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Advances in lignocellulosic feedstocks for bioenergy and bioproducts

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Lignocellulose, an abundant renewable resource, presents a promising alternative for sustainable energy and industrial applications. However, large-scale adoption of lignocellulosic feedstocks faces considerable obstacles, including scalability, bioprocessing efficiency, and resilience to climate change. This Review examines current efforts and future opportunities for leveraging lignocellulosic feedstocks in bio-based energy and products, with a focus on enhancing conversion efficiency and scalability. It also explores emerging biotechnologies such as CRISPR-based genome editing informed by machine learning, aimed at improving feedstock traits and reducing the environmental impact of fossil fuel dependence.

The reliance on fossil fuels has become increasingly unsustainable in light of climate change. Fossil fuels, such as coal, oil, and natural gas, have been the primary drivers of environmental pollution and anthropogenic greenhouse gas (GHG) emissions worldwide[1]. Unprecedented levels of atmospheric carbon dioxide (CO_2) are leading to a rise in global average temperatures[2], which in turn, has triggered a cascade of environmental challenges, including more frequent and severe weather events, rising sea levels, disruptions to ecosystems and biodiversity, and have materialized into significant threats to the well-being of humanity[3].

Consequently, interest in renewable natural resources and alternative energy solutions has surged, driving increased efforts into climate change mitigation. This shift is endorsed by international policies and regulatory frameworks, including the United Nations Paris Agreement, which set a target to keep global warming at well below 2 °C, with an ideal ceiling of 1.5 °C relative to pre-industrial levels[2,4]. Many nations have also committed to achieving net zero GHG emissions by 2050[2,5]. Renewable energy sources, such as wind, solar, and hydroelectric power, offer cleaner, sustainable alternatives to fossil fuels by converting natural energy into electricity without emitting GHG during operation. These technologies are vital for reducing carbon emissions, but their widespread adoption faces obstacles, including geographical constraints, intermittency, and technological readiness to up-scale[6,7]. Moreover, they cannot fully replace fossil fuels in all sectors, such as petrochemical production for industries traditionally reliant on fossil fuels, including maritime transport and aviation[7]. According to the U.S. Department of Energy's (DOE) 2023 Billion-Ton Report, despite significant advances in battery technology for electrification, biomass feedstocks will continue to be critical for renewable energy production and bioproducts, particularly for biofuels and industrial applications that cannot easily transition to electrification[8].

One promising source of biomass energy is lignocellulosics derived from bioenergy crops and woody plants. Lignocellulose is the most abundant form of biomass on Earth[9], accounting for approximately 57% of the planet's biogenic carbon[10]. Innovation toward deploying biomass energy has been projected to displace 30% of fossil fuel consumption in the near future with biofuels, biochemicals and biomaterials[7]. Unlike fossil fuels, which release CO_2 into the atmosphere from permanently sequestered subterrestrial carbon sinks, sustainably managed lignocellulose biomass can be carbon-neutral while alleviating food-security concerns of starch-based bioenergy by not competing with agricultural land used for food production[11]. Despite its tremendous potential, the development and utilization of lignocellulosic feedstocks have not yet met industrial expectations. Bottlenecks include production scalability[12], long production cycles and timelines, bioprocessing efficiency, and cost-effectiveness[13,14], as well as concerns over the adaptation and resilience of feedstocks to climate change.

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Biotechnological advances to modifying lignocellulosic feedstocks offer enormous potential to enhance biomass yield, improve bioenergy traits, reduce agricultural inputs, and increase resilience to climate change and pathogens. Combined with innovations in bioprocessing methods and feedstock pretreatments, this integrative approach creates transformative opportunities for scaling up bio-based applications to meet the global demands for sustainable energy and materials. This holistic approach not only addresses critical environmental challenges but also broadens the scope of feedstock utilization into emerging and diverse sectors[15].

Here, we review current efforts and future perspectives in harnessing lignocellulosic feedstocks for bio-based energy and products, with a focus on reducing environmental impacts of fossil fuel dependence. Advances in feedstock applicability and utilization across various bioprocessing and feedstock engineering strategies to enhance conversion efficiency and scalability are described. Additionally, we explore the potential of emerging biotechnologies to accelerate trait modification and expanding feedstock capabilities in response to the rapidly evolving challenges of climate change, at a time when CRISPR-based genome editing technologies are informed by democratized artificial intelligence (AI) resources and sophisticated machine learning (ML) models that enable next-generation mutagenesis and breeding in lignocellulose feedstocks.

Existing and future lignocellulosic-based applications

Lignocellulosic feedstocks have a long and impactful history in human civilization and remain deeply embedded in modern society. Traditionally, their use has centered on applications such as heat and energy production, lumber for construction and furniture, and pulping of cellulosic fibers. However, recent advances in biomaterials science have opened the door to a wide array of emerging applications. Lignocellulosic biomass predominantly consists of the thickened secondary cell walls of vascular plants, which are mainly composed of cellulose and hemicelluloses, impregnated with lignin[16,17]. This section provides an overview of both current and emerging applications for the individual components of lignocellulose (Fig. 1).

Cellulose based applications

Cellulose is the most abundant biopolymer on earth. It is primarily used in a fibrous form for paper-based products and textiles, extracted through pulping. Fiber characteristics vary widely across biomass sources, providing flexibility for different applications. Softwood fibers, known for their length and coarseness, are ideal for packaging, sanitary, and hygiene products due to their bulk and absorbency. In contrast, hardwood fibers are preferred for soft tissues[18]. Highly pure cellulose fibers (> 90%), known as dissolving pulp, are used in clothing (textiles) and are also processed into cellulose derivatives. These derivatives have broad applications, including the production of bioplastics for food and medical industries[19].

The exploitation of cellulose at the nano- to micro-scale has started a green revolution, with the successful implementation of large-scale manufacturing of cellulose nanomaterials. Renewable nanomaterials[20] represent the next generation of nanotechnology, outperforming their synthetic analogs owing to their sustainability features. Nanocelluloses not only retain the inherent properties of cellulose but also exhibit key characteristics of nanomaterials, such as a large surface area, versatile reactive sites, and stable scaffolding for hosting nanoparticles[21]. Applications of nanocelluloses are being explored across various fields, including energy storage, photovoltaic devices, thin-film transistors, medical applications like biomedical scaffolds and drug delivery, functional barrier packaging, and mechanical reinforcement in composites or concrete, among others[22,23].

Recently, top-down methods of wood engineering leveraging cellulose fibrils while maintaining wood anisotropic structure has gained increasing interest, such as densified, cooling, thermal insulating, and transparent wood[24,25]. These advanced wood materials are part of the broader push towards sustainable and energy-efficient building solutions, combining the natural benefits of wood with enhanced physical and chemical properties that could replace traditional materials in various applications.

Finally, the conversion of cellulose into biofuels and renewable chemicals has gained worldwide attention due to climate change and the projected 6% increase in total energy demand by 2030, as estimated by the International Energy Agency[26]. The significance of cellulose-based applications, derived from biomass through sustainable technologies, in addressing social and environmental challenges cannot be overstated, as they offer solutions for reducing dependence on fossil fuels, minimizing waste, and promoting resource efficiency while contributing to the development of a circular economy and mitigating climate change impacts.

Hemicellulose based application

Unlike cellulose, hemicelluloses are amorphous and heterogenous in chemical composition[27]. In the pulping processes, the majority of hemicelluloses are degraded and burnt to produce energy. Meanwhile, in biorefinery, hemicelluloses are often partially extracted in pretreatments to ensure efficient enzymatic digestion of cellulose, and the residual part is converted to pentoses and hexoses by hemicellulases[28]. The monomeric sugars from hemicelluloses can be further processed into bio-ethanol by fermentation or catalytically converted into platform chemicals, such as sorbitol, xylitol, and 2,3-Butanediol[28]. Hemicelluloses have been shown to improve the mechanical strength of paper when used as a wet-end additive[29], enhance food properties[28], and serve as an alternative source for pharmaceutical and healthcare products[30]. Other emerging hemicellulose-based applications include films for packaging, absorbent material, aerogels, raw materials for carbon quantum dots and porous carbons[27,28,31].

Lignin based application

Remarkably, lignin represents the relatively highest carbon content (~ 60%) of the lignocellulosic biomass[32] due to its polymer size and naturally branched structure. However, it remains largely underutilized. To date, lignin is principally burned for heat and energy production (e.g., in pulp and paper mills)[33]. Research efforts have been directed towards valorization of the lignin stream. However, the complexity and heterogeneity of the lignin polymer, along with its recalcitrance to depolymerization, pose considerable challenges to lignin valorization for producing biochemicals and bioproducts.

Promising conversion methods, including hydrogenolysis, acidolysis, and depolymerization, have been exploited to convert lignin into value-added products[34,35]. The use of specific solvents, organic compounds (e.g., formaldehydes) and metal formates have been investigated to promote lignin depolymerization through C-O bond cleavages, ring opening, and subsequent oxidation, meanwhile preventing the recondensation reactions of the lignin fragments. Among the possible lignin derivatives that can be produced, vanillin has been the major phenol aldehyde, industrially manufactured from the guaiacyl and syringyl units of lignin[36]. While chemical approaches have been the primary means to lignin valorization, the use of bacteria such as *Rhodococcus*[37] and *Pseudomonas*[38] species have also shown promise in the production of biopolymers (e.g., polyhydroxyalkanoates, PHAs) or polymer precursor molecules (e.g., nylon[39]) from depolymerized lignin. Recently, studies are investigating the potential of the filamentous white-rot fungi for lignin valorization[40].

Processing and treatments

Conversion of lignocellulosic feedstocks into biofuels and bioproducts involves a series of successive, often integrated steps, from feedstock selection, preparation and pre-processing to conversion and valorization through principally, thermochemical and biological means. Advances in

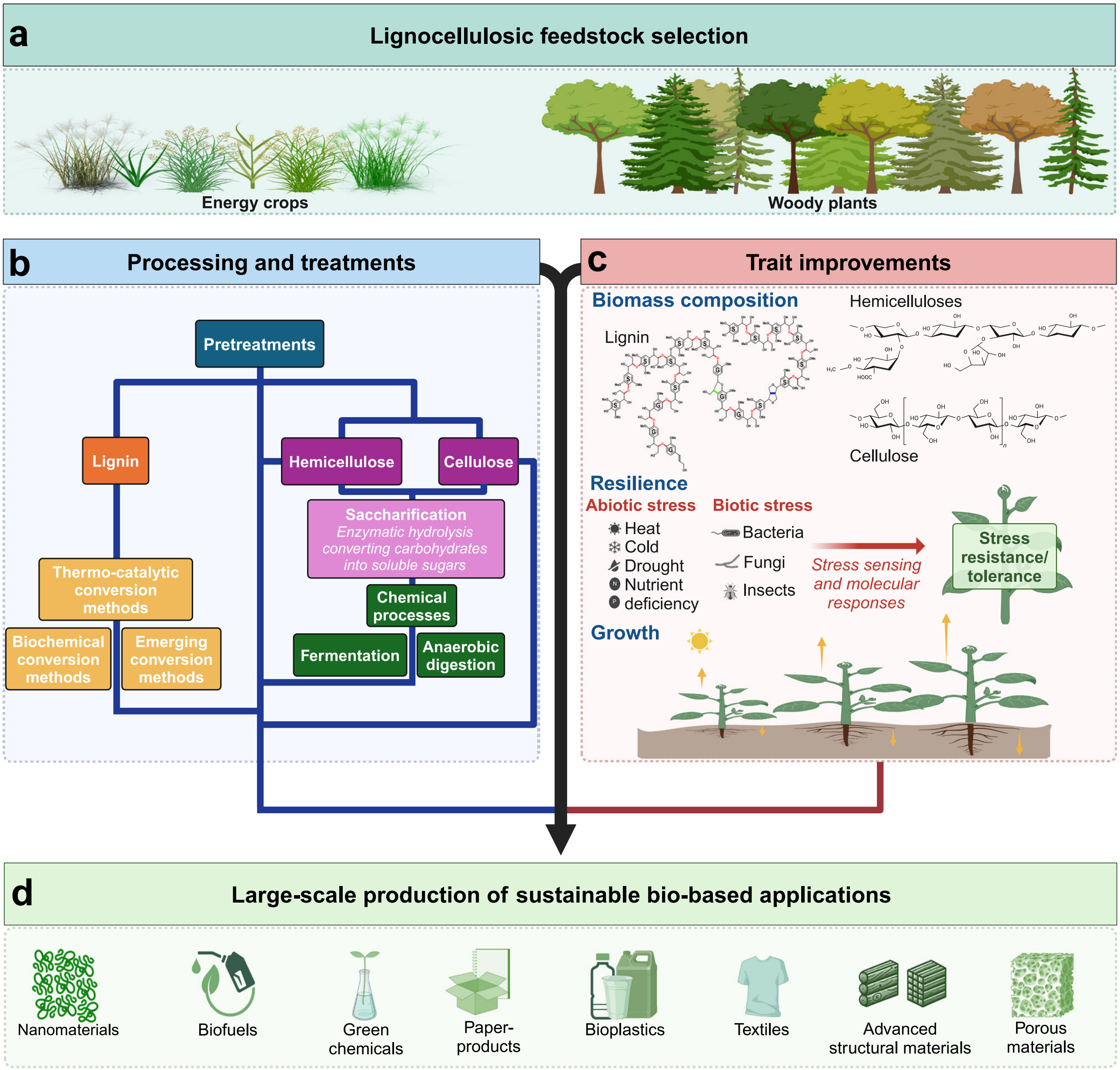

Fig. 1 | Pathway to large-scale bio-based production. **a** The choice of biomass (e.g., energy crops or woody plants) depends on factors like availability and recalcitrance level, which in turn influence the required bioprocessing methods, treatment approaches, and trait improvements; **b** Overview of the processing and treatment of lignocellulosic biomass; **c** Optimizing feedstock composition for efficient conversion, improving plant growth, and increasing resilience to biotic and abiotic stresses are critical for ensuring a stable and sustainable biomass supply; **d** Large-scale production of bio-based applications can be achieved by integrating optimal feedstock selection, processing methods, treatments, and trait improvements, contributing to sustainable solutions in the face of climate change.

technologies and sustainable practices continue to drive efficiency and productivity improvements of these processing and treatment steps, while expanding the range of economically viable biomass-derived, value-added products. Yet, the conversion of lignocellulosic feedstocks into biofuels and bioproducts still faces major challenges that curb enthusiasm for industrial deployment, given the inherent difficulty of breaking down this complex and recalcitrant polymer.

The development of biorefineries[12,41], or integrated systems that combine farming and conversion processes to produce bioenergy and biomaterials, has been proposed as a sustainable industrial alternative to fossil-based refineries. These biorefineries aim to enhance scalable production across industrial sectors, address climate change, reduce dependence on fossil fuels, and stimulate job creation and development in rural areas. The conversion of biomass into fuels, chemicals, and energy occurs through various processes, including liquefaction, fractionation, hydrolysis, pyrolysis, gasification, catalysis, and fermentation. While there have been several examples of lab-scale and industrial-scale implementation, expanding research and development at scale is needed to overcome biomass conversion bottlenecks and techno-economic challenges, while minimizing environmental impact[12].

Feedstocks is the starting point of a biorefinery

Starch and lignocellulosic biomass in all its forms remain a large reserve of fixed carbon that could be used to displace petroleum as a source of fuels and chemical products. The source, availability, type and recalcitrance level of the biomass dictate the selection, runnability and productivity of the main converting operations[42] (Fig. 1a). First-generation biorefineries[43] rely on food-grade feedstocks for biofuel production, which has led to growing socio-economic and environmental concerns. Also, their excessive use of nitrogen fertilizers can result in the emission of nitrous oxide (N_2O), a GHG more potent than CO_2[34]. Alternatively, lignocellulosic feedstocks, such as wood and energy crops, have driven the development of second-generation biorefineries[44]. These feedstocks could lower GHG emissions, may be cultivated on marginal lands that are unsuitable for food crops, while also minimizing the strain on scarce water resources. However, the type of lignocellulosic biomass and its intrinsic properties significantly impacts the cost, productivity, and efficiency of the conversion process[44]. Studies show that feedstock supply logistics and handling can account for nearly 50% of the total cost of biofuels and bioproducts[45].

Pretreatment strategies of lignocellulosic biomass

The pretreatment step is an essential part of the biomass conversion process[34,42] (Fig. 1b). It aims to breakdown the recalcitrant structure of the lignocellulosic biomass to enzymatic or microbial deconstruction and allows for separation and further treatment of each of the biomass components (i.e., cellulose, hemicelluloses and lignin). This step is also key to maximize transportation efficiency through biomass size reduction, drying, and compaction. Ideally, the pretreatment process should result in maximum recovery with minimum degradation of the biomass components, minimum capital and operating costs, and should be applicable to a variety of lignocellulosic materials. However, most conventional pretreatment techniques are limited by their high energy demand and high cost and can form inhibitory –potentially toxic– intermediates and by-products, resulting in low productivity and the need for additional costly steps[42].

Pretreatment methods can be categorized into physical, chemical, physicochemical, and biological methods[34,42,46]. Physical pretreatment uses mechanical forces like friction and shear to reduce biomass size, increase surface area, and improve reaction rates, such as for saccharification. When combined with fractionation methods (e.g., sieving), particle size control can be achieved. However, these methods require significant mechanical and electrical energy, making them costly, time-consuming, and eventually damaging the lignocellulose. Although large-scale equipment is available, particularly in the pulp and paper, and polymer industries (e.g., refiners, ball-milling, extrusion), these processes still face efficiency challenges.

Chemical pretreatments require less energy but involve chemicals and digesters, which can raise costs and environmental concerns. Originally established in the pulp and paper industry, processes like Kraft, sulfite, organosolv or dissolving pulping remove controlled amount of lignin for further biomass conversion[47]. While effective for paper, packaging, and cellulose products, these methods are less suitable for biofuels. Acid pretreatment removes hemicellulose and produces inhibitors, while alkali pretreatment can dissolve too much biomass. Residual lignin also limits sugar yield, and alternative methods like organosolv and ionic liquids are costly and not yet scalable due to limited solvent/chemical recovery systems.

Physicochemical pretreatments commonly employ high pressure and temperature to alter biomass structure by disrupting intra- and inter-molecular linkages. These pretreatments often require specialized reactors or costly setups. Steam explosion works well on hardwood and agricultural residues, but liquid hot water pretreatment is ineffective at removing lignin from hardwood and softwood. Wet oxidation effectively removes lignin but requires oxygen or air and may use catalysts, further increasing costs[42].

Alternatively, biological pretreatments offer sustainable, cost-effective alternatives, operating under mild conditions without chemicals or high energy input. Specialized enzymes and fungi can facilitate lignin degradation and partially remove hemicelluloses without producing toxic byproducts, eliminating the need for a neutralization step before saccharification. This process can be integrated with enzymatic hydrolysis for sugar conversion, promoting a safer, more sustainable biorefinery. However, improving reaction rates, titer, and sugar yields, along with large bioreactors and continuous monitoring, is needed for large-scale use[42].

Combining different pretreatment methods has been explored to improve sugar yields and conversion efficiency. While this synergy accelerates biomass breakdown and enhances conversion rates, they still results in high operational costs. Moreover, since conversion efficiency depends on biomass type and structure, finding a universal pretreatment method for all lignocellulosic materials remains elusive. Pretreatment of biomass is estimated to contribute approximately 40% of the total production costs in biomass conversion, with these costs depending on the specific pretreatment method used. The remaining 60% includes costs for enzymatic hydrolysis (the saccharification step), which alone can account for nearly 25% of the overall production costs due to enzyme prices, along with additional costs for energy, chemicals, and reactor operation[41,42]. Substantial research efforts are needed to optimize the pretreatment step, ensuring higher yield and productivity at lower costs.

Over the last few decades, bioconversion of lignocellulosic feedstocks has primarily focused on the carbohydrate components, resulting in the development of methods to remove lignin, the primary recalcitrance to biomass utilization[33]. However, for specific bioproducts like cellulose nanomaterials, the presence of lignin in the biomass can, instead, show benefits for endowing the carbohydrates with additional performance such as hydrophobicity and resistances against biological degradation[48,49]. It is essential to select pretreatment methods that align with the desired properties of specific biofuels and bioproducts.

Saccharification of lignocellulosic biomass

Biomass pretreatment is usually followed by enzymatic hydrolysis that converts the extracted carbohydrates into soluble sugars (Fig. 1b). Enzymatic hydrolysis has proven to be quite effective and cost-efficient with reported conversion rates that can exceed 80%[41]. This step utilizes a cocktail of enzymes that selectively hydrolyze either hemicelluloses or cellulose. The high cost of enzymes, especially for large-volume sugar productions, remains a barrier for industrial scalability of high-efficiency biorefineries[50]. While some enzymatic cocktails can be an economically viable solution for ethanol production, the enzymatic hydrolysis step only converts the hexose (C6) sugars, leaving behind the pentose (C5) sugars along with proteins and other carbon sources as residues. Given the growing need for a more sustainable carbon solution, the complete utilization of both hexoses and pentoses would significantly improve the overall biorefinery carbon efficiency. Genetic engineering possibilities, for instance, are being investigated to improve the fermentation performance of microorganisms[51,52], while other novel contributions involve the development of statistical tools and computational models to design, predict, and validate the performance and stability of enzyme cocktails[33]. From an economic perspective, combining enzyme hydrolysis with pretreatment or fermentation could create a path towards improving the process economics of other fermentation products (e.g., butanol), since such a procedure has been successful with ethanol production[50].

Feedstock utilization challenges for industrial use

Cutting-edge technologies, in bioprocessing and feedstock genetic modification, have the potential to overcome key challenges related to the economic viability and environmental impacts of lignocellulosic feedstock, enabling their industrial implementation for commercial applications. According to the Billion-Ton report by the U.S. Department of Energy, the future supply of the lignocellulosic biomass for the U.S. could achieve 174 million dry metric tons per year for bioenergy use[53], highlighting the significant potential for large-scale deployment. Life-cycle analysis, encompassing steps from raw material extraction to end-of-life[54,55], has demonstrated reduced carbon footprints and environmental impacts compared to traditional industrial methods in several bio-based applications, including biofuels[56], hydrogen[57], renewable natural gas[58], sustainable aviation fuel[59,60], electricity[58], biogas[61], nanocelluloses[62], platform chemicals[63], and structural materials[64]. Additionally, techno-economic assessments looking at economic indicators like minimum selling price, internal rate of return, and net present value are key to overcome the issues in a sustainable manner for large-scale practices. The expansion of the circular bioeconomy enhances environmental sustainability and economic welfare, besides providing social benefits for future generations[65].

Among the ongoing challenges is the need for effective detoxification methods to neutralize fermentation-inhibitory compounds generated during biomass pretreatment[66]. These methods are crucial for enhancing conversion rates and efficiency, such as achieving higher biofuel yields. However, these benefits are accompanied by additional costs. The recovery of enzymes and chemicals/solvents is another aspect to address for decreasing the environmental footprint of a biorefinery and improve its economics[67]. The use of green solvents and chemicals (i.e., ionic liquids), development of solvent recovery systems, implementation of on-site enzyme production technologies and integrated conversion steps are all strategies being investigated to minimize environmental impacts and improve process economics[12]. In addition, cost-effective strategies for management and exploitation of each of the generated residual streams into viable, value-added products are critical for commercial viability. A plethora of other novel value-added products from lignocellulosic feedstocks are within reach. For instance, biochar, a carbonizing material with a high carbon content, can be derived from the thermo-chemical decomposition of lignocellulosic materials by pyrolysis or gasification in the absence of oxygen[68]. Biochar can be used as a soil supplement, a water treatment or for CO_2 capture and sequestration[68]. Co-production of renewable nanomaterials, biochemicals and biofuels is a promising way to offset some of the processing costs for biofuel and biochemical production by generating new revenues through market diversification and waste residues reduction[69].

Finally, an important driver for the industrial development of lignocellulosic bioenergy and bioproduct industries is the development of strategic policies and regulatory frameworks. Over the years, many countries have initiated national policies and mandates to reduce their dependence on conventional sources of energy and combat climate change. The United States biofuels initiative was kicked off in 2001 with the authorization of blending E10 and E15 (i.e., 10% and 15% ethanol, respectively) with gasoline for all cars[70]. The US Energy Independence and Security Act (EISA) set up a target production of 136 billion liters of biofuels by 2022 to meet 18% of the nation's transportation fuel demands[71]. Similar initiatives have been implemented by the European Union, China, India, and others with the intend of achieving percent biofuel blending mandates and strengthening the global development of biorefineries[68].

Modifying feedstocks to optimize biomass conversion efficiency

Alongside advancements in bioprocessing technologies, optimizing the composition of lignocellulosic feedstocks for efficient conversion is essential for ensuring a sustainable feedstock supply, particularly in the context of climate change (Fig. 1c).

Lignin represents a significant impediment in the processing of lignocellulosic biomass for various downstream applications[16,17,72]. Lignin is formed by free radical polymerization of three major monolignol precursors, *p*-coumaryl, coniferyl, and sinapyl alcohols, which form the *p*-hydroxyphenyl, guaiacyl, and syringyl (in angiosperms) units in lignin. The monolignols are biosynthesized from phenylalanine through a series of enzymatic reactions in a metabolic grid consisting of at least 24 metabolites and 11 enzyme families. The pathway is regulated in a multilayer manner, encompassing transcriptional, post-transcriptional, and post-translational modifications, protein-protein interactions, and metabolic regulations[72]. A hierarchical transcription regulatory network, involving transcription factors like MYBs and NACs, controls secondary cell wall formation in plants by coordinating the spatial-temporal expression of lignin biosynthetic genes[73–76]. Non-coding RNAs (ncRNAs) like miRNAs and lncRNAs[77,78], along with alternative splicing mechanisms[77], further regulate lignin biosynthesis by targeting TFs and enzyme-encoding genes involved in the pathway. Protein-protein interactions[79] and post-translational modifications[80], such as phosphorylation and ubiquitination, modulate the metabolic fluxes, and alter the activity or stability of enzymes, resulting in changes in lignification. Understanding how these multilayer regulations respond to developmental and environmental stimuli is crucial for engineering plants with modified lignocellulosic properties, which could improve biomass utilization for industrial applications.

Given the importance of lignin for biomass conversion efficiency, its modification has been a major focus for lignocellulosic feedstock engineering[81]. Downregulation of monolignol biosynthesis genes can lead to significant changes in lignin content, subunit composition, and lignin–carbohydrates linkages. These alterations have been demonstrated to enhance biomass deconstruction, saccharification, and fermentation efficiencies in numerous energy crops and woody species such as *Populus spp.*, *Pinus spp.*, *Panicum virgatum*, *Miscanthus sinensis*, and *Mendicago sativa*[82]. In *P. virgatum* and *M. sinensis*, the downregulation of *aldehyde O-methyltransferase* (*AldOMT*) led to a reduction in lignin content by up to 15% and 63%, respectively[83,84]. Low lignin transgenic *P. virgatum* showed up to 38% increase in saccharification efficiency and 42% increase in ethanol yield per unit of biomass[83]. *In P. trichocarpa*, Wang et al. generated ~2000 engineered *P. trichocarpa* trees, downregulating 21 monolignol genes individually, in pairs, or entire gene families. Some transgenic lines showed substantial changes in wood properties, leading to improvements in glucose and xylose release by up to 351% and 828%, respectively, compared to wild-type trees[17]. These transgenic poplars also increased biomass solubilization via microbial digestion by *Caldicellulosiruptor bescii*, from 20% in wild-type trees to 79% in transgenic trees, resulting in a 7.6-fold improvement in bioethanol production[52].

Modifications to lignin composition and its interactions with other cell wall components can lead to architectural changes in the plant cell walls. The heterologous expression of *feruloyl–coenzyme A monolignol transferase* (*FMT*) and *3-dehydroshikimate dehydratase* (*QsuB*) in *P. alba* × *grandidentata* resulted in the incorporation of non-canonical conjugate subunits into lignin[85,86]. These subunits introduce readily cleavable ester bonds in the lignin backbone and facilitate the deconstruction of feedstocks and subsequent release of sugars[85,86]. The overexpression of the *callose synthase* gene in *P. tremula* × *tremuloides* induces callose deposition in the secondary cell walls, which is not typically observed in most plant species. Callose deposition modulated cell wall porosity, water, and lignin contents, and increased the lignin–cellulose distance. These modifications ultimately resulted in a substantial reduction in biomass recalcitrance, evidenced by improvements in enzymatic hydrolysis[87].

Research on modifying cell wall polysaccharides has been less extensive than lignin. Perturbations to cellulose and hemicellulose biosynthesis have been achieved by manipulating secondary cell wall TFs, cellulose synthases (CesA), sucrose synthases (SuSy), and glycosyltransferases[88,89]. The downregulation of *glycosyltransferases* from the *GT8*, *GT43*, and *GT47* families decreases xylan content, the predominant hemicellulose in angiosperms, resulting in reduced feedstock recalcitrance in woody species[90–92]. In *P. virgatum* and *P. deltoides*, the downregulation of *Galacturonosyltransferase 4* (*GAUT4*), a key enzyme in pectin biosynthesis, reduced cell wall pectin content and the molecular weight of hemicelluloses, while altering lignin composition, lignin inter-unit linkages, and lignin-polysaccharide cross-linkages. These alterations in the plant cell wall likely synergistically contribute to the reduced feedstock recalcitrance[92,93].

Advances in feedstock modifications for biofuels and bioproducts

Despite notable advances in the genetic improvement of bioenergy and woody plants, challenges remain in developing lignocellulosic feedstocks with optimal bioprocessing, growth, and resilience to climate change. Altering the expression of cell wall-related genes often impairs growth and reduces biomass yield. Moreover, RNA interference (RNAi) and artificial microRNAs (amiRNAs) techniques to gene perturbation can lead to unpredictable outcomes, such as off-target effects or variability in transgene expression across different individuals and environmental conditions. These challenges contribute to inconsistent feedstock performance, restricting the practical application of trait improvements.

Additionally, most studies have concentrated on individual traits (e.g., cell wall composition), with less attention given to the understanding the broader impacts of how bioenergy trait modifications on plant development and resilience. Climate change is exposing plants to more frequent and severe abiotic stresses, such as prolonged droughts and nutrient limitations[94]. Rising temperatures, increased CO_2 levels, and extended droughts also have been shown to increase the spread of pathogens and the vulnerability of crops and trees to diseases[95]. These environmental stresses ultimately lead to reduced growth and higher plant mortality and are projected to reduce plant productivity and decrease the global planted area by 2–9%[96]. Furthermore, agricultural expansion and urbanization are forcing non-food crops and tree plantations into less fertile, non-arable lands, limiting water and nutrient availability[97,98]. Given the clear interplay between biomass recalcitrance and feedstock scalability, fine-tuning characteristics of resilience, growth, and bioprocessing are essential for practical applications (Fig. 1c).

Expanding lignocellulosic trait enhancement by genome editing

CRISPR-Cas genome editing technology has emerged as a powerful tool for inducing precise and heritable mutagenesis in lignocellulosic feedstocks, allowing for predictable modification of traits for bio-based utility. CRISPR-modified lignocellulosic feedstocks with loss-of-function mutations of single genes were successfully developed in energy crops and woody plants, leading to enhancements in feedstock composition[72,99], bioprocessing[72,100], engineered wood[101], growth[102], plant architecture[103] and resilience to climate stress[104]. However, the potential of CRISPR extends far beyond canonical single-gene loss-of-function mutations. Recent advancements in CRISPR now allow for more complex and powerful editing modalities, encompassing multiple-gene mutations, whole plants or tissue-specific alterations, the transcriptional regulation of thousands of genes, and even editing entire chromosomes. This versatility expands the scope of modifications possible for enhancing feedstocks, particularly in response to challenges posed by climate change (Fig. 2).

Targeted editing and spatial regulation in feedstock modifications

Tissue-specific CRISPR editing has been demonstrated in *A. thaliana*, *Lycopersicon esculentum*, and *Gossypium hirsutum* using tissue-specific promoters to drive Cas9 expression, enabling editing of specific cell types and tissues (e.g., stomatal lineage, fibers, root-caps, lateral roots and fruits)[105–108]. While this approach has not yet been demonstrated in bioenergy crops and woody feedstocks, it holds promise for enhancing woody tissues while preserving other cell types to preserve plant growth and development. Recent studies have demonstrated spatial modulation of lignin biosynthesis. Target suppression of *LTF1*, a transcriptional repressor of lignin biosynthesis, was achieved in a fiber-specific manner. Concurrent loss-of-function editing of *Cinnamoyl-CoA reductase* 1 (*CCR1*) while overexpressing a modified version of the same gene in vessel cells, resulted in significant improvements in wood bioprocessing and saccharification efficiency[16,109]. These findings highlight the potential for spatial regulation of lignin biosynthesis, avoiding disruptions to vessel lignification that could lead to vessel collapse and preventing plant developmental problems.

Targeted editing of regulatory sequences

Recent efforts have increasingly explored the mutagenesis of non-coding sequences to fine-tune gene expression patterns. Manipulating cis-regulatory elements within promoters or 5′UTR regions can yield diverse quantitative changes across traits of interest, from photosynthetic efficiency to disease resistance.

Introducing indels and sequence inversions into the 5′ untranslated region (UTR) of the *photosystem II* (PSII) *subunit S* (*OsPSBS1*) in transgene-free *O. sativa* CRISPR lines produced photoprotective phenotypes[110], opening the possibility of applying similar strategies across plant species to optimize photosynthetic efficiency and minimize energy loss during light transitions. Significant losses in CO_2 fixation during transitions from full light to shaded conditions are attributed to energy dissipation as heat by nonphotochemical quenching (NPQ)[111,112]. Overexpression of NPQ-related genes has been shown to enhance photosynthetic efficiency in transgenic *Nicotiana tabacum* and *Glycine max*, resulting in a 15% biomass increases and a 33% grain yield increase during light transition in greenhouse and field conditions[111,112]. However, unlike CRISPR-edited plants, this approach relied on transgene expression, which is susceptible to gene silencing or expression instability. By editing the regulatory sequences (e.g., suppressors) of other *NPQ* genes in combination with *PSII*, and extending this strategy to lignocellulosic feedstocks (NPQ genes are present in all plants[110]), could offer promising opportunities for improving yield and resilience under field environments.

Similarly, gene expression modulation through promoter editing has shown great potential in enhancing disease resistance in plants. *Xanthomonas spp*. use transcription activator-like effectors (TALEs) to transactivate susceptible host genes, promoting pathogen growth and symptom development[113–115]. Editing the promoter of the susceptibility gene *CsLOB1* by introducing indels to disrupt the bacterial effector binding elements has enhanced resistance to canker disease in CRISPR-edited lines of *Citrus sinensis* and *Citrus x paradisi*[113,114]. Alternatively, introducing TAL effector binding elements from susceptibility genes into the promoter of dysfunctional resistance genes has enabled inducible TALE-dependent resistance in *O. sativa*[115]. Critical cis-elements in the promoters of pathogen-responsive genes have been identified in *Populus ssp*[116]. By editing these *cis*-elements, introducing new copies, or disrupting transcriptional regulator sites, gene expression patterns can be modulated to enhance disease resistance in lignocellulosic feedstocks, offering another promising avenue for improving resilience.

Building on the strategies of editing regulatory sequences for gene expression modulation, recent innovations have expanded the potential of CRISPR-based systems to achieve more sophisticated modifications. One such approach involves fusing Cas9 or gRNA with transcriptional regulators (either activators or repressors) to simultaneously edit the genome and modulate gene expression[117]. The development of a CRISPR-Combo system exemplifies this dual functionality by enabling the concurrent activation of endogenous genes and targeted gene mutation[118]. This system was used to demonstrate enhanced plant regeneration and propagation of *P. alba* × *tremula* and *O. sativa* by activating morphogenic genes *WUS* and *WOX11* while editing a lignin biosynthesis gene. The multiplex functionality of CRISPR-Combo is a step towards more complex metabolic engineering and sophisticated modulation of signaling pathways that require simultaneous gene editing and activation.

Altogether, the integration of these techniques provides a versatile toolkit for optimizing plant traits through precise gene expression control.

Modulating genetic linkages for the inheritance of desired traits

Chromosome rearrangement is a promising approach for the development of feedstocks with novel traits. Plant attributes, including

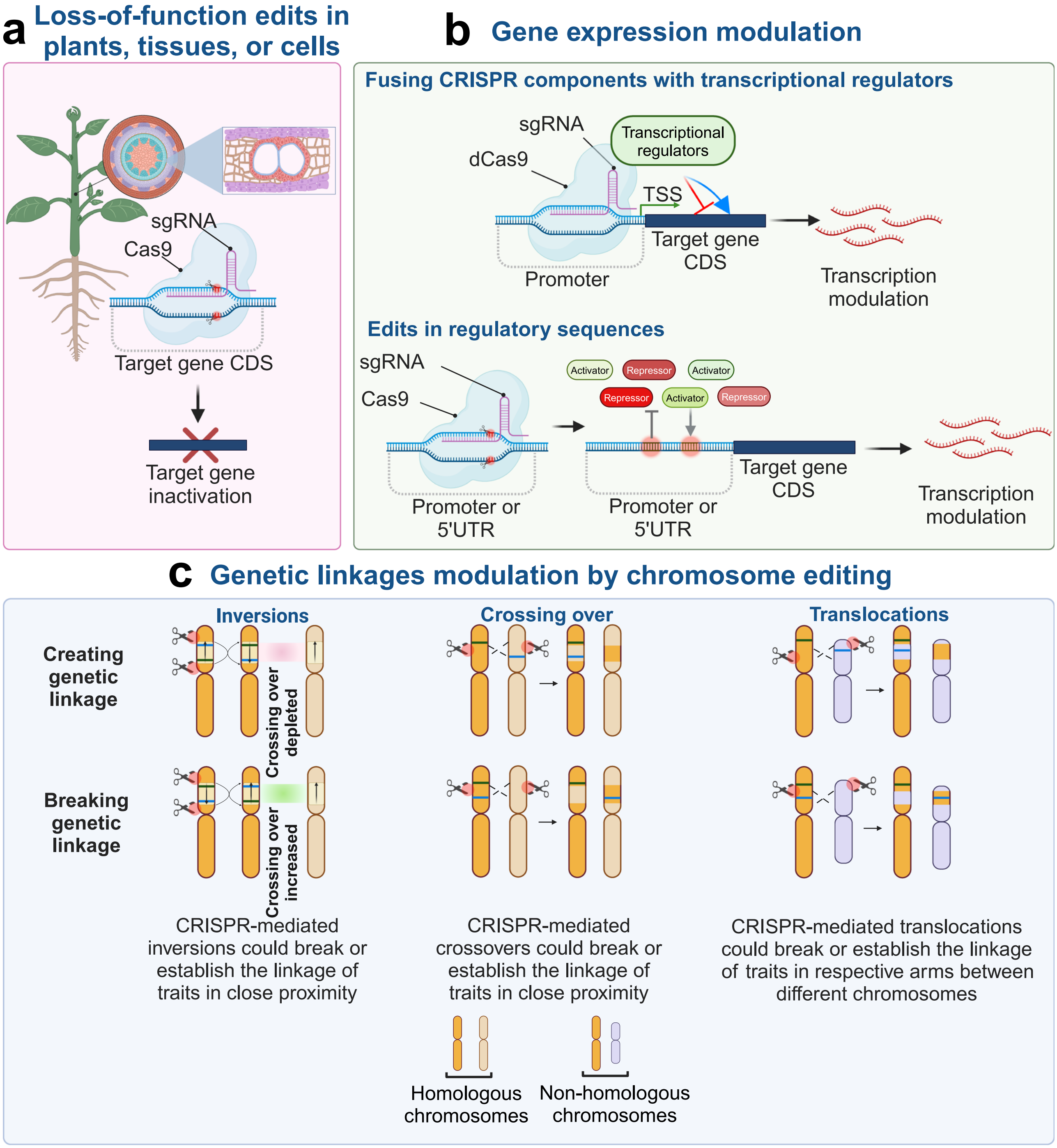

Fig. 2 | Advanced CRISPR-based genome editing technologies for expanding the range of possible lignocellulose feedstock modifications. **a** CRISPR-mediated gene knockout at the whole-plant, tissue-specific, or cell-type-specific levels. **b** Gene expression modulation achieved through transcriptional regulators fused to a catalytically dead Cas9 (dCas9) or by editing gene regulatory sequences. **c** CRISPR-mediated chromosome rearrangements to modulate genetic linkages for the inheritance of desired traits. CDS Coding sequence, UTR Untranslated region, TSS Transcription start site.

resilience to pathogens, adaptation to climate conditions, biomass yield, and quality, have been associated with single or multiple quantitative trait loci (QTLs) in different species, including woody plants like *Populus ssp.* and *Salix ssp.* and energy crops such as *M. sinensis* and *P. virgatum*[119,120]. The core genes associated with those traits are not always fully characterized, posing significant challenges for canonical approaches to target gene manipulation, especially at non-model plants like lignocellulosic feedstocks.

Modulating the genetic linkages between a gene or QTL and other QTLs is crucial for ensuring the inheritance of desired traits across generations. This is particularly relevant for woody species with long generation cycles, where this approach could support effective breeding programs by enabling the development of strategic progeny genotypes that achieve consistent phenotypic outcomes more rapidly. Chromosomal inversions, permutations, and translocations can disrupt or stabilize genetic linkage groups through physical proximity in specific chromosome regions[121]. In *A. thaliana*, the utilization of frequent-cutter restriction enzymes induced multiple DNA double-strand breaks across the genome, resulting in chromosome rearrangements (inversions and translocations) rates up to 17.4% and 66.6% in

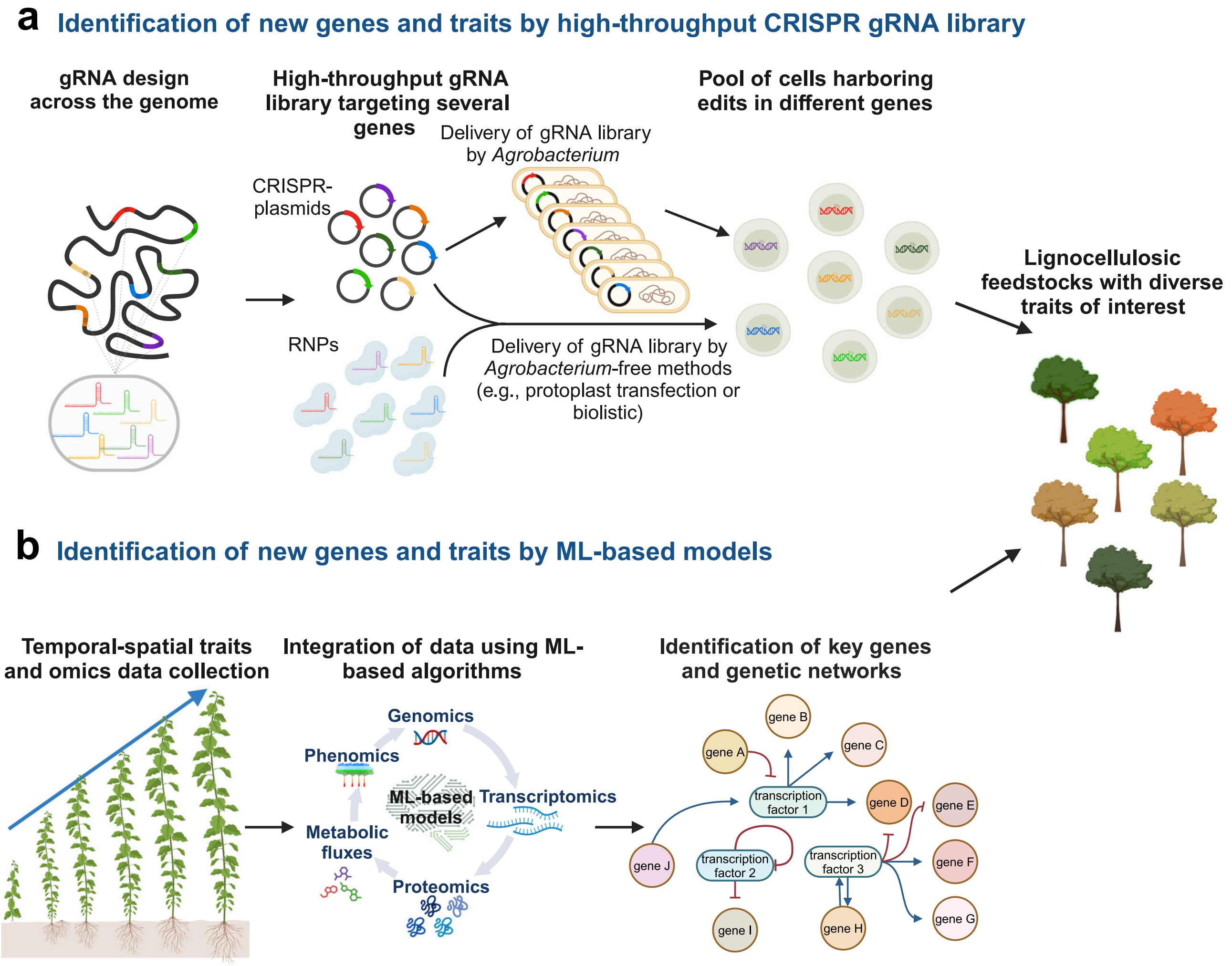

Fig. 3 | Identification of new genes and traits of interest for feedstock modifications. High-throughput CRISPR gRNA libraries targeting multiple genes, individually or in combination (**a**), along with the utilization of ML-based models (**b**), enable the identification of key genes and regulatory networks associated with desirable traits.

diploid and tetraploid plants, respectively. Chromosome rearrangements have been used to improve biomass yield and tolerance to high-salinity[122,123]. Once QTLs for traits of interest have been identified, we can now inform the manipulation of the plant genome precisely using CRISPR-Cas to induce chromosome rearrangement at the desired locations. The first CRISPR-Cas-mediated inversions were recently achieved in plants and involved heritable inversions of 75.5-Mb and >17-Mb-long chromosome fragments in *Zea mays* and *A. thaliana*[124,125], leading to suppressed meiotic recombination across nearly the entire chromosome in *A. thaliana*[125]. Similarly, targeted induction of heritable reciprocal chromosomal translocations was achieved in *A. thaliana* between chromosomes 1 and 2, and between 1 and 5, involving fragments of approximately 0.5 Mb and 1 Mb-long, respectively[126]. These pioneering studies offer optimism that comparable achievements may be replicated in energy crops and woody plants[121,124–126], potentially eliminating or stabilizing linkage drags to enhance growth, yield, resilience, and bioprocessing efficiency. However, challenges may arise for lignocellulosic species without adequate genotypic and phenotypic data, and more studies will be necessary to identify relevant QTLs.

Revealing complex genetic traits using CRISPR library and ML

High-throughput forward genetic screens are powerful tools for deciphering novel genes and functions without prior knowledge. Even in *P. trichocarpa*, the first woody species with a sequenced genome, the number of annotated genes validated by experimental data remains limited. The functions of many genes are still speculative, often inferred through comparative genomics with distant model plants like *Arabidopsis*, underscoring gaps in functional genomic studies related to economically and ecologically significant traits in lignocellulosic feedstocks.

Large-scale CRISPR gRNA library screening has been used for loss-of-function screens on a genome-wide scale to establish causal links between genotypes and phenotypes (Fig. 3a). Despite their potential, it has been underutilized in plants, limited to a few food crops and model species[127–132]. Remarkably, a library of gRNAs targeting 34,234 genes (83% of all genes) in *O. sativa* was generated, resulting in the production of 91,004 loss-of-function mutants[127]. *O. sativa* and *Z. mays* lines generated from CRISPR libraries exhibited phenotypic changes across several traits, including growth, plant architecture, leaf morphology, and disease susceptibility[127,128,132]. Since the regulation of complex plant traits are typically multigenic, a multiplex CRISPR library can generate mutants that harbor concurrent edits in multiple genes, uncovering phenotypes that are obscured by genetic redundancy or epistasis. In *A. thaliana*, a CRISPR library of 59,129 gRNAs targeting concurrently two to ten genes within a gene family were designed for over 16,152 genes. This library enabled the identification of novel mutant phenotypes masked by genetic redundancy[131]. These CRISPR screens were based on loss-of-function mutagenesis. However, it would be very useful to apply CRISPR-based gain-of-function screens for trait engineering, using CRISPR activation systems such as CRISPR-Act3.0[133]. High-throughput CRISPR library of lignocellulosic feedstocks could significantly advance breeding programs by increasing genetic diversity and enhance bioenergy traits. Notably, further research is critical for expanding the availability of genome sequences for more species, maximizing the potential of new genome editing technologies in lignocellulosic feedstocks.

ML is a powerful tool to rapidly uncover the non-linear relationship between genotypes and phenotypes and enable the identification of key genes and genetic networks involved in complex traits, when conventional genetic approaches fail to capture these regulatory complexities (Fig. 3b). In *P. trichocarpa*, a predictive model based on ML for lignin biosynthesis was used to explore 69,123 multigenic editing strategies to identify optimal gene targeting combinations for improving the bioprocessing characteristics of wood for kraft pulping. CRISPR-edited trees showed significant alterations in wood properties, resulting in more efficient fiber pulping, and could increase productivity on an industrial scale, while reducing CO_2 emissions from pulp mills, thereby supporting a more sustainable and efficient fiber bioeconomy[72]. Feedstocks with properties tailored for applications other than pulping, such as bio-based fuels and products can be generated by integrating advanced computational tools with CRISPR-based applications to address environmental, social, and industrial needs[134]. A new generation of predictive models will likely be crucial for enhancing predictability and advancing feedstock improvements by integrating multi-omics data with powerful ML-based algorithms while accounting for the complexity, plasticity, and dynamic spatial-temporal responses of plants to environmental stimuli[135].

Challenges and frontiers in lignocellulose utilization

CRISPR-Cas genome editing offers significant potential for identifying and targeting genes linked to valuable traits in lignocellulosic feedstocks. However, it faces notable challenges, primarily due to the variable feasibility of genetic transformation across different lignocellulosic species and genotypes. The most common method for transforming woody plants and energy crops is *Agrobacterium tumefaciens*-mediated transformation and particle bombardment[120,136]. Despite its effectiveness, *A. tumefaciens* infection is restricted to certain host species and genotypes[136]. Particle bombardment, though less genotype-specific, often introduces high copy numbers of DNA, and is overall inefficient. Another widely used method is PEG-mediated transformation of protoplasts (plant cells lacking cell walls) for the transient delivery of CRISPR-derived plasmids and ribonucleoprotein complexes (RNPs). With the plant cell walls removed, a major barrier to transformation is eliminated, allowing for high transformation rates and enabling precise, targeted changes introduced by CRISPR at desired locations. However, it requires well-developed protoplast regeneration systems.

Regeneration of transformed plant cells to entire plants through tissue culture techniques remains a major obstacle, as many species and genotypes show limited response to conventional hormone-based regeneration systems. This limitation hampers the broader application of techniques such as genome editing, particularly in diverse plant groups like lignocellulosics[137]. Recently, various approaches have been explored to overcome the genotype dependence and low transformation rates associated with the limited regeneration capacity of some plants, alongside public initiatives from the U.S. Department of Energy (DOE) to define transformation and editing needs and barriers focused on bioenergy crops[120]. The utilization of less genotype-dependent explant tissues (e.g, meristematic tissues), screening and engineering of *Agrobacterium* strains and other non-pathogenic bacteria, exogenous application of phytohormones to pretreat explants before transformation, and ectopic expression of genes encoding morphogenic transcription factors (e.g., *Bbm* and *Wus2*) were demonstrated to increase the substantially the transformation rates of recalcitrant genotypes and species[138]. Concomitantly, tissue culture-free transformation methods, such as electroporation, cell-penetrating peptides, carbon nanotubes, viral vectors, and inoculation *Agrobacterium* strains suspensions onto the wound sites also holds promise for delivering cargos directly into plant tissues without the need for in vitro plant regeneration, though they are not yet universally effective.

Enhancing transformation efficiencies in plant species now extends beyond traditional screening of culture media compositions and in vitro growth conditions[138]. Optimizing existing methods and developing new transformation techniques[138], combined with insights into how differentiated cells reprogram to achieve pluripotency or totipotency for regeneration[139], are essential for making genome editing technologies accessible across diverse plant species, and to provide sustainable lignocellulosic feedstock in a timely manner.

While efforts toward biofuel innovations continue to advance, exciting new frontiers are emerging, including renewable nanomaterials, advanced wood products, bioplastics, and platform chemicals. These innovations broaden the versatility of lignocellulosic materials, offering sustainable alternatives across various industries. The future utility of lignocellulosic biomass hinges not only on improving existing applications but also expanding into new, high-value sectors, paving the path for a circular bioeconomy.

New bioprocessing technologies are vital for industrial-scale implementation of lignocellulosic feedstocks. Improving pretreatment methods, enzyme recovery, and integrated biorefineries are essential to overcoming the economic and environmental challenges associated with utilizing lignocellulosic biomass. Life-cycle analyses demonstrated that these innovations could significantly reduce the carbon footprint of bio-based products, from sustainable aviation fuel to advanced nanomaterials. However, persistent challenges remain, including the removal of undesirable inhibitors, recovering valuable byproducts, and enhancing process efficiency to ensure the economic viability of biorefineries. Additionally, governmental policy and regulatory frameworks play a pivotal role in driving the large-scale adoption of these sustainable technologies. Without strong political endorsement and public support, scaling these innovations will remain constrained.

Modifications to cell wall composition have significantly improved bioprocessing efficiency in energy crops and woody plants. However, these feedstocks have primarily been tailored for biofuels and platform chemical production, with a relatively limited focus on developing sustainable biomaterials for broader applications, including medical, electronic, and structural materials. Recently, CRISPR-mediated mutagenesis tailoring wood composition by reducing lignin content has enabled the production of densified woody materials without the need for chemical delignification, a step typically required in traditional wood engineering processes. Engineered wood is increasingly being explored as a sustainable alternative to conventional structural materials like steel, cement, glass, and plastic. These materials, produced through a combination of genetic and wood engineering, have the potential to lower costs and support the development of a CO_2-negative bioeconomy by providing renewable, environmentally friendly alternatives to traditional materials[101].

In addition, more work is needed to ensure feedstocks thrive under changing climate conditions and elevated environmental stresses. As drought, nutrient deficiency, and pathogen infections intensify, new traits must be explored to enhance feedstock resilience. CRISPR-Cas technology offers promising tools for accelerating plant breeding by enabling precise mutagenesis of the genome. However, the potential of CRISPR-Cas remains to be broadly democratized across lignocellulosic feedstocks. Beyond canonical applications, CRISPR-Cas can be expanded to edit regulatory sequences, perform tissue-specific mutations, use chromosome editing to induce the inheritance of desired traits, or conduct library screening for gene discovery. Furthermore, CRISPR-derived precise genome editing techniques like base editors, prime editors, and homology direct repair will augment our capabilities to introduce desirable gain-of-function traits[140], which remain challenging to achieve with traditional CRISPR-Cas tools in plants. Additionally, beyond editing the genomic sequence, there are also opportunities to manipulate the transcriptome and epigenome by exploring the sophisticated CRISPR toolbox encompassing Cas proteins fused with diverse effectors such as transcriptional activators and repressors, as well as methylases and acetylases[117]. Similarly, integrating ML into breeding programs can accelerate the discovery of new genes and genetic networks, complementing these advanced CRISPR-Cas techniques.

Looking ahead, interdisciplinary research, technological innovation, and policy support will be critical in overcoming these obstacles and fully unlocking the potential of lignocellulosic biomass. Collaboration between academia, industry, and policymakers is essential to creating an environment that fosters the growth of this sector, at speed and at scale. As the world strives to meet climate goals and transition to more sustainable energy and materials sources, lignocellulosic feedstocks offer a promising and perhaps the only path for reducing reliance on fossil fuels and advancing a sustainable bioeconomy.

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Author contributions

D.B.S., J.P.W., and R.B. contributed to the manuscript's conception, design, preparation, and review. N.L., H.S., X.J., B.M.M., K.L., and C.C.V. contributed to manuscript preparation and review. All agreed on the final content of the manuscript.

Competing interests

The authors declare no competing interests.

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