

Review

Integration of advanced biotechnology for green carbon

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ABSTRACT

Carbon neutralization has been introduced as a long-term policy to control global warming and climate change. As plant photosynthesis produces the most abundant lignocellulosic biomass on Earth, its conversion to biofuels and bioproducts is considered a promising solution for reducing the net carbon release. However, natural lignocellulose recalcitrance crucially results in a costly biomass process along with secondary waste liberation. By updating recent advances in plant biotechnology, biomass engineering, and carbon nanotechnology, this study proposes a novel strategy that integrates the genetic engineering of bioenergy crops with green-like biomass processing for cost-effective biofuel conversion and high-value bioproduction. By selecting key genes and appropriate genetic manipulation approaches for precise lignocellulose modification, this study highlights the desirable genetic site mutants and transgenic lines that are raised in amorphous regions and inner broken chains account for high-density/length-reduced cellulose nanofiber assembly *in situ*. Since the amorphous regions and inner-broken chains of lignocellulose substrates are defined as the initial breakpoints for enhancing biochemical, chemical, and thermochemical conversions, desirable cellulose nanofibers can be employed to achieve near-complete biomass enzymatic saccharification for maximizing biofuels or high-quality biomaterials, even under cost-effective and green-like biomass processes *in vitro*. This study emphasizes the optimal thermal conversion for generating high-performance nanocarbons by combining appropriate nanomaterials generated from diverse lignocellulose resources. Therefore, this study provides a perspective on the potential of green carbon productivity as a part of the fourth industrial revolution.

1. Introduction

Excessive consumption of fossil fuels, in parallel with its environmental consequences, has become a stern hindrance to economic expansion worldwide. Therefore, the generation of sustainable biofuels and valuable bioproducts is considered a promising solution [1–5]. Plant photosynthesis produces enormous amounts of lignocelluloses through the uptake of CO₂, providing a sustainable path for carbon fixation of approximately 50 billion tons per year [6–8]. Consequently, lignocelluloses can be converted into renewable biofuels and valuable biomaterials to reduce net carbon release [9–12]. Therefore, attention is increasingly being paid to produce carbon-fixed materials with economic benefits from diverse plant biomass resources such as biochar and nanocarbon [13–15].

Lignocellulose recalcitrance inevitably leads to expensive processes coupled with secondary waste release into the environment [16]. In principle, lignocellulose recalcitrance arises from the intricate structures and miscellaneous functions of plant cell walls, and thousands of genes involved in cell wall polymer biosynthesis and network construction have been identified [17–19]. Lignocellulose recalcitrance can be reduced by the genetic manipulation of one or more genes in bioenergy crops [20–22]; therefore, the genetic modification of lignocellulose is a valuable green-like strategy for cost-effective cellulosic ethanol conversion and value-added bioproduction with zero biomass release.

Provided that biofuel conversion is effective in reducing net carbon liberation, biocarbon assembly has more advantages [23–25]. Carbon

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materials, such as graphene, carbon nanotubes, graphdiyne, and full-ene have been considered for use in the fields of energy [26–28], medical sensing, chemical catalysis [29–32], environmental remediation [33], and others [34,35]. Nevertheless, these carbon materials are mostly generated from petroleum oil and coal, and their processes usually require harsh and energy-intensive conditions, including arc discharge [36], laser ablation [37], chemical vapor deposition [38], and pyrolysis [39–43]. Alternatively, lignocellulose-based biocarbon production is economical and environmentally friendly in terms of the carbon cycle and carbon neutrality.

Here, we review recent progress in advanced biotechnology for biofuels and bioproduction. The study presents five portions consisting of diverse biomass resources in agriculture and forestry, desirable lignocellulose substrates from the genetic engineering of bioenergy crops, near-complete biomass saccharification for cost-effective biofuels, efficient catalysis for well-performing biocarbon, and the perspective of integrated biotechnology for green carbon. This study proposes a novel strategy for the full utilization of lignocelluloses as cost-effective biofuels and high-value biocarbon.

2. Diverse biomass resources in dedicated bioenergy crops

During their evolution, plant species have had to construct plant cell walls with complicated structures and multiple biological functions, accounting for the characteristic lignocellulose recalcitrance [44,45]. As the most abundant bioresource on Earth [46,47], lignocelluloses are derived from three major types of plants: agricultural crops, forest trees, and herbaceous plants (Fig. 1). Besides the food supply, agricultural crops can provide enormous lignocellulose-rich straws, and rice, wheat, and maize cover approximately 70% of the total agricultural lignocellulose residues worldwide [48]. In comparison, forest trees, such as poplar, willow, pine, and eucalyptus are considered ideal bioenergy plants because of their fast growth, low land occupation, high disease resistance, and low management costs [49]. Among the herbaceous plants, *Miscanthus* and switchgrass are the leading lignocellulose feedstocks because of their high biomass yield, good adaptation to various environments, and low water and fertilizer requirements [50].

Plant cell walls are mainly composed of 30%–50% cellulose, 20%–40% hemicellulose, and 15%–35% lignin [51]. The enzymatic saccharification of agricultural crop straws for biofuel production is highly efficient because of the relatively high proportions of wall

polysaccharides in this material, and the remaining residues rich in lignin favor the production of biosorbents and biochemicals [52–55]. For comparison (Fig. 1), forest trees are primarily used for the productions of functional biomaterials and biocarbon because of their highly crystalline cellulose and rich lignin deposition [56,57], whereas herbaceous plants, which are subject to diverse lignocellulose compositions, are selected for both biofuels and bioproducts [58–60]. Although diverse biomass resources are available, desirable lignocelluloses with low recalcitrant properties for high enzymatic saccharification remain to be identified.

3. Biological synthesis of desirable lignocellulose

To obtain the desired lignocellulose, genetic engineering of bioenergy crops has been performed to improve the cell wall recalcitrance in multiple plant species (Table 1). However, it is challenging to maintain the inherent characteristics of plant cell walls, which are primarily designed to provide mechanical strength and protection against environmental stress [61,62]. Therefore, the identification of appropriate genes is a vital step in the genetic engineering of plant cell walls in bioenergy crops, as described below.

3.1. Synthesis of cellulose nanofibers at high density

Cellulose is composed of β -1,4-glucan chains that are assembled in parallel to form microfibrils via inter- and intramolecular hydrogen bonds and other forces (Fig. 2) [106]. Cellulose synthase (CesAs) genes have been identified in various plant species, including *Arabidopsis*, rice, barley, and *Populus* (Table 1). In rice, OsCESA1, OsCESA3, OsCESA8, and other OsCESAs have been shown to form the CESA complexes for cellulose biosynthesis in primary cell walls, whereas the essential isoforms involved in secondary cell wall formation are OsCESA4, OsCESA7, and OsCESA9 [107]. As the macromolecular structure of CESA complexes is favored for clustering of β -1,4-glucan chains to form crystalline microfibrils, the crystalline property of cellulose is a major factor in the recalcitrance of lignocellulose to biomass enzymatic saccharification [19,108].

In terms of the CesAs gene family identified, the overexpression of CesA6-like genes can increase biomass yield by enhancing primary cell wall biosynthesis in plants, but lignocellulose recalcitrance is not significantly improved in transgenic *Arabidopsis* [59]. Although the overexpression of other CesAs may reduce lignocellulose recalcitrance, it causes plant growth defects and biomass yield penalties in different plant species [109,110]. Alternatively, site mutation of the *OsCesA9* gene cannot only maintain plant growth and raise biomass yield in a natural rice *Osfc16* mutant, but also significantly reduce lignocellulose recalcitrance for largely enhanced enzymatic saccharification, which is attributed to a reduction in the cellulose crystallinity index (CrI) and degree of polymerization (DP), two major factors affecting lignocellulose recalcitrance [108]. Recently, advanced atomic force microscopy (AFM) technology was developed to observe length-reduced cellulose nanofibers (CNFs) assembly, which is accountable for high-density amorphous/non-crystalline cellulose chains in the *Osfc16* mutant, providing direct evidence of the amorphous chains as breakpoints for initiating and completing cellulose enzymatic hydrolysis or chemical catalysis of lignocellulose conversion (Fig. 2) [111]. By performing site-specific mutations and knockouts of OsCESA4, A7, and A9 isoforms in rice, it was confirmed that the number of amorphous regions and inner broken chains increases in the mutants that are effective for biomass enzymatic saccharification and nanomaterial conversion (Table 1), suggesting a novel strategy for high-quality and diverse bioproduction by integrating precise genetic modification of cellulose with efficient biomass processing technology. The amorphous regions and inner-broken chains may cause efficient CO₂ penetration into plant cell walls to enhance plant photosynthesis capacity, which can explain why biomass yields are increased in the site mutants. Hence, despite the reduced cellulose levels, the site mutants have significantly increased hemicellulose content,

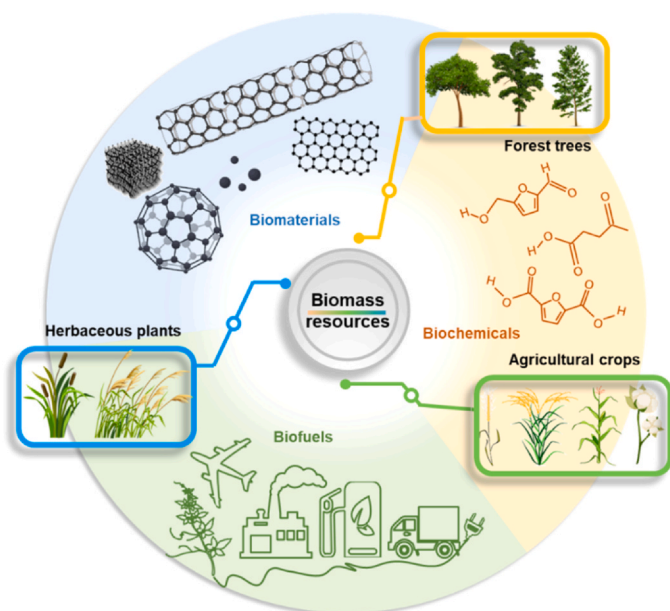


Fig. 1. An overview of the three major biomass resources for the productions of sustainable biofuels and valuable bioproducts.

Table 1

Genetic engineering of bioenergy crops for reducing lignocellulose recalcitrance.

Gene	Approach	Phenotype	Reference
<i>AtCesA2</i> , <i>AtCesA5</i> , <i>AtCesA6</i>	Overexpression	Increase cellulose content	[63]
<i>AtCesA1</i> , <i>AtCesA3</i>	Point mutations	Reduce cellulose content and crystalline index; increase enzymatic digestibility	[64]
<i>BdCesA4</i> , 7	Single gene mutations	Reduce cellulose content and crystallinity index; affect growth; reduce biomass yield	[65]
<i>OsCesA4</i>	Point mutation	Reduce cellulose content; affect growth; reduce biomass yield	[66]
<i>OsCesA7</i>	Mutations by insertion of <i>Tos17</i>	Reduce cellulose content; affect growth; reduce biomass yield	[67]
<i>AtGH9C2</i>	Downregulation	Reduce cellulose content and crystallinity index; increase biomass yield	[68]
<i>OsGH9B1</i> , <i>OsGH9B3</i>	Overexpression	Reduce cellulose degree of polymerization and lignocellulose crystallinity index; increase saccharification efficiency	[69]
<i>AtCel1</i>	Gene silencing	Reduce cellulose content; affect growth; reduce biomass yield	[70]
<i>GhCOBL9A</i>	Overexpression	Increase cellulose content; increase biomass yield	[71]
<i>AtKORRIGAN1</i>	Point mutation	Reduce primary cellulose content; increase crystallinity index; affect growth; reduce biomass yield	[72]
<i>OsMYB103L</i>	Overexpression	Increase secondary wall thickness	[73]
<i>AtRX10G283D</i> , <i>AtRX10E293Q</i> (<i>GT47</i>)	Point mutation	Reduce xylose content; increase hexose/pentose ratio; increase enzymatic digestibility	[74]
<i>AtMUR3/AtMURUS3</i> (<i>GT47</i>)	Point mutation	Reduce xyloglucan; reduce biomass yield	[75]
<i>AtESK1</i> (<i>DUF231</i>)	Point mutation	Reduce xylan acetylation; affect growth; reduce biomass yield; increase enzymatic digestibility	[76]
<i>AtTBL129</i>	Point mutation	Reduce acetate content; reduce enzymatic digestibility	[77]
<i>AtGXMT</i> (<i>DUF579</i>)	Point mutation	Reduce content of 4-O-methyl-GlcA substituents; increase enzymatic digestibility	[78]
<i>AnAXE1</i>	Overexpression	Reduce xylan content; cell wall polymer composition remains unaltered; increase enzymatic digestibility	[79]
<i>OsXAX1</i> (<i>GT61</i>)	Point mutation	Reduce xylose, ferulic, and coumaric acid content; reduce biomass yield; increase enzymatic digestibility	[80]
<i>AtAXY9</i> (<i>TBL</i>)	Gene mutation	Decrease xyloglucan content; reduce biomass yield	[81]
<i>XynB</i> , <i>ThiXynB</i> (<i>GH11</i>)	Point mutation	Affect growth; increase enzymatic digestibility	[82]
<i>AtHCT</i> (<i>At5g48930</i>)	RNAi	Repress lignin synthesis and reduce biomass yield	[83]
<i>CsHCT-1</i> , <i>CsHCT-2</i>	Overexpression	Increase phenolic acids content; increase lignin content	[84]
<i>BdHCT-1</i> , <i>BdHCT-2</i>	RNAi	Reduce lignin levels	[85]
<i>PtC3'H3</i>	Point mutation	Reduce total lignin content; increase H lignin monomer content	[86]
<i>PtC3H3</i>	RNAi	Reduce total lignin content; increase H lignin monomer content	[87]
<i>EgrC3'H3</i>	Downregulation	Reduce total lignin content	[88]
<i>EgrC4H1</i>	Downregulation	Reduce total lignin content	[88]
<i>BnF5H-1</i> , <i>BnF5H-4</i> , <i>BnF5H-6</i> , <i>BnF5H-7</i>	Point mutation	Decrease S/G lignin compositional ratio	[89]
<i>AtF5H</i>	Overexpression	Decrease G lignin monomer content	[90]
<i>OsCCR9</i>	Overexpression	Increase lignin content	[91]
<i>OsCCR9</i>	Point mutation	Reduce lignin content	[91]
<i>MdCCR</i>	Overexpression	Increase lignin content	[92]
<i>MdCCR</i>	Transient gene inhibition	Reduce lignin content	[92]
<i>OsCCoAOMT-1</i> , <i>OsCCoAOMT-2</i>	Single gene mutations	Reduce lignin content	[93]
<i>ZmCoA</i>	RNAi	Reduce lignin content	[94]
<i>brm12</i>	Single gene mutations	Reduce lignin content	[95]
<i>CsCSE-1</i>	Overexpression	Increase expression of <i>CsLAC</i> , <i>CsCOMT</i> , <i>CsCCR</i> , <i>CsCAD</i> ; increase lignin content	[96]
<i>AtCSE</i>	Single gene mutations	Reduce lignin content	[97]
<i>MOMT4</i> , <i>MOMT9</i>	Overexpression	Decrease G and S lignin monomer content	[98]
<i>FuPAL1</i>	Overexpression	Increase lignin content	[99]
<i>GhACL30</i>	Gene silencing	Decrease lignin and S lignin monomer content; increase G monomer content and G/S lignin ratio	[100]
<i>OsPMT-1</i> , <i>OsPMT-2</i>	Point mutation	Alterations in S/G lignin compositional ratios and inter-monomeric linkage patterns	[101]
<i>MSBP-1</i> , <i>MSBP-2</i>	RNAi	Impair stability and activity of the <i>C3H</i> , <i>C4H</i> , <i>F5H</i> ; reduce lignin content	[102]
<i>AsFMT</i>	Overexpression	Increase ferulic acid conjugation, ester bonding, and β -ether bond degradation; increase enzymatic digestibility	[103]
<i>PvMYB4</i>	Overexpression	Reduce lignin G and S unit content; increase enzymatic digestibility	[104]
<i>OsSWN2S</i>	Overexpression	Reduce xylose content; affect growth; increase enzymatic digestibility	[105]

leading to many more pentose sugars being determined in biomass pretreatment and sequential enzymatic hydrolysis of crop straws. In terms of the accumulation of soluble sugars in rice site mutants and other crop mutants [53,59,112], they are directly extractable and fermentable for cost-effective bioethanol production, which should be mainly derived from excess hexoses that are not used in cellulose biosynthesis, revealing a potential genetic engineering approach for altered carbohydrate metabolism and carbon partitioning regulation in bioenergy crops.

Furthermore, the overproduction of endo- β -1,4-glucanase (EG II) secreted by fungi has also led to slightly affected plant growth and

greatly enhanced saccharification under mild chemical pretreatments in transgenic rice lines, which suggests length-reduced CNFs assembly scaled by the amorphous cellulose chains raised on the surface cellulose microfibrils [113,114]. Similar findings have been observed in transgenic rice lines that exhibit overexpression of native *OsGH9B1* and *OsGH9B3* genes [69,115] or down-regulation of the *OsMYB103L* transcription factor [116]. As site mutations of *CesAs* and dual expression of other genes could alter cellulose biosynthesis for length-reduced CNFs assembly (Table 1), it would be interesting to determine an integrated biotechnology strategy for optimal CNFs substrates by performing

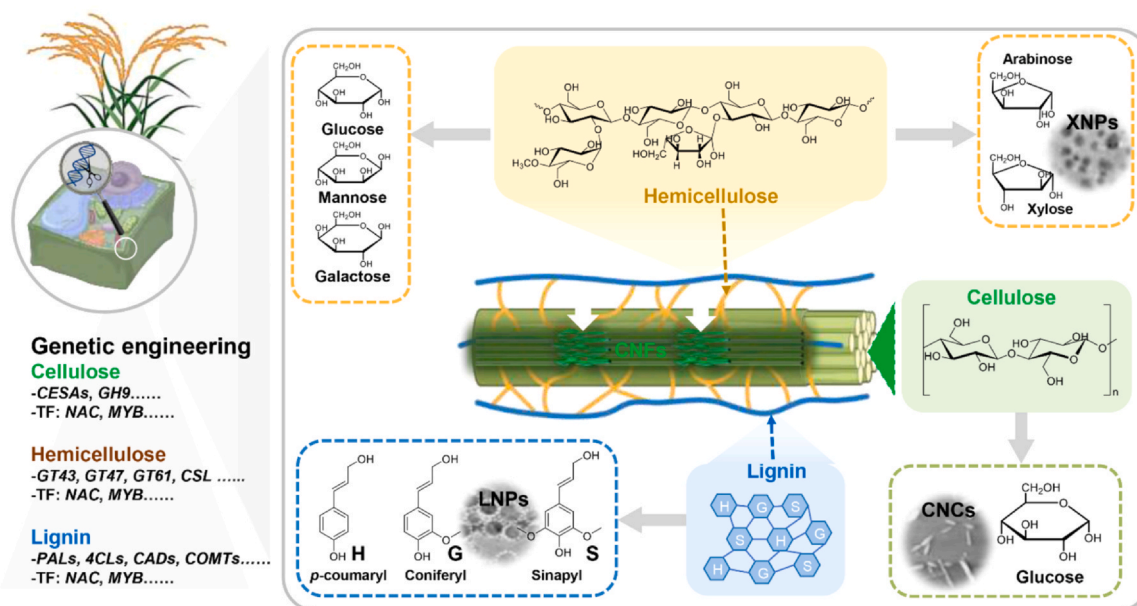


Fig. 2. Precise lignocellulose modification of bioenergy crops through genetic engineering. Amorphous cellulose density, which is accountable for length-reduced cellulose nanofibers (CNFs) assembly *in situ* and dimension-lessen nanomaterial (CNCs, XNPs, LNPs) production *in vitro*, is upgraded. The amorphous cellulose regions are indicated by white arrows scaled for CNF length. CNCs, cellulose nanocrystals; XNPs, xylan nanoparticles; LNPs, lignin nanoparticles. The representative genes applicable for optimal genetic manipulation are indicated as shown in Table 1.

classic genetic crossing among these mutants and transgenic lines or genetic manipulation of multiple genes in future studies.

3.2. Synthesis of xylan with high-branched arabinose

Hemicellulose is composed of pentoses (xylose and arabinose), hexoses (mannose, glucose, and galactose), and sugar acids (4-O-methyl-glucuronic, galacturonic, and glucuronic acids). As the major type of hemicellulose, xylan is composed of the β -(1,4)-xylopyranose backbone substituted with arabinose and small amounts of other monosaccharides in plant cell walls, which are associated with cellulose microfibrils and lignin to form a complicated cross-linking network (Fig. 2) [117]. Given that xylan deposition reduces cellulose crystallinity for high-biomass enzymatic hydrolysis [118], arabinose chains branched at the minor non-KOH-extractable xylan have recently been assumed to interact tightly with the amorphous regions of cellulose microfibrils [111,119], which may play an important role in CNFs assembly, as described above.

With respect to xylan biosynthesis, two significant glycosyltransferase family genes have been identified for the elongation of xylan backbone chains, i.e., GT43/IRX9, 14 and GT47/IRX10 [74,120]. In contrast, the GT61/XAT and GT8/GUX enzymes catalyze side-chain substitutions on xylan [80,121] (Table 1). As overexpression of GT61 genes may enhance the degree of arabinose substitution in xylan for high biomass enzymatic saccharification, transgenic lignocellulose substrates could be used to determine whether the branched arabinose chains of nano-xylan are entrapped in the amorphous regions of cellulose microfibrils, which may aid in determining how xylan biosynthesis regulates CNFs assembly in plant cell walls. It should also reveal an integrated biotechnology for the generation of optimal cellulose nanocrystals (CNCs) and xylan nanoparticles (XNPs) by genetic co-manipulation of CesAs and GT61 genes.

3.3. Synthesis of lignin with low S/G ratio

Lignin is a heteropolymer consisting of three major phenylpropane units joined by carbon–carbon and aryl–ether linkages (Fig. 2). Since lignin is a major polymer in plant secondary cell walls, lignin deposition is a crucial factor in lignocellulose recalcitrance [122] because it provides impermeability and protection against microbial invasion and

enzymatic hydrolysis [123]. Lignin is derived from the polymerization of its precursors, *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, which form three main monolignols, i.e., *p*-hydroxyphenyl/H, guaiacyl/G, and syringyl/S [124,125]. Therefore, lignin biosynthesis requires multiple types of enzymes, including phenylalanine ammonia lyase (PLA), shikimate hydroxycinnamoyl transferase (HCT), caffeoyl-CoA 3-O-methyltransferase (CCoAOMT), 4-coumarate-CoA ligase, coumarate 3-hydroxylase (C3H), cinnamate 4-hydroxylase (C4H), caffeic acid/5-hydroxyferulic acid O-methyltransferase (COMT), cinnamyl alcohol dehydrogenase (CAD), ferulate 5-hydroxylase (F5H), and cinnamoyl-CoA reductase (CCR), to synthesize three monomer units.

Genetic engineering has been widely used in plants to affect lignin biosynthesis by reducing lignin levels and altering monolignol proportions or their interlinkages (Table 1). However, transgenic crops mostly exhibit defects in growth and biomass yield owing to severely reduced lignin levels and affected cell wall integrity [126]. To address this issue, site mutation of a specific region in exon 3 of *OsCAD2* has caused a slightly reduced lignin levels using CRISPR/Cas9 editing technology; however, it significantly improves lignocellulose recalcitrance by reducing the lignin S/G ratio and increasing cell wall porosity [127,128]. As G monomers are mainly involved in interlinking with xylan and S monomers are mostly assembled for lignin chain elongation, genetic reduction of the S/G ratio is advantageous for the effective generation of lignin nanoparticles (LNPs) or efficient co-extraction of hemicellulose-lignin complexes (Fig. 2), leading to valuable nanomaterial production or high biomass enzymatic saccharification under mild chemical processes [128,129].

3.4. Construction of dynamic wall networks

Wall polymers are coordinately deposited and interlinked into wall networks (Fig. 2), and this process is dynamically mediated by several transcription factors, such as MYB, NAC, SWN, VND, and SND (Table 1). Although overexpression or silencing of these transcription factor genes can significantly cause defective plant growth and reduced biomass yield, site-specific mutations in MYB results in improved biomass digestibility with a slight impact on plant growth. Whether the integration of two or more site mutants of appropriate transcription

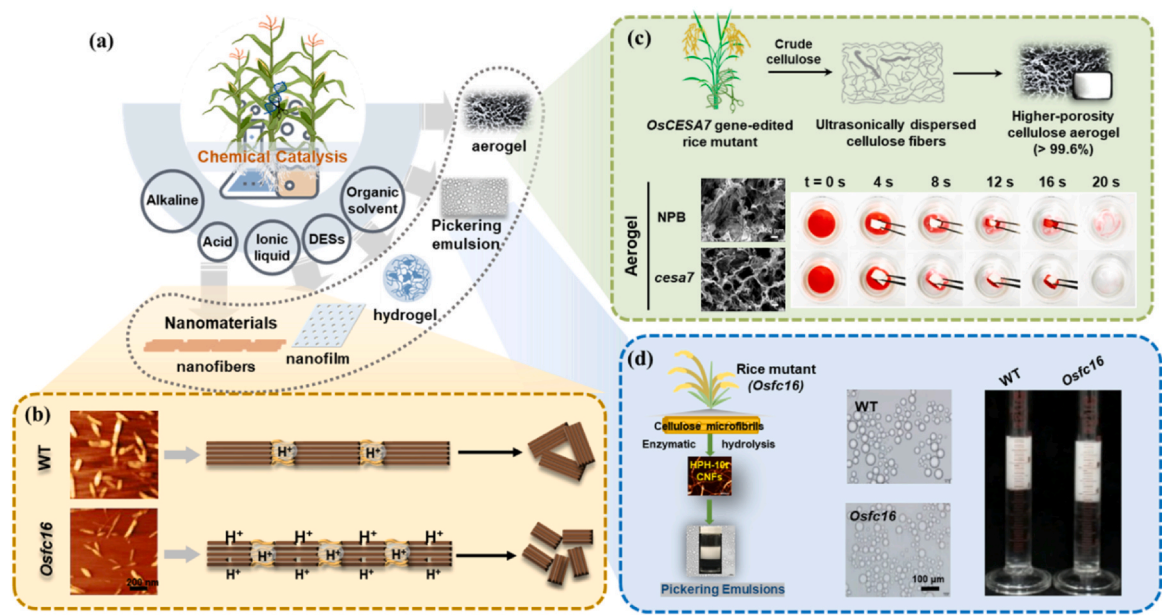


Fig. 3. Combined biotechnology for the diverse bioproducts from desirable lignocelluloses. (a) Conventional and emerging chemical processing of genetically modified lignocellulose for the valuable nanomaterials; (b) Acidic catalysis of CNFs for size-reduced CNCs using the rice *OsCESA9* site mutant. Reproduced with permission [111]. Copyright 2023, The authors, Springer Nature; (c) Combined physical and chemical treatments of modified lignocellulose to generate high-porosity cellulose aerogel for efficient oil adsorption (stained in red) using the rice *OsCESA7* site mutant. Reproduced with permission [153]. Copyright 2023, Elsevier; (d) CNFs are generated as an excellent stabilizer for high-quality Pickering emulsions in a rice natural site mutant (*Osf16*). Reproduced with permission [154]. Copyright 2022, RSC.

factors would minimize lignocellulose recalcitrance with a slight impact on plant growth and biomass yield remains an open question.

Construction of wall networks can also be modified by genetically altering wall polymer interlinkage styles [130], such as ester-, ether-, and C–C bonds, but the difficulty of targeting specific linkages among wall polymers and avoiding the consequent impacts on plant cell wall integrity and biomass production are issues that persist. Alternatively, as xylan plays a crosslinking role in wall network construction, xylan post-modification may slightly alter wall integrity and plant growth and increase biomass porosity for reduced recalcitrance by using specific promoters for genetic manipulation of glycoside hydrolase (GHs) family genes. In addition, it remains challenging to integrate distinct site mutants and transgenic lines to enable cost-effective biofuel conversion and high-value bioproduction using green-like processes.

4. Chemical catalysis of desirable lignocellulose for the diverse nanomaterials

Although mild and green-like pretreatments are sufficient to achieve near-complete biomass enzymatic saccharification to maximize biofuel production in genetic mutants and transgenic crops, desirable lignocelluloses are increasingly considered to efficiently generate functional nanomaterials, and the lignin-rich residues remaining from biofuel refineries are particularly applicable for value-added bioproducts with zero biomass release (Fig. 3; Table 2). However, it has become more important to develop advanced technologies for specific bioproductions by employing an appropriate lignocellulose substrate at a relatively low cost, as described below.

Table 2
Conventional and green-like biomass pretreatments used for the distinct reduction of lignocellulose recalcitrance.

Pretreatment	Chemicals	Function	Advantage	Disadvantage	Reference
Acid	Inorganic acids including H ₂ SO ₄ , H ₃ PO ₄ , HNO ₃ , and HCl; organic acids such as CH ₃ COOH, HCOOH, and HOOCOOH	Hemicellulose and lignin fractionation	Lower reaction time; lower costs	Environmental pollution; high water consumption; formation of inhibitory by-products	[131–133]
Alkaline	NaOH, KOH, Ca(OH) ₂	Lignin and hemicellulose removal	Cause less sugar degradation than acid pretreatment; higher efficiency in lignin removal	Not easy to recycle; not environmentally compatible; high water consumption	[131,132,134]
Organic solvent	Ethanol, methanol, acetone, glycols, and phenols	Lignin removal	It allows their recycling and reuse	Solvents need to be separated, recovered, and reused because of their high cost	[135]
Ionic liquid	Cholinium lysinate, ethanolamine acetate	Reduced cellulose crystallinity and partial removal of hemicellulose and lignin	Mild operating conditions; efficient lignin recovery; easy to recover and recycle	High cost owing to complex synthesis and purification; poor biodegradability; inhibitory effects on enzyme activity	[136,137]
Deep eutectic solvents	Choline chloride and lactic acid, ChCl/Glycerol	Fractionation of hemicellulose and partial removal of lignin	Green solvent, biodegradable and biocompatible; low energy requirement	Low stability under high temperature	[138,139]

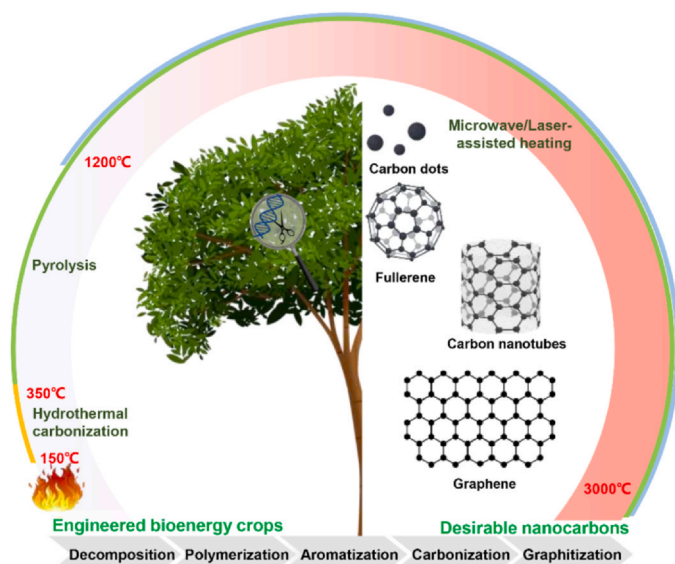


Fig. 4. Mechanisms of biomass carbonization using genetically modified lignocellulose.

4.1. Conventional chemical processing

Classic chemicals, such as acids, alkalis, and organic solvents, have been broadly utilized for lignocellulose pretreatment. In principle, acid pretreatment uses sulfuric, phosphoric, and acetic acids to break the van der Waals forces and hydrogen bonds of hemicellulose [140,141], whereas alkali pretreatment is effective for the co-extraction of lignin and hemicellulose with potassium, sodium, ammonium, and calcium hydroxides [142]. However, because of lignocellulose recalcitrance, alkali and acid pretreatments mostly require high-temperature incubation and a large dosage input, which favors the formation of toxic compounds, such as levulinic, acetic, furfural, formic, and furfural acids, as well as phenolic and aliphatic hydroxyl groups [143–145]. Organic solvents, such as methanol, ethanol, acetone, and ethylene glycol, are generally used for organosolv pretreatment. Although organosolv is an effective pretreatment method, it has the disadvantages of high cost, volatility, flammability, and difficulty in recovery, thereby increasing the overall process energy consumption and costs [146].

4.2. Green-like chemical processing

The concept of “green chemistry” has attracted attention in the development of applicable technologies for carbon neutralization and environmental protection [147]. To achieve this goal, natural materials should be treated more efficiently by avoiding the use of toxic and hazardous chemicals, with less waste released into the environment. Recent advances in chemistry have led to the development of environmentally friendly and sustainable alternatives such as ionic liquids (ILs) and deep eutectic solvents (DESs). ILs are green alternatives to volatile organic solvents, whose physicochemical characteristics (polarity, conductivity, viscosity, and hydrophobicity) can be tailored by adding various combinations of cationic and anionic components [148]. More recently, imidazolium-based ILs have attracted attention [149] because the dissolution of cellulose and lignin during biomass pretreatment is affected by both anions and cations. High-purity cellulose can be collected by the addition of an anti-solvent such as ethanol or acetone, whereas ILs can be recovered by salting out, pervaporation, ion exchange, or reverse osmosis. Therefore, the recovery capabilities of ILs improve the economic efficiency of their applications. However, IL pretreatment has obvious limitations because ILs are obtained from petroleum sources, and their processes cause water pollution [150].

Alternatively, DESs can be synthesized by mixing hydrogen-bond donors and acceptors at certain ratios. As DESs are economical, non-toxic, and biodegradable solvents, they are being increasingly applied to selectively remove lignin from lignocellulose complexes, and are regarded as green-like pretreatments of lignocellulosic biomass under mild reaction conditions. However, the high viscosities of DESs adversely affect the delignification of biomass feedstocks owing to the associated mass transfer limitations [151].

4.3. Selective chemical processing

With the development of nanotechnology, lignocelluloses have been progressively employed to generate functional nanomaterials such as CNFs, CNCs, LNPs, and XNPs [152,153]. As a breakthrough, advanced AFM technology has been applied to the *in situ* observation of distinct length-reduced CNFs assemblies in the plant cell walls of *Osfc16* [111], revealing how dimension-reduced CNCs are generatable under mild and cost-effective chemical processes (Fig. 3(b)). Remarkably, distinct CNFs assemblies are observed in genetic site mutants and knockout lines of rice *OsCESA4*, *A7*, and *A9* isoforms, leading to size-minimized CNCs generated from the transgenic knockout lines and size-varied CNCs from the genetic site mutants [119]. These findings have not only demonstrated advanced biotechnology for CNFs and CNCs production at high quality, but have also determined the mechanism of chemical catalysis for the efficient generation of valuable nanomaterials at a relatively low cost. For instance, Hu et al. have reported that a rice *Oscesa7* mutant can produce aerogels with high porosity and specific surface area [154]. Owing to the higher proportion of nanoscale fibers and increased specific surface area of the honeycomb-like structure in the mutant sample, it could effectively adsorb oil in a shorter processing time than the wild-type sample (Fig. 3(c)). Furthermore, Peng et al. have demonstrated that the distinct CNFs generated in a natural rice mutant (*Osfc16*) could act as efficient stabilizers to improve the parameters of Pickering emulsions (Fig. 3(d)), indicating that emulsions prepared with the CNFs of *Osfc16* samples are more stable in coalescence or phase separation [155].

5. Optimal thermal conversion for highly valuable nanocarbons

The invention of carbon materials has driven the advancement of human civilization because it has propelled social and technological developments [156]. Therefore, nanocarbon materials such as fullerenes, graphene, and graphdiyne have been increasingly explored for potential applications in energy [26–28], sensing [29,30], catalysis [32], environmental monitoring [33]. Nevertheless, most nanocarbon materials depend on fossil fuel-based precursors (e.g., phenol, methane, and pitch) and energy-intensive synthesis processes (e.g., chemical vapor deposition, electric arc discharge, and laser ablation), which consequently causes environmental pollution [157]. Since the utilization of lignocellulosic biomass resources as carbon precursors is potentially affordable and sustainable [158], biomass-derived nanocarbons are promising candidates for industrialization [159–161]. More recently, biomass-derived nanocarbons with distinct structures and properties have been generated by choosing different carbonization strategies (Fig. 4). Various biomass feedstocks have been employed to synthesize different types of nanocarbons [162], providing a selective strategy for green carbon production.

5.1. Classic synthesis of biomass-derived carbon materials

During carbonization, biomass undergoes several reactions accompanied by the transport of heat and chemicals to form a variety of nanocarbon materials. Carbonization methods primarily determine the physical and chemical properties of nanocarbon materials such as their morphology, surface area, porosity, elemental components, functional groups, and degree of graphitization. The representative methods are briefly described below (Table 3).

Table 3

Optimal thermochemical treatments used for the specific nanocarbons from diverse lignocellulose resources.

Biomass resource	Strategies	Function	Parameter	Product	Reference
Cellulose	Pyrolysis	KAc	400–800 °C, 2 h	Porous carbon	[163]
<i>Miscanthus</i>	Pyrolysis	-	1200 °C, 1 h	Graphene oxide (GO)	[164]
Mangrove	Pyrolysis	-	600 °C, 4 h	Biochar	[165]
Bamboo sticks	Pyrolysis	-	700 °C, 30 min	Biochar	[166]
Sawdust	Pyrolysis	Ni, HCl	800–900 °C, 30 min	Graphene	[167]
Alginate	Pyrolysis	RuCl ₃	900 °C, 1 h	Graphene	[168]
Cellulose/Lignin	Microwave + Pyrolysis	SiC	600 °C, 2000 W, 60 min	Carbon nanotube	[169]
Wheat straw	Pyrolysis	K ₂ CO ₃	750 °C, 1 h	Biochar	[170]
Sugarcane bagasse	Hydrothermal	-	200 °C, 18–20 h	Biochar	[171]
Rice husk	Hydrothermal	EDA/ascorbic acid	190 °C, 12 h	Carbon quantum dots	[172]
Wheat straw	Hydrothermal + Pyrolysis	NH ₄ Cl, KOH	1) 120 °C, 10 h; 2) 800 °C, 2 h	Graphene-like nanosheet	[173]
Mangosteen peel	Microwave	ZnCl ₂	800 W, 20 min	Activated carbon	[174]
Jujube fruit shell	Microwave	-	150 W, 20 min	Carbon quantum dots	[175]
Orange peels	Microwave	-	600–850 W, 30 min	Carbon quantum dots	[176]
Raisins	Laser	-	Nd:YAG laser at 1064 nm, 1 Hz, 3–266 J/cm ²	Graphene	[177]
CNFs	Laser	H ₂	10.6 μm pulsed CO ₂ laser (75 W)	Graphene	[178]

Pyrolysis, which involves heating and decomposing organic biomass under an inert gas, is a common method for the preparation of porous carbon materials. The key factor affecting the pyrolysis product is the heating rate [179,180], whereas the carbonizing temperature influences the elemental composition, degree of graphitization, and heteroatom state of the carbon materials. At low temperatures, lignocellulosic biomass is mostly converted to amorphous carbon consisting of aromatic oligomers [181]. At high temperatures, carbon materials have a high crystal mass and perfect lattice [182]. In addition, the annealing time is an important factor, and a long annealing time leads to fewer heteroatoms and a higher degree of graphitization in carbon materials [183].

Hydrothermal carbonization (HTC), which uses biomass as a raw material and an aqueous solution as a medium, is a green synthesis method. The principle of HTC is to simulate the natural coalification process under the synergistic effects of high temperature and pressure over a short period of time, which involves three types of reactions: biomass dehydration, fragment polymerization, and intermolecular dehydration. As temperature and residue time are the two main factors affecting HTC, longer carbonization times are beneficial for the formation of spherical carbon nanoparticles, and temperature has a significant influence on the structure of hydrocarbons. Although HTC is a simple and low-pollution method, an additional activation process is required to further improve the pore structure of the carbon material.

Laser-induced graphitization (LIG) is an attractive approach to convert biomass into carbon materials. In contrast to HTC and classical pyrolysis, LIG can produce conductive graphitic carbon within a selective area of biomass. For instance, polyimide can be converted into graphene under CO₂ laser irradiation, enabling the writing of conductive porous graphitic patterns on different materials [178]. Without the influence of O₂, LIG can easily convert crosslinked lignocellulose into high-quality graphene. Microwave-assisted carbonization (MAC) is an emerging technology for generating biomass-based carbon materials. MAC converts electromagnetic energy into heat within the irradiated material, which has a high safety level and quick start-up and stopping compared to conventional heating.

5.2. Activation of biomass carbonization

As carbonization is the main process for the conversion of biomass into carbon-based products, activation techniques are typically used to modify the pore structure of the carbon material during the carbonization process (Table 3). Physical activation is a major method that includes two steps, i.e., biomass material is pyrolyzed to produce

biochar (400–850 °C) and then activated to create the carbon material with pore structure by regulating gasification (600–1200 °C) [184]. CO₂ is commonly used as a gaseous activator because of its ease of handling and high activation efficiency. Furthermore, prolonging the activation time favors the formation of micropores and mesopores; however, an overly long activation time can lead to pore collapse and deterioration. Similarly, steam activation is an economical and environmentally friendly method that has been used to optimize the pore structures of carbon materials.

In contrast to physical activation, carbonization and activation processes occur simultaneously during chemical activation owing to the addition of activator reagents. Activator reagents are inserted into the interior of biomass materials during activation, which leads to cross-linking polycondensation reactions between the carbon skeleton and activator reagents [185]. Commonly used chemicals include KOH, ZnCl₂, and H₃PO₄, which help dissolve cellulose for the effective generation of carbon materials rich in pore structures and surface functional groups. Although physical activation improves the pore structures and specific surface areas of biomass-derived carbon materials to a certain extent, chemically activated carbon materials possess relatively high specific surface areas and total pore volumes mainly because of the highly effective carbon-etching reaction. Physical activation is a simple, clean process that causes minimum pollution to the environment [186]. Therefore, appropriate activation methods depend on the reaction rate and activation time. For different types of biomass substrates, appropriate activation and process conditions should be comprehensively evaluated to achieve excellent performance in their applications. However, it is important to note that controlling the formation of ordered porous structures remains a challenge in activation methods.

5.3. Applications of biomass-derived nanocarbons

Owing to their excellent physical and chemical properties, graphene-like nanocarbons are being increasingly used in supercapacitors, fuel and solar cells, H₂ storage devices, sensors, adsorbents, and catalysts. For instance, nanocarbon materials have been used in electrode/electrolyte interface devices for energy storage because of their long lifespan, high energy density, and superior pulse charge-discharge performance [187]. Because biomass-derived carbon materials are sustainable, low-cost, and non-toxic, they are considered for application in supercapacitors to improve the graphitization degree, specific surface area (SSA), and surface properties of electrode materials. For example, Liu et al. converted *T. reesei*-undigested lignocellulosic residue

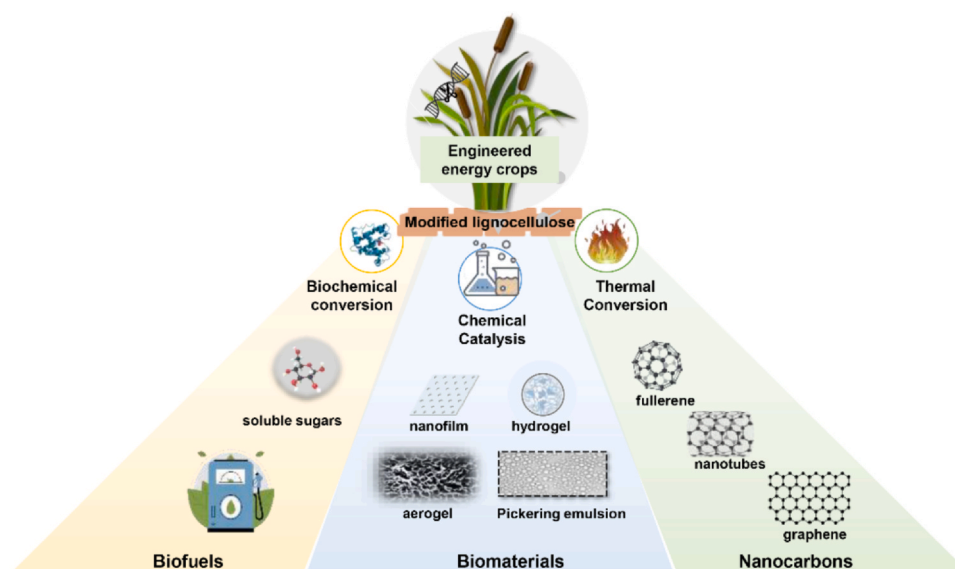


Fig. 5. A novel strategy for the productions of cost-effective biofuels, value-added nanomaterials, and high-performance nanocarbons based on the integration of genetically modified lignocelluloses with green-like biomass processes.

into highly porous carbon in a thermochemical process with a specific electroconductivity raised by 3–12-fold for a supercapacitor by supplying 1:2 (w/w) FeCl_3 [188].

In recent years, the rapid development of dye manufacturing technology has led to an increase in the discharge of dye wastewater from the textile industry. Most dyes, such as Congo red, rhodamine, methylene blue (MB), and methyl orange, are difficult to biodegrade because of the complex molecular structure and chemical stability of aromatic compounds and/or amines. Therefore, the development of biomass-derived materials for dye adsorption is an ecofriendly and inexpensive approach [189]. For example, Yu et al. have combined a yeast-fermented rice mutant residue with pectin extracted from citrus peels to generate active biosorbents that significantly enhance Cd and MB adsorption [190], thus proposing an environmentally friendly and cost-effective strategy for the removal of industrial dyes without liberating any biomass residue.

Furthermore, graphene-like nanocarbons can be generated from various lignocellulose resources. Owing to their varied compositions and complicated structures, lignocellulose substrates are commonly employed in classic thermochemical processes to generate amorphous biochar, which is applied as an efficient sorbent for the elimination of industrial dyes, toxic chemicals, and heavy metals, or as functional materials for electrochemical conductivity and other purposes. Therefore, advanced biotechnology for biomass-derived nanocarbon production by integrating genetically modified lignocellulose with optimal thermochemical processes needs to be explored. In particular, based on the different types of lignocellulose substrates in grassy and woody plants, advanced biotechnology needs to be developed for the desirable nanocarbons with high quality and excellent performance via simple and cost-effective operations.

6. Conclusion and perspective remarks

Based on recent advances in plant cell wall engineering, biomass process technology, and carbon nanotechnology, this study proposes a novel approach that dynamically integrates genetically engineered bioenergy crops with green-like biomass conversion, selective for the production of cost-effective biofuels or value-added bioproducts, such as nanomaterials and nanocarbons (Fig. 5). To achieve these goals, the precise modification of plant cell walls can be implemented by selecting key genes and appropriate genetic manipulation methods. Thus, the integration of desirable genetic site mutants and transgenic lines should be considered to produce optimal lignocellulose substrates that have minimized recalcitrance, which is

attributed to the amorphous regions and inner-broken chains raised for length-reduced cellulose nanofibril accumulation in plant cell walls. Since the amorphous regions and inner-broken chains are defined as the initial breakpoints for enhancing the three types of biomass conversions (biochemical, chemical, and thermochemical), length-reduced cellulose nanofibers can be employed with mild biochemical conversion to achieve near-complete enzymatic saccharification for high-yield biofuels at a relatively low cost. Alternatively, efficient chemical catalysis can be achieved using desirable cellulose nanofibers for high-quality biomaterials. Furthermore, optimal thermochemical conversion can be achieved to generate high-performance graphene and nanocarbons using specific nanomaterials generated from appropriate lignocellulose substrates. This study provides a novel strategy for a green carbon revolution that consists in the integration of advanced biotechnology with nanotechnology.

Declaration of competing interests

Liangcai Peng is an associate editor for Green Carbon and was not involved in the editorial review or decision to publish this article. The authors declare that they have no known competing financial interests or personal relationships that have appeared to influence the work reported in this paper.

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