



Harnessing Genetic Engineering for Enhancing Lignocellulose Biomass Production

Ankit Joshi and Madhulika Gupta*

Computational Biophysics Lab, Department of Chemistry and Chemical Biology, Indian Institute of Technology (Indian School of Mines) Dhanbad, Dhanbad, Jharkhand, India

Theerawut Phusantisampan

Biorefinery and Process Automation Engineering Center, Department of Biotechnology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand

* Corresponding author. E-mail: madhulikagupta@iitism.ac.in

DOI: 10.14416/j.asep.2024.09.013

© 2024 King Mongkut's University of Technology North Bangkok. All Rights Reserved.

Currently, around 80–85% of the energy demands around the world are met by non-renewable fossil fuels that have finite reserves [1], [2]. The burning of fossil fuels also majorly contributes to increased greenhouse gas (GHG) emissions [3], urging the need to develop renewable energy systems and associated technologies to meet worldwide energy consumption. However, their widespread practical implementation is limited by intermittent availability, lack of storage capability, high cost, and demographical factors. In this regard, biomass has emerged as a promising renewable and low-carbon energy sources that include wood and woody biomass, agricultural residues, energy crops, animal waste, industrial residues, and algae. The plant matter also referred to as lignocellulosic biomass (LCB), has been identified as a crucial feedstock to produce biofuels directly. LCB can also be used to generate other bioproducts, including chemicals such as bioethanol and bioplastics, biodegradable polymers and composites, and bio-based fuels.

First-generation biofuels are sourced from edible food crops such as vegetable oils, sugarcane, and grains, while second-generation biofuels are sourced from LCB, which typically includes non-edible feedstocks such as forestry residues, side products like wheat straw, and woody biomass [4], [5]. LCB is the main component of the plant cell walls and primarily comprises aligned bundles of cellulose fibrils, embedded within a disordered matrix of hemicellulose and lignin [6]. Cellulose is a linear polysaccharide composed of β -(1 \rightarrow 4)-linked glucose units that form strong, crystalline microfibrils. Hemicellulose is a branched polymer made up of various sugar monomers,

including xylose, arabinose, and mannose, which interact with cellulose and lignin to provide structural flexibility to plant cell walls [7], [8]. Lignin is a complex, aromatic polymer that confers rigidity to plant cell walls and maintains its structural integrity by acting as a protective shield for cellulose and hemicellulose against enzymatic degradation. The recalcitrant nature of lignin limits the accessibility of cellulose and hemicellulose to enzymatic hydrolysis, thereby reducing the overall biomass conversion efficiency to biofuels. Thus, the complex and intricate structure of different constituents of LCB poses significant challenges in converting biomass into fermentable sugars and further into biofuels for industrial applications.

The traditional pretreatment methods include energy-intensive and costly chemical pretreatments, which may further produce inhibitory by-products, decreasing the downstream process efficiency [9]. Thus, formulation of new chemical methods or modification of existing ones is essential for the effective isolation of cell wall constituents for sustainable biofuel production. In the past decade, the use of genetic engineering to make LCB more processable at its very source by directly altering the genetics of the plant has gained substantial attention. This technology involves precisely altering the plant cell walls, reducing the content or modifying the composition of lignin, improving the accessibility of cellulose, and optimizing hemicellulose for enzymatic breakdown.

Genetic engineering to downregulate genes coding for important enzymes during the biosynthesis of lignin, such as cinnamyl alcohol dehydrogenase and



cinamate 4-hydroxylase (C4H) was conducted to produce plants with reduced lignin levels or a different lignin structure that resulted in a better yield of biofuels through enhanced saccharification [10]. The modified lignin levels through genetic engineering for poplar, alfalfa, tobacco, and corn have also helped in reducing the pretreatment process [11], [12]. Another study involves the editing of genes responsible for the biosynthesis of lignin using CRISPR/Cas9 technology [13]. This technology allows for specific editing in the genome of the plant, whereby selective genes can be knocked out or their expression levels can be altered. CRISPR/Cas9 has been applied to knock out the CCR1 gene in poplar, which resulted in the reduction of lignin content without compromising plant growth.

The applications of genetic engineering have also proven to be effective in improving the cellulose content by reducing its crystallinity to make it more accessible for enzymatic hydrolysis. A recent study used overexpression of cellulose synthase-like genes to engineer plants to over-deposit cellulose with reduced lignin content in rice leading to improved digestibility [14]. One of the studies showed that the introduction of a bacterial gene encoding ferulic acid esterase in switchgrass cleaved the cross-linking between lignin and hemicellulose [15]. This modification resulted in an increased release of 33% in fermentable sugar during enzymatic hydrolysis, indicating the effectiveness of directed genetic modifications. Many studies have highlighted promising avenues such as improved biomass digestibility by yielding enhanced amounts of fermentable sugars on altering biosynthesis pathways of hemicellulose [16], [17]. However, a limited understanding of these pathways constrains the broad implementation of inducing changes in biosynthetic pathways in LCB through genetic engineering.

Although the modifications induced in plants through genetic engineering appear promising, they face several challenges and controversies. One of the debilitating issues is the choice between improving LCB characteristics through genetic engineering at the cost of inducing detrimental changes in plant health and agricultural productivity. For instance, reductions in lignin content may cause weakness in plant structure, thereby making plants more susceptible to environmental stresses such as wind and pathogens. The gene flow from genetically modified (GM) crops into wild relatives may also lead to a set of unintended ecological consequences. In addition, there is immense public resistance to GM crops, particularly in regions with strong opposition to genetically

modified organisms (GMOs). However, the adoption of biomass GM crops can be normalized by using transparent research practices and public consultation.

It is thus evident that although abundantly available, biomass has not been utilized to its full potential to fuel a sustainable economy due to the recalcitrant nature of its constituents. The technological advancements aimed at enhancing biomass yields using genetic engineering is an emerging area that requires deeper investigation to obtain a cohesive understanding of the economic viability and efficiency of biomass production. The scalability of such genetically engineered biomass modifications, along with associated environmental concerns and ethical implications, also need to be scrutinized to ensure their adaptability and long-term sustainability. The production of biofuels from microalgae and cyanobacteria, categorized as third-generation biofuels, is also under study. The use of genetic engineering on these microorganisms can also result in increased growth rates and biomass yields which is still under investigation. Future research studies based on the combination of genetic engineering with other biotechnological methods may also be investigated to improve the accessibility of cellulose and hemicellulose within LCB. For example, genetic engineering combined with advanced breeding or synthetic biology will likely produce bioenergy crops with superior properties for a wide range of ecological circumstances or processing requirements [18], [19]. The ongoing technology development to improve genome editing, including but not limited to CRISPR/Cas9, with targeted modifications of traits pertaining to biomass production may further contribute to better extraction efficiency of different components.

References

- [1] J. L. Holechek, H. M. E. Geli, M. N. Sawalhah, and R. Valdez, "A global assessment: Can renewable energy replace fossil fuels by 2050?," *Sustainability*, vol. 14, no. 8, p. 4792, 2022, doi: 10.3390/su14084792.
- [2] M. Sriariyanun and B. Dharmalingam, "From waste to wealth: Challenges in producing value-added biochemicals from lignocellulose biorefinery," *Journal of Applied Science and Emerging Technology*, vol. 22, no. 3, 2023, doi: 10.14416/JASET.KMUTNB.2023.03.001.
- [3] T. Kundu, S. Suyash, M. Gupta, and B. Chowdhury, "Introduction to greenhouse gases

- composition and characteristics,” in *Advances and Technology Development in Greenhouse Gases: Emission, Capture and Conversion*. Amsterdam, Netherlands: Elsevier, 2024, pp. 3–18, doi: 10.1016/B978-0-443-19066-7.00008-4.
- [4] M. Sriariyanun, M. P. Gundupalli, V. Phakeenuya, T. Phusamtisampan, Y.-S. Cheng, and P. Venkatachalam, “Biorefinery Approaches for production of cellulosic ethanol fuel using recombinant engineered microorganisms,” *Journal of Applied Science and Engineering*, vol. 27, no. 2, pp. 1985–2005, 2023.
- [5] D. Jose, S. Vasudevan, P. Venkatachalam, S. K. Maity, A. A. Septevani, M. Gupta, P. Tantayotai, H. E. Bari, and M. Sriariyanun, “Effective deep eutectic solvent pretreatment in one-pot lignocellulose biorefinery for ethanol production,” *Industrial Crops and Products*, vol. 222, 2024, Art. no. 119626, doi: 10.1016/j.indcrop.2024.119626.
- [6] B. Paramasivam, R. Mensah, and M. Sriariyanun, “Advantages and significance of acid and alkali pretreatment of lignocellulose biomass in biorefining process,” *Applied Science and Engineering Progress*, vol. 17, no. 1, 2024, Art. no. 6913, doi: 10.14416/j.asep.2023.05.004.
- [7] M. Gupta, P. Dupree, L. Petridis, and J. C. Smith, “Patterns in interactions of variably acetylated xylans with hydrophobic cellulose surfaces,” *Cellulose*, vol. 30, no. 18, pp. 11323–11340, 2023, doi: 10.1007/s10570-023-05584-z.
- [8] M. Gupta, T. B. Rawal, P. Dupree, J. C. Smith, and L. Petridis, “Spontaneous rearrangement of acetylated xylan on hydrophilic cellulose surfaces,” *Cellulose*, vol. 28, pp. 3327–3345, 2021, doi: 10.1007/s10570-021-03706-z.
- [9] M. Gundupalli and M. Sriariyanun, “Recent trends and updates for chemical pretreatment of lignocellulosic biomass,” *Applied Science and Engineering Progress*, vol. 16, no. 1, 2023, Art. no. 5842, doi: 10.14416/j.asep.2022.03.002.
- [10] P. Wang, N. Dudareva, J. A. Morgan, and C. Chapple, “Genetic manipulation of lignocellulosic biomass for bioenergy,” *Current Opinion in Chemical Biology*, vol. 29, pp. 32–39, 2015, doi: 10.1016/j.cbpa.2015.08.006.
- [11] K. L. Kadam and J. D. McMillan, “Availability of corn stover as a sustainable feedstock for bioethanol production,” *Bioresource Technology*, vol. 88, no. 1, pp. 17–25, 2003, doi: 10.1016/S0960-8524(02)00269-9.
- [12] O. Shoseyov, Z. Shani, and I. Levy, “Carbohydrate binding modules: Biochemical properties and novel applications,” *Microbiology and Molecular Biology Reviews*, vol. 70, no. 2, pp. 283–295, 2006, doi: 10.1128/mmbr.00028-05.
- [13] D. Konar, R. Saha, D. Bhattacharya, and M. Mukhopadhyay, “Present status and future prospect of genetic and metabolic engineering for biofuels production from lignocellulosic biomass,” in *Genetic and Metabolic Engineering for Improved Biofuel Production from Lignocellulosic Biomass*. Amsterdam, Netherlands: Elsevier, 2020, pp. 171–192, doi: 10.1016/B978-0-12-817953-6.00003-8.
- [14] P. Phitsuwan, K. Sakka, and K. Ratanakhanokchai, “Improvement of lignocellulosic biomass in planta: A review of feedstocks, biomass recalcitrance, and strategic manipulation of ideal plants designed for ethanol production and processability,” *Biomass and Bioenergy*, vol. 58, pp. 390–405, 2013, doi: 10.1016/j.biombioe.2013.08.027.
- [15] T. van der Weijde, C. L. A. Kamei, A. F. Torres, W. Vermerris, O. Dolstra, R. G. F. Visser, and L. M. Trindade, “The potential of C4 grasses for cellulosic biofuel production,” *Frontiers in Plant Science*, vol. 4, p. 107, 2013, doi: 10.3389/fpls.2013.00107.
- [16] N. Xu, W. Zhang, S. Ren, F. Liu, C. Zhao, H. Liao, Z. Xu, J. Huang, Q. Li, Y. Tu, B. Yu, Y. Wang, J. Jiang, J. Qin, and L. Peng, “Hemicelluloses negatively affect lignocellulose crystallinity for high biomass digestibility under NaOH and H₂SO₄ pretreatments in *Miscanthus*,” *Biotechnology for Biofuels*, vol. 5, pp. 1–12, 2012, doi: 10.1186/1754-6834-5-58.
- [17] Y. Wang, C. Fan, H. Hu, Y. Li, D. Sun, Y. Wang, and L. Peng, “Genetic modification of plant cell walls to enhance biomass yield and biofuel production in bioenergy crops,” *Biotechnology Advances*, vol. 34, no. 5, pp. 997–1017, 2016, doi: 10.1016/j.biotechadv.2016.06.001.
- [18] M. R. Allwright and G. Taylor, “Molecular breeding for improved second generation bioenergy crops,” *Trends in Plant Science*, vol. 21, no. 1, pp. 43–54, 2016, doi: 10.1016/j.tplants.2015.10.002.
- [19] P. M. Shih, Y. Liang, and D. Loqué, “Biotechnology and synthetic biology approaches for metabolic engineering of bioenergy crops,” *The Plant Journal*, vol. 87, no. 1, pp. 103–117, 2016, doi: 10.1111/tj.13176.



Ankit Joshi



Asst. Prof. Dr. Madhulika Gupta



Asst. Prof. Dr. Theerawut Phusantisampan