

REVIEW

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Effective valorisation of cereal lignocellulosic waste: a review of pretreatment techniques to enhance microstructural modification

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Food waste biomass from agro-food processing, such as grain husks, represents an abundant and renewable resource with significant potential for valorisation through enzyme hydrolysis. The enzyme hydrolysis process can convert lignocellulosic waste into valuable end products like biofuel, bioethanol, biochemicals, bioplastics, and bio-fertilisers. However, the complex structure of lignocellulose, including tightly bonded cellulose, hemicellulose, and lignin, poses significant barriers to enzymatic hydrolysis and conversion into valuable products. Hence, structural modification, as a pretreatment step before hydrolysis, is crucial in facilitating the efficient conversion of lignocellulosic waste biomass into valuable end products. This review examines the role of various pretreatment methods, including milling, extrusion, chemicals (acid & alkali), steam explosion, ammonia expansion, and biological processes (bacteria, fungi, and enzymes), in breaking down the recalcitrant nature of lignocellulose through structural modification in cereal husk. This review also discusses the resulting changes in microstructure (crystallinity, porosity, and surface morphology) due to various pre-treatments and their aligned effects on the hydrolysis rate and production of high-value fermentable sugars. The novelty of this review lies in the focus on incorporating microstructure-based pretreatment strategies for the underexplored cereal husk, offering new insights into the structure–function relationship that influences the enzyme hydrolysis and valorisation potential. By identifying the gaps in current research and highlighting the fermentation potential of pretreated cereal husks, this review provides a guide to biomass-specific, synergistic and nanotechnology approaches and environmentally sustainable valorisation strategies that support circular economy goals.

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Sustainability spotlight

Cereal husks are generated in significant quantities as by-products of grain processing and are often underutilised or disposed of as low-value waste. Addressing this challenge is vital for reducing environmental impacts and promoting resource efficiency. This review highlights different pretreatment strategies to valorise cereal husks that enhance structural modification and conversion into high-value products such as fermentable sugars, functional food ingredients, and bio-packaging. Reducing agricultural waste through value-added industrial applications contributes to UN Sustainable Development Goals (SDGs) 9: Industry, Innovation and Infrastructure, 12: Responsible Consumption and Production, and 13: Climate Action, supporting the development of sustainable food systems and bio-based industries.

1. Introduction

Agro-food processing wastes, such as straw, pulp, peels and husks, represent a significant proportion of the total food waste, accounting for approximately 12.9%.^{1,2} These wastes are generated from agricultural activities during harvest (leaves, roots, stalks, straws, stems) and industrial processing (husks,

peels, pulps).³ All these wastes share a common composition, primarily made up of cellulose, hemicellulose, and lignin, collectively referred to as lignocellulose biomass.² Lignocellulose waste biomass (LWB) is readily available, cost-effective, and renewable, and therefore has long been exploited commercially, as seen in the production of bioethanol from sugarcane bagasse by companies like Raizen and GranBio.^{4,5} Other notable examples include the conversion of corn stover into ethanol by Poet-

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DSM and the processing of wheat straw by Beta Renewals, as well as Clariant and Valmet.⁶ Additionally, woodchips are used commercially in the paper industry by International Paper and Stora Enso,⁷ and DuPont uses corn stover to transform it into biochemicals and ethanol.⁸ Recent advancements in lignocellulose biorefinery are paving the way for the sustainable production of energy, biofuels, biochemicals, bio-packaging, bioethanol, biofertiliser, biochar, and bio-enzymes.^{9–11} Whilst recognising the broad spectrum of LWB and its extended applications, this review will focus primarily on the cereal husk structure, chemical composition, and valorisation potential.

Around 63.5–73.5 million metric tonnes of cereal husks from wheat, rice, oats, and barley are produced yearly as processing waste.¹² The amount of husk residues produced from cereal

processing depends on environmental, economic, processing and cultural factors.^{13,14} Lignocellulosic waste accumulated during the manufacturing and processing of cereals, if improperly disposed of, incinerated, or landfilled, can have environmental effects due to greenhouse gas (GHG) emissions.¹⁵ This leads to global warming, soil acidification and water contamination and hence, the sustainable development of waste valorisation is compromised.^{15,16} Agricultural waste has been reported to be the second-largest (19.9%) contributor to greenhouse gases in the environment.¹⁷ The adverse effects of improper waste management, particularly lignocellulose waste, can be addressed through the implementation of effective and eco-friendly valorisation processes to produce simple fermentable sugars.³



Smriti Ale

Smriti Ale is a PhD researcher at Curtin University. Her academic journey is driven by a strong interest in food sustainability and the valorisation of food processing by-products. Her research focuses on waste upcycling, addressing industry challenges related to waste and contributing to sustainable food innovations. She also holds a Master's Degree in Food Science and Technology from Curtin University and has 3

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Pramesh Dhungana

Dr Pramesh Dhungana has over 20 years of experience working in the food industry and academia. He completed his PhD in Food Process Engineering from the University of Queensland (UQ) in 2019, where he investigated the fat globule size and functionality of dairy emulsions. Later, he worked as a postdoctoral fellow on gaseous nanobubbles in dairy processing and as a Senior Technical Officer at UQ. At Curtin Univer-

sity, his research focuses on future food innovation, particularly in dairy and plant-based systems. Pramesh has expertise in food process engineering, as well as in process and equipment design.



Janet Howieson

Dr Janet Howieson completed an Honours degree in microbiology at the University of Western Australia (1984), MSc in municipal water supply microbiology (1988) and a PhD in algal biotechnology at Murdoch University (2001). She has held research positions in public health microbiology, algal biotechnology and fisheries policies. For the past nine years at Curtin University, she has led post-harvest seafood research

projects across multiple seafood sectors, supply chain monitoring and intervention, value chain analysis, new product development, market strategy development, traceability and provenance, and waste and byproducts utilisation. She has supervised numerous postgraduate research students and recently expanded her work in horticulture. Dr Howieson has received multiple awards for her research and industry impact.



Rewati Raman Bhattarai

Dr Rewati completed his PhD from the University of Queensland, Brisbane, in 2018, elucidating the structure–property relations of plant cell walls. He then worked at the Australian Export Grains Innovation Centre, focusing on wheat quality for bread and Asian noodles. At Curtin, his research is focused on alternative plant proteins derived from cereals and legumes for food and nutraceutical applications.

including vegan, gluten-free, soy-free, and non-GMO protein ingredients. Likewise, he is exploring innovative technologies to enhance techno-functionalities, viscoelastic properties, and bioactive legume proteins, such as γ -conglutin, for maintaining healthy blood glucose levels.



Table 1 Biochemical composition of lignocellulose materials in cereal husks

			Lignocellulose					
Cereals		Scientific names	Cellulose	Hemicellulose	Lignin	Ash	Extractives	References
By-product (husk)	Oats	<i>Avena sativa</i>	23–48%	16–35%	16–25%	2–10%	2.1–13.94%	22–25
	Wheat	<i>Triticum aestivum</i>	36%	18%	16%	1–19%	20.89%	23 and 26–28
	Rice	<i>Oryza sativa</i>	28–35%	12–33%	15–23%	20%	6.8%	2, 9 and 29–34
	Corn	<i>Zea mays</i>	28–38%	28–35%	9–20%	5–7%	13.9–19.55%	35–38
	Barley	<i>Hordeum vulgare</i>	34–39%	23–30%	12–20%	5.35%	3.82–16.53%	24, 39 and 40

Cereal grains are inherently designed to remain physically, chemically, and biologically inert until proper conditions prompt germination. This is primarily due to the protective hull that shields cereal seeds from adverse environmental conditions. If consumed unprocessed, the seed can go through the digestive tract undigested.¹⁸ Food Processing operations like dehulling and milling are essential and play a pivotal role in transforming agricultural commodities into consumer-friendly foods, enhancing shelf life, nutrient bio-accessibility, colour, flavour, economic value, and ease of production.¹⁹ Consequently, a large quantity of by-products is generated, especially in the form of husks, which are lignocellulosic in nature.²⁰ Lignocellulose in cereal husk is primarily composed of cellulose, hemicellulose and lignin with varying proportions depending on the type of biomass source, growth conditions, climatic conditions, harvesting and storage processes.^{5,21} Variances in the lignocellulose composition of different cereal crop husks are depicted in Table 1.

The microfibrils of crystalline and amorphous cellulose are interconnected by hydrogen bonds, along with amorphous chains of hemicellulose embedded within the lignin matrix, providing a compact and stable structure.^{41,42} Lignocellulose biomass generally requires breakdown to convert into simpler forms of sugars for its commercial use in a biorefinery.⁴³

In recent years, the potential applications of lignocellulose-derived sugars to produce biofuels, alcohols, biogas, and bio-packaging have been discussed.¹⁰ Likewise, previous investigations have revealed various potential uses of cereal husk waste, including the preparation of biodegradable packaging,^{44,45} production of biofuels^{46,47} and treatment of wastewater.^{48–52} However, the lignocellulose biomass forms a complex, polymerised structure through intermolecular hydrogen bonds, covalent linkages, and interactions between its monomers and polymers, contributing to the recalcitrance property and making the isolation of key components challenging.

Recent studies described the correlation between micro-structural properties and possible valorisation techniques for different LWBs, highlighting the feasibility of various applications in the food industry. The feasibility of using fungal-assisted valorisation of lignocellulose due to structural degradation or depolymerisation, the role of chemical pretreatment, and the potential of using acid or alkali to break down the complex lignocellulose structure have been discussed in various research articles.^{53–58}

This review will describe the fundamental aspects of different lignocellulosic cereal husk waste, its structural constituents, the underlying challenges in depolymerisation and the potential for valorisation through modification of microstructural properties. This review will also discuss the treatments carried out to modify the structural and chemical properties of lignocellulose, thereby converting it into valuable products. Finally, it will provide an overview of waste valorisation efficiency and profitability through microstructural modification.

The scope of this paper focuses on exploring how micro-structural modifications can improve the efficiency of waste valorisation while addressing the challenges associated with treatments.

2. Composition and general structure of lignocellulose

Lignocellulose biomass from cereal waste is mainly composed of structural polymers – cellulose, hemicellulose and lignin with varying proportions depending on the type of biomass source, growth conditions, climatic conditions, and harvesting and storage processes.^{5,59} Minor proportions of pectin, proteins, extractives and inorganic compounds may also be present.⁶⁰

Cellulose ($C_6H_{10}O_5$)_n is the dominant polymer in cereal husks (see Table 1). Cellulose is an amphiphilic polysaccharide composed of linear chains of D-glucopyranose units linked together by β-(1-4) glycosidic bonds. This structure forms a compact microfibril in crystalline and amorphous states.⁶¹ The other common components, hemicellulose and lignin (see below), envelop cellulose microfibrils through non-covalent hydrogen bonding. These microfibrils are insoluble and are bound together by hydrogen bonds and van der Waals forces, comprising 36 glucan chains, each containing 4000–14 000 glucose molecules.⁵ The hydroxyl group at the axis forms intra- and intermolecular hydrogen bonds, resulting in a stable microfibril with a hydrophobic core and a hydrophilic exterior.⁶² Cellulose is relatively heat-stable, insoluble in water, alcohol, and ether, and cannot be digested by the human body.⁶³ This suggests that highly crystalline forms of cellulose can resist physical, chemical, or enzymatic treatment, requiring a pretreatment to reduce cellulose's crystallinity and increase its digestibility.¹⁵

Hemicellulose ($C_5H_8O_4$)_n is a heterogeneous polymer with randomly branched structures with a degree of polymerisation



Table 2 H : G : S lignin composition in cereal husks

Cereals		Scientific names	H : G : S composition	References
By-product (husk)	Oats	<i>Avena sativa</i>	15 : 49 : 36	73
	Wheat	<i>Triticum aestivum</i>	—	
	Rice	<i>Oryza sativa</i>	7 : 81 : 12	69
	Corn	<i>Zea mays</i>	51.3 : 40.4 : 8.3	
	Barley	<i>Hordeum vulgare</i>	—	

ranging from 50–300 monosaccharide units. Unlike cellulose, hemicellulose lacks crystallinity due to its branching and the presence of acetyl groups, making it more easily degradable.^{60,62,64} The structure and chemical composition of hemicellulose, however, vary among different plant species with varying proportions of arabinose, xylose, rhamnose, mannose, glucose, and galactose, along with glucuronic acid side chains.⁶⁵ These chains are linked together by β -(1-4) and β -(1-3) glycosidic bonds. The hemicellulose acts as a binding agent between cellulose and lignin, providing strong structural support.⁶⁶ It is hydrophilic due to hydroxyl groups, allowing for high water absorption and influencing mechanical properties.⁶⁷

Lignin consists of phenylpropane units: *p*-coumaric alcohol ($C_9H_{10}O_2$), coniferyl alcohol ($C_{10}H_{12}O_3$) and sinapyl alcohol ($C_{11}H_{14}O_4$), each differing in the number of (OCH_3) methoxyl group substitutions on the aromatic ring.^{68,69} They are linked together by ether (C–O) and carbon–carbon (C–C) bonds, creating lignin's three-dimensional structure.⁶² Upon polymerisation, these alcohol units form *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units, respectively, linked through β -O-4 and α -O-4 linkages.^{62,70} The structure of lignin, including its type and proportion of H, G & S units (H : G : S ratio), plays a crucial role in its degradability.⁷¹ Lignin rich in syringyl (S) units is more soluble in organic solvents, whereas guaiacyl (G) and *p*-hydroxyphenyl (H) rich lignin is recalcitrant due to higher crosslinking.⁷² The relative ratios of H, G, and S units in different cereal husks are summarised in Table 2; however, no literature data were available for wheat and barley husks.

Lignin provides strength and rigidity to the lignocellulose structure, acting as a defence against microbial and enzymatic breakdown. The hydrophobic nature and amorphous matrix of lignin interact with cellulose and hemicellulose, providing high mechanical strength and recalcitrance by limiting the accessibility of chemical or enzyme attack.^{41,55} This structural complexity of lignocellulose makes the separation of individual components difficult, and the recovery of glucose is often challenging, requiring a pretreatment of lignocellulose biomass.⁷⁵

3. Microstructural properties of husk lignocellulose

Besides the broader compositional characteristics, lignocellulosic cereal husk biomass possesses distinct microstructural characteristics that can significantly influence its recalcitrance property⁷⁶ and, ultimately, the enzymatic hydrolysis process and valorisation potential.^{77,78} Therefore, it is essential to

understand the microstructures of lignocellulosic biomass and how these structures contribute to its recalcitrance behaviour.⁷⁹ Traditionally, commercial enzymes such as cellulase, hemicellulase and ligninase have been used to break down the complex lignocellulose biomass into fermentable sugars for downstream valorisation processes.⁸⁰ The following sections provide a brief overview of each physical property, its implications for recalcitrance behaviour and subsequent enzyme hydrolysis.

3.1. Crystallinity

Cellulose in cereal husks can exist in both crystalline and amorphous forms.⁸¹ Cellulose with high crystallinity is less susceptible to enzyme degradation as opposed to those in amorphous form, and this crystalline region in cellulose microfibrils is formed through hydrogen-bonded β -(1-4) glycosidic bonds, leaving little space for amorphous nature.⁸² The amorphous cellulose is 3–30 times faster to hydrolyse than the crystalline forms of cellulose.⁷⁶

In lignocellulosic cereal husk biomass, cellulose is the only component responsible for the crystalline contribution, whereas hemicellulose and lignin are considered amorphous components.^{83,84} The multilayered structures of the cell wall, inter- and intramolecular hydrogen bonds, and the strong hydrophobic nature of cellulose give it a recalcitrant property, increasing its resistance to breakdown methods (*e.g.*, enzymes) and thereby hindering the effective breakdown of cellulose into fermentable sugars.^{68,78} The crystalline structure of cellulose can be distorted when subjected to chemical or physicochemical pretreatments.^{85–88} These treatments cause cellulose to swell and transition into a disordered state, reducing the crystallinity index (CrI), a measure of cellulose crystallinity. The CrI can be calculated using eqn (1):

$$\text{CrI (\%)} = (I_{002} - I_{\text{am}})/I_{002} \times 100\% \quad (1)$$

where I_{002} is the maximum intensity value of diffraction of the peak for the crystalline cellulose at $2\theta \approx 22^\circ$, while I_{am} is the intensity value for the amorphous cellulose at $2\theta \approx 18^\circ$. The crystallinity index can vary between cereal crops, as shown in Table 3.

3.2. Degree of polymerisation

Cellulose is the homopolymer of glucose units linked together by β -1,4 glycosidic bonds, and the number of these glucose units is the degree of polymerisation (DP). The DP of cellulose plays a pivotal role in providing mechanical strength to the



Table 3 Crystallinity index of cereal lignocellulose biomass

Cereal lignocellulose biomass	Crystallinity	References
Corn husk	21–30.1%	36, 83 and 89
Rice husk	39.3–46.8%	31 and 90
Rice husk nanocellulose	62–72%	91
Wheat bran	40.18%	92
Oat husk	38%	93
Oat husk nanocellulose	49–86%	93 and 94

biomass.⁹⁵ Long cellulose chains contain more hydrogen bonds, making them resistant to hydrolysis.⁹⁶ Conversely, short cellulose chains possess fewer hydrogen bonds and form a weaker network, making them more susceptible to enzyme hydrolysis.⁹⁷ Therefore, reducing the degree of polymerisation provides more binding sites for enzymes to act, exposes the cellulose ends, and speeds up the enzyme hydrolysis rate.⁷⁷ Altering the DP changes structural properties like crystallinity and porosity.⁹⁸ The DP can be modified through various pretreatment methods, physical, chemical, physicochemical, and biological, resulting in varying lengths of cellulose chains and supporting the production of soluble and insoluble polymer chains. Soluble chains have a DP ranging from 2–12, whereas insoluble chains can have a DP between 100 and 20 000.^{96,99} Additionally, cellulose with a DP > 1000 possesses a rigid crystalline structure, requiring a high enzyme load to break through its crystalline matrix. In contrast, cellulose with medium DP (300–1000) combines crystalline and amorphous regions, balancing resistance and accessibility characteristics.¹⁰⁰ The lower DP cellulose (<300) is more easily accessible, requiring minimal pretreatment and lower enzyme concentration due to increased substrate availability.⁹⁸

3.3. Available surface area

The available surface area of lignocellulose biomass is a critical factor affecting digestibility as it largely depends on the biomass particle size and porosity. A reduction in particle size or an increase in porosity increases the available surface area.^{76,101,102} The available surface area can be classified into two types: exterior surface area, influenced by length and width and interior surface area, influenced by pores and cracks within biomass.¹⁰³ Studies have revealed that the interior surface area of cellulose is 2-fold larger compared to the exterior.^{104,105} The higher the available surface area, the higher the adsorption rate of enzymes onto the substrate, resulting in higher hydrolytic activity.⁷⁶

3.4. Porosity

The pore size of lignocellulose biomass can vary widely, influencing the enzyme–cellulose interaction.¹⁰⁶ The narrow pores can restrict enzyme–substrate interaction, affecting the hydrolysis rate.⁷⁷ Enzymes like cellulases are trapped if the pore size is too small, affecting hydrolysis.⁹⁹ Research indicates that the pore sizes in the 5–10 nm range are too narrow for the enzymes to diffuse. In contrast, biomass with a pore size greater than 10 nm showed a positive correlation with the hydrolysis rate, allowing better penetration and diffusion of enzymes.¹⁰⁶

3.5. Hemicellulose composition and amorphous structure

Hemicellulose has a lower DP (100–200 units) than cellulose but contains many complex substitutes like xylans, xyloglucans, mannans, and glucomannans.^{107,108} Although hemicellulose is more readily hydrolysable than cellulose, it protects cellulose fibres from enzyme attack through hydrogen bonds and covalently links with lignin.^{82,109} Also, the acetyl groups in the hemicellulose structure limit enzyme activity by interfering with the formation of hydrogen bonds between cellulose and cellulose-binding domains of an enzyme.⁷⁶ Hemicellulose is also covalently linked to lignin, forming a lignin-carbohydrate complex that gives a rigid wood-like structure and acts as a barrier limiting enzyme access to cellulose.^{110,111} Removal of hemicellulose during pretreatment processes has been reported to improve fibre porosity and surface area.¹¹²

3.6. Lignin content and distribution

Lignin is the outermost structural component of lignocellulose biomass, containing heteropolymeric phenylpropanoid units of *p*-coumaryl, coniferyl, and sinapyl alcohol.⁶⁸ The presence of lignin hinders enzyme activity by limiting physical contact between the enzyme and cellulose^{77,98} and provides mechanical support by covalently linking to hemicellulose.¹¹³ The lignin structure and its proportion distributed throughout lignocellulose biomass determine the extent of recalcitrance and the rate of enzyme hydrolysis. The rate of enzyme hydrolysis in the presence of lignin also depends on the type of enzyme used for treatment, with cellulase being the least effective compared to xylanase and glucosidases.¹¹⁴ Lignin adsorbs enzymes irreversibly, thereby reducing enzyme activity and hydrolysis efficiency.⁸² Removal of lignin can disrupt the lignin-hemicellulose matrix, increase porosity, reduce the number of lignin-enzyme binding sites, and expose the hemicellulose surface area, thereby facilitating the hydrolysis rate.^{112,115}

4. Strategies for microstructural modification to enhance valorisation

The recalcitrant nature of lignocellulosic biomass towards enzymatic and microbial breakdown, the crystalline structure of cellulose, and the hemicellulose matrix embedded within highly polymerised phenolic lignin present a significant barrier to potential industrial valorisation.¹¹⁶ As discussed in the previous section, the extent of lignocellulose recalcitrance is influenced by its chemical composition, complex cross-linking between polymers, physical interactions, structural heterogeneity, including crystallinity, degree of polymerisation (DP), accessible surface area, and porosity.¹¹⁷

Pretreatment of lignocellulose biomass is considered an effective strategy to overcome the microstructural barriers and recalcitrance for valorisation by disrupting the physical structure, breaking the chemical bonds within the lignocellulose biomass or eliminating the hemicellulose and lignin content.^{118–121} The damage to the crystal structure increases the accessibility of enzymes (both microbial and commercial), enhancing the breakdown of components and facilitating the





Table 4 Pretreatment techniques, effect on microstructure, advantages and limitations

Treatment methods	Type	Mode of action	Biomass type	Modifications	Advantages	Disadvantages	References
Physical pretreatment	Milling	Reduces the size and cellulose lowers DP, and increases surface area	Corn stover	- Particle size 53–75 μm vs. 425–710 μm : 1.5-fold increase in enzyme hydrolysis rate - Exposed surface area: 1.3 $\text{m}^2 \text{g}^{-1}$ (untreated) vs. 2.2 $\text{m}^2 \text{g}^{-1}$ (treated) - DP: 191.6 units (untreated) vs. 136.6 units (treated) & CrI 77.5% (untreated) vs. 0.59% (treated) - Enzyme hydrolysis rate: 93.8% - Total reducing sugar yield: 83.7% & 60–73% respectively	No toxic or inhibitor formation, eco-friendly	High power consumption, high capital cost of equipment	129–131
			Commercial cellulose				132
	Extrusion	Defibrillation	Rice straw, wheat bran		Short processing time, no inhibitor formation, easy scale-up	High energy consumption	133–135
	Microwave irradiation	Molecular collision disrupts the hydrogen bond	Corn stover	- Enzyme hydrolysis rate: 59.96% (untreated) vs. 85.4% (treated) - CrI 62.42% (untreated) vs. 61.36% at 200 W & 54.56% at 700 W (treated) - CrI 62.5% (untreated) vs. 68.3% (treated) - Husk surface corrosion with exposed surface area: 2.42 $\text{m}^2 \text{g}^{-1}$ (untreated) vs. 2.82 $\text{m}^2 \text{g}^{-1}$ (treated) vs. 3.22 $\text{m}^2 \text{g}^{-1}$ (treated) at 20 kHz frequency for 0.5 h and 1 h, respectively - Glucose yield: 76.28% and 59.63% respectively - Enzyme hydrolysis rate: 60% vs. CrI: 39.3% (untreated) vs. 42% at 1 M HNO_3 (treated), 37% for 1 M HCl (treated), 63.9% for 2 M HNO_3 (treated), and 52.2% for 2 M HCl (treated) - Glucose yield: 83%, pore volume: 0.02 $\text{cm}^3 \text{g}^{-1}$ (untreated) vs. 0.04 $\text{cm}^3 \text{g}^{-1}$ (treated) - Total reducing sugar yield: 37.1%	Short processing time, high uniformity and selectivity, and less energy input Clean process, short extraction time, simple operation	High temperature and energy requirement, high operation cost Prolonged treatment can induce unwanted changes, and high equipment cost	136–139
Chemical pretreatment	Acid	Hemicellulose depolymerisation, swelling of fibres, and redistribution of lignin	Wheat straw				140–142
			Rice husk				143 and 144
			Barley hulls and oat hulls Rice hulls Rice hulls cellulose		High efficiency, low energy consumption	Toxic, corrosive, and hazardous, inhibitor formation	145–147 148 90
	Alkali	Fractionation of hemicellulose and lignin	Rice straw Rice hulls		Effective in lignin hydrolysis or removal, partially hydrolysis of	Time-consuming and requires a large amount of alkali, treated biomass	149 150–153

Table 4 (Contd.)

Treatment methods	Type	Mode of action	Biomass type	Modifications	Advantages	Disadvantages	References
Ozonolysis		Dissolves lignin and hemicellulose	Wheat straw	<ul style="list-style-type: none"> - Lignin reduction: 55% (untreated) vs. 14.9% (treated) - Hemicellulose hydrolysis: 10.7% (untreated) vs. 33.1% (treated) - Increased cellulose concentration: 32.65% (untreated) vs. 51.65% (treated) - CFI: 45% (untreated) vs. 69% (treated) 	hemicellulose requires simple reactor equipment	requires a neutralisation step, and proper waste disposal management is also required	137, 154 and 155
				<ul style="list-style-type: none"> - Lignin reduction rate: 29% & 60% respectively - Enzyme hydrolysis rate: 57% & 5-fold higher 	No inhibitor formation, water soluble, can be performed at ambient conditions	Expensive, as a large amount of ozone is required	
				<ul style="list-style-type: none"> - Lignin removal: 77.5% - Lignin recovery: 49.8% - Xylan removal: 86% - Xylose recovery: 67.6% - Enzyme hydrolysis rate: 54.8% - Glucose yield: 70% 	Short processing time, recyclable, easy recovery of solvents, separation of pure lignin	Expensive solvents, the recovery process is energy-intensive, highly flammable and volatile	
Organosolv		Acts on α -aryl and β -aryl ether linkages in lignin	Rice husk	<ul style="list-style-type: none"> - Enzyme hydrolysis rate: 54.8% 	Non-toxic, non-volatile, thermal stability, green solvent, recyclable	Inactivates the cellulase enzyme, expensive solvent, and is energy-intensive to recycle	137 and 155–157
Ionic liquid		Dissolves H-bond in cellulose, ether/ester bond between lignin-carbohydrate, and β -O-4 bonds within lignin	Wheat straw rice husk				
Steam/CO ₂ /ammonia		Swelling of fibres, hydrolysis of lignin & hemicellulose	Rice husk	<ul style="list-style-type: none"> - Glucose yield: 53.87% 	Steam – minimal energy and chemical usage, cost-effective	Steam – formation of toxic component	134, 155, 160 and 161
Bacteria/fungi		Depolymerisation of lignin	Corn stover	<ul style="list-style-type: none"> - Hemicellulose hydrolysis: 38.81% (untreated) vs. 2.6% (treated) - Lignin hydrolysis: 24.48% (untreated) to 15.65% (treated) 	CO ₂ – no inhibitor formation, cost-effective, environment-friendly, non-flammable, easy recovery	CO ₂ – requires a facility to withstand high-pressure conditions	162
				<ul style="list-style-type: none"> - 2.5-fold increase in glucose concentration - Reducing sugars yield: 447.95 mg mL⁻¹ g⁻¹ & 103 mg g⁻¹ respectively 	Ammonia – no inhibitor formation for downstream processes	Ammonia – not efficient for biomass with high lignin, corrosive, and high operating costs	
					No chemical requirement, environment-friendly, low energy input	Time-consuming, large space requirement, and need for continuous monitoring of microorganism growth	



separation of individual components into useful forms for upcycling.^{122,123} The pretreatment process is also reported to improve saccharification by up to 90%, a key step in sugar-based biorefinery operations.^{124–126} Different pretreatment strategies like physical, chemical, physicochemical and biological have been developed to act on cellulose crystallinity, DP, porosity and surface area, and lignin-hemicellulose matrices, thereby enhancing the enzyme accessibility and breakdown of lignocellulose biomass.^{127,128} To illustrate, a comparative summary of strategic methods to induce microstructural modification and their impact on hydrolysis yields is presented in Table 4, including their advantages and limitations.

4.1. Physical pretreatment

4.1.1. Milling/grinding. Physical methods such as size reduction, grinding, shredding and milling of lignocellulose biomass can reduce the crystallinity of lignocellulose through particle size reduction and cell wall rupture.^{116,165} A reduction in particle size of 0.2 mm through grinding or milling is associated with an increased reactive surface area, reduced crystallinity of cellulose and a decrease in the degree of polymerisation, resulting in reduced recalcitrance and increased enzymatic hydrolysis yields.^{15,166} Furthermore, particle size reduction modifies the flow behaviour of the resulting powder, making it more compact, denser and uniformly shaped.¹⁶⁵

Research has shown that corn stover with a particle size of 53–75 μm is approximately 1.5 times more susceptible to enzymatic hydrolysis than material with a larger particle size of 425–710 μm .¹²⁹ Further evidence demonstrates that superfine grinding of steam-exploded rice straw to 60 μm improved the hydrolysis rate, with a significant yield of reducing sugar concentration of 61.4%.¹⁶⁷ Another study, which combined grinding, heating, and chemical treatment (using a 10 mm particle size, 110 °C, and 2% ammonia) on rice straw, resulted in a 17.5% increase in biogas yield compared to untreated straw.¹⁶⁸

Different milling techniques, like ball milling, two-roll milling, hammer milling, and disk milling, are commonly used to reduce the particle size of lignocellulosic biomass.¹⁶⁹ Ball milling is reported to yield higher fermentable sugars than wet milling.¹³⁷ This is consistent with the findings that compared the enzymatic hydrolysis of rice straw pretreated with both wet and ball milling methods, reporting glucose and xylose yields of 78.5% and 41.5% for wet milling, and 89.4% and 54.3%, respectively, for ball milling.¹⁷⁰ Ball milling of oil palm empty fruit bunch for 120 minutes significantly reduced the crystallinity index from 56.1% to 9.3%, resulting in high glucose (67.5%) and xylose (80.1%) yields after enzymatic hydrolysis at 50 °C for 72 h. In comparison, the untreated sample yielded only 15.9% glucose and 5.4% xylose.¹⁷¹ In another study, 90 minutes of ball milling significantly increased the surface area of cellulose from 1.3 $\text{m}^2 \text{g}^{-1}$ to 2.2 $\text{m}^2 \text{g}^{-1}$, decreased the degree of polymerisation (DP) from 191.6 to 136.6 units, and reduced the CrI from 77.5% to 0.59%.¹³² The same study also demonstrated a strong correlation between structural properties, CrI, DP, surface area, and the enzymatic hydrolysis rate, which

improved by 93.8%.¹³² Despite the effectiveness of grinding pretreatment, one of the major drawbacks of this process is its high energy consumption and the high capital cost of the equipment.^{130,155,165} Energy consumption of 0.5–2.5 kWh kg^{-1} for 7–30 minutes of ball milling at 270 rpm has been reported, to increase glucose yield by 59.02% and xylose yield by 49.95% during the enzymatic hydrolysis of wood fibre.¹⁷² Similarly, the size reduction of lignocellulosic biomass, such as corn stover, required considerable energy with reported values of up to 0.011 kWh kg^{-1} , highlighting the energy-intensive nature of the milling pretreatment to reduce crystallinity by 73%.¹⁷³

4.1.2. Extrusion. Extrusion is another physical method that disrupts the internal structure of biomass by applying mechanical force.¹⁷⁴ This method involves mixing, heating, and shearing to induce physical and chemical changes in the biomass.¹⁷⁵ When biomass with a moisture content of 10–80% is rotated in an extrusion machine at a screw speed of 50–150 rpm and a barrel temperature of 50–150 °C, the internal structure of the biomass is disrupted, increasing its surface area, and both physical and chemical orientations are altered.¹⁷⁶ This method is rapid, can be carried out at moderate temperatures, and produces no harmful derivatives.¹³⁵ Different extruders, including single screw and twin screw, have been widely used to process lignocellulosic biomass.¹⁷⁷ Several researchers have reported using single or twin-screw extruders combined with chemical pretreatments of different biomasses, like rice straw,¹³³ wheat bran¹³⁴ and soybean hulls¹³⁴ with total reducing sugar yields of 83.7%, 60–73% and 25–36% respectively. Likewise, a comparative study which involved treatment of soybean hulls using acid, alkali and twin screw extrusion before enzymatic hydrolysis, reported an increase in glucose yield by 69.6%, 128.7%, and 132.2%, respectively.¹⁷⁵

4.1.3. Microwave irradiation. Microwave irradiation is widely used in the structural modification of lignocellulose biomass due to ease of use and high heat efficiency. This method uses electromagnetic waves to generate heat through the oscillation/collision of molecules.¹⁷⁸ This technique alters the crystalline structure of cellulose, depolymerises lignin and hemicellulose, increases the surface area, disrupts the waxy surface and enhances enzyme susceptibility.^{179,180} One study demonstrated that microwave-acid-alkali-hydrogen peroxide pretreated rice straw is effective in recovering hydrolysate with a high glucose content of 35.6%.¹⁸¹ Another study reported an increase in the enzymatic hydrolysis rate of corn stover from 59.96% to 85.4% when microwaved for 45 minutes at 100 °C, 600 W power, and 5% NaOH prior to hydrolysis.¹³⁶ Additional research has highlighted the enhanced enzyme-substrate reaction and increased yield through microwave treatment, making it a promising approach to processing lignocellulose biomass.¹⁸² The advantages of microwave irradiation as a pretreatment method include a short processing time, high uniformity and selectivity, and lower energy input (480–800 W) compared to conventional heating.^{137,139,183,184}

4.1.4. Ultrasound pretreatment. Ultrasound waves with frequencies ranging from 10 to 100 kHz can significantly affect the physical and chemical properties of lignocellulose biomass. The formation and collapse of microscopic bubble cavities due



to ultrasound vibrations lead to the development of fissures, disrupting the hemicellulose and cellulose structures within the material.¹⁸⁵ These fissures arise from the breakdown of β -O-4 and α -O-4 linkages in lignin caused by vibrational forces.¹⁸⁶ It has been proposed that the cavitation effect disrupts the husk cell wall and displaces the middle layer, thereby enhancing the accessibility of cellulose fibres to cellulase enzymes.^{187,188} Furthermore, ultrasound irradiation has been found to reduce the degree of polymerisation in cellulose.¹⁸⁸ For instance, increasing the ultrasound power from 200 W to 700 W (at 20 kHz for 15 minutes) resulted in a decrease in cellulose crystallinity, from 62.42% in untreated biomass to 61.36% at 200 W and 54.56% at 700 W.¹⁴⁰

Ultrasound pretreatments are often used in conjunction with chemical pretreatment methods to achieve more effective results.¹⁸⁹ Reported evidence includes a 45% removal of lignin from coffee husk when ultrasonicated at 47 kHz for 20 minutes in combination with 4% potassium permanganate, and 80–100% lignin removal from groundnut shells, coconut coir, and pistachio shells, which were dipped in a 1% NaOH solution, followed by ultrasonication at 20 kHz.¹⁹⁰

4.1.5. Pulsed electric field. The pulsed electric field method involves subjecting lignocellulosic biomass to high voltage, typically between 5 and 20 kV cm⁻¹, for a short time (less than 1 s).¹⁹¹ During this short exposure, pores are created in the plasma membrane, exposing the cellulose, which becomes accessible for hydrolysis.¹⁸⁵ An increase in pore size facilitates the movement of hydrolytic enzymes, resulting in an enhanced hydrolysis rate.¹⁸⁶ A study reported an increase in saccharification yield from 22.8% to 32.6% when rapeseed straw was treated with a pulsed electric field strength of 12 V m⁻¹.¹⁹² Lignocellulose biomass, such as switchgrass and wood chips, was also successfully treated using a pulsed electric field at 10 kV cm⁻¹, with reports of increased porosity.¹⁹³ The increase in pore size of switchgrass and wood chips was associated with the faster and greater absorption of natural red dye.¹⁹³

4.2. Chemical pretreatment

Chemicals like acids, alkalis, organic solvents, and ionic liquids have been widely used to enhance the biodegradation of complex lignocellulosic biomass, resulting in significant structural modifications.^{116,194}

4.2.1. Acid hydrolysis. Acid pretreatment involves the use of acids like sulphuric acid (H₂SO₄), hydrochloric acid (HCl), phosphoric acid (H₃PO₄) and nitric acid (HNO₃), either in dilute (<4%) or concentrated form.¹¹⁶ The process involves soaking in concentrated or dilute acids (0.2–2.5% w/w), followed by constant mixing for a few minutes to several hours, depending on the acid concentration and the complexity of the lignocellulose structure.^{195,196} Acid pretreatment breaks down the hydrogen and covalent bonds as well as van der Waals forces within the lignocellulose structure, leading to a redistribution of lignin in condensed structure and the depolymerisation of hemicellulose.¹⁹⁷ As a result, cellulose becomes more accessible to enzymes, enhancing decrystallisation and swelling of microfibrils, which leads to increased breakdown into

oligosaccharides and monosaccharides.^{197–199} A significant advantage of using acid is its ability to solubilise hemicellulose into fermentable sugars like xylose, galactose, mannose, and glucose.¹⁴⁸ Hydrolysis yield was reported to be 60% in rice hulls when subjected to acid hydrolysis at 1% v/v H₂SO₄ and a temperature of 140 °C.¹⁴⁸ However, at moderately and highly acidic conditions, fermentation inhibitors like furfural and hydroxymethyl furfurals (HMFs) are produced *via* a dehydration reaction, which then affects yield, thereby suggesting that the pretreatments are required to perform at low temperatures (<110 °C) and low acid concentrations (0–5% w/w).^{15,200,201} This aligns with an insignificant hydrolysis yield of 60.7% when rice husk was treated with 5% v/v H₂SO₄ at 120 °C, compared to the 60% yield from rice husk treated with 1% v/v H₂SO₄ at 140 °C.^{148,202}

Barley husk, oat husk and rice straw pretreated with 1.86% (v/v) [1 : 8% w/v, 130 °C, 30 min], 1% (v/v) [1.8% w/v], (130 °C, 19 min), and 1% (w/w) [160–180 °C for 1–5 min] H₂SO₄ resulted in a maximum glucose yield of 76.28%, 59.63% and 83% respectively, after enzyme hydrolysis.^{145,149} Additionally, the pore volume of rice straw was observed to increase from 0.02 cm³ g⁻¹ to 0.04 cm³ g⁻¹ with the associated sugar yield and the rearrangement of lignin structure, as well as C–H deformation at an elevated temperature of 190 °C, consistent with the findings of previous studies.^{203,204}

FTIR investigation of the pretreated rice husk with HCl (0.5% w/v, 125 °C, 1.5 h) also exhibited a high cellulose content, with intensified bands at 1046–2924 cm⁻¹, increased crystallinity, and a smooth surface, as observed through SEM micrographs.⁵⁸ The crystallinity index of rice husk has been reported to vary with different acid treatments, showing values of 39.3% for untreated, 42% for 1 M HNO₃-treated, 37% for 1 M HCl-treated, 63.9% for 2 M HNO₃-treated, and 52.2% for 2 M HCl-treated samples.⁹⁰ This increase in crystalline values was attributed to the solubilisation of amorphous cellulose regions by acid.⁹¹ Structural characterisation using C-NMR demonstrated that treatment of rice husk cellulose with H₂SO₄ (64% 45 °C, 30 min) yielded a rod-like cellulose nanocrystal and increased crystallinity from 22.63% to 67.16%, which was reflected as a sharp peak in the XRD pattern.²⁰⁵

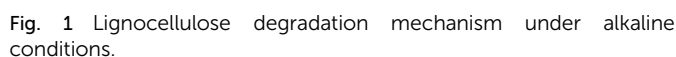
Both dilute and concentrated sulphuric acids are hazardous, toxic and corrosive, requiring corrosive-resistant non-metallic equipment.^{43,131,206}

4.2.2. Alkaline hydrolysis. The alkaline method mostly involves the use of alkaline solutions like sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium hydroxide (Ca(OH)₂), and ammonium hydroxide (NH₄OH) to disrupt lignin structure and enhance enzyme accessibility to cellulose and hemicellulose. The process involves soaking biomass for a targeted time–temperature combination, followed by neutralisation and enzyme hydrolysis.²⁰⁷ The first two simultaneous reactions that occur during alkali treatment are solvation and saponification, causing the biomass to swell, increase its available surface area, porosity, and reduce its crystallinity and degree of polymerisation.¹ The ester bond cross-linking lignin and xylan is broken, leading to delignification and depolymerisation.²⁰⁸ In addition, the hydroxyl radical (OH) from the alkali



4.2.3. Ozonolysis. Ozonolysis is an effective strategy for converting lignocellulose biomass. The process uses ozone, a powerful oxidising agent, to degrade lignin and hemicellulose, releasing acetic acid and formic acid, while leaving the cellulose completely unaffected.²¹⁶ Ozone pretreatment was reported to remove up to 60% of lignin, followed by a 5-fold increase in enzyme hydrolysis of wheat straw.¹⁵⁴ Removal of 29% lignin and improved hydrolysis rate of 57% is reported due to ozonolysis in poplar sawdust.¹⁵⁴ A major advantage is that this process does not produce toxic residues and improves the *in vitro* digestibility of treated biomass. It can also be carried out under room

The major drawback of the organosolv pretreatment is its high flammability, volatility, and low boiling point, which limit



its use in high-pressure applications. This necessitates the recovery and recycling of the process to reduce operational costs and prevent inhibitory effects on microbial fermentation.²²⁵ Additionally, residual lignin condensation is an undesirable side reaction that can lower delignification efficiency by re-polymerising the dissolved lignin fragments onto the biomass surface.²²¹

4.2.5. Ionic liquids pretreatment. Ionic liquids (ILs), also known as green solvents, have gained recent interest due to their unique applications. The method uses salts of large organic cations and small inorganic anions in liquid form at ambient temperatures. These solvents are thermally stable and non-flammable, have low vapour pressures, and are environmentally friendly.²²⁶ ILs with anionic activity dissolve complex lignocellulose through hydrogen bond formation between chloride ions and the hydroxyl group of sugars. The non-covalent interactions among cellulose, hemicellulose, and lignin are disrupted.⁷⁷ The effectiveness of the ionic liquid treatment on lignocellulose materials like wheat straw¹⁵⁹ and wood²²⁷ has been successful, yielding a sugar content of 54.8% at 130 °C for 30 minutes. The treatment also led to highly porous and amorphous structures, as reported in this study. Similar treatment of rice husk yielded 70% sugar¹⁵⁸ and 38% sugar²²⁸ at the ionic liquid-to-water ratios of 2:5 and 1:5, respectively. Considerable changes in morphology were noticed in the form of wave-shaped, amorphous cellulose, porous and disorganised microfibrils, as opposed to the compact structures of the untreated rice husk when observed through a scanning electron microscope.^{228–230}

4.3. Physicochemical pretreatment

This strategy combines factors like temperature or pressure with chemical processes to break down the lignocellulosic waste through approaches like steam explosion, CO₂ explosion, and ammonia pretreatment.¹⁶⁹ The use of steam or CO₂ is a hybrid approach that affects both physical parameters (discussed in Section 3), chemical bonding (bond cleavage) and intermolecular interactions to fractionate the lignocellulose components effectively.^{121,231}

4.3.1. Steam explosion. The steam explosion process uses high-pressure, short-time (a few seconds to a few minutes) saturated steam (160–240 °C, 0.7–4.8 MPa), followed by rapid decompression to break down the lignocellulose structure.²³² During high-pressure steam exposure, the hemicellulose and lignin are hydrolysed, with hemicellulose deacetylated into organic acids, making them readily degradable. Likewise, C=C bonds in the aromatic rings of lignin are weakened.²³³ When the pressure is released and the hydrolysate is cooled down, the dissolved lignin and hemicellulose transition to the liquid phase while cellulose remains in the solid phase, thereby exposing surface area to enzymes and ultimately increasing the digestibility of cellulose.¹ Additionally, acid is generated during the explosion through partial hydrolysis of cellulose, which then acts on hemicellulose to hydrolyse into smaller components,⁴³ a process known as autohydrolysis.¹⁴⁶ The effectiveness of steam explosion is influenced by moisture content, steam

temperature, and substrate size, with high moisture content requiring longer treatment times.^{234–236} A pretreatment study on rice husks using a steam pressure of 2.5 MPa reported maximum lignin reduction from 24.48% to 15.65%, along with significant structural damage to lignin, affecting its barrier functionality.¹⁶⁰ Another study reported an increase in cellulose and lignin content, but a reduction in hemicellulose content, from 38.81% to 2.6%, in rice husk treated at a steam temperature of 220 °C for 10 minutes.²³⁷ This study also observed significant changes in structure, where the smooth, lengthy, and ordered microfibril arrangement became rough, irregular, and distorted, with porous holes, due to the removal of hemicellulose, pectin, wax, and other impurities. This structural modification enhanced enzyme saccharification and improved sugar yield by 53.87%.²³⁷

4.3.2. Carbon dioxide explosion. The mechanism involved in the pretreatment of lignocellulose using carbon dioxide (CO₂) (200 °C, 28 MPa) is similar to a steam explosion. When the biomass is subjected to the explosion, CO₂ penetrates through the structure and dissolves in the moisture inside to form carbonic acid, which then hydrolyses hemicellulose. The high-pressure release also alters biomass structure, thereby increasing porosity, surface area, and susceptibility to enzymatic action.^{238,239} A study involving the supercritical CO₂ treatment of soybean hulls at an optimal condition of 6.62 MPa and 130 °C yielded 97% glucose, 98% xylose, 59% arabinose, and 89% total reducing sugars.²⁴⁰ Another study reported a 2.5-fold increase in glucose concentration of corn stover when treated with CO₂ at 160 °C and 24 MPa.¹⁶²

4.3.3. Ammonia pretreatment. Ammonia pretreatment is performed at high temperatures and pressure, with the most common methods being Ammonia Fibre Expansion (AFEX) and Ammonia Recovery Process (ARP). The AFEX method involves mixing biomass with anhydrous ammonia (1–2 kg ammonia per kg dry biomass), followed by heating at 60–120 °C and a pressure of 2 MPa for 5–30 min. Rapid decompression causes the ammonia to evaporate quickly, and the change in pressure leads to the swelling of cellulose fibres with increased porosity as well as surface area and rupture of the crystalline structure.^{241,242}

Unlike the AFEX, the ARP method uses an aqueous ammonia solution (5–15%) to run through a packed biomass bed in a column reactor at elevated temperatures (150–180 °C).²³⁹ The aqueous ammonia solution flows through the biomass at a rate of 1–5 mL min^{−1} with a retention time of 10–90 min.²⁴¹ This process hydrolyses lignin and hemicellulose, reducing the crystallinity of cellulose. The excess ammonia solution can be recovered and reused, and the resulting hydrolysates are free from fermentation inhibitors due to the mild process conditions (approximately 100 °C, pH below 12, and a short retention time of 10–90 min).²⁴³

Pretreatment with ammonia selectively removes lignin and hemicellulose, increasing the cellulose hydrolysis rate even at low enzyme loadings, compared to other pretreatment methods.¹⁵⁵ The effectiveness of ammonia treatment is superior to dilute acid treatment due to its ability to achieve maximum sugar recovery at low enzyme loading, minimal sugar loss, and



minimal formation of inhibitors.²⁴⁴ For example, the cellulose content was higher in rice husk samples treated with ammonia than in those treated with sodium hydroxide. This variation is attributed to the sodium hydroxide dissolving cellulose into β -cellulose and γ -cellulose, whereas the ammonia treatment preserves the cellulose integrity through selective lignin removal.⁶³

4.4. Biological pretreatment

Structural modification of lignocellulose biomass through biological strategies relies on the enzymes produced by microorganisms (bacteria/fungi) to degrade lignin and hemicellulose, while offering the advantages of low operational cost and environmental friendliness.²⁴⁵ This method does not require high temperatures or pressure, does not generate any inhibitory products, and neutralisation after treatment is not required.²⁴⁶ The process, however, is time-consuming and requires a sterile environment.²⁴⁷ Various bacteria and fungi have been reported to be involved in the biological treatment of lignocellulose biomass. Bacteria such as *Paenibacillus campinasensis*, *Thermomonospora fusca*, *Bacillus subtilis*, and *Azospirillum lipoferum* can degrade lignocellulose biomass. *Thermomonospora fusca* and *Cellulomonas fimi* are cellulolytic bacteria capable of secreting cellulase enzymes predominantly.²⁴⁸ Likewise, *Paenibacillus campinasensis* can tolerate harsh environmental conditions and break down the lignocellulosic biomass.²⁴⁹ Cellulase enzymes produced by some anaerobic cellulolytic bacteria, such as *Bacteroides cellulosolvens* and *Clostridium thermocellum*, possess high activity, although they are secreted in smaller amounts.²⁵⁰ Few bacterial strains, like *Bacillus subtilis* and *Azospirillum lipoferum*, have been widely reported to produce bacterial laccases, which cause lignin depolymerisation.²⁵¹ At least 30 predominant rumen cellulolytic bacterial species are known to be used in pretreatment through their own specific mechanisms of adhesion to cellulose and hydrolysis.²⁰⁶ However, the rate of hydrolysis by bacteria depends on the substrate type, incubation time, temperature, nutrients, aeration, pH, and type of bacterial strains used in delignification.²⁵²

Fungal species like white rots (*Pleurotus ostreatus*, *Trametes versicolor*, *Phanerochaete chrysosporium*), brownrots (*Gloeophyllum trabeum*, *Coniophora puteana*) and soft rots (*Trichoderma reesei*) are the most commonly used microorganisms in the treatment of lignocellulose biomass owing to their ability to release lignin-degrading enzymes like lignin peroxidase, manganese peroxidase and laccase.^{246,252} Brown rots mainly attack cellulose, whereas white and soft rots attack cellulose and lignin.¹⁷⁸ During the fungal treatment of lignocellulose biomass, mycelium penetration increases pore size and breaks down the bonds between lignin, hemicellulose, and cellulose, thus facilitating structural degradation.²⁵³ However, the rate of hydrolysis by fungi depends on the substrate type, incubation time, temperature, nutrients, aeration, pH, and the type of fungal strains used in delignification.²⁵²

Lignin degradation by fungi can occur through selective and non-selective mechanisms. Selective degradation involves the localised breakdown of lignin and hemicellulose with minimal

degradation on the cellulose fraction, whereas non-selective degradation involves the simultaneous breakdown of all the lignocellulosic components.²⁵² This mechanism is particularly characteristic of white-rot fungi, which first degrade lignin during their vegetative phase, followed by a productive phase in which polysaccharides are broken down.^{242,254,255} Non-selective degradation simultaneously breaks down all the lignocellulosic components.²⁵²

In a fungal pretreatment of rice husk, significant enzyme activity was observed in lignin-degrading enzymes, yielding 447.95 mg mL⁻¹ g⁻¹ of reducing sugars. The pretreatment of rice husk and sorghum husk with *Phanerochaete chrysosporium* not only yielded a reducing sugar content of 447.95 mg g⁻¹ and 103 mg g⁻¹, respectively, but also induced morphological changes like reduced crystallinity and increased pore size due to lignin removal.^{54,163} These changes in the surface morphology are due to the activity of ligninolytic enzymes produced by *P. chrysosporium*. Additionally, pretreatment with *Pleurotus ostreatus* has been reported to increase the enzymatic hydrolysis of rice straw through partial lignin degradation.²⁵⁶ Thus, the biological pretreatment process can bring extensive structural modification whilst improving the efficiency of enzymatic hydrolysis and increasing the yield of fermentable sugars.

Biological pretreatment also includes the use of commercial microbial enzymes to break down crystalline cellulose and hemicellulose into fermentable sugars, while minimising inhibitor formation and energy use.^{43,131} Commercial cellulase, hemicellulase and ligninase enzymes are commonly used to hydrolyse cellulose, hemicellulose, and lignin. Cellulase, like endoglucanase, exoglucanase and β -glucanase/ β -glucosidase, adsorbs onto the cellulose surface and degrades it into simple sugars, whereas hemicellulase, like xylanase, acts on glycosidic linkages in xylan during saccharification, producing xylose.^{131,257,258} Ligninases, such as laccase peroxidases, serve as accessory enzymes in the effective hydrolysis of lignocellulosic biomass by removing the xylan layer, facilitating the dissociation of cellulose–hemicellulose, and disrupting the crystalline structure of cellulose.^{75,259,260}

Although the primary focus has been on utilising cellulose through enzyme breakdown, current research is shifting towards the aligned use of hemicellulase enzymes to enhance theoretical yield and significantly improve process feasibility.²⁶¹ A combination of enzymes is used for efficient breakdown, as these enzymes work synergistically to degrade the substrate, achieving higher performance than when acting individually. This phenomenon is called enzyme synergy.²⁶² One study reported a significant synergistic effect of a cellulase–xylanase enzyme cocktail on dilute acid-pre-treated sorghum stover, yielding glucose and ethanol concentrations of 40.7% and 41.1%, respectively.²⁶³

The degree of synergy (DS) can be measured mathematically as:

$$DS = \frac{\text{combined enzyme activity on the substrate}}{\text{the sum of individual enzyme activity on the same substrate}}$$



A $DS > 1$ indicates synergistic action, while $DS = 1$ means enzymes degrade substrate independently, and a $DS < 1$ indicates enzymes are not synergistic but inhibit each other by competing for the same binding sites.²⁶⁴ However, a high DS does not always result in the maximum conversion yield. For example, the combination of enzymes laccase and ferulic acid esterase gave the highest DS of 88, but the lowest conversion yield of 17% in oat hulls. Conversely, the highest conversion yield of 57.2% was achieved in oat hulls when subjected to ferulic acid esterase + xylanase + arabinoxylanase with a DS of 1.52.²⁶⁵

5. Cereal husk applications, future opportunities and perspectives

The valorisation of cereal husk biomass extends beyond its pretreatment stage, with growing research focusing on sustainable strategies to repurpose agro-processing waste into renewable biofuels, value-added products through bioconversion, and eco-friendly solutions to minimise environmental pollution.²⁶⁶ Once cereal husks undergo pretreatment, the resulting modified biomass has different applications in the food industry. Post-treatment applications focus on utilising the structurally modified cellulose, hemicellulose, and lignin fractions to create safe, functional, and sustainable food ingredients and materials.¹²³ These applications support the principles of a circular food economy by reducing waste, improving sustainability, and generating high-value products from underutilised by-products.

One of the most direct applications of treated cereal husk is as a dietary fibre ingredient.²⁶⁷ The micronised cereal husks are suitable for incorporation into bakery products, extruded snacks, plant-based meat and functional beverages.^{268–271} The fibres are associated with health benefits, including cholesterol reduction, improved gut health, and blood sugar regulation.²⁶⁷ Cereal husk can also serve as a substrate for bioethanol production. Enzymatic saccharification of cellulose and hemicellulose releases fermentable sugars that can be converted into ethanol.²⁷² Hydrolysed hemicellulose can also be used to produce prebiotic oligosaccharides, which selectively stimulate beneficial gut bacteria, such as *Bifidobacterium* and *Lactobacillus*, making them suitable for incorporation into functional foods.²⁷³ Additionally, hemicellulose can also be used to produce flavour enhancers, such as furfural, and sweeteners, including xylitol, as well as nutraceuticals and food packaging.^{274–276} Lignin also offers opportunities for upcycling due to its antioxidant, antibacterial, and UV-blocking properties.^{277,278}

In addition to food ingredients, cereal husk has significant potential in the development of bioplastics and edible coatings, an environmentally friendly alternative to conventional plastics.^{21,279} These bioplastics, prepared by extracting cellulose fibres from cereal husks, are biodegradable, durable, and have improved tensile strength.^{12,280,281} Considering the wide availability, low cost, and underutilised nature of agro-processing residues, there is potential to add value to cereal husk waste through sustainable processing pathways.²⁸² These innovations

highlight the versatile applications of cereal husks, including the production of renewable biofuels, the development of functional foods, and environmentally friendly packaging. Such advancements reinforce the role of cereal husk valorisation in achieving a circular economy while reducing the environmental footprint associated with husk waste.

The diversity of pretreatment strategies discussed (Section 5) clearly demonstrates that no single method is effective across all biomass types. Moreover, the lignocellulose biomass derived from different plant sources greatly varies in composition and structure. Therefore, the success of down-streaming waste byproducts depends on aligning pretreatment with the dominant microstructural barriers of each biomass. For instance, lignin-rich biomass benefits from a delignification-focused pretreatment method such as alkali, while hemicellulose-rich biomass is best liberated through acid or steam explosion.

A biomass-specific structure-guided framework can significantly improve the efficiency of enzymatic hydrolysis and down-stream conversion. This framework will provide a basis for pretreatment design, where combinations of methods will be selected to overcome the multiple barriers simultaneously. Such an approach not only enhances hydrolysis yields but also reduces the energy and chemical inputs, as well as the formation of inhibitors often associated with non-targeted pretreatments. Additionally, future studies could employ a meta-bibliographic analysis approach to systematically map emerging trends in pretreatment strategies, identify research clusters, and reveal knowledge gaps across the unexplored cereal husks.

6. Challenges and limitations

Commercially feasible utilisation of lignocellulose biomass requires addressing technical, operational and economic challenges. These include the high cost of pretreatment technologies, energy input, biomass composition and quality, the formation of inhibitory compounds, the environmental impact of the processing technique and the energy requirement.⁶⁷ Additionally, the high cost of hydrolytic enzymes and the complexity of developing efficient enzyme cocktails for cellulose and hemicellulose hydrolysis further complicate the process.^{10,283}

As the lignocellulose biomass derived from different plant sources greatly varies in composition, standardising the pretreatment technique for each biomass type is difficult.²⁸⁴ Lignin recalcitrance also poses a major barrier to biomass conversion, as its rigid structure resists enzymatic degradation and necessitates higher energy and chemical inputs during pretreatment. Operational costs associated with energy-intensive equipment, expensive chemicals, and enzymes are a significant bottleneck in many industrial applications.⁶⁷

During chemical pretreatment, undesirable by-products or derivatives, such as furans, hydroxymethylfurfural, organic acids, soluble sugars, and phenols, are released, negatively affecting microbial growth, substrate utilisation, fermentation, and impacting the environment.^{82,124,285,286} Due to their small size, these inhibitors can easily penetrate cell membranes, causing damage to internal structures, altering cell morphology and inhibiting RNA and protein synthesis. They are also



reported to reduce sugar consumption by microbes, subsequently reducing the production of end products.^{124,287}

During enzyme hydrolysis, accumulated sugars such as cellobiose and glucose can inhibit enzyme activity, resulting in feedback inhibition.²⁸⁸ During fermentation, substrate inhibition occurs due to high feedstock concentrations, resulting in low water activity, increased osmotic pressure and cell lysis. End-product inhibition occurs when high substrate concentration penetrates microbial cell membranes, disrupting their intracellular processes and leading to cell death.¹⁰

7. Conclusion

A large amount of organic waste is generated in the agro-industrial cereal processing cycle. The efficient valorisation of lignocellulosic components present in cereal biomass is pivotal in advancing sustainable biorefinery practices. However, structural recalcitrance of lignocellulose hinders the valorisation into high-value products. Pretreatment methods can effectively break down the recalcitrant structure of biomass, thereby enhancing further enzymatic hydrolysis and converting it into fermentable sugars and other high-value products.

This review highlights the importance of pretreatment strategies in enhancing the hydrolysis efficiency of lignocellulose biomass by targeting microstructural barriers, including crystallinity, degree of polymerisation, porosity, surface area, and the lignin–hemicellulose ratio. A biomass-specific structure-guided framework can significantly improve the efficiency of enzymatic hydrolysis and downstream conversion.

However, challenges such as scalability, process integration, reducing energy consumption, processing cost and the environmental impact of certain pretreatments exist. Despite extensive research on developing numerous pretreatment techniques, only a few methods, like dilute acid, have been commercialised for bioconversion.

Future research should focus on developing synergistic pretreatment approaches to address the limitations of individual treatment methods. However, significant research specific to target raw materials is still required to establish and optimise these combined techniques for optimal efficiency. While numerous novel pretreatment methods have been proposed, scaling these processes from laboratory research to industrial applications remains a major challenge. Addressing this issue should be another key focus for future studies. Structural modification of lignocellulosic biomass holds significant potential for reducing waste and transforming it into valuable resources, supporting economic growth and the achievement of the United Nations Sustainable Development Goals.

Author contributions

Smriti Ale: writing – original draft, writing – reviewing & editing, conceptualization, visualization, Pramesh Dhungana: writing – reviewing & editing, conceptualization, supervision, validation. Janet Howieson: writing – reviewing & editing, supervision, validation, funding acquisition, Rewati Raman Bhattarai:

writing – reviewing & editing, conceptualization, supervision, validation.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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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