulolytic enzymes | Endoglucanases | Randomly cleave internal β -1,4-glycosidic bonds within the amorphous regions of cellulose, which create smaller polymers with new chain ends | Crucial for breaking down cellulose to smaller fragments, which is the first step for producing fermentable sugar (glucose) and the cello-oligosaccharide prebiotics |
| | Exoglucanases | Cleave the reducing and non-reducing ends of the cellulose chains, to release the glucose dimer, cellobiose | Produce cellobiose, which is the main glucose precursor. Cellobiose also has a variety of food applications |
| | β -Glucosidases | Hydrolyse cellobiose to produce glucose monomers | Ensure formation of monomeric glucose, the main carbon source for fermentation |
| Hemicellulolytic enzymes | Endoxylanases | Cleave internal β -1,4-glycosidic bonds of xylan, producing shorter xylo-oligosaccharides | Main enzyme for producing XOS prebiotics and initiating xylan breakdown |
| | β -Xylosidases | Act on the non-reducing ends of xylo-oligosaccharides to release xylose monomers | Crucial in producing biomass derived xylose, which is used as food additives and also serve as precursor for xylitol production |
| | Mannanases and mannosidases | Degrade β -1,4-mannosidic bonds and hydrolyse manno-oligosaccharides, to produce mannose | Manno-oligosaccharide is prebiotic, and mannose has potential application as functional food |
| | Debranching enzymes (arabinofuranosidases, acetyl xylan esterases, feruloyl esterases <i>etc.</i>) | Remove various side-chain substitutions and release hemicellulose from lignin, which facilitate the action of the main-chain-degrading enzymes | Play important role in hemicellulose degradation and overall breakdown of LCB |
| Ligninolytic enzymes | Laccases | Oxidize a range of phenolic compounds and facilitate lignin breakdown | Considered as the key enzymes for LCB delignification and may play crucial role in eco-friendly production of lignin-derived phenolics, including vanillin |
| | Peroxidases | Depolymerize lignin through oxidative cleavage using hydrogen peroxide | |

cocktails have been reported to degrade hemicellulose and lignin individually,^{82,86–88} there has been limited success in the rational formulation of enzyme cocktails for whole LCB degradation.⁸⁹ Several LCB-degrading enzyme cocktails have been derived from microbial secretomes, either from isolated microorganisms or from microbial consortia; however, most are not fully characterized.^{90–93}

3.1.3. Engineered enzymes. Despite the availability of several cellulase, hemicellulase and ligninase enzymes, enzymatic LCB depolymerization still falls short of industrial requirements.^{94–96} Key challenges include high cost, low catalytic activity, low selectivity to complex substrates, product inhibition, and incompatibility between different enzyme groups. To address these issues, various enzyme engineering strategies have been employed.^{97–101} Enzyme engineering is a powerful technology for enhancing enzymes' catalytic efficiency, expanding substrate scope, and improving physical properties like thermostability, solvent tolerance, and pH resistance. The three main enzyme engineering approaches are rational mutagenesis, directed evolution, and semi-rational enzyme engineering approaches. As the name implies, in rational mutagenesis, a few most-probable mutations are

rationally designed based on the prior knowledge of structure and mechanism of action of the enzyme. Various bioinformatics tools, such as *in silico* modelling and molecular dynamics simulations, are often employed for this purpose.^{102,103} The actual mutants are generated by site-directed mutation of one residue at a time or through combinatorial mutagenesis to create multiple mutations at once. In directed evolution, a large number of mutants (collectively called mutant library) are generated through random mutagenesis, and the best mutants for the desired trait are selected by high throughput screening.^{104,105} The effectiveness of a directed evolution platform is influenced by several factors such as library size, mutation rate, and diversity. However, its success largely relies on the efficiency of the high-throughput screening system, since the library size can easily reach a few thousand or even millions. While rational mutagenesis generally focuses on the proximity of an active site, directed evolution can identify mutations near as well as distant to the active site. The semi-rational enzyme engineering approach combines the benefits of both rational and random mutagenesis, leading to the creation of a smaller library (also called focus library) based on the structural and functional knowledge of the enzyme. Focus



Table 3 Comparison of lignocellulosic biomass (LCB) degradation through microbial, enzymatic, and chemical routes

| Parameters | Microbial degradation | Enzymatic degradation | Chemical degradation |
|-------------------------------|---|--|--|
| Mechanism | Microorganism/s are cultured directly on biomass to break down the polymers by synergistic action of diverse enzymes present in the microbial secretome | Purified lignocellulolytic enzyme/s are used to degrade lignocellulosic polymers in pretreated biomass | Strong acid, alkali, or ionic liquids are used under high temperature and pressure to break down lignocellulosic structure |
| Substrate (starting material) | Crude or pre-treated biomass; often supplemented with additional nutrients | Pre-treated biomass; may need to remove contaminants (inhibitors) | Crude biomass |
| Products | Partially decomposed biomass (with enhanced enzymatic accessibility), fermentable sugars or other target products. Contaminated with microbial cells and spent growth medium | Fermentable sugars (monosaccharides), oligosaccharides, and lignin derived phenolic compounds | De-structured LCB with high enzyme accessibility and/or fermentable sugars. Often contains enzyme inhibitors that need to be separated |
| Speed | The process is slow as it depends on microbial growth and enzyme production. May take days to weeks | The reaction is moderate to fast depending on enzyme and biomass composition; inhibitors can slow the process. Typical time is hours to days | The reactions finish very fast, typically within minutes to hours |
| Environmental impact | Ecofriendly process without any hazardous byproducts, as it mimics a natural biological process | Very low to no byproduct as the enzyme works on specific substrate. Minimum energy requirement and the process is highly ecofriendly if enzymes are sourced sustainably | Environmentally hazardous; need high energy and hazardous chemicals. Also, produces toxic waste streams that may cause soil and water pollution |
| Cost and scalability | The most cost-effective method due to on-site enzyme production. Scale-up requires careful optimization of fermentation and reaction parameters | Scalable but high enzyme production and purification expense is the major barrier; ongoing research in cost reduction | Can be costly due to energy demands and the need for specialized, corrosion-resistant equipment. The process is established at large scale |
| Control | Less control on the process as it relies on complex microbial metabolic activities | High control over reaction conditions (pH and temperature), enzyme activity, and specificity | Little control on product profile, although reaction parameters (temperature, pressure, chemical concentration) could be adjusted |
| Application | Suitable for biological pretreatment of LCB for enhanced enzymatic accessibility | A key step for producing fermentable sugars | Widely used for biomass pretreatment to break down lignocellulose structure and isolate the components |
| Major advantages | (1) Cheap and ecofriendly option (2) Minimal inhibitory by-product formation for subsequent fermentation step (3) Saccharification and fermentation steps can be integrated | (1) Highly specific and efficient route for saccharification (2) Almost no inhibitory by-products formation (3) The process is mild and eco-friendly | (1) Highly efficient and fast reactions (2) Highly effective in disintegrating the structure of recalcitrant lignocellulose (3) Suitable for handling broad range of crude and contaminated feedstocks |
| Major disadvantages | (1) Long processing time due to slow reaction rates (2) Complex system; difficult to manipulate and control | (1) High cost of enzyme production (2) Lack of cost-competitiveness at industrial scale (3) Sensitive to fluctuations in feed (substrate) composition, temperature and pH, requiring precise operational control | (1) Not ecofriendly (2) Additional pH neutralization and separation steps prior to subsequent bioprocessing (3) Generate toxic by-products detrimental to subsequent fermentation step |

libraries are created mostly at the vicinity of the enzyme's active site and are generally screened using low to medium throughput techniques.

In the past few years, machine learning has made a significant impact in the advancement of protein engineering techniques. Recent advancement in machine learning is being

leveraged for enhancing speed and improving the outcome of enzyme engineering.¹⁰⁶⁻¹⁰⁸ Integrating machine learning in enzyme engineering workflows not only reduces the experimental burden of mutant screening but also improves the library quality and increases probability of obtaining better hits. The success of rational design largely relies on accurately



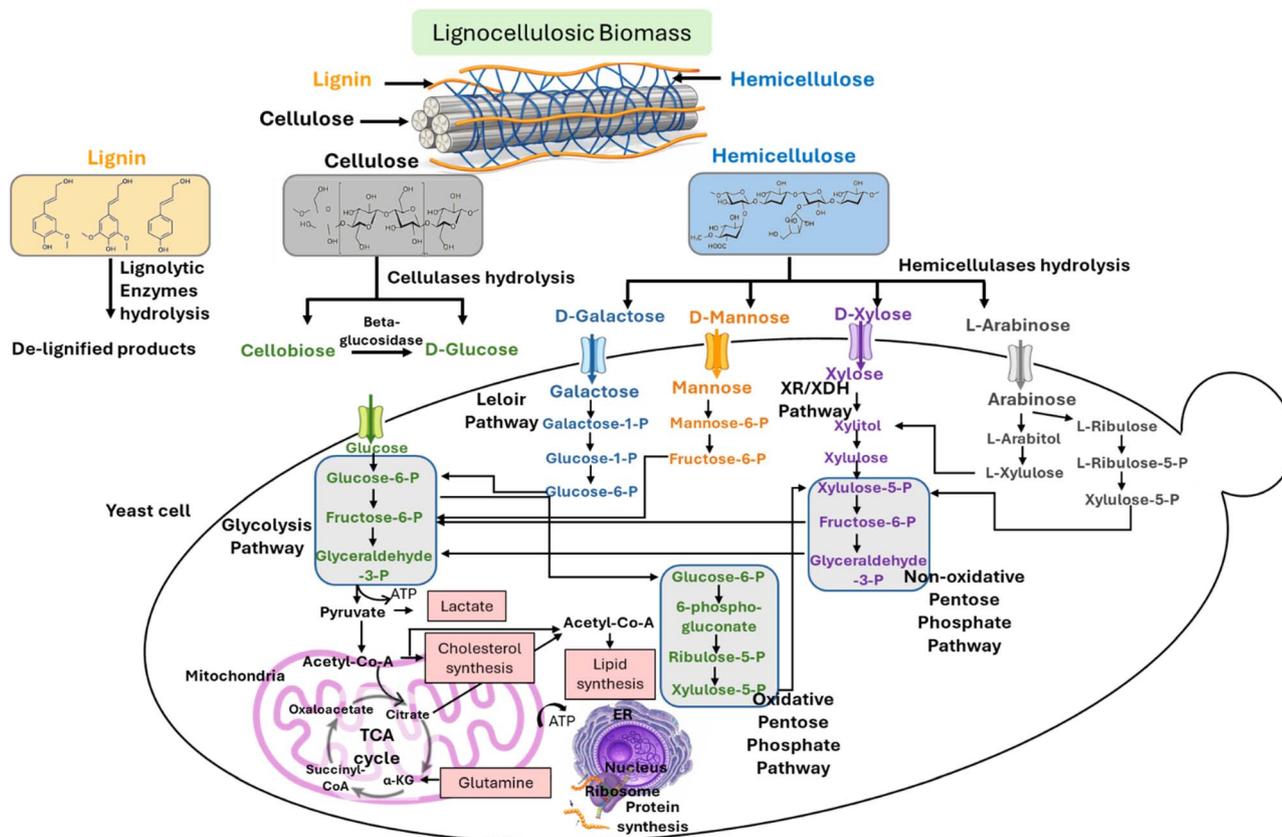


Fig. 4 Schematic overview of yeast metabolic pathways involved in the valorization of lignocellulosic biomass (LCB) derived sugars into lipids and protein-rich biomass. Cartoon icons representing lignocellulosic biomass, yeast mitochondria, the nucleus, and ribosomal protein synthesis were sourced from FigureLabs (<https://chat.figurelabs.ai/>) and manually assembled.

predicting enzyme activity and selectivity from their sequence, structure, and catalytic mechanisms.¹⁰⁹ Although numerous computational tools have been developed, achieving high predictive accuracy remains a challenge. Machine learning offers a promising solution by recognizing patterns in amino acid sequences and linking them to catalytic activity and physicochemical properties, thereby enabling more reliable predictions of enzyme activity, stability, selectivity, and other desired characteristics.^{110,111} Structure guided combinatorial libraries are often burdened with large number of mutants that need a high throughput screening strategy. Machine learning guided combinatorial mutagenesis improved overall capability and performance of this rational enzyme engineering approach.^{112–114} Directed evolution workflows have been accelerated substantially using machine learning based predictions.^{115,116} Machine learning models have been trained using the change (increase or decrease) in properties (such as activity and stability) of characterized mutant enzymes from the first round of directed evolution, to predict new variants that are likely to display improved catalytic activity or desired physical properties. Beyond strengthening the traditional enzyme engineering approaches, machine learning has also taken a lead in *de novo* designing of new enzyme scaffolds, predicting their structures, physical properties and biocatalytic functionalities.^{117–119}

3.1.4. Immobilized enzymes. Enzymatic lignocellulose degradation is a slow process, and enzymes need to be stable for a longer time at the reaction condition, which could be achieved using immobilized enzymes. An immobilized enzyme is a preparation in which a soluble enzyme is fixed within a solid matrix while retaining catalytic activity. This strategy not only helps with enzyme stabilization but also makes enzymes reusable in a batch or continuous process. Compared to enzymes in solution, immobilized enzymes often show higher catalytic activity and tolerance to harsh environmental conditions such as high temperature, extreme pH, and the presence of organic solvents. There are various techniques for enzyme immobilization; the common methods include covalent binding, adsorption, cross-linking, and physical entrapment. In covalent binding and adsorption, the enzymes chemically or physically bind to a solid matrix (such as a synthetic resin, an inorganic polymer, or a biopolymer), while the physical entrapment involves entrapping an enzyme in a polymeric network, during its formation. In cross-linking, a cross-linking agent is used to interconnect several enzyme molecules without the help of any carrier or solid support. These techniques have been used to immobilize various enzymes for recycling a range of LCB, including sugarcane bagasse, corncobs, barley straw, rice straw, and wheat straw.¹²⁰ The immobilized enzymes generally showed high stability and, in a few cases, were re-used for up to 10



Table 4 Comparison of microbial, enzymatic and chemical methods for oligosaccharide based dietary fiber production from lignocellulosic biomass (LCB)

| Parameters | Microbial | Enzymatic | Traditional/chemical |
|--|---|--|---|
| Mechanism | Partial degradation of polysaccharides (<i>e.g.</i> cellulose, xylan) through microbial fermentation | Controlled hydrolysis using isolated enzyme/s | Partial hydrolysis using strong acids or alkalis |
| Substrate | Isolated polysaccharides or crude/pretreated biomass | Isolated polysaccharides | Crude biomass |
| Product (purity and reproducibility) | Susceptible to produce other fermentation products and may need complex purification steps. Relatively low batch to batch reproducibility of oligomer composition | Highly pure oligosaccharides with minimal byproducts, usually with narrow distribution of the oligomer length. Relatively defined oligomer composition suitable for food applications; high batch to batch reproducibility | Generates non-specific degradation products (<i>e.g.</i> monomeric sugars, furfural, phenolic compounds) that require extensive and difficult purification. Due to lack of selectivity, oligomer composition may vary extensively from one batch to another |
| Reaction conditions and environmental impact | Mild and eco-friendly condition is needed for microbial growth and reaction No hazardous waste produced | Mild reaction condition as most enzymes work at near-neutral pH and moderate temp Minimum waste generation | Reaction needs harsh conditions, such as high temp, high pressure, strong acids/alkalis Less ecofriendly due to production of hazardous chemical wastes |
| Efficiency/cost | The process is time-consuming, and the microorganism might utilize the oligosaccharides as carbon source | Higher enzyme cost is the major bottleneck | The process is well-established but faces high equipment and operating costs due to use of corrosive agents. Product isolation and hazardous waste management add-up extra processing cost |
| Major advantages | (1) Enzyme production and hydrolysis are integrated into one step (2) Avoid expensive enzyme purification step (3) Often works on crude biomass | (1) The process operates under mild temperatures and pH (2) No toxic byproduct formation (3) Produces high quality oligosaccharides suitable for use in food industry | (1) Well-established process (2) Fast and simple reactions |
| Major disadvantages | (1) The process is time-consuming compared to enzymatic and chemical methods (2) Oligosaccharide consumption by the fermenting strain often hinders the oligosaccharide accumulation (3) Process optimization is challenging due to lack of understanding in polysaccharide degrading reactions in microbes | (1) Often requires additional pretreatment step (2) High cost of commercial enzymes is a major barrier | (1) The reaction lacks specificity (2) The process is environmentally detrimental due to the use of strong acid/alkali (3) High concentrations of monomeric sugar production (4) Generates harmful contaminants, requiring extensive purification for food-grade use |

cycles. Immobilization also helped to overcome enzyme inhibition and inactivation by furans and phenolic compounds produced during pretreatment.^{78,121} In addition to immobilizing single enzymes, enzyme cocktails were also immobilized for LCB degradation as its breakdown often requires the catalytic activity of multiple enzymes.¹²²

Despite their potential advantages, the use of immobilized enzymes in LCB conversion faces significant challenges. As highlighted by Patti *et al.*, a key limitation is mass transfer; immobilization often restricts substrate accessibility to the active sites, which becomes more pronounced when working with complex, heterogeneous LCB substrates.¹²³ As a result, many studies report superior hydrolysis efficiency with free enzymes compared to their immobilized counterparts. Another

major challenge is scalability, since most reported work has been carried out in model systems or at very low solid loadings. At practical substrate concentrations, the system is hindered by high viscosity, poor mixing, and reduced mass transfer, all of which further limit the effectiveness of immobilized enzymes. Consequently, while immobilization may offer benefits such as enzyme reusability and stability, overcoming these operational barriers is essential before it can be widely applied to industrial-scale LCB bioconversion.

3.2. Microorganisms

Research has shown that a wide range of microorganisms can degrade lignocellulosic materials. However, due to complex and



Table 5 Examples of lignocellulosic agricultural wastes valorization for food applications

| Biomass source | Product/s | Pre-treatment/s | Bio-based tools involved | | | Reference | |
|-------------------|--|--|---|---|---|-----------|-----|
| | | | Enzyme/s | Microbe/s | Fermentation | | |
| Sugarcane bagasse | Lipid | — | — | <i>Mortierella wolfii</i> AH12 | Batch fermentation (shake flask) | 197 | |
| | Lipid | Acid hydrolysis | — | <i>Yarrowia lipolytica</i> Po1g | Batch fermentation (shake flask) | 19 | |
| | Dietary fiber (cello-oligosaccharides) | Delignification by sodium chlorite treatment followed by alkali treatment | 1,4-β-Endoglucanase | — | — | — | 16 |
| | Dietary fiber (xylooligosaccharides) | Delignification by sodium hypochlorite, sodium chlorite, aqueous ammonia, or combination of hydrogen peroxide and acetic acid, followed by xylan extraction by alkali hydrolysis, acid hydrolysis, or coupled alkali and thermal treatment | Isolated endoxylanase from <i>Aspergillus flavus</i> MG-7 | — | — | — | 22 |
| | Dietary fiber (xylooligosaccharides) | Xylan extraction by alkali hydrolysis | Recombinant GH10 endoxylanase from <i>Thermoascus aurantiacus</i> | — | — | — | 216 |
| | Dietary fiber (xylooligosaccharides) | Hemicellulose extraction by alkali hydrolysis | Crude xylanase complex | <i>Aspergillus fumigatus</i> M51, and <i>A. fumigatus</i> U2370 | Batch fermentation (shake flask) | — | 226 |
| Corn stover | Palmitoleic acid | Steam explosion | Cellic Ctec2 enzyme blend (Novozymes) | <i>Saccharomyces cerevisiae</i> YS10 | Batch fermentation (shake flask) | 201 | |
| | Lipid | Alkali hydrolysis | Cellulase and hemicellulase | <i>Schizochytrium</i> sp. HX-308 | Two-stage fermentation: batch followed by fed batch in shake flasks | 202 | |
| | Lipid | Steam explosion | Cellulase, β-glucosidase, and xylanase | <i>Cryptococcus podzolicus</i> SCTCC300292 | Batch fermentation (shake flask) | 203 | |
| | Vanillin | Alkali hydrolysis | Accelerase 1500 (DuPont) and Multifect xylanase (DuPont) | Engineered <i>Saccharomyces cerevisiae</i> | Batch fermentation (shake flask) | 233 | |
| Corn cob | Dietary fiber (xylooligosaccharides) | Alkaline hydrolysis, followed by hydrothermal pretreatment | Two endoxylanases from GH10 and GH11 families, and one <i>exo</i> -xylosidase from GH11 family | — | — | 218 | |
| Rice straw | Lipid | Acid hydrolysis | — | <i>Trichosporon fermentans</i> | Batch fermentation (shake flask) | 198 | |
| Wheat straw | Lipid | Acid hydrolysis | Cellic CTec3 enzyme cocktail (Novozymes) | <i>Rhodotorula babjevae</i> DBVPG 8058, or <i>Lipomyces starkeyi</i> CBS 1807 | Batch fermentation (in 0.7 litre bioreactor) | 196 | |
| | Lipid | Deep eutectic solvent (DES) (citric acid : choline chloride : ethylene glycol in a molar ratio of 1 : 1 : 2) | — | <i>Trichosporon cutaneum</i> ACCC 20119 | Batch fermentation (shake flask) | 261 | |
| Barley straw | Dietary fiber (xylooligosaccharides) | Steam explosion | Enzyme cocktail containing <i>endo</i> -β-(1,4)-D-xylanase, α-L-arabinofuranosidase, feruloyl esterase, and acetyl xylan esterase | — | — | 220 | |
| Wheat husk | Dietary fiber (xylooligosaccharides) | — | Crude extracellular xylanase preparation | <i>Aspergillus fumigatus</i> R1 | — | 223 | |



Table 5 (Contd.)

| Biomass source | Product/s | Pre-treatment/s | Bio-based tools involved | | | Reference |
|----------------|--|--|--|--|---|-----------|
| | | | Enzyme/s | Microbe/s | Fermentation | |
| Soybean fiber | Dietary fiber (xylooligosaccharides) | — | <i>endo</i> - β -1,4-Xylanase | Recombinant <i>Escherichia coli</i> | Batch fermentation (shake flask) | 227 |
| | Dietary fiber (xylooligosaccharides) | — | Recombinant xylanase and arabinofuranosidase | Engineered <i>Aspergillus nidulans</i> A773 | Batch fermentation (shake flask) | 224 |
| | Dietary fiber (xylooligosaccharides) | — | Crude enzyme preparation containing xylanase, cellulase, β -xylosidase, and β -glucosidase | <i>Aspergillus brasiliensis</i> BLf1 | Batch fermentation (solid-state and submerged cultures) | 225 |
| Grape pomace | Dietary fiber (cello-oligosaccharides) | — | 1,4- β -Endoglucanase | — | — | 215 |
| | Dietary fiber (xylooligosaccharides) | Acetylated xylan extraction by two-step hydrothermal treatment | <i>endo</i> - β -1,4-Xylanase, and deacetylase | — | — | 221 |
| Rye straw | Lipid | — | Endogenous xylitol dehydrogenase, and xylulose kinase | Engineered <i>Yarrowia lipolytica</i> A101 and AJD | Batch fermentation (shake flask) | 17 |
| Kraft lignin | Vanillin | — | None | Mixed indigenous bacterial | Batch fermentation (shake flask) | 20 |

recalcitrant structure, efficient lignocellulose degradation requires the synergistic action of diverse microorganisms secreting a broad spectrum of enzymes.¹²⁴ Both aerobic and anaerobic microorganisms contribute to this process, though their strategies differ.¹²⁵ Aerobic lignin degradation has been extensively studied, with microorganisms typically employing free radicals and oxidative enzymes such as peroxidases and laccases to dismantle lignin. In contrast, supplying oxygen in industrial fermentation is costly, making oxygen-free anaerobic fermentation an attractive alternative for large-scale applications. Anaerobic lignin degradation, however, remains less understood, with evidence suggesting that some microbes metabolize only lignin's methyl groups, while others require external electron donors.¹²⁶ Collectively, microorganisms play a central role in LCB conversion in nature.¹²⁷ A comprehensive summary of various organisms utilizing different substrates for food and feed production is reported by Li *et al.* (2024).¹²⁸ In this review, we specifically focus on pretreated LCB as substrate, highlighting recent advances in lignocellulose-degrading microorganisms, particularly fungi and bacteria, and their associated cellulolytic and hemicellulolytic enzyme systems.

3.2.1. Bacteria. Bacteria are emerging as key players in lignocellulose degradation due to their metabolic diversity, adaptability, and ease of genetic manipulation. Recent studies show they contribute more to lignocellulolytic enzyme activity than fungi in synthetic communities, making them strong candidates for engineered biomass conversion systems.¹²⁹ A

diverse range of bacterial species are able to degrade lignocellulosic biomass, employing different enzymatic systems depending on their environmental conditions. A recent review outlines various bacterial genera involved in the degradation of lignocellulose, highlighting their enzymatic capabilities.¹³⁰ Aerobic bacteria such as *Thermobifida fusca*, *Cellulomonas* spp., *Bacillus* spp., *Thermomonospora* spp., and *Microbispora* spp. utilize free cellulolytic enzymes to hydrolyze cellulose, while anaerobic bacteria like *Clostridium thermocellum*, *Ruminococcus* spp., *Bacteroides* spp., and *Acetivibrio* spp. employ complex multienzyme structures such as cellulosomes for efficient cellulose degradation. Several bacteria also exhibit ligninolytic activity, notably members of the Actinobacteria such as *Streptomyces viridosporus* T7A, *Streptomyces* spp., *Nocardia* spp., and *Rhodococcus jostii* RHA1, which produce oxidative enzymes capable of lignin depolymerization. Among Proteobacteria, *Sphingobium* sp. SYK-6 and *Pseudomonas putida* mt-2 are well-characterized for their ability to catabolize lignin-derived aromatic compounds. Additionally, thermophilic bacteria such as *Caldicellulosiruptor bescii* can degrade both lignin and cellulose under extreme conditions. These bacteria play essential roles in the global carbon cycle and offer promising potential for industrial biomass conversion processes.¹³⁰ In addition, lactic acid bacteria species, particularly *Lactobacillus plantarum*, which is commonly involved in vegetable fermentation, have been identified for their potential in lignocellulose breakdown, contributing to the growing number of bacterial



genera involved in biomass degradation.¹³¹ Moreover, these bacteria can be effectively integrated into co-culture systems with fungi or yeasts to improve substrate utilization, enhance fermentation performance, and increase the yield of targeted food-grade products.¹³²

3.2.2. Fungi. Fungal degradation of lignocellulosic biomass is a complex, yet crucial process influenced by the complex structure of lignocellulose. Strong physical association of lignin and hemicellulose and the crystalline nature pose major challenges to enzymatic cellulose hydrolysis, with lignin removal being particularly critical for improving access to cellulose. Filamentous fungi, especially wood-degrading species, play a vital ecological role in degrading plant biomass through the secretion of diverse extracellular enzymes. These fungi utilize both hydrolytic and oxidative enzymes to break down polysaccharides and lignin, respectively. Depending on their degradation strategies and lignin-targeting efficiency, fungi are broadly categorized into soft-rot, brown-rot, and white-rot groups.¹³³

Soft-rot fungi, mainly *Aspergillus* and *Neurospora* spp., are ascomycetes that degrade plant surface polysaccharides. They produce laccases and peroxidases that modify lignin, though these enzymes are less specific and effective than those of white-rot and brown-rot fungi. The mechanisms behind their lignocellulose degradation are still not well understood.¹³⁴

Brown-rot fungi, such as *Gloeophyllum trabeum*, *Coniophora puteana*, and *Postia placenta*, are basidiomycetes that efficiently degrade cellulose and hemicellulose but only slightly alter lignin. They use a non-enzymatic oxidative mechanism called the chelator-mediated Fenton system, which generates hydroxyl radicals to break down the lignocellulose matrix. This process leads to cube-shaped, brown-colored wood residues due to oxidized lignin.¹³⁵

White-rot fungi are basidiomycetes that can break down all major components of lignocellulose such as lignin, cellulose, and hemicellulose. They are particularly effective at degrading lignin, even converting it completely to CO₂. This unique ability makes them promising sources of commercial lignocellulose-active enzyme cocktails. The major white-rot fungi for lignocellulose degradation include *Aspergillus niger*, *Trichoderma reesei*, *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Pycnoporus cinnabarinus*, and *Trametes versicolor*.¹³⁶

Although traditionally explored for their environmental applications in biopulping, bioremediation, and enzyme production, the interest in ligninolytic basidiomycetes in food-related bioprocesses is increasing steadily. Their potential lies in enhancing the digestibility of lignocellulosic agricultural residues, thus improving their suitability as animal feed or fermentation substrates.¹³⁷ Additionally, the ligninolytic activity of these fungi facilitates the release and recovery of bound phenolic compounds from plant biomass. These phenolics, once liberated, can act as natural antioxidants with potential health-promoting and functional food applications.¹³⁸ *Pleurotus ostreatus* and *Ganoderma lucidum*, both edible mushrooms, offer dual benefits of biomass degradation and production of bioactive compounds such as polysaccharides, triterpenoids, and antioxidant phenolics, aligning with the growing interest in

fungal fermentation for production of functional food ingredients.^{139,140}

Filamentous fungi such as *Aspergillus*, *Trichoderma*, *Rhizopus*, and *Penicillium* spp. are extensively studied for their lignocellulose-degrading capabilities due to their ability to secrete a range of extracellular enzymes, including cellulase, hemicellulase, ligninase, and other enzymes.¹³³ *Trichoderma reesei* is considered as industrial fungi species for cellulase production and is extensively used in the bioconversion of lignocellulosic substrates.¹⁴¹ *Aspergillus niger* is widely used in the food industry to produce organic acids such as citric acid and gluconic acid, and is also known for its hemicellulase activity.¹⁴² These fungi can grow directly on lignocellulosic substrates and are capable of transforming them into value-added products, including single cell proteins, organic acids, and flavor-enhancing enzymes for food and feed applications. Edible filamentous fungi such as *Neurospora intermedia* and *Rhizopus oligosporus* have been evaluated for solid-state fermentation of agro-industrial residues, including wheat bran, fruit pulp, and rice straw, to produce food-grade fungal biomass rich in protein, dietary fiber, and bioactive compounds. Their potential in alternative protein development is of growing interest, especially in the context of sustainable and functional food production.¹⁴³

Yeasts also serve as promising hosts for the production of lignin-degrading enzymes, particularly through heterologous expression systems.¹⁴⁴ Species such as *Saccharomyces cerevisiae*, *Yarrowia lipolytica*, *Pichia pastoris*, *Pichia methanolica*, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, and *Cryptococcus* spp. have been successfully engineered to express laccases and peroxidases using exogenous genes derived not only from fungal sources (Ascomycota and Basidiomycota) but also from plants, oomycetes, and bacteria. Yeast platforms offer several advantages for enzyme production, including ease of cultivation, cost-effective substrates, rapid growth rates, and well-established tools for genetic manipulation.¹⁴⁵ Moreover, yeasts possess the cellular machinery necessary for complex post-translational modifications such as glycosylation, disulfide bond formation, and proteolytic processing which are critical for the proper folding and activity of many ligninolytic enzymes. These features make yeasts attractive hosts for scalable and efficient production of lignin-degrading enzymes in industrial biotechnology applications.¹⁴⁶ Beyond their role as enzyme factories, yeasts also represent a valuable source of protein-rich biomass.¹⁴⁷ Their high protein content, favorable nutritional profile, and strong safety record position them as promising candidates for single-cell protein applications, further enhancing their value in sustainable bioprocessing.

3.2.3. Microalgae. Microalgae are another class of organisms that are gaining more attention as production host, capable of generating proteins, lipids, pigments, bioactive compounds, and recombinant enzymes with applications in food and feed industries. They are the base of the food chain in aqueous environments and are renowned for their ability to produce and accumulate polyunsaturated fatty acids such as docosahexaenoic acid (DHA). These fatty acids are essential and need to be obtained through diet, as mammals are incapable of



synthesizing them by themselves. This also makes them industrially relevant with applications such as nutraceuticals, but also in cosmetic products, or polymer precursors. Microalgae can grow under photosynthetic or heterotrophic conditions. Their heterotrophic metabolism is especially interesting, as the light independent utilization of different sugars (hexoses and pentoses) makes them candidates for producing value-added chemicals at industrial scale.¹⁴⁸

Species such as *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Spirulina platensis* (cyanobacterium), are increasingly explored for their potential in food applications due to their high protein content, and presence of essential fatty acids, vitamins, and antioxidant compounds.^{149,150} Currently, research focuses on finding cost-effective carbon sources, including lignocellulosic biomass to make lipid production processes economically viable.^{151,152}

Chlorella spp. and *Scenedesmus* spp., in particular, have been shown to grow on media derived from enzymatically hydrolyzed lignocellulosic materials or anaerobically fermented hydrolysates, converting these into biomass rich in protein and pigments such as chlorophylls and carotenoids.^{153–155} The incorporation of microalgae-derived biomass in functional foods and animal feed not only valorizes agro-industrial residues but also contributes to sustainable protein and bioactive compound production, aligning with circular bioeconomy principles.¹⁵⁶

3.2.4. Engineered microorganisms. A key aspect in valorization of lignocellulosic agricultural wastes for food applications is the ability of a production host to utilise a wide variety of different sugars. Hydrolysis of lignocellulosic biomass usually results in large amounts of glucose (C6) and xylose (C5) sugar. Besides, arabinose, galactose and mannose are commonly present in the hydrolysate. In addition, degradation products of lignin often have inhibitory effects. Advancements in engineering biology have enabled the development of microorganisms that can cope with inhibitory compounds, overcome natural limitations in substrate utilisation and can lead to improved yields of relevant high value compounds. Engineering methylotrophic yeast *Komagataella phaffii* (formerly known as *Pichia pastoris*) is a successful case study in this respect. This industrially relevant yeast is naturally incapable of utilizing C5 sugars such as xylose as sole carbon source.¹⁵⁷ After significant efforts, it has been successfully engineered to utilise xylose efficiently.^{158,159}

Despite progress in metabolically engineering host strains for broader substrate utilization, few studies have examined the long-term stability of engineered traits under industrial conditions. Moreover, engineered strains often exhibit reduced growth rates or increased metabolic burden, which can negatively affect overall performance and process economics.¹⁶⁰

Besides recombinant proteins, lipids are an interesting target for food and feed applications. Several methylotrophic yeasts, including *Ogataea polymorpha* (formerly *Hansenula polymorpha*) have been engineered to increase xylose utilisation for the production of free fatty acids using hydrolyzed lignocellulose as feedstock.¹⁶¹ Additionally, oleaginous yeasts, such as *Y. lipolytica* has also been an engineering biology target as

this yeast naturally accumulate large amounts of lipids, and could be exploited for the efficient production of single-cell oils or essential omega-3 fatty acids such as DHA.¹⁶² For example, DuPont developed a *Y. lipolytica* strain that produces eicosa-pentaenoic acid (EPA), comprising 25% of cell dry weight and over 50% of total lipids. The process uses glucose as feedstock, that indicates that glucose derived from hydrolyzed lignocellulose could also be a viable alternative to be used.¹⁶³ Ge *et al.* demonstrated this by constructing a *Y. lipolytica* strain that is capable of co-utilizing glucose and xylose derived from lignocellulosic biomass to produce succinic acid.¹⁶⁴

3.3. Fermentation

3.3.1. Fermentation medium and feedstock. Microbial fermentation is an established technology for producing valuable products utilizing a range of feedstocks. Fermentation has long been used for food production, and with the advancement of precision fermentation, it is taking centre stage in sustainable production of food ingredients. Traditional fermentation process uses naturally occurring microorganisms to anaerobically metabolise substrates (typically, carbohydrates) to desired products (*e.g.* alcohol and organic acids), while in precision fermentation, engineered microorganisms are used for producing a predefined molecule in a highly controlled process.

Microorganisms require a cultivation medium that provides all necessary nutritional elements for growth and metabolism. For most bioprocesses, it is essential to provide a carbon source (*e.g.* sugar, oils, alcohols or organic acids), a nitrogen source (*e.g.* ammonium salts, protein hydrolysates *etc.*), a phosphorous source (*e.g.* phosphate salts or phosphoric acid), vitamins (*e.g.* biotin or riboflavin), a sulphur source (*e.g.* sulphate salts or amino acids) and trace elements (*e.g.* Mg, Ca, K, Fe *etc.*). The choice of carbon source heavily affects production costs as it is required at large quantities. First-generation feedstocks such as grains, sugar crops, and oilseeds have long been used as fermentation substrates, which is now raising concerns as this practice may lead to direct competition between fermentation feedstock and human food.^{165–167} To address this issue, second-generation feedstocks *i.e.* lignocellulosic biomass such as sugarcane bagasse, corn stover, rice straw or grape pomace are being explored as potential fermentation substrates. The use of these low-cost, agro-industrial byproducts will reduce production costs and enhance the sustainability of fermentation processes.

Hydrolyzed lignocellulosic materials can be used as a fermentation feedstock. However, hydrolyzed lignocellulosic materials do not only contain the desired glucose, but also various lignin degradation products that may inhibit microbial growth or impair productivity and yield of the fermentation process. Therefore, hydrolyzed lignocellulosic material often requires further purification which add ups to the substrate costs. Alternatively, the material needs to be used in diluted form in complex media. The use of complex media has certain advantages as they contain nutrients that provide trace elements, vitamins and amino acids that are beneficial for cell growth. Presence of these nutritional components also reduces



stress on cellular metabolism, as these components do not need to be synthesized anew.¹⁶⁸ Thus, complex media can help to overcome the inhibitory effects of lignin degradation products produced during lignocellulose pre-treatments. In some cases, the lignin monomers themselves can serve as fermentation substrate. Separating them after hydrolysis might therefore be beneficial as the by-products can be of further use. Lee *et al.* reported biological funnelling of lignin into 2-pyrone-4,6-dicarboxylic acid *via* electrocatalytic depolymerization, followed by biotransformation using genetically engineered *Pseudomonas putida*.¹⁶⁹

Lignin and its degradation products possess antimicrobial properties and the ability to block UV-radiation, which could be beneficial for their use in food packaging.^{170,171} Phenolic compounds from lignin degradation also have several potential health benefits due to their antioxidant, antimicrobial, and anti-inflammatory properties. Some lignin degradation products are even being studied for potential pharmaceutical applications.¹⁷² The composition and abundance of phenolic compounds heavily depend on the lignin degradation. The effect of these compounds on different food products and the potential toxicity in some individuals are not fully explored. This underscores the need for further investigation into toxicity and biosafety aspects before using hydrolyzed lignocellulosic biomass as a fermentation feedstock for food production.

3.3.2. Submerged and solid-state fermentation. Besides feedstock selection, the mode of operation is of importance for the efficiency of fermentation processes. Generally, two types of operation are used *viz.* submerged fermentation and solid-state fermentation. Submerged fermentation is characterized by the substrate being dissolved in a liquid, and microorganisms such as bacteria, yeast and fungi are suspended within that mixture. This allows a very high degree of process control, as pH, temperature, gas-liquid mass transfer, power input, mixing and substrate availability can be tightly regulated in a bioreactor containing a homogenous mixture. There are different types of bioreactor and various modes of operation in biotechnological production processes, using submerged fermentation. The bioreactor type is selected depending on the expression host (*e.g.* bacteria, single cell yeast or filamentous fungi) and the desired product. The continuously stirred tank reactor (CSTR) is regarded as the work horse in aerobic biotechnological processes. The well-characterised nature of this type of bioreactor is important for scale-up, as transport and mixing processes can be mathematically described and modelled. The CSTR also offers the highest flexibility in handling lignocellulosic biomass, as the impeller type can be varied in order to meet process needs.¹⁷³

On the contrary, solid-state fermentation (SSF) is characterized by the use of solid substrates with low amounts of free water, where microbial growth essentially occurs on moist particles.¹⁷⁴ This strategy is frequently used for valorizing agro-industrial by-products such as fruit peels.¹⁷⁵ This process predominantly uses filamentous fungi as it allows low shear forces (*e.g.* introduced by continuous stirring). SSF has a few advantages, including high product concentration and relatively smaller reaction volume for the same amount of

substrate, as the substrate and microorganisms are not diluted in an aqueous mixture. Nonetheless, this technology has not been widely established at industrial scale as it brings a few limitations. The processes usually suffer from inefficient mixing, high substrate heterogeneity and low heat transfer efficiencies. This also leads to poor batch to batch reproducibility. Additionally, it is hard to recover the microbial biomass as it is tightly connected to insoluble particles and the process may not be suitable for production of microbial biomass as source of protein unless the insoluble particles are safe for consumption.¹⁷⁴ However, if the fungus produces an extracellular product (*e.g.* enzymes or organic acids), SSF is a suitable fermentation method as these components can be easily recovered.¹⁷⁶ There is ongoing research to increase the reproducibility of SSF processes to make them feasible at industrial scale.¹⁷⁷

3.3.3. Precision fermentation. Fuelled by recent advancement in synthetic biology, precision fermentation is being developed for sustainable production of food ingredients, including flavouring and colouring agents. Precision fermentation is a process of converting a specific substrate to a target compound, using an engineered microorganism. The microorganism is generally programmed with a genetic circuit for performing a series of reactions to convert the substrate to a target product. The primary benefit of precision fermentation is microbial production of compounds that would otherwise be too expensive or too complex to extract from natural sources. Its key advantage lies in the ability to engineer microbial systems for high-yield production of target ingredients, while also enabling the use of low-cost, renewable feedstocks, thereby contributing towards a circular bioeconomy. Furthermore, recombinant protein expression allows to tailor proteins for desired applications such as gelling, foaming, and emulsification, while also enabling protein fortification in bakery products, beverages, and snacks.¹⁷⁸ Among the most promising developments is the precision fermentation enabled production of recombinant animal proteins. Notable successes include egg white proteins, dairy proteins like β -lactoglobulin, and functional ingredients such as human lactoferrin and soy leg haemoglobin.^{179,180}

3.4. Conclusions and future perspectives

Among the biological LCB deconstruction techniques, enzymatic techniques are the most advanced. There are commercial cellulolytic-hemicellulolytic enzyme cocktails that can hydrolyse the cellulose and hemicellulose components of LCB. Nevertheless, the effectiveness of these enzyme preparations depends on the degree of complexity and lignification of the LCB. The more complex the LCB is, the less effective these enzyme cocktails are regardless of the pre-treatment process applied prior to the enzymatic hydrolysis step. Moreover, there are no effective delignification enzyme cocktails in the market and as such pre-treatment using thermochemical or thermophysical techniques are required for delignification, which make the LCB conversion process energy intensive and costly. Thus, there is still a need for more efficient enzyme preparations that



enable efficient delignification and saccharification of LCB regardless of their degree of complexity. In this regard, advances in enzyme engineering – particularly the integration of machine learning – potentially offers a more streamlined enzyme engineering workflow with faster design and screening of enzyme mutants. Reliable prediction of enzymes' catalytic activity and stability will reduce experimental burden and enable faster development of effective enzymes and enzyme cocktails for efficient delignification and saccharification processes, ideally without the need for intensive physicochemical pre-treatment regimes. The cost of enzymes will still affect the economic viability of the process and in this regard, immobilised enzymes or enzyme scaffolds may offer better cost-effectiveness. However, this requires significant advancement in enzyme immobilisation technologies to overcome the high viscosity and mass transfer limitations associated with complex LCB suspensions at solid loadings high enough to render the process economically viable. Microorganisms are the ultimate recyclers of carbon and nitrogen in nature. As such, the use of microbes for LCB deconstruction is a promising alternative to enzymatic approaches. It is potentially more cost-effective as it enables simultaneous enzyme production and LCB degradation as well as conversion to target products. However, microbial deconstruction of LCB is intrinsically a very slow process involving complex consortia of several microorganisms, which are not well characterised. So far, microbial valorization approaches mainly use hydrolyzed LCB as the starting material, which suffer from challenges such as the inability of microbes to utilise C5 carbons such as xylose and the inhibitory effects of lignin degradation products. Genetic engineering of microbial strains of interest for co-utilisation of C5 and C6 sugars as well as resistance to the inhibitory effects of lignin degradation products is increasingly being investigated to overcome these challenges. Nevertheless, this comes with regulatory hurdles for food-related applications, especially in jurisdictions where the use of genetically engineered microorganisms is restricted. Advances in AI assisted genome scale modelling is resulting in better understanding of the metabolic interaction between members of microbial consortium at genome scale. This may ultimately lead to the development of microbial consortia for target applications including efficient microbial saccharification of LCB and direct fermentation to desired products. This has the potential to substantially improve the economic viability of LCB bioconversion and valorization processes. Table 3 summarizes advantages and limitations of enzymatic, microbial, and chemical methods for LCB degradation.

4 Food ingredients through biobased lignocellulose valorization

4.1. Proteins

There is a growing demand for alternative protein sources globally, mainly driven by the need to sustainably feed the increasing population in a resource constrained world.¹⁸¹ Microbial proteins, often called single cell proteins (SCP), are potentially sustainable alternatives to traditional proteins as

they provide equivalent nutritional benefits and can be produced using cheap and abundant resources such as lignocellulosic agri-food waste as feedstock. They generally include whole microbial cells such as yeast, fungi, algae and bacteria. The inactivated cells can directly be used in animal feed (*e.g.* fishmeal substitute) or can be further processed to isolate proteins for human consumption.¹²⁸ Microbial proteins are often regarded as an additional source of protein for humans and animals to meet their dietary requirements. Especially in the context of a growing world population whose protein demand is steadily increasing, SCP holds the promise of being able to sustainably contribute to meeting the rising protein demand.¹⁸¹ In this regard, conversion of cheap carbon feedstock into edible biomass with a high protein content and a favourable amino acid profile, through microbial fermentation using suitable strains of yeast, bacteria or filamentous fungi is crucial.¹⁸² Depending on the microorganism selected, the composition of the resulting biomass changes, particularly in terms of its protein, fat, ash, and nucleic acid content.¹²⁸ The first example for commercially produced SCP is the animal feed Pruteen. It was produced during the 1970s by Imperial Chemical Industries (ICI, Billingham, UK), using the largest pressure cycle fermenter ever built to grow the methylotrophic bacterium *Methylophilus methylotrophus* consuming cheap and excessively available methanol.¹⁸³ Example of another commercially available SCP product is Quorn®. This product uses the filamentous fungi *Fusarium venenatum* and was released for human consumption in 1985.¹⁸⁴

The focus of recent research in single cell protein production is on the use of alternative, non-food feedstocks such as agricultural waste streams (*e.g.* fruit waste, molasses, dairy waste and lignocellulosic crop waste). Hydrolyzed lignocellulosic biomass is being considered as an alternative feedstock and can contribute towards a cheaper and more sustainable production process whilst enabling up-cycling of waste material into a valuable food or feed product. Proteins derived from lignocellulosic biomass are not limited to those intrinsically produced by microbes as SCP, or those released from agricultural residues through fermentation and pre-treatment. Increasingly, the production of recombinant proteins using genetically modified microorganisms is being researched as alternative avenue for bio-based valorization of lignocellulose biomass for food applications.¹⁸⁵ Enzymes that are used as processing aids in food or feed processing or those included in feed formulation to improve the digestibility or bioavailability of nutrients can be recombinantly produced using hydrolyzed LCB as feedstock. One such enzyme is phytase that is added to plant-based animal feed to release phosphate that is stored as phytic acid in plants.¹⁸⁶ Nonruminant animals, that lack or have limited endogenous phytase, can benefit from this enzyme supplementation. The enzyme is typically produced recombinantly using fungi or bacteria.¹⁸⁷ Similarly, lipases find broad application in the food industry, for example in dairy and meat processing.¹ Here, they can be added to accelerate cheese ripening, engineer texture and develop specific flavour profiles.¹⁸⁸ Further, pectinases are produced using microbial hosts for processing pectin-rich food products such as fruit



juices. Pectinases catalyse the hydrolysis of the polysaccharide pectin in the plant cell wall, making the material more accessible, thereby improving juice yield and clarity.¹⁸⁹ Currently, recombinant proteins are industrially produced using refined carbohydrates as the main carbon source. The use of hydrolyzed lignocellulosic biomass can potentially make the production of these proteins cheaper and more sustainable.

Biochemical pathways underlying microbial protein formation from LCB hydrolysates involve the assimilation of lignocellulose-derived sugars into central metabolic intermediates that serve as carbon skeletons for amino acid biosynthesis. Glucose and xylose produced by LCB hydrolysis are channelled through glycolysis and the pentose phosphate pathway (PPP), producing key precursors such as 3-phosphoglycerate, pyruvate, α -ketoglutarate and oxaloacetate.¹⁹⁰ These intermediates form the foundation for all major amino acid families, including the glutamate, aspartate, serine and pyruvate groups. Nitrogen, whether present in pretreated LCB or supplied as ammonium, urea, or complex nutrients, is incorporated *via* the GS-GOGAT pathway or NADPH-dependent glutamate dehydrogenase, generating glutamate, the universal amino donor for transamination reactions.^{191,192} Under balanced C/N ratios, nutrient-sensing systems such as TOR and SNF1 promote ribosomal biogenesis, biomass accumulation and amino acid synthesis rather than storage formation. These metabolic routes collectively explain how a broad range of yeasts, fungi and bacteria translate LCB-derived carbohydrates into high-quality microbial protein, supporting single-cell protein production across diverse industrial chassis.^{193,194}

4.2. Fats and oils

Single Cell Oil (SCO) is an important target molecule that can be produced *via* fermentation using LCB as feedstock. Various LCB hydrolysates are rich in fermentable pentose or hexose sugars that can be utilized by microorganisms like oleaginous yeasts and microalgae to produce SCO. For example, wheat straw was hydrolyzed using dilute sulphuric acid to generate high amounts of pentose (24.3 g L⁻¹) and hexose (4.9 g L⁻¹) sugars. Five different oleaginous yeasts were able to grow on this detoxified hydrolysate.¹⁹⁵ Oleaginous yeasts like *Lipomyces starkeyi*, *Yarrowia lipolytica*, *Mortierella wolfii*, *Trichosporon* spp., and *Rhodotorula babjevae* have been used to produce lipids from a variety of LCB hydrolysates like those of wheat straw, sugarcane bagasse, corncob and rice straw *etc.*^{17-19,196-199} Non-pretreated LCB can also be used as carbon source. The isolated indigenous strain *Mortierella wolfii* AH12 was able to grow on such non-pretreated sugarcane bagasse in a solid-state fermentation setup and produced lipids.¹⁹⁷ Some hydrolysates like corncob acid hydrolysate contain high amounts of sugar (~45.7 g L⁻¹) leading to higher lipid yield (7.7 g L⁻¹).¹⁸ Sometimes, the hydrolysates need to be detoxified for use. For example, rice straw acid hydrolysate was detoxified using low-cost activated carbon, and a lipid titre of 12.1 g L⁻¹ was achieved.¹⁹⁸ Ternary deep eutectic solvent (citric acid : choline chloride : ethylene glycol in a molar ratio of 1 : 1 : 2) have also been used for pretreatment of wheat straw to achieve non-

condensed lignin and high-yield fermentable sugars. *Trichosporon cutaneum* cultivated in undetoxified solvent waste liquid (hemicellulose-rich) and residue hydrolysate (cellulose-rich) produced 8.7 g total lipid per 100 g of wheat straw. *Trichosporon dermatis* has been cultivated on corncob acid hydrolysate to achieve a lipid titre of 7.0 g L⁻¹.¹⁹⁹ High amounts of unsaturated lipids including omega-3 fatty acids were obtained from *Lipomyces starkeyi* and *Rhodotorula babjevae* utilizing furfural extracted wheat straw hydrolysate.¹⁹⁶ One benefit of using microbes like oleaginous yeasts is that it naturally contains xylose utilization pathways which can be induced during fermentation.²⁰⁰ Growing *Y. lipolytica* on rye straw hydrolysate by overexpression of native xylose utilization genes enabled production of 2.19 g per L lipids.¹⁷ *Y. lipolytica* PO1g strain was grown in sugarcane bagasse hydrolysate and peptone as nitrogen source, which yielded 6.68 g per L lipids.¹⁹

Non oleaginous yeasts such as *Saccharomyces cerevisiae* have also been used to produce lipids from LCB. Engineered strains were shown to produce specific fatty acids like palmitoleic acid using corn stover hydrolysate. Even though the yeast growth was inhibited by the hydrolysate, the overall palmitoleic acid production was higher than when pure glucose was used. A titre of 6.56 g L⁻¹ was obtained by using a combination of strategies, like using a C/N ratio of 120, two-stage cultivation in a 5 L bioreactor and addition of 1 g L⁻¹ of lysine.²⁰¹

Apart from yeasts, microalgae like *Schizochytrium* spp. have been grown on LCB for lipid productions. Growth of *Schizochytrium* sp. HX-308 on corn stover hydrolysate mixed with glucose was slower than that of pure glucose, but the lipid profile indicated that corn stover hydrolysate led to higher proportion of polyunsaturated fatty acids. Two stage cultivation technique was also used to improve lipid production in this strain.²⁰² Fungi like *Cryptococcus podzolicus* was assessed for lipid production using a novel pretreatment technique on corn stover. Ammonium carbonate-steam explosion and recirculation of enzymatic hydrolysate was used to reduce enzyme load.²⁰³ Zhang *et al.* (2004) combined different strategies like engineering the cellobiohydrolase and delta 6 desaturase enzymes in an oleaginous fungus *Mucor circinelloides* to use cellulose and produce gamma linolenic acid.²⁰⁴ Two stage temperature control (32 °C for the first 48 hours and 28 °C for the next 144 hours) and addition of 1.5% cellulase increased lipid production.²⁰⁴

In the yeast cells, there are various pathways to utilize sugars derived from LCB. Glucose, galactose, and mannose can be directly transported into the cells²⁰⁵ whereas other sugars like xylose and arabinose might require specific transporters produced in the yeast cells.²⁰⁶ Glucose is converted to pyruvate through glycolysis, which is further converted to acetyl-CoA. The acetyl-CoA can enter TCA cycle, or it is used in the fatty acid biosynthesis pathway. Under nitrogen limiting conditions, the TCA intermediate citrate is transported from the mitochondria to the cytoplasm and converted back to acetyl-CoA where it serves as precursor for lipid synthesis.²⁰⁷ In some yeasts, special hexose transporters are required to import xylose into the cells. Xylose can be converted to xylulose-5-phosphate, which enters the non-oxidative pentose phosphate pathway and is converted



into fructose-6-phosphate and glyceraldehyde-3-phosphate, thereby feeding into glycolysis.²⁰⁸ Efforts on utilizing arabinose has focused on expressing GAL2 permease for arabinose uptake. Bacterial arabinose utilization pathway comprises of genes encoding L-arabinose isomerase (*araA*), L-ribulokinase (*araB*), and L-ribulose-phosphate epimerase (*araD*). The expression of these genes is needed to catalyse the conversion of arabinose into xylulose-5-phosphate, which enters the xylulose assimilation pathway.²⁰⁹ Mannose can be metabolized by most yeast species and is converted to mannose-6-phosphate and then isomerised to fructose-6-phosphate, which enters the glycolysis pathway.²¹⁰ Another sugar from LCB is galactose. Galactose is converted to glucose-1-phosphate *via* Leloir pathway (GAL genes). Glucose-1-phosphate is isomerized to G6P, which subsequently enters the glycolysis pathway.²¹¹

In summary, a range of strategies has been explored for producing SCOs from LCB. The major research focus has centred on depolymerizing LCB into fermentable sugars, typically through acid hydrolysis or novel pretreatment techniques. In parallel, process optimization, yeast strain engineering and enzyme engineering have been applied to enhance sugar utilization and increase lipid productivity. Therefore, a combination of approaches is required to effectively utilize the target LCB and channel the LCB derived fermentable sugars into lipid biosynthesis pathways.

Fig. 4 summarizes the various sugar assimilation pathways in yeasts towards protein and lipid production. Following delignification and enzymatic hydrolysis, cellulose and hemicellulose are converted into fermentable sugars, including glucose, galactose, mannose, xylose, and arabinose, which are transported into the yeast cells. Hexose sugars are primarily metabolized *via* glycolysis, while pentose sugars enter the pentose phosphate pathway through native or engineered assimilation routes. Carbon flux through glycolysis and the tricarboxylic acid cycle generates key precursors, including pyruvate and acetyl-CoA, supporting fatty acid biosynthesis and lipid accumulation. In parallel, nitrogen assimilation coupled with central carbon metabolism directs carbon skeletons toward amino acid biosynthesis, enabling rapid accumulation of protein-rich single-cell biomass. NADPH generated *via* the oxidative pentose phosphate pathway provides reducing power for anabolic reactions essential for both lipid synthesis and single-cell protein production.

4.3. Dietary fibers

Dietary fibers provide prebiotic health benefits by modulating the gut microbiota. LCB is a good source of dietary fibers. While cellulose, hemicellulose and lignin work as insoluble dietary fibers, oligosaccharides from xylose and cellulose are soluble dietary fibers. Both groups have shown significant prebiotic benefits.^{212,213} Xylan is hydrolyzed to non-digestible pentose sugar oligomers called xyloligosaccharides (XOS), which promote growth of beneficial microbes in human gut microbiome. High xylan content makes LCB an ideal feedstock for producing XOS. Corn cob and sugarcane bagasse have been successfully used for commercial XOS production. Use of

various LCB material in XOS production has been summarized in a recent review.²¹⁴ Cello-oligosaccharides (COS) are short chain oligosaccharides derived from cellulose. Soluble COS are typically composed of six or fewer glucose units and are particularly interesting because of their prebiotic properties. LCB can also be used as a source of arabino-oligosaccharides (AOS), galactooligosaccharides (GOS), and maltooligosaccharides (MOS). For instance, the treatment of LCB with fungal *Aspergillus* spp. enables the production of these compounds.²¹ These oligosaccharides have a variety of applications including prebiotic activities.

The LCB polysaccharides, cellulose and hemicellulose, need to be partially hydrolyzed to produce oligosaccharides suitable as dietary fibers, which could be achieved by controlled hydrolysis of the pre-treated biomass. Physicochemical property and physiological activity of oligosaccharides largely depend on their molecular weight, degree of polymerization, and chemical structure. However, fine-tuning the hydrolysis condition is crucial to obtain the desired oligosaccharide composition. Beside chemical, physicochemical, and thermochemical methods, enzymatic hydrolysis has been widely studied for XOS and COS production from LCB.^{16,79} Enzymatic hydrolysis has advantages over other methods due to the specificity of enzymatic reactions and milder reaction conditions, which minimize monosaccharide production by complete hydrolysis of polysaccharides, as observed in relatively uncontrolled chemical reactions. 1,4- β -Endoglucanase enzyme has been used to successfully develop a three-stage enzymatic hydrolysis process for producing COS from cellulose extracted from coffee husk and sugarcane straw.¹⁶ This process yielded 85.43 mg COS from each gram of cellulose after 48 hours reaction. In another study, this enzyme was used to hydrolyse grape marc for producing water soluble COS, including cellotriose and cellopentaose.²¹⁵ The COS oligosaccharides showed prebiotic effects when assessed using human probiotic monocultures and *in vitro* human faecal fermentation.

Xylan from a range of agrifood wastes such as sugarcane bagasse, corncob, wheat straw, and coffee peels, was enzymatically valorized for XOS production. Gupta *et al.* (2022) used an endoxylanase from *Aspergillus flavus* strain to hydrolyze xylan from sugarcane bagasse.²² The hydrolysate was a mixture of 69.57% xylobiose, 13.17% xylotriose, and 8.38% xylotetrose, which exhibited antioxidant activity and growth-stimulating property on a probiotic *Lactobacillus plantarum* strain. In another study, a GH10 endoxylanase from *Thermoascus aurantiacus* was recombinantly produced in *Pichia pastoris* and used for hydrolysing sugarcane bagasse xylan, which produced a XOS mixture containing primarily xylobiose, xylotriose and xylotetraose.²¹⁶ The XOS demonstrated prebiotic activity by stimulating growth of multiple probiotic lactobacillus strains that was able to produce several beneficial organic acids, including acetic acid.

Numerous endoxylanase enzymes were reported to cleave polymeric xylan, but the substrate specificity varies significantly between enzyme families and even between individual enzymes in the same family. Depending on the substitution pattern, individual enzymes cleave xylan chains at specific cleavage sites,



and thus reaction with each enzyme produces unique oligosaccharide compositions.²¹⁷ Co-incubating xylans with endoxylanases from different families helps to produce smaller oligomers. Multiple studies showed beneficial effects of using endoxylanase cocktails for XOS production. Interestingly, including some exoxylanases has also proven to be beneficial for XOS production. In a study, XOS was produced from pretreated corncob biomass by combined action of recombinant *endo*- and *exo*-acting xylanolytic enzymes.²¹⁸ Two endoxylanases, PxXyn10A and PxXyn11B from GH10 and GH11 families, respectively and one *exo*-xylosidase MetXyn11 from GH11 family were used in the study. The authors experimentally confirmed synergistic activity of PxXyn10A and PxXyn11B enzymes, which act at two different sites based on xylan substitutions. Addition of MetXyn11 demonstrated enhanced XOS production when combined to the PxXyn10A enzyme or to the mixture of PxXyn10A and PxXyn11B enzymes. Although MetXyn11 is an *exo*-acting enzyme, it produces xylobiose as sole xylan hydrolysis product, high amount of xylose production otherwise may inhibit XOS formation. Comparison of two pretreatment strategies showed higher XOS production from hydrothermally pretreated biomass than the alkali pretreated biomass.²¹⁸ While most of the enzyme cocktails contain only different xylanase enzymes, mixing other lignocellulolytic enzymes enhances complete biomass valorization. Esterases, including feruloyl esterase and acetylxytan esterase break down ester bonds between plant polymers, and thus play a key role in improving accessibility for xylanase enzymes to the xylan inside LCB.²¹⁹ When co-incubated, these accessory enzymes were reported to act synergistically with xylanase for xylan degradation. Álvarez *et al.* (2017) developed an enzyme cocktail for optimum XOS production from hemicellulase fraction of pretreated barley straw, which contains three accessory enzymes: α -L-arabinofuranosidase, feruloyl esterase, and acetylxytan esterase, along with different combinations of *endo*- β -(1,4)-xylanase.²²⁰ Combined action of these enzymes produced XOS with a low degree of polymerization, xylobiose, xylotriose, xylotetrose, and xylopentose being the primary component. Using DoE modelling, Fuso *et al.* tailored degree of polymerization of XOS produced from acetylated xylans through enzymatic hydrolysis using xylanase and deacetylase enzymes.²²¹ The order of the xylanase and deacetylase addition greatly influenced the XOS distribution in the hydrolysate, while pH showed significant impact on the total yield. The method was tested on an acetylated xylan derived from grape stalks biomass, and the model was successfully extended to study enzymatic hydrolysis of non-purified xylans in the biomass.

In some studies, engineered and natural microbes were used to produce xylanolytic enzymes through fermentation, often using lignocellulosic biomass such as wheat bran, soybean fiber, corn stover, or sugarcane bagasse as carbon sources, which stimulate the enzyme production.²²² While natural isolates secrete multiple lignocellulolytic enzymes, one or two targeted extracellular hydrolytic enzymes are expressed in recombinant microbes, for biomass hydrolysis. A crude enzyme formulation is generally prepared from the secreted proteomes and used for partial hydrolysis of the biomass, to obtain the

soluble oligosaccharides. In one such study, extracellular xylanase was produced by submerged fermentation of *Aspergillus fumigatus* R1 using wheat husk as carbon source, and the crude enzyme was used for XOS production through hydrolysis of xylan-rich wheat husk biomass without any pretreatment.²²³ The XOS mixture exhibited prebiotic activity on six probiotic *Bifidobacterium* and *Lactobacilli* strains; antioxidant activity of the XOS was also demonstrated by a chemical test (DPPH assay). In another study, recombinant xylanase and arabinofuranosidase were produced by solid-state fermentation of soybean fiber by a genetically modified *Aspergillus nidulans* A773, and XOS was produced from the same biomass by the action of these enzymes.²²⁴ 28% (w/w) XOS yield was achieved by optimizing the fermentation condition and the enzymatic reaction; each gram of xylan was converted to 138.36 mg xylobiose, 96.96 mg xylotriose, and 53.04 mg xylotetraose. Da Silva Menezes *et al.* screened environmental filamentous fungi for xylanase and XOS production, using spent malt, soybean hull, and rice husk in submerged and solid-state fermentations.²²⁵ The microbes produced various lignocellulolytic enzymes, including xylanase, cellulase, β -xylosidase, and β -glucosidase. A new strain *Aspergillus brasiliensis* BLf1 has been identified for maximum xylanase activity, and XOS production were optimized using the crude enzyme preparation. Using a similar approach, after screening 138 fungi, two xylanolytic *Aspergillus fumigatus* strains were identified for XOS production from sugarcane bagasse.²²⁶ These strains were used for xylanolytic enzyme production through solid-state fermentation in a media containing sugarcane bagasse as carbon source, and the crude enzymes were used for hydrolysing the bagasse. The authors reported higher XOS production using the crude enzymes than a commercially available xylanase enzyme.²²⁶ Using an engineered *E. coli*, Liu *et al.* produced XOS by a one-step fermentation of wheat bran, without isolating the crude enzymes.²²⁷ The *E. coli* exhibited xylanolytic activity through recombinant production and secretion of a thermostable *endo*- β -1,4-xylanase enzyme from *Bacillus agaradhaerens*. The recombinant *E. coli* directly hydrolyzed de-starched wheat bran in the fermentation media to produce XOS mixture consisting of 23.1% xylose, 37.3% xylobiose and 39.6% xylotriose.

While both follow the same enzymatic reactions, XOS production by isolated enzyme and microbial fermentation have distinct pros and cons. Isolated enzymes offer purity and high yield in a controlled multi-step process that can produce a desired degree of polymerization. In contrast, microbial fermentation offers cost-reduction and simplicity, but compromises in XOS yield and purity. In spite of the higher production costs, the enzymatic route is preferred for XOS production in the food industry, as there are strong regulatory requirements for the products intended for human consumption.²²⁸ XOS production through microbial fermentation is generally a one-step process avoiding the enzyme preparation step and often can use crude biomass as feedstock, which makes the process less expensive.²²³ However, several challenges make this approach unfavourable. Product quality is often compromised due to formation of undesirable by-products, including xylose, lignin derivatives, and microbial



metabolites. Critical factors affecting XOS production often remain unknown, limiting the scope to improve XOS yields in microbial fermentation. In addition, several fermentation strains were reported to utilize XOS as carbon source, which jeopardise XOS accumulation in the medium.²²⁷ This challenge could be overcome by strain engineering. In some strains, β -xylosidase gene was knocked out to enhance XOS production by preventing the further breakdown of XOS into xylose.²²⁹ Table 4 compares the advantages and disadvantages of microbial, enzymatic, and chemical approaches for dietary oligosaccharide production from LCB.

4.4. Other food ingredients

4.4.1. Vanillin. Vanillin is a widely used flavor in food and beverages, and about 15% of industrial vanillin is produced from kraft lignin, which is a major byproduct of the paper and pulp industry.^{230–232} Various efforts have focused on converting Kraft Lignin (KL) into vanillin primarily through alkaline oxidation. Apart from KL, other lignocellulosic feedstocks such as corn stover have also been explored for vanillin production. Two approaches that have been used, (i) chemical oxidation, which remains the only commercially established process, and (ii) microbial biotransformation, which are either using microbes that can naturally utilize these substrates and produce vanillin^{20,24} or genetically engineering host microbes in which the vanillin synthesis pathway is incorporated.²³³ Natural microbes used for vanillin production, include organisms like *Lactobacillus acidophilus*²³⁴ and *Staphylococcus lentus*.²⁰ Yeasts such as *Saccharomyces cerevisiae* has been engineered for vanillin production from LCB.²³³ Although difficult to scale-up, study suggests that vanillin yield may increase significantly through enzymatic treatment compare to the current industrial practice of extraction through liginosulfonate pulping process.⁶⁴

4.4.2. Gluconic acid. Gluconic acid (GA) is an important carbonic acid of interest in food, pharmaceutical and concrete industries. GA was produced using *Gluconobacter oxydans* grown on enzymatic hydrolysate of pretreated corncob and pretreated sugarcane bagasse.²³⁵ Other strategies used for improving GA production are treatment of enzymatic hydrolysate with activated carbon and high-tension oxygen supply during fermentation. This helped to reduce the viscosity caused by the enzymatic hydrolysate and improve the oxygen transfer rate in the fermentation broth. *Aspergillus niger* has also been cultured in pretreated corn stover hydrolysate without any detoxification to produce GA up to a titer of 76.67 g L⁻¹.²³⁶

4.4.3. Lactic acid. Lactic acid is an important organic acid in the food industry. Sugars derived from LCB have been used as a substrate for lactic acid production. Metabolic engineering was conducted on *Saccharomyces cerevisiae*, *Candida* spp. and *Kluyveromyces* spp. to obtain lactic acid producing yeast strains.²³⁷ *S. cerevisiae* has been engineered to use xylose as carbon source and lactate dehydrogenase genes introduced along with deletion of *CYB2*, *ERF2*, and *GPD1* genes. This led to production of 93 g L⁻¹ of lactic acid when grown on xylose and 0.75 g lactic acid per g sugar consumed in synthetic lignocellulosic hydrolysate medium.²³⁸ Apart from common yeast

strains, other microorganisms have been identified such as *Bacillus coagulans* LA204 which are able to ferment glucose, xylose, and cellobiose to lactic acid. Several sodium hydroxide pretreated corn stover were used to grow this strain to reach a yield of up to 97.59 g per L lactic acid.²³⁹ Other aerobic bacterium strains include thermophilic strain of *Geobacillus stearothermophilus* 2H-3,²⁴⁰ *Lactobacillus pentosus* LB-1 and *Lactobacillus plantarum* LB-1.²⁴¹ *Geobacillus stearothermophilus* was grown in several hydrolysates including corncob, corn stover and wheat straw at 60 °C and pH 6.5 to produce up to 51.36 g L⁻¹ of lactic acid. Interestingly, *Lactobacillus pentosus* LB-1 could co-utilize glucose, galactose, arabinose, xylose, and mannose as carbon source. A third approach evaluated for production of lactic acid using hydrolyzed LCB is through fermentation of fungal-bacterial consortium, which includes aerobic fungus like *Trichoderma reesei* and anaerobic lactic acid bacteria to produce up to 34.7 g per L lactic acid. Such a consortium grown on hydrolyzed LCB leads to the consumption of both hexose and pentose sugars simultaneously and *in situ* degradation of acetic acid, leading to higher purity products.²⁴²

4.4.4. Propionic acid. Propionic acid and its salts have antifungal activities and are widely used as food preservative to prevent growth of molds in bread and bakery products.²⁴³ Although industrial propionic acid is primarily derived from petrochemicals, bio-based production through microbial fermentation is gaining interest due to its potential for sustainable production and increasing consumer demand for green and natural ingredients. Hemicellulose hydrolysate from corncob molasses was used to culture *Propionibacterium acidipropionici* using fed-batch fermentation to produce up to 71.8 g L⁻¹ of propionic acid.²⁴⁴ Various media and fermentation conditions have also been optimized for propionic acid production in this organism grown on corn stover hydrolysate.²⁴⁵ Since it is difficult to engineer *P. acidipropionici*, other *Propionibacterium* spp. that are amenable to metabolic engineering have been used to express xylose utilizing genes from *P. acidipropionici*.²⁴⁶ The studies so far indicate that, various approaches and their combinations like fermentation optimization and metabolic engineering could be used to further improve biobased production of propionic acid.

4.4.5. Citric acid. Citric acid has a variety of applications in food, pharmaceutical and chemical industries.²⁴⁷ Hydrolyzed pretreated straw was used to culture *Yarrowia lipolytica*. Three-cycle fed-batch cultivation of *Y. lipolytica* led to 42.4 g per L citric acid production.²⁴⁸ Metabolic engineering approaches like overexpression of TCA cycle genes to improve citric acid production, deletion of mitochondrial transporter for isocitric acid to reduce byproduct formation and gene deletion (*ACL*, *DGA1* and *DGA2*) to inhibit citric acid consumption in the cell led to improvement of citric acid production in *Y. lipolytica*. A titre of 83.6 g L⁻¹ of citric acid was achieved in a 3 L bioreactor using fed-batch fermentation and 35% loading of corn stover hydrolysate.²⁴⁹ Apart from *Y. lipolytica*, other microbial species such as *Penicillium funiculosum* have also been evaluated for citric acid production using hydrolyzed LCB as feedstock.^{250,251} *Aspergillus niger* is commonly used for industrial production of



citric acid production both through submerged and solid-state fermentation from sugarcane molasses.^{252,253}

4.4.6. Succinic acid. Succinic Acid (SA) is a precursor to many chemicals in the agriculture and food processing industries.²⁵⁴ Its production from hemicellulose hydrolysate fraction of sugarcane bagasse using *Actinobacillus succinogenes* was demonstrated with a titer of 22.5 g L⁻¹.²⁵⁵ Addition of glycerol to LCB like Napier grass hydrolysate for growing *Actinobacillus succinogenes* have shown to improve production of SA and reduction of the unwanted acetic acid byproduct.²⁵⁶ Other bacterial strains such as *Basfia succiniciproducens* have also been shown to produce succinic acid using LCB (high xylose hydrolysates) due to their broad substrate utilization capability.²⁵⁷ Furfural is an inhibitor found in LCB hydrolysates that hinder SA production. Metabolic engineering of *Y. lipolytica* to over-express glutathione synthetase gene led to detoxification of furfural and improvement of SA production to 45.34 g L⁻¹.²⁵⁸

4.4.7. Carotenoids. Carotenoids have a range of health benefits due to their antioxidant, anti-diabetic, and anti-inflammatory activities. Hydrolyzed pretreated wheat straw was used to grow a *Rhodospiridium toruloides* strain to maximize the production of carotenoids and lipids.²⁵ Adaptive laboratory evolution was used to improve the performance of this oleaginous yeast on LCB hydrolysates. The evolved strains had better tolerance to inhibitors present in the LCB.²⁵⁹ The growth of the bacterium *Novosphingobium aromaticivorans* on alkaline pretreated and hydrolyzed LCB was also assessed for carotenoid production.²³ A recent review summarized the efforts on the production of high-value pigments from yeasts grown on LCB.²⁶⁰

4.5. Conclusions and future perspectives

From the foregoing discussion, it is clear that microbial fermentation and enzymatic approaches individually or in combination can be used for valorizing LCB into a range of food ingredients including single cell proteins, edible oils, dietary fibers, organic acids and enzymes (Table 5). The questions are whether those processes are scalable and economically viable to achieve cost parity with traditionally produced food ingredients, whether safety concerns and regulatory hurdles around the use of such ingredients for human consumption could be overcome and whether broad consumer acceptance of these products is achievable in the foreseeable future while traditional food ingredients are still available at reasonable cost. The current valorization processes often involve energy intensive unit operations such as size reduction, steam explosion or acid or alkaline treatment combined with heat to render the LCB amenable to enzymatic hydrolysis and subsequent microbial fermentation. Post-processing of the pre-treated LCB may also be required to remove lignin degradation products that inhibit microbial growth. The pretreatment and the post-processing steps together with the high cost of enzymes and other processing inputs may render the valorization process cost prohibitive. Nevertheless, advances in LCB degradation technologies through the development of more efficient enzyme cocktails and scaffolds or microbial consortia may improve the

efficiency and the economic viability of LCB valorization processes. This coupled with the need for more food for a growing and more affluent population in a resource constrained world will possibly lead to broader acceptance of LCB based valorization technologies for food applications. Some of the current hurdles to the commercial adoption of LCB valorization technologies are discussed in the next section.

5 Challenges and major considerations

The valorization of lignocellulosic biomass for food applications through enzymatic hydrolysis and microbial fermentation offers a promising pathway toward sustainable and circular bioeconomy. However, successful implementation at commercial scale is contingent upon overcoming a series of technical, logistic, safety, and economic challenges. These considerations are particularly critical when the final products are intended for food use, where stringent safety and quality standards apply.

5.1. Continuous supply throughout the year

A consistent and reliable supply of lignocellulosic biomass is critical for the uninterrupted operation of valorization facilities. Unlike other fermentation feedstocks, biomass availability is influenced by seasonal agricultural cycles, harvesting windows, and weather conditions, which can cause fluctuations in feedstock supply and, consequently, processing schedules.²⁶² Long-term storage represents a key unit operation in the biomass logistics supply chain, enabling biorefineries to operate year-round despite daily, monthly, and seasonal variations in feedstock availability. For food applications, stockpiling is further complicated by the need to maintain biomass quality and prevent microbial spoilage or mycotoxin development during storage.²⁶³ Appropriate preservation techniques and storage facilities are necessary to preserve biomass integrity, as uncontrolled microbial degradation can lead to significant losses. Strategies such as contractual arrangements with multiple suppliers, blending biomass from diverse agricultural residues, and implementing drying or ensiling techniques can help ensure a continuous feedstock supply.²⁶⁴ Nevertheless, these measures increase the complexity and cost of the supply chain.

5.2. Large volumes, transportation costs, and supply chain logistics

Lignocellulosic biomass is inherently bulky and has a low bulk density, which makes transportation both inefficient and costly. Transportation and associated logistics often account for a significant portion of the total feedstock supply cost as well as overall energy consumption. The ideal solution is locating lignocellulose biorefineries near the source of the lignocellulose biomass, which is not always practical. Thus, optimizing transport strategies is the next best thing that can be implemented to improve the cost-competitiveness and economic viability of the bioenergy and biomass valorization sector.²⁶⁵ Effective transport logistics planning, including the



development of models to evaluate regional production and distribution networks, is essential to manage seasonal peaks in biomass availability that can strain infrastructure and storage capacity.²⁶⁶ For food applications, additional logistics considerations include ensuring that transport vehicles and storage systems prevent cross-contamination and preserve feedstock integrity. The consistent supply of high-quality and reliable quantities of lignocellulosic feedstocks to biorefineries is critical to the success of the biomass industry. However, raw biomass often lacks desirable properties for storage, handling, transportation, and consistent chemical composition. Collocation of processing facilities near biomass sources, or deployment of decentralized pre-processing units such as pelletization, drying, separation, or on-site partial hydrolysis, can help reduce transport costs and improve supply chain efficiency.²⁶⁷ Therefore, there is a need to develop advanced preprocessing and pretreatment technologies that render biomass more conversion-ready, ensuring it meets the quality, quantity, and reliability requirements necessary for operating biorefineries at their designed capacities.

5.3. Quality and composition variability across sources and seasons

The chemical composition of lignocellulosic biomass varies significantly depending on plant species, growth conditions, soil type, climate, and harvest time. Seasonal changes can alter not only the proportion of structural carbohydrates but also the content of phenolic compounds, ash, and minor components that may influence enzymatic digestibility and microbial fermentability.²⁶⁸ This variability poses challenges for process standardization. Enzyme cocktails optimized for one batch of biomass may be less effective for another, potentially leading to reduced sugar yields or inconsistent fermentation performance.²⁶⁹ For food-related production, variations in nutrient profile can also influence sensory properties, nutritional content, and regulatory compliance of the final product.²⁷⁰ To address these challenges, adaptive biomass valorization processes, thorough feedstock characterization protocols, and real-time process control strategies are essential to ensure consistent product quality and operational efficiency. Recently, artificial intelligence and machine learning tools have also been applied to predict biomass composition,²⁷¹ optimize enzyme cocktail,^{272,273} and adapt fermentation parameters in real time, offering a powerful means to overcome variability and enhance the robustness of lignocellulose valorization.

5.4. Contamination and food safety risks

With biomass intended for food applications, contamination risk is a major concern. Agricultural byproducts can carry pesticide residues, heavy metals (*e.g.*, cadmium, lead, arsenic), and other environmental contaminants that must be removed or reduced to permissible limits.²⁷⁴ Additionally, biomass stored under poor conditions can develop microbial contamination, including pathogenic bacteria or mycotoxin-producing fungi, which may be concentrated during hydrolysis or fermentation, creating potential hazards in the final product. Feedstock may

also harbor endogenous plant toxins (*e.g.*, glycoalkaloids, cyanogenic glycosides) or accumulate soil-derived contaminants that require removal to reduce these toxicants to safe levels for food applications. Furthermore, the heterogeneity of agricultural residues introduces variability in contaminant profiles, increasing the need for both traditional and novel food-processing techniques to support effective detoxification.²⁷⁵

Consequently, pre-processing steps often require integrated cleaning, washing, or detoxification stages, coupled with rigorous testing to ensure compliance with food safety regulations.²⁷⁶ Several novel decontamination techniques, such as cold plasma, ozone treatment, photocatalysis, nanoparticle adsorbents, and microbial enzymes, have shown high efficacy in laboratory studies, but their industrial-scale application remains challenging and requires careful assessment of feasibility.²⁷⁷

In addition to feedstock-derived hazards, pretreatment and hydrolysis steps can generate process-derived inhibitors such as furfural, HMF, acetic acid and phenolic fragments, which may persist into downstream operations if not effectively neutralized or removed. These compounds not only inhibit microbial performance but may pose toxicological risks if present at elevated levels in food ingredients.²⁷⁸ Mitigation strategies typically include optimization of pretreatment processes to minimize inhibitor formation, followed by detoxification steps such as overliming, activated carbon adsorption, ion-exchange resins, or membrane separation.²⁷⁹

Biological detoxification using specific microbes or oxidoreductases capable of converting furans and phenolics into less toxic derivatives is also increasingly explored. Process integration strategies such as pH control, washing of pretreated solids, or employing tolerant microbial strains can further reduce inhibitor carryover.²⁸⁰ Routine monitoring of inhibitor concentrations throughout the process is essential to ensure both fermentation performance and compliance with food-safety thresholds.

The valorization process must also be designed to prevent recontamination during handling, transport, and storage. Traceability systems from feedstock source to final product are crucial for meeting regulatory and consumer safety expectations. Other considerations in the consumption of single-cell protein (SCP) derived from the microbial valorization of lignocellulosic biomass include the levels of nucleic acids present in microbial biomass.^{281,282}

Furthermore, the microbes that are employed in the biobased valorization of LCB for food and applications need to be safe for human consumption. In other words, the need to have generally regarded safe status (GRAS) or need to be part of the EU's QPS (Qualified Presumption of Safety) list. This is applicable for microbial biomass protein production or microbial production of value-added food ingredients using hydrolyzed LCB as feedstock. The use of non-GRAS microorganisms even for processing LCB into hydrolysate, if the hydrolysate is used directly as feedstock for food and feed production, is not practically feasible as it would require extensive downstream processing of the hydrolysate to remove unsafe metabolites as well



as the microbial cell. Non-GRAS strains may produce secondary metabolites, toxins, or cell wall components (*e.g.*, endotoxins, mycotoxins) that are incompatible with food applications, further reinforcing the need for pre-approved, food-safe microbial chassis.^{283,284} Regulatory constraints therefore strongly guide chassis selection toward organisms with established safety profiles.

This limits the range of microbes that can be used for biobased valorization of LCB for food application to GRAS organisms, which may not be necessarily the best from a biotransformation efficiency perspective.²⁸⁵ Despite this limitation, the use of GRAS or QPS organisms remain essential to ensure regulatory compliance, reduce purification burdens, and maintain consumer confidence in LCB-derived food ingredients.

5.5. Technoeconomic considerations

While lignocellulosic biomass is often perceived as a low-cost raw material, the costs associated with collection, transport, storage, pretreatment, enzymatic hydrolysis, microbial fermentation, and downstream purification can be substantial. In many cases, the economics of enzymatic and microbial valorization are challenged by the high cost of enzymes, energy requirements for pretreatment, and the need for additional purification to meet food-grade specifications.²⁸⁶

Technoeconomic analysis (TEA) is usually performed to evaluate the commercial viability of a production process. A commonly calculated metric is the minimum selling price (MSP), representing the product price required for the project to break even over the facility's lifetime, assuming a specific internal rate of return (IRR).

MSP is also a useful benchmark for comparing production costs of food ingredients derived from fermentation processes using lignocellulose-derived sugars against global market prices. MSP values for LCB derived products including lactic acid (US\$ 0.5–1.9 per kg), xylitol (US\$ 1.5–3.1 per kg), and succinic acid (US\$ 1.5–6.9 per kg) have been reported. These compounds are widely used as food additives, flavoring agents, alternative sweeteners, acidity regulators and precursors of emulsifiers. The review by Patel *et al.* provides a comprehensive list of studies examining the major factors influencing their production costs.²⁸⁶

All products share the potential for improved economic feasibility through better integration of material and energy flows. Key strategies include reusing by-products, recycling process streams, capturing waste heat, and utilizing residual materials for electricity generation.^{287–289} Additionally, the integration of lignocellulosic valorization into existing agro-industrial operations, the valorization of co-products (*e.g.*, lignin fractions for bioactive compounds or functional food additives), and optimization of enzyme recycling can improve technoeconomic viability.^{290,291}

Upstream operation costs (fermentation) are highly process-dependent, with significant differences between aerobic and anaerobic processes. In aerobic processes, oxygen transfer into the fermentation broth often becomes rate-limiting, requiring

substantial power input for agitation and active aeration, which increases processing costs. To address these challenges, alternative bioreactor designs are being explored to enable process intensification.²⁹² Besides, shifting away from batch and fed-batch operation in industrial settings is being explored. Continuous manufacturing is evaluated as a strategy to reduce both capital and operating costs.²⁹³

It is important to note that the major cost drivers are the downstream processing steps, particularly purification. For example, separating lactic acid from fermentation broth can consume approximately 20–50% of the total process energy during the separation stage alone.²⁹⁴ Similarly, for succinic acid, purification accounts for about 50–75% of the total production cost.²⁹⁵ Overall, achieving competitiveness with conventional food ingredients remains a significant hurdle, especially given that food-grade compliance adds costs compared to non-food applications.

6 Conclusions

The enzymatic and microbial valorization of lignocellulosic biomass for food applications presents both an opportunity and a challenge. This approach aligns strongly with sustainability principles by converting agricultural residues and food processing waste into high-value food ingredients, thereby reducing environmental burdens and diverting biomass from landfill or low-value uses. It also represents the principles of circular economy, where waste streams are transformed into valuable resources, enhancing resource utilization efficiency, contributing towards food security, and a zero-waste economy. Moreover, by unlocking new sources of sustainable proteins, dietary fibers, and bioactive compounds, lignocellulosic valorization can contribute to global food security, particularly in regions where traditional agricultural expansion is limited by land and water constraints. Clearly, valorization of LCB waste into food ingredients makes strong environmental sustainability sense and can even be considered essential for ensuring future food security. However, the ultimate test is whether existing valorization approaches make economic sense. In that regard, success entails addressing supply continuity, transportation and logistics constraints, feedstock variability, contamination risks, and technoeconomic hurdles. Integrating such systems into local and regional agro-industrial networks can not only improve economic viability but also reduce the carbon footprint associated with long-distance transport of feedstocks. Holistic strategies that combine supply chain management, process adaptability, rigorous safety protocols, environmental impact minimization, and cost optimization will be key to realizing the full potential of this sustainable resource for the food industry while meeting both nutritional needs and global ecological limits. Among these, cost optimisation and ensuring product safety and regulatory approval are the main drivers that would facilitate widespread commercial adoption of LCB valorization technologies for food application. With respect to cost optimization, sustained research efforts are required for developing better enzyme cocktails and microbial consortia, tailored for efficient deconstruction of a target LCB



under mild conditions, taking advantage of advances in computational biology and machine learning tools. LCB supply chain logistics, which significantly contribute to LCB valorization cost, can be addressed by government-private partnerships facilitating the establishment of regional LCB processing hubs in proximity to sources of LCB generation. To mitigate safety concerns, in-depth research is needed throughout the LCB value chain, including detailed analysis of raw material composition and the byproducts formed at various stages of valorization. Consolidating these findings will equip policymakers, regulators, and consumers with robust evidence to evaluate potential food safety risks associated with LCB-derived food ingredients for human consumption.

Author contributions

MMH contributed to conceptualization, original draft preparation, reviewing, and editing the manuscript. NS contributed to original draft preparation, reviewing, and editing the manuscript. DW contributed to original draft preparation, reviewing and editing the manuscript. NST contributed to original draft preparation, critical reviewing, and editing the manuscript. BS contributed to conceptualization, original draft preparation, reviewing, and editing the manuscript.

Conflicts of interest

There are no conflicts to declare.

Data availability

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

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