

Lignocellulosic Biomass Decomposition and Bioconversion

Zhina Lian and Xin Zhou *

Jiangsu Co-Innovation Center of Efficient Processing and Utilization of Forest Resources, College of Chemical Engineering, Nanjing Forestry University, Nanjing 210037, China; lianzhina@njfu.edu.cn

* Correspondence: xinzhou@njfu.edu.cn

Lignocellulosic biomass (LCB) offers numerous advantages as a sustainable energy resource, such as its abundance, degradability, environmental compatibility, non-toxic nature, and cost-effectiveness [1]. It primarily comprises cellulose, hemicellulose, and lignin, with minor quantities of additional components such as acetyl groups, minerals, and phenolic substituents. In these fractions, the cellulose is identified as a polysaccharide comprising glucose units connected via β -1,4-glycosidic bonds, while hemicelluloses are a collection of polysaccharides coexisting with cellulose within plant cell walls. In contrast to cellulose, hemicelluloses exhibit branching and are composed of diverse sugar monomers such as xylose, mannose, and glucose [2]. Overall, the polysaccharide (cellulose and hemicellulose) components can be potentially converted into fermentable sugars, such as glucose and xylose, which can then be further processed into biofuels and biochemicals via fermentation [3]. Thus, due to its abundance and high concentration of non-fossil carbon, LCB is viewed as a viable alternative to fossil resources to produce sustainable biofuels and biochemicals [4].

However, given the inherent resistance of LCB, pretreatment plays a crucial role in altering the native lignin structure to expose cellulose and hemicellulose for enzymatic hydrolysis. LCB must be pretreated before it can be converted into fermentable monosaccharides, a process that requires high levels of energy and expensive chemicals; therefore, the choice of an appropriate pretreatment method is very critical. Overall, an effective pretreatment process should be economically viable, require minimal energy consumption, and be designed to achieve optimal sugar yields by utilizing a diverse array of lignocellulosic feedstocks. Currently, various pretreatment methods, such as mechanical, chemical, physicochemical, and biological approaches, as well as their combinations, have been categorized [5,6]. In detail, numerous techniques have been employed to pretreat LCB for fractionating, solubilizing, hydrolyzing, and separating the cellulose, hemicellulose, and lignin constituents of biomass, such as milling, irradiation, microwave treatment, steam explosion, and ammonia fiber explosion (AFEX), ozonolysis, acid hydrolysis, and biological pretreatments, or their combination [7–9]. The shared objective of these methodologies is to decrease the biomass volume and enhance its structural accessibility. Each of these approaches has been documented to possess unique merits and drawbacks [10,11]. Therefore, investigating efficient and cost-effective techniques for breaking down cellulose and hemicellulose into fermentable sugars is also imperative [12–14].

Although the fermentable sugars (mainly glucose and xylose) liberated by saccharification of pretreated LCB can be fermented into various biofuels and biochemicals, the fermentation efficiency and final product content are still unsatisfactory due to the complexity of hydrolysates that usually contain inhibitors, colored substances, proteins, and colloid from the pretreatment process [15,16]. Thus, improving the tolerance of microorganisms for fermentation inhibitors derived from the degradation of LCB and enhancing the fermentation efficiency of LCB hydrolysate are still urgent for the industrialization of the biorefinery process [17].

This editorial refers to the Special Issue “Lignocellulosic Biomass Decomposition and Bioconversion”. This Special Issue seeks to showcase cutting-edge research on the biorefin-



Citation: Lian, Z.; Zhou, X. Lignocellulosic Biomass Decomposition and Bioconversion. *Fermentation* **2024**, *10*, 480. <https://doi.org/10.3390/fermentation10090480>

Received: 3 September 2024
Revised: 11 September 2024
Accepted: 12 September 2024
Published: 15 September 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

ery process of lignocellulosic biodegradation and bioconversion, focusing on technological advancements. A total of 16 manuscripts were submitted for review for this Special Issue, all of which underwent a rigorous review process by the journal *Fermentation*. Ultimately, 10 papers were selected for publication and inclusion in this Special Issue. The contributions are detailed below [18–27].

In summary, the contributions covered pretreatment technology, biochemical production, functional saccharide products, and fermentation process optimization. The main research contents in this Special Issue are as follows:

Contribution 1 explores the feasibility of cultivating shiitake mushrooms on lignocellulosic biomass, specifically sawdust and wheat straw, treated with various heat treatments using six different strains of shiitake mushrooms (DMRO-35, 51, 297, 388s, 410, 412). The study revealed a robust correlation between the productivity of the examined strains and the degradation of lignin in the growth medium, underscoring the importance of pretreatment for lignin conversion and its utilization in solid-state fermentation. The results of this study indicate that DMRO-388s exhibited the highest productivity among all strains tested, regardless of the substrate and heat treatment method employed. Furthermore, the findings suggest that subjecting the substrate to pre-heat treatment via high-pressure sterilization can significantly enhance the yields of shiitake mushrooms.

Contribution 2 conducted the assays of gluconic acid production using the enzymatic hydrolysate from pretreated LCB material (Corncob) by bio-oxidation of *Gluconobacter oxydans* (*G. oxydans*) and investigated the influences of the pH for the *G. oxydans*. The findings suggest that maintaining a low pH environment (2.5–3.5) can inhibit the metabolism of gluconic acid and enhance its yield to over 95%. This resulted in the production of 98.8 g/L of gluconic acid from concentrated corncob enzymatic hydrolysate with an initial glucose concentration of 100 g/L. The implementation of a low pH stress strategy demonstrates potential for optimizing gluconic acid production and fermentation control, particularly when utilizing lignocellulosic biomass materials.

Contribution 3 examines the potential utility of dielectric measurements at various stages of plant-based biomass utilization, including the enzymatic hydrolysis process of native and microwave-preprocessed corncob residues, the ethanol fermentation process of the resulting hydrolysates, and the anaerobic co-digestion process with meat-industry wastewater sludge. The findings indicate a significant linear relationship between the dielectric constant and the concentrations of sugars, ethanol, and the success of anaerobic co-digestion. The results demonstrate that dielectric measurements offer a viable alternative for monitoring and controlling biomass utilization processes.

Contribution 4 presents a novel approach for the simultaneous production of xylooligosaccharides (XOS) and humic-like acid (HLA) using the vinegar residue as feedstock through a two-step hydrothermal pretreatment process. The first step involves the production of XOS with a yield of 36.2% using a combination of hydrothermal pretreatment at 170 °C for 50 min and endo-xylanase hydrolysis. The second step focuses on the production of HLA with a yield of 15.3% through a second-step hydrothermal pretreatment process under the condition of 0.6 mol/L of KOH at 210 °C for 13 h. As the results of this study indicate, lignocellulosic biomass could be used to make XOS and HLA in a promising and comprehensive way.

Contribution 5 involved the isolation and identification of a cellulase-producing bacterial strain, *Paenibacillus peoriae* (*P. peoriae*) MK1, from soil. Furthermore, the gene of the cellulase enzyme from *P. peoriae* MK1 was successfully cloned and expressed in *Escherichia coli*. The expressed cellulase after the purification process with a yield of 19% demonstrated a specific activity of 77 U/mg for carboxymethyl-cellulose, and this enzyme existed as a metal-independent monomer with a molecular weight of 65 kDa. In the presence of Ca²⁺ ions, this purified recombinant cellulase exhibited optimal activity at pH 5.0 and 40 °C, with a half-life of 9.5 h. This study will aid in securing high-quality cellulase resources for industrial use.

Contribution 6 employed six distinct deep eutectic solvent (DES) systems, which were prepared using lactic acid, acetic acid, and levulinic acid as hydrogen bond donors, in conjunction with two independent hydrogen bond acceptors (benzyl triethyl ammonium chloride and benzyl triphenyl phosphonium chloride) for the purpose of assessing their efficacy in pretreating sugarcane bagasse. The utilization of the benzyl triethyl ammonium chloride: lactic acid (TEBAC:LA)-based DES under mild operating conditions demonstrated significant effectiveness in lignin removal, achieving 85.3% for lignin removal rate and 98.7% for cellulose recovery rate, respectively. Thus, the findings offer a potentially effective pretreatment method for selectively removing lignin and converting sugarcane bagasse into bioethanol.

Contribution 7 also evaluated the influence of pretreatment with different DES systems for biogas production with halophyte *Atriplex crassifolia* as material. The DES systems were prepared by using choline chloride as hydrogen bond acceptors and carboxylic acids, amine/amide, and polyols/glycols as hydrogen bond donors, and the DES system of choline chloride and lactic acid displayed the highest delignification values. The results confirmed *Atriplex crassifolia*'s suitability as a feedstock for biogas production and demonstrated the effectiveness of deep eutectic solvents (DES) containing choline chloride (ChCl) and lactic acid (LA) in pretreatment, which successfully removed the lignin barrier. This process ultimately enhanced the biodegradability of cellulosic sugars, making them more accessible to anaerobic microorganisms.

Contribution 8 successfully prepared galactomannan oligosaccharides (GMOS) through the enzymatic degradation of *Gleditsia microphylla* (GM) and evaluated the effects of GMOS as a prebiotic on human intestinal bacteria. The findings of the study demonstrate that GMOS can be efficiently produced at a notable yield of 72.8% through the enzymatic degradation of GM using the β -mannanase enzyme under specific conditions (50 °C, pH 5, 20 U/g-GM, and 40 g/L). Furthermore, GMOS were found to promote the proliferation of the phylum Bacteroidetes, inhibit the growth of the phylum Fusobacteria, and significantly inhibit *Fusobacterium*. These results suggest that GMOS derived from *Gleditsia* as prebiotics have the potential to serve as a dietary supplement for enhancing gut health and potentially preventing or treating certain diseases.

Contribution 9 elucidated the discovery of a previously unidentified aldehyde dehydrogenase gene, W826-RS0111485, within *G. oxydans* DSM2003, which plays a crucial role in the conversion of aldehyde inhibitors. Through heterologous expression in *E. coli*, this gene was successfully transformed into an aldehyde dehydrogenase capable of directly converting highly toxic aldehyde inhibitors into less harmful acids, notably demonstrating the efficient conversion of furfural to furoic acid. These findings highlight the potential of W826-RS0111485 as a promising candidate gene for detoxification purposes. This study established a theoretical framework for improving the degradation capacity of inhibitors in industrial strains. By doing so, the detoxification process can be attenuated, leading to a reduction in production costs and facilitating the advancement of the biorefinery industry in the future.

Contribution 10 introduced a biphasic organic solvent, consisting of phenoxyethanol and maleic acid, as an efficient method for pretreating and fractionating vinegar residue into glucan, xylan, and lignin under mild conditions. The utilization of this biphasic system (0.5% maleic acid, 60% phenoxyethanol) resulted in the retention of 80.9% of cellulose in the solid residue, the removal of 75.4% of hemicellulose, and the removal of 69.3% of lignin after cooking vinegar residue at 140 °C for 1 h. Furthermore, the process yielded xylooligosaccharides (XOS) with a 47.3% yield and glucose with an 82.7% yield. In summary, the utilization of phenoxyethanol–maleic acid pretreatment has demonstrated the potential for the simultaneous production of XOS and glucose from vinegar residue, highlighting its viability as a valuable biorefinery feedstock.

In this Special Issue, different pretreatment methods, such as DES, two-step hydrothermal, and biphasic organic solvent, were put forward (Contributions 4, 6, 7, 10). Contribution 4 achieved the preparation of multiple value-added products (XOS, HLA, and hydrochar)

by the two-step hydrothermal pretreatment. Contributions 6 and 7 have proved that the use of an acidic DES system (LA as hydrogen bond donors) can efficiently achieve the removal of lignin under mild operating conditions, improving the enzymatic hydrolysis efficiency of cellulose and bioconversion efficiency for bioethanol and biogas. Contribution 10 presented an effective and facile biphasic fractionation system (phenoxyethanol–maleic acid) to pretreat LCB, enhancing the efficiency of enzymatic hydrolysis and achieving the production of XOS.

Studies about the preparation of functional oligosaccharides were also reported in this Special Issue, which involved two types of prebiotic functional oligosaccharides (XOS and GMOS) derived from the different LCB materials. Contribution 8 has successfully prepared GMOS through enzymatic degradation of GM and proved that GMOS is beneficial to human intestinal bacteria. XOS are usually prepared by hydrolysis of the xylan-type hemicellulose and are gaining increasing attention due to their high market price. Contributions 4 and 10 combined the pretreatment and XOS production processes, and XOS as value-added products can partly offset the pretreatment cost.

The hydrolysate of LCB materials, which contained various monosaccharides, can be used as feedstock for fermentation of biochemicals, such as ethanol, gluconic acid, and LA. *G. oxydans* showed a high tolerance ability for inhibitors from the degradation of LCB during the pretreatment process (contribution 9), and it has been proven by contribution 2 that it is able to be used for the highly efficient production of gluconic acid by the pH control strategy. Exceptional productivity and abundant yield facilitated the large-scale bio-production of gluconic acid from cellulosic materials in industrial settings.

As a final note, it is well known that the efficient utilization of LCB materials is critical for the implementation of a circular economy. This Special Issue reported on novel studies in the biorefinery field, including pretreatment, enzymatic hydrolysis, and fermentation [18–27]. All these results can contribute to improving the resource utilization and profitability of lignocellulosic biomass.

Funding: This research received no external funding.

Acknowledgments: We appreciate the support of the Special Issue “Lignocellulosic Biomass Decomposition and Bioconversion” in *Fermentation* and thank all authors whose valuable work was published under this issue and thus contributed to the success of the edition.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Li, R.; Zheng, Y.; Zhao, X.; Yong, Q.; Meng, X.; Ragauskas, A.; Huang, C. Recent advances in biomass pretreatment using biphasic solvent systems. *Green Chem.* **2023**, *25*, 2505–2523. [\[CrossRef\]](#)
2. Li, R.; Zhang, D.; Shen, B.; Lai, C.; Yong, Q. Alleviating lignin repolymerization facilitated the combined acid and deep eutectic solvent pretreatment of *Populus*. *Ind. Crops Prod.* **2024**, *207*, 117778. [\[CrossRef\]](#)
3. Wang, X.; Huang, C.; Fu, X.; Jeon, Y.; Mao, X.; Wang, L. Bioactivities of the popular edible brown seaweed sargassum fusiforme: A Review. *J. Agric. Food Chem.* **2023**, *71*, 16452–16468. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Wang, X.; Huang, C.; Yang, F.; Wang, K.; Cha, S.; Mao, X.; Jeon, Y.; Wang, L. Fucoidan isolated from the edible seaweed *Sargassum fusiforme* suppresses skin damage stimulated by airborne particulate matter. *Algal Res.* **2024**, *77*, 103339. [\[CrossRef\]](#)
5. Yao, M.; Liu, B.; Qin, L.; Du, Z.; Wang, Z.; Qin, C.; Liang, C.; Huang, C.; Yao, S. Preparation of homogeneous lignin nanoparticles by efficient extraction of lignin and modification of its molecular structure using a functional deep eutectic solvent containing γ -valerolactone. *Green Chem.* **2024**, *26*, 4528–4543. [\[CrossRef\]](#)
6. Periyasamy, S.; Karthik, V.; Senthil Kumar, P.; Isabel, J.B.; Temesgen, T.; Hunegnaw, B.M.; Melese, B.B.; Mohamed, B.A.; Vo, D.N. Chemical, physical and biological methods to convert lignocellulosic waste into value-added products. A review. *Environ. Chem. Lett.* **2022**, *20*, 1129–1152. [\[CrossRef\]](#)
7. Chen, H.; Liu, J.; Chang, X.; Chen, D.; Xue, Y.; Liu, P.; Lin, H.; Han, S. A review on the pretreatment of lignocellulose for high-value chemicals. *Fuel Process Technol.* **2017**, *160*, 196–206. [\[CrossRef\]](#)
8. Cheng, J.; Huang, C.; Zhan, Y.; Liu, X.; Wang, J.; Huang, C.; Fang, G.; Ragauskas, A.J.; Xie, Z.; Meng, X. A novel mineral-acid free biphasic deep eutectic solvent/ γ -valerolactone system for furfural production and boosting the enzymatic hydrolysis of lignocellulosic biomass. *Bioresour. Technol.* **2023**, *387*, 129653. [\[CrossRef\]](#)

9. Marks, C.; Viell, J. Solvents and ions for pretreatment in lignocellulosic biorefineries. *Process Biochem.* **2022**, *113*, 241–257. [\[CrossRef\]](#)
10. Beig, B.; Riaz, M.; Naqvi, S.R.; Hassan, M.; Zheng, Z.; Karimi, K.; Pugazhendhi, A.; Atabani, A.E.; Chi, N.T.L. Current challenges and innovative developments in pretreatment of lignocellulosic residues for biofuel production: A review. *Fuel* **2021**, *287*, 119670. [\[CrossRef\]](#)
11. Mankar, A.R.; Pandey, A.; Modak, A.; Pant, K.K. Pretreatment of lignocellulosic biomass: A review on recent advances. *Bioresour. Technol.* **2021**, *334*, 125235. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Lai, C.; Jia, Y.; Yang, C.; Chen, L.; Shi, H.; Yong, Q. Incorporating lignin into polyethylene glycol enhanced its performance for promoting enzymatic hydrolysis of hardwood. *ACS Sustain. Chem. Eng.* **2020**, *8*, 1797–1804. [\[CrossRef\]](#)
13. Zhou, M.; Tian, X. Development of different pretreatments and related technologies for efficient biomass conversion of lignocellulose. *Int. J. Biol. Macromol.* **2022**, *202*, 256–268. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Zhao, L.; Sun, Z.; Zhang, C.; Nan, J.; Ren, N.; Lee, D.; Chen, C. Advances in pretreatment of lignocellulosic biomass for bioenergy production: Challenges and perspectives. *Bioresour. Technol.* **2022**, *343*, 126123. [\[CrossRef\]](#)
15. Zhang, Y.; Xia, C.; Lu, M.; Tu, M. Effect of overliming and activated carbon detoxification on inhibitors removal and butanol fermentation of poplar prehydrolysates. *Biotechnol. Biofuels* **2018**, *11*, 178. [\[CrossRef\]](#)
16. Jin, X.; Liu, P.; Li, H.; Yu, H.; Ouyang, J.; Zheng, Z. Sustainable wheat straw pretreatment process by self-produced and cyclical crude lactic acid. *Bioresour. Technol.* **2024**, *402*, 130788. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Qiu, Z.; Han, X.; He, J.; Jiang, Y.; Wang, G.; Wang, Z.; Liu, X.; Xia, J.; Xu, N.; He, A.; et al. One-pot d-lactic acid production using undetoxified acid-pretreated corncob slurry by an adapted *Pediococcus acidilactici*. *Bioresour. Technol.* **2022**, *363*, 127993. [\[CrossRef\]](#)
18. Annepu, S.K.; Sharma, V.P.; Barh, A.; Kamal, S.; Shirur, M.; Kumar, S.; Bairwa, R.K.; Gupta, S.; Gupta, M.; Dutta, U.; et al. Influence of heat treatment and solid-state fermentation on the lignocellulosic fractions of substrates supporting *Lentinula edodes* (Berk.) Pegler cultivation: Implications for commercial production. *Fermentation* **2023**, *9*, 130. [\[CrossRef\]](#)
19. Dai, L.; Lian, Z.; Fu, Y.; Zhou, X.; Xu, Y.; Zhou, X.; Kuznetsov, B.N.; Jiang, K. Low pH Stress Enhances Gluconic Acid Accumulation with Enzymatic Hydrolysate as Feedstock Using *Gluconobacter oxydans*. *Fermentation* **2023**, *9*, 278. [\[CrossRef\]](#)
20. Jákó, Z.P.; Lemmer, B.; Dobózi, R.; Hodúr, C.; Beszédes, S. Using dielectric constant measurement to monitor ethanol fermentation and anaerobic co-digestion of lignocellulosic biomass. *Fermentation* **2023**, *9*, 902. [\[CrossRef\]](#)
21. Jiao, N.; Zhu, Y.; Li, H.; Yu, Y.; Xu, Y.; Zhu, J. Two-step hydrothermal pretreatments for co-producing xylooligosaccharides and humic-like acid from vinegar residue. *Fermentation* **2023**, *9*, 589. [\[CrossRef\]](#)
22. Kim, S.J.; Shin, K.; Kim, D.W.; Kim, Y.; Park, C. Cloning and Characterization of Cellulase from *Paenibacillus peoriae* MK1 Isolated from Soil. *Fermentation* **2023**, *9*, 873. [\[CrossRef\]](#)
23. Li, B.; Qiu, Z.; Huang, J.; Xian, X.; Zheng, X.; Lin, X. Application of aromatic ring quaternary ammonium and phosphonium salts–carboxylic acids-based deep eutectic solvent for enhanced sugarcane bagasse pretreatment, enzymatic hydrolysis, and cellulosic ethanol production. *Fermentation* **2023**, *9*, 981. [\[CrossRef\]](#)
24. Nawaz, A.; Chaudhary, R.; Haq, I.U.; Fu, X.; Huang, R.; Mukhtar, H.; Jiang, K. Delignification of halophyte *atriplex crassifolia* by green recyclable deep eutectic solvents for enhanced production of biogas. *Fermentation* **2023**, *9*, 314. [\[CrossRef\]](#)
25. Wang, H.; Lai, C.; Tao, Y.; Zhou, M.; Tang, R.; Yong, Q. Evaluation of the enzymatic production and prebiotic activity of galactomannan oligosaccharides derived from *Gleditsia microphylla*. *Fermentation* **2023**, *9*, 632. [\[CrossRef\]](#)
26. Zhang, H.; Jiang, J.; Quan, C.; Zhao, G.; Mao, G.; Xie, H.; Wang, F.; Wang, Z.; Zhang, J.; Zhou, P.; et al. Identification of a novel dehydrogenase from *Gluconobacter oxydans* for degradation of inhibitors derived from lignocellulosic biomass. *Fermentation* **2023**, *9*, 286. [\[CrossRef\]](#)
27. Zhu, Y.; Tang, R.; Yu, Y.; Yu, Z.; Wang, K.; Wang, Y.; Liu, P.; Han, D. Efficient co-production of xylooligosaccharides and glucose from vinegar residue by biphasic phenoxyethanol-maleic acid pretreatment. *Fermentation* **2023**, *9*, 61. [\[CrossRef\]](#)

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.