Ionic Liquids as an Effective Medium for Enzymatic Saccharification of Lignocellulosic Biomass: A Review

Wen Jun Dong,[a] Li Shuang Wang,[a] Dan Rui Wang,[a] Shi Jia Dong,[b] Zhi Yuan Xu,[a,*] and Xiao Mei Hu [a,*]

The efficient utilization of lignocellulosic biomass for biofuel production represents a significant challenge. As effective solvents, ionic liquids (ILs) have demonstrated considerable potential in laboratory-scale studies for the pretreatment of biomass, thereby enabling successful enzymatic saccharification. However, critical issues should be resolved, for instance, the remaining tolerance or activity of microorganisms or cellulase in the presence of ILs. This review aims to study the impact of ILs on microorganisms and cellulase during ILs-assisted biomass degradation, to investigate the interactions between ILs and microorganisms/enzymes, and to explore the feasible mechanism of ILs on enzymatic activity. This study emphasizes ILs-assisted enzymatic saccharification systems for the successful biomass degradation. Future research will focus on developing composite catalytic systems of ILs and microorganisms/enzymes and also the recycling and reusing of ionic liquids for industrial applications.

DOI: 10.15376/biores.20.4.Dong

Keywords: Ionic liquids; Biomass; Cellulase; Tolerance

*Contact information: a: Northeast Agricultural University, Harbin, 150030, P.R. China; b: Harbin University, Harbin, 150086, P.R. China; *Corresponding author: xmhu@neau.edu.cn; xzywd@sina.com*

INTRODUCTION

Reduced reserves of fossil fuel and increased importance of environmental protection have immensely influenced the search for biofuel production and biomass management. Lignocellulosic biomass is a renewable resource for biofuel production and a principal material to produce biosurfactants and a variety of chemicals (Xu *et al.* 2014). Enormous efforts have been devoted to the microbial and enzymatic hydrolysis of lignocellulosic materials. However, these processes are limited due to the complex structure of lignocelluloses (Bose *et al.* 2010). In general, the hydrolysis of lignocellulosic biomass requires a crucial pre-treatment process. Strong acid and base solutions are unsuitable due to compositional challenges (Gunny and Arbain 2013).

Ionic liquids (ILs) are ionic compounds consisting of cations and anions that remain in a liquid state at room temperature, typically demonstrating minimal volatility, exceptional thermal stability, and outstanding solubility (Gunny *et al.* 2014). ILs have been recognized as viable eco-friendly solvents for the treatment of lignocellulosic biomass (Mäki-Arvela *et al.* 2010). ILs have been demonstrated to be valid in the liberation of cellulose from the complicated structure of lignocelluloses (Moniruzzaman and Ono 2011). Sugarcane straw as an abundant waste material is subjected to pre-treatment with protonic ILs 2-hydroxyethylammonium acetate ([Mea][Ac]) and 2-hydroxyethylammonium hexanoate([Mea][Hex]) mixtures. Through the optimization of operational conditions and the use of specialized biomass reactors, deacetylated sugarcane straw was achieved

(Gonçalves *et al*. 2024). The saccharification and fermentation process of biomass pretreated with the low-cost protic IL ethanolamine acetate ([EOA][OAc]) can substantially decrease the economic cost of the production plant. The bagasse of agave pretreated with this IL achieved optimized saccharification under the action of enzyme mixtures (Pérez-Pimienta *et al*. 2024).

The utilization of ILs facilitates the efficient decomposition of lignocellulose, leading to the overall efficiency of cellulose saccharification with high efficient microorganisms and cellulase. The application of ILs in lignocellulosic biomass is inseparable from the assistance of microorganisms. Studies have demonstrated that certain microorganisms exhibit tolerance to ILs while preserving enzyme activity. For instance, various yeast strains display notable tolerance to the IL 1-ethyl-3-methylimidazolium acetate ([C_2C_1Im][OAc]) (Sitepu *et al*. 2020). It is proposed that cellulose-degrading enzymes can maintain their catalytic activity even in the presence of certain ILs, that suitably high rates and extents of hydrolysis can be achieved, and that near quantitative recovery of used ILs can be obtained at the end of the reaction.

Numerous articles indicate that ILs assist in biomass degradation, yet few reviews focus on the impact of ILs on microorganisms or enzymes. This review not only elucidates ILs as a medium for the enzymatic hydrolysis of lignocellulosic biomass but also explores the specific ILs-tolerant microorganisms and cellulases. Furthermore, it highlights the potential of ILs to simplify the saccharification process *via* integrated treatment, enabling the production of high-value products, and offers a promising strategy for advancing enzymatic saccharification in industrial applications.

THE DISTINCT CHARACTERISTICS OF ILS

ILs have gained significant attention in the fields of environmental applications in recent years due to their distinctive physicochemical properties. The initial type of ILs, known as a heptachloroaluminate salt, can be defined as a "salt with a melting temperature below the boiling point of water (Wilkes 2002)". ILs exhibit a wide fluid-like range, encompassing a significant temperature span between their freezing and boiling points, and the melting points are typically characterized by a relatively low value, enabling them to maintain their liquid state across a broad spectrum of temperatures (Arthur 2021).

The Pros and Cons of ILs

Firstly, the non-volatility of ILs significantly reduces solvent volatilization losses, while simultaneously safeguarding the environment. Secondly, their exceptional solubility enables them to dissolve a wide range of organic and inorganic substances, making them suitable for diverse reaction systems. Additionally, the remarkable thermal stability exhibited by ILs is particularly crucial for high-temperature enzyme-catalyzed reactions. Lastly, the tunability of ILs enables their properties to be finely adjusted by manipulating the combination of cations and anions, thereby exhibiting remarkable versatility in enhancing solvent polarity, miscibility, hydrophobicity, among other aspects (Brogan and Hallet 2016). Meanwhile, ILs also encounter substantial challenges. Owing to the diversity of IL types, the myriad combinations of their cations and anions might interfere with metabolic pathways in biological cells, thereby influencing the synthesis of intracellular substances. Additionally, due to the unique properties of ILs, conventional methods such as distillation, extraction, and membrane separation are insufficient for their complete recovery at present (Ovejero-Pérez *et al*. 2024). Consequently, further investigation is required to enhance their recovery and reuse.

The Utilization of ILs as Biocompatible Solvents

Due to their diverse structure and functionality, ILs exhibit a broad spectrum of potential applications with microorganisms and enzymes.

In particular, the advantageous properties of ILs have rendered them extensively employed in biocatalysis (Sheldon *et al.* 2002). ILs are used as reaction mediums, leading to a substantial enhancement in both esterification and transesterification efficiency of lipase, thereby extending the enzyme's operational lifespan. Moreover, ILs can serve as a catalyst in biodiesel production to enhance efficacy, minimize expenses, and facilitate recyclability (Zhang *et al.* 2024). Most importantly, ILs serve as solvents for cellulase degradation, carbohydrate conversion, and other biocatalytic processes, thereby enhancing reaction kinetics and product yields while also exerting a pivotal role in protein stability and enzyme preservation (Yu *et al.* 2015). In recent decades, ILs have emerged as a viable and environmentally sustainable alternative to hazardous organic solvents, exhibiting remarkable efficacy in various chemical reactions (Kragl *et al.* 2002).

ILS – PRETREATMENT FOR BIOMASS DEGRADATION

Recently, an increasing number of studies have demonstrated ILs have been carried out related to the pretreatment of lignocellulosic biomass. The hemicellulose and lignin in wheat husks and rice husks pretreated with 1-ethyl-3-methylimidazolium acetate (EMIM[OAC]) were effectively decomposed (John and Selvarajan 2024). Under optimized enzymatic hydrolysis conditions, the biomass exhibited the highest catalytic hydrolysis rate on the third day. The corn cobs and corn stalks were pretreated with a 20 wt% tetrabutyl phosphorus hydroxide (TBPH) aqueous solution. The cellulose separated out could be directly enzymatically hydrolyzed to produce sugar. The sugar production of the pretreated biomass was 4 to 5 times that of the untreated biomass (Sun *et al*. 2022), laying a foundation for the efficient conversion of other biomass resources into sugar. Furthermore, the pretreatment of corn stalks using the [BHEM] mesy-ethylene glycol co-solvent system resulted in the cellulose content and the lignin removal both exceeding 90%, thereby establishing a robust foundation for subsequent enzymatic hydrolysis to efficiently produce glucose (Zhu *et al*. 2024). Additionally, the results confirmed the successful production of high-concentration bioethanol *via* the fed-batch method. These studies highlight the potential of ILs as valuable biological solvents for facilitating biomass-to-biofuel conversion.

ILS – IMPACT ON MICROORGANISMS

According to investigations and analyses, imidazolium-based ILs such as 1-octyl-3-methylimidazolium chloride ([C_8MIM]Cl), 1-octyl-3-methylimidazolium bromide ([C_8MIM]Br), and 1-octyl-2,3-dimethylimidazolium bromide ([C_8DMIM]Br) can inhibit the growth of soil microorganisms (Chu *et al*. 2025) and influence the activity of soil enzymes. However, numerous microorganisms have been found to survive in environments containing ILs (Jace *et al.* 2016), including commonly used imidazolium-based ILs. For instance, *Lactobacillus plantarum* SKL-22 has been found to exhibit high tolerance in 1-butyl-3-methylimidazolium methane-sulfonate ([BMIM][MeSO$_4$]) and 1-butyl-3-methylimidazolium chloride ([BMIM][Cl]) (Yadav *et al*. 2020). *Aspergillus terreus* NEAU-7 is capable of growing in 1-ethyl-3-methylimidazolium chloride ([EMIM][Cl]) (Sun *et al.* 2022). *Bacillus paralicheniformis* was isolated and screened from [Emim][Cl] and [Bmim][Cl] using an adaptive evolution method (Ali *et al*. 2024). Furthermore, choline-based ILs choline alaninate [Ch][Ala] and choline glycinate [Ch][Gly] demonstrate strong antibacterial activity against *Bacillus cereus* and *Pseudomonas fluorescens* (Pereira *et al*. 2022), offering a sustainable alternative for biofilm control. *Penicillium oxalicum* HC6 exhibits notable salt tolerance, with its crude enzyme showing potential resilience to ILs and yielding a significant amount of reducing sugar (Sun *et al.* 2018). The tolerance of these microorganisms provides a solid foundation for further research into the application mechanisms of ILs (Table 1).

Table 1. ILs Impact on Microorganisms and Cellulase

	Strain	ILs	Tolerance situation
ILs impact on microorganisms	*Lactobacillus plantarum* SKL-22	[BMIM][MeSO$_4$] and [BMIM][Cl]	Having tolerance
	Bacillus paralicheniformis	[Emim][Cl] and [Bmim][Cl]	Having tolerance
	Bacillus cereus and *Pseudomonas fluorescens*	[Ch][Ala] and [Ch][Gly]	Antibacterial property
	Penicillium oxalicum HC6	/	Salt tolerance
ILs effect on cellulase	Strain	ILs	Enzymatic activity
	Pseudoalteromonas sp	5% [EMIM]Br	115%
		20% [EMIM]Ac	94.37%
	Aspergillus fumigatus	30% [Emim][DMP]	127%
	Penicillium oxalicum GS	20%[Dmim][(OCH$_3$)$_2$PO$_2$]	105%

ILS – IMPACT ON ENZYME ACTIVITY AND STABILITY

To further elucidate the advantages of ILs in lignocellulosic biomass, it is important to enhance the compatibility between enzymes and ILs. The main characteristics of ILs towards enzymes are stability and tolerance. The activity of enzymes is intricately linked to the structural and conformational changes occurring at their active sites within the microenvironment. The microenvironment surrounding the enzymatic active site is influenced by ions, with large organic cations exhibiting distinct interactions with enzyme anions and displaying complex solvation characteristics (Wawoczny *et al.* 2023). Consequently, ensuring stability of enzymes in ILs becomes paramount.

Cellulase exhibits a certain degree of tolerance towards ILs (John and Selvarajan 2024) (Table 1). Cellulase produced by the marine bacterium *Pseudoalteromonas* sp. exhibits high activity and stability in various ILs, including 1-ethyl-3-methylimidazolium bromide ([EMIM]Br), 1-ethyl-3-methylimidazolium acetate ([EMIM]Ac), ([BMIM]Cl), 1-ethyl-3-methylimidazolium methanesulfonate ([C$_2$MIM][CH$_3$SO$_3$]), 1-butyl-3-

methylimidazolium trifluoromethanesulfonate ([BMIM][OTF]), and 1-butyl-1-methylpyrrolidinium trifluromethanesulfonate ([BMPL][OTF]) (Trivedi *et al.* 2013). At a concentration of 5% (v/v) for these six ILs, cellulase activity remained above 90%, with the highest activity (115%) observed in [EMIM]Br. When the concentration was increased to 20% (v/v), the highest enzyme activity (94.37%) was recorded in [EMIM]Ac. Notably, the cellulase demonstrated significant stability in all six ILs at 5% (v/v). After incubation for both 24 and 36 h, its activity exceeded that of commercial cellulase. *Aspergillus fumigatus* isolated from chemical pollution showed increased enzymatic activity at higher concentrations (30% v/v) of 1-ethyl-3-methyl-imidazolium dimethylphosphate ([Emim][DMP]), 1-allyl-3-methylimidazolium chloride ([Amim][Cl]), and 1-ethyl-3-methylimidazolium methylammonium-methylsulfate ([Emim][MA]), with [Emim][DMP] (127%) > [Amim][Cl] (111%) > [Emim][MA] (109%). The half-life of cellulase in ILs was studied, demonstrating the stability of the cellulase in ILs. At the same time, an IL-cellulase system was established for in situ saccharification of rice straw (Xu *et al.* 2014). The enzyme activity of *Penicillium oxalicum* GS was increased by 105% in 20% (w/v) dimethylimidazolium methylphosphonate ([Dmim][$(OCH_3)_2PO_2$])(Xu *et al.* 2016). A novel IL-cellulase system was developed, which successfully disrupted the compact structure of rice straw and significantly enhanced the saccharification efficiency of rice straw. The key characteristics of cellulase activity and stability in ILs are crucial for the subsequent development and optimization of IL-cellulase systems. The proposed system not only significantly enhances the saccharification rate of straw but also effectively mitigates the enzymatic saccharification process of lignocellulosic biomass. These findings hold substantial implications for addressing the issue of surplus straw and advancing the development of a streamlined lignocellulosic enzymatic saccharification process flow.

Through the practical application of theoretical principles and continuous technological innovation, in certain instances, the catalytic efficacy can be augmented through synergistic combinations of ILs with other co-factors. Significant progress has been made in the production of biofuel-ethanol. The synergistic effect of sodium hydroxide and [BMIM]Cl pretreatment demonstrated remarkable efficacy in the enzymatic saccharification process for sunflower stalk biomass, enabling efficient conversion to bioethanol within a single container (Nargotra *et al.* 2018). The stable cellulase/xylanase produced by *Aspergillus aculeatus* PN14, which exhibited resistance against ILs, achieved complete saccharification of *Parthenium hysterophorus* biomass, pretreated with a surface-active [Emim][$MeSO_3$] using the enzyme. The multi-step pretreatment simplified the enzymatic saccharification process, enhanced sugar yield, and ultimately facilitated bioethanol fermentation (Nargotra *et al.* 2019).

MECHANISM OF ILS' EFFECTS ON MICROORGANISMS AND ENZYMES

The types and concentrations of ILs exert varying effects on microorganisms, which is closely linked to their metabolic processes. ILs can penetrate lipid bilayers, thereby modifying the properties of cell membranes and consequently impacting microbial cell membrane integrity, metabolism, and community structure (Kumari *et al.* 2020).The toxicity and lipophilicity of ILs are influenced by the hydrocarbon chains of their cations (Pallavi *et al.* 2020). These chains can penetrate the cell membrane, disrupt the organization of phospholipids, modulate membrane proteins and enzymes, and alter signaling pathways, ultimately leading to indirect effects on the biochemical functions of microorganisms.

The various enzyme activity may be attributed to the salt concentration of the ILs, whereby the high salinity of ILs can impede proper polypeptide folding of the enzyme, consequently leading to its deactivation, and achieving inhibitory effects (Salvado *et al.* 2010). The tolerance of cellulase in ILs is attributed to its high conformational stability, resulting in a longer half-life in ILs compared to buffer solutions. ILs can interact with amino acid residues on the surface of enzymes, inducing conformational changes that modulate enzyme activity and stability. Additionally, the polarity and hydrophobicity of ILs play a critical role in influencing substrate binding and the efficiency of catalytic reactions (Arakelyan *et al*. 2024).

APPLICATION OF ILS WITH OTHER ENZYMES

Lipases are the most extensively studied enzyme in ILs. They are frequently employed in hydrolysis and esterification reactions. Numerous lipases, such as porcine pancreatic lipase, exhibit remarkable activity and stability when utilized in ILs (Debajyoti 2022). Comparative studies reveal that CaLB demonstrates enhanced stability in 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]), 1-ethyl-2,3-dimethylimidazolium bis(trifluoromethylsulfonyl)imide ([EdMIM][Tf_2N]), and 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([EMIM][Tf_2N]) in comparison to organic solvents such as toluene, isooctane, and 3-pentanone at 80 °C (Ha *et al.* 2008).

Pectinase is primarily employed in the food industry for pectin degradation, exhibiting remarkable resilience within certain ILs environments. Simultaneously, the utilization of pectin as a coating agent presents a novel approach towards calcium carbonate-based microcapsule development. The newly synthesized prochloraz IL(PRO IL) serves as a nucleating agent to induce *in situ* precipitation of $CaCO_3$ onto PRO IL micellar surfaces (Zhiyuan *et al.* 2022). This straightforward and eco-friendly methodology aims to significantly enhance pesticide utilization efficiency by augmenting their photostability and environmental robustness.

The xylose enzymes are primarily utilized for the degradation of xylans, and these enzymes play an essential role in biomass conversion. Through synergistically combining microwave radiation with [EMIM][Cl], the enzymatic isomerization of xylose to xylulose was achieved rapidly and efficiently (Yu *et al.* 2012), thereby enhancing both enzyme activity and the yield of xylulose. This innovative approach not only advances industrial production but also contributes significantly to the field. Using corncob biomass as the substrate, xylose production can be achieved through pretreatment with an IL mixture followed by hydrolysis using xylanase. This process efficiently converts agricultural by-products into valuable biochemical products (Tinh *et al.* 2024).

CONCLUSIONS AND FUTURE PROSPECTS

Lignocellulosic biomass serves as a critical alternative biochemical resource for biofuel production. However, achieving efficient degradation of large-scale lignocellulosic biomass remains a significant challenge. This review has highlighted the application of ILs as media for enzymatic saccharification of waste lignocellulosic biomass, offering unique perspectives and methodologies for biofuel production and environmental management. In addition, ILs have been extensively investigated as effective solvents in biomass conversion processes, yet their impacts on microorganisms and enzymes in facilitating biomass degradation has not been summarized. Therefore, this review confirms that cellulase can retain catalytic activity in specific ILs, elucidates the interactions between ILs and microorganisms or enzymes, and describes the development of efficient enzyme-ionic liquid systems through the ILs-assisted biomass degradation, thereby enabling biofuel production. The potential of ILs to enhance enzymatic saccharification has been preliminarily validated through studies on microorganisms and enzyme activity; however, selecting appropriate types and concentrations, as well as evaluating toxicity and side effects, poses substantial challenges for industrial applications. Future research directions could focus on developing composite catalytic systems and achieving efficient, low-cost recyclability to fully realize their catalytic potential.

ACKNOWLEDGMENTS

The authors are grateful for the support of the Natural Science Foundation of Heilongjiang Province of China (No. YQ2022C027) and Heilongjiang Spring Goose Program for Innovative Talents (No.CYCX24005)

REFERENCES CITED

Ali, T. A., Sattar, Q. A., Imrana, K., Nabi, J. A., Yusuf, C., Muhammad, N., and Qamer-u-Nisa (2024). “Ionic-liquid-tolerant enzymes from an adapted *Bacillus paralicheniformis* for one-pot conversion of lignocellulosic substrates to ethanol,” *Industrial Crops and Products* 209(4), article 117988. DOI: 10.1016/J.INDCROP.2023.117988

Arakelyan, L. A., Arkhipova, D. M., Seitkalieva, M. M., Vavina, A. V., Sahharova, L. T., Kurbanalieva, S. K., Posvyatenko, A. V., Egorova, K. S.., and Ananikov, V. P. (2024). “A comprehensive dataset on cytotoxicity of ionic liquids,” *Scientific Data* 11(1), article 1379. DOI:10.1038/S41597-024-04190-3

Arthur, S. R. (2021). “Biocatalysis in ionic liquids: State-of-the-union,” *Green Chemistry* 23(21), 8406-8427. DOI: 10.1039/D1GC03145G

Bose, S., Armstrong, D. W., and Petrich, J. W. (2010). “Enzyme-catalyzed hydrolysis of cellulose in ionic liquids: a green approach toward the production of biofuels,” *The Journal of Physical Chemistry: B* 114(24), 8221-8227. DOI: 10.1021/jp9120518

Brogan, A. P. S., and Hallett, J. P. (2016). “Solubilizing and stabilizing proteins in anhydrous ionic liquids through formation of protein-polymer surfactant nanoconstructs,” *Journal of the American Chemical Society* 138(13), 4494-4501. DOI: 10.1021/jacs.5b13425

Chu, L., Zheng, W., Zhao, X., and Song, X. (2025). “Effects of different ionic liquid s on microbial growth and microbial communities’ structure of soil,” *Bulletin of Environmental Contamination and Toxicology* 114(2), 19. DOI:10.1007/S00128-024-03997-5

Debajyoti, G. (2022). “Lipase catalysis in mixed micelles,” *ChemBioEng Reviews* 9(4), 409-418. DOI: 10.1002/CBEN.202100045

Gunny, A. A. N., and Arbain, D. (2013). “Ionic liquids: Green solvent for pretreatment of lignocellulosic biomass,” *Advanced Materials Research* 2409(701-701), 399-402. DOI: 10.4028/AMR.701.399

Gunny, A. A. N., Arbain, D., Gumba, R. E., Jong, B. C., and Jamal, P. (2014). “Potential halophilic cellulases for *in situ* enzymatic saccharification of ionic liquids pretreated lignocelluloses,” *Bioresource Technology* 155(2014), 177-181. DOI: 10.1016/j.biortech.2013.12.101

Gonçalves, I. S., Teixeira Franco, T., and Soores Forte, M. B. (2024). “Cello-oligosaccharides and fermentable sugars production: An integrated deacetylation-delignification process to biomass pretreatment by using a mixture of protic ionic liquid,” *Food and Bioproducts Processing* 148(6), 95-107. DOI: 10.1016/J.FBP.2024.08.013

Ha, S. H., Lee, S. H., Dang, D. T., Kwon, M. S., Chang, W.-J., Yu, Y. J., Byun, S., and Koo, Y.-M. (2008). "Enhanced stability of *Candida antarctica* lipase B in ionic liquids," *The Korean Journal of Chemical Engineering* 25(2), 291-294. DOI: 10.1007/S11814-008-0051-0

John, A. J., and Selvarajan, E. (2024). “Ionic liquid-assisted pretreatment of lignocellulosic biomass using purified *Streptomyces* MS2A cellulase for bioethanol production," *International Journal of Biological Macromolecules* 270(1), article ID 132149. DOI: 10.1016/J.IJBIOMAC.2024.132149

Kragl, U., Eckstein, M., and Kaftzik, N. (2002). “Enzyme catalysis in ionic liquids,” *Current Opinion in Biotechnology* 13(6), 565-571. DOI: 10.1016/S0958-1669(02)00353-1

Kumari, P., Pillai, V. V. S., and Benedetto, A. (2020). “Mechanisms of action of ionic liquids on living cells: The state of the art,” *Biophysical Reviews* 12(5), 1-29. DOI: 10.1007/s12551-020-00754-w

Mäki-Arvela, P., Anugwom, I., Virtanen, P., Sjöholm, R., and Mikkola, J. P. (2010). “Dissolution of lignocellulosic materials and its constituents using ionic liquids—A review,” *Industrial Crops and Products* 32(3), 175-201. DOI: 10.1016/j.indcrop.2010.04.005

Moniruzzaman, M., and Ono, T. (2011). “Ionic liquid assisted enzymatic delignification of wood biomass: A new ‘green’ and efficient approach for isolating of cellulose fibers,” *Biochemical Engineering Journal* 60(1), 156-160. DOI: 10.1016/j.bej.2011.11.001

Nargotra, P., Sharma, V., and Bajaj, B. K. (2019). “Consolidated bioprocessing of surfactant-assisted ionic liquid-pretreated *Parthenium hysterophorus* L. biomass for bioethanol production,” *Bioresource Technology* 289(19), article ID 121611. DOI: 10.1016/j.biortech.2019.121611

Nargotra, P., Sharma, V., Gupta, M., Kour, S., and Bajaj, B. K. (2018). “Application of ionic liquid and alkali pretreatment for enhancing saccharification of sunflower stalk biomass for potential biofuel-ethanol production,” *Bioresource Technology* 267(22), 560-568. DOI: 10.1016/j.biortech.2018.07.070

Ovejero-Pérez, A., Nakasu, P. Y. S., Hopson, C., Costa, J. M., and Hallett, J. P. (2024). “Challenges and opportunities on the utilisation of ionic liquid for biomass pretreatment and valorisation,” *NPJ Materials Sustainability* 2(1). DOI: 10.1038/S44296-024-00015-X

Pereira, A. R., Gomes, I. B., and Simõnes, M. (2022). “Choline-based ionic liquids for planktonic and biofilm growth control of *Bacillus cereus* and *Pseudomonas* *fluorescens*," *Journal of Molecular Liquids* 346(2), article 117077. DOI: 10.1016/J.MOLLIQ.2021.117077

Pérez-Pimienta, J. A., Castillo-Preciado, D. J., González-Álvarez, V., and Méndez-Ac osta, H. O. (2024). "Optimization of cost-effective enzymatic saccharification usin g low-cost protic ionic liquid as pretreatment agent in Agave bagasse," *Waste M anagement* 175(3), 204-214. DOI: 10.1016/J.WASMAN.2024.01.001

Salvado, A. C., Santos, M. d. C., and Saraiva, J. A. (2010). "Effect of the ionic liquid [bmim]Cl and high pressure on the activity of cellulase," *Green Chemistry* 12(4), 632-635. DOI: 10.1039/B918879G

Sheldon, R. A., Lau, R. M., Sorgedrager, M. J., Rantwijk, F. V., and Seddon, K. R. (2002). "Biocatalysis in ionic liquids," *Green Chemistry* 4(2), 147-151.

Sitepu, I., Enriquez, L., Nguyen, V., Fry, R., Simmons, B., Singer, S., and Boundy-Mills, K. L. (2020). "Ionic liquid tolerance of yeasts in family Dipodascaceae and genus *Wickerhamomyces*," *Applied Biochemistry and Biotechnology* 191(4), 1580-1593. DOI: 10.1007/s12010-020-03293-y

Sun, Y. X., Shen, B. B., Han, H. Y., Lu, Y., Zhang, B. X., Gao, Y. F., and Hu, X. M. (2018). "Screening of potential IL-tolerant cellulases and their efficient saccharification of IL-pretreated lignocelluloses," *RSC Advances* 8(54), 30957-30965. DOI: 10.1039/c8ra05729j

Sun, Y., Li, P., Sun, Q., Liu, X., Wang, Y., Zhang, B., Dong, S., and Hu, X. (2022). "Insights into ionic liquids-resistance mechanism and lignocellulose-degradation model of *Aspergillus terreus* in 1-ethyl-3-methylimidazolium acetate," *Industrial Crops and Products* 178, article ID 114593. DOI: 10.1016/J.INDCROP.2022.114593

Sun, J.-F., Ding, R., and Yin, J.-Z. (2022). "Pretreatment of corn cobs and corn stalks with tetrabutyl phosphate hydroxide ionic liquid to enhance enzymatic hydrolysis process," *Biochemical Engineering Journal* 177(1), article ID 108270. DOI: 10.1016/J.BEJ.2021.108270

Tinh, N. T., Bao, L. M., Nhan, D. D., Bao, P. P., Hoang, T. M., Nam, N. T. H., and Nguyen, H. H. (2024). "Green process prorogue by xylanase enzyme and cellulose-derived sulfonated graphene oxide-like reinforcement in xylose and furfural production from corncob biomass," *Renewable Energy* 232(13), article ID 121039. DOI: 10.1016/J.RENENE.2024.121039

Trivedi, N., Gupta, V., Reddy, C. R. K., and Jha, B. (2013). "Detection of ionic liquid stable cellulase produced by the marine bacterium *Pseudoalteromonas* sp. isolated from brown alga *Sargassum polycystum* C. Agardh," *Bioresource Technology* 132(6), 313-319. DOI: 10.1016/j.biortech.2013.01.040

Wawoczny, A., Przypis, M., and Gillner, D. (2023). "Enzymatic valorization of lignocellulosic biomass—The influence of deep eutectic solvents and ionic liquids on the activity of cellulolytic enzymes," *Sustainability* 15(24), article ID 16726. DOI: 10.3390/SU152416726

Wilkes, J. S. (2002). "A short history of ionic liquids-from molten salts to neoteric solvents," *Green Chemistry* 4(2), 73-80. DOI: 10.1039/B110838G

Xu, J., He, B., Wu, B., Wang, B., Wang, C., and Hu, L. (2014). "An ionic liquid tolerant cellulase derived from chemically polluted microhabitats and its application in *in situ* saccharification of rice straw," *Bioresource Technology* 157(7), 166-173. DOI: 10.1016/j.biortech.2014.01.102

Xu, J., Wang, X., Liu, X., Xia, J., Zhang, T., and Xiong, P. (2016). "Enzymatic in situ saccharification of lignocellulosic biomass in ionic liquids using an ionic liquid-tolerant cellulases," *Biomass and Bioenergy* 93(10), 180-186. DOI: 10.1016/j.biombioe.2016.07.019

Yadav, N., Pranaw, K., and Khare, S. K. (2020). "Screening of lactic acid bacteria stable in ionic liquids and lignocellulosic by-products for bio-based lactic acid production," *Bioresource Technology Reports* 11(3), article ID 100423. DOI: 10.1016/j.biteb.2020.100423

Yu, D.-H., Wang, Y.-Y., Wang, C.-M., Ma,D.-X., and Fang, X.-X. (2012). "Combination use of microwave irradiation and ionic liquid in enzymatic isomerization of xylose to xylulose," *Journal of Molecular Catalysis B: Enzymatic* 79(7), 8-14. DOI: 10.1016/j.molcatb.2012.04.005

Yu, S., Chen, P., Liu, X., and Li, L. (2015). "Degradation of chitosan by modified cellulase in the ionic liquid system," *Catalysis Letters* 145(10), 1845-1850. DOI: 10.1007/s10562-015-1596-2

Zhang, Y., Li, Y., and Sun, S. (2024). "Unveiling the innovation: Optimized biodiesel production from emerging *Acer truncatum* Bunge seed oil using novel and highly effective alkaline ionic liquid catalyst," *Chemical Engineering Journal* 487(9), article ID 150603. DOI: 10.1016/J.CEJ.2024.150603

Zhiyuan, Z., Yunhao, G., Gang, T., Yuyang, T., Yan, L., Huachen, W., and Yongsong, C. (2022). "Facile preparation of pH/pectinase responsive microcapsules based on $CaCO_3$ using fungicidal ionic liquid as a nucleating agent for sustainable plant disease management," *Chemical Engineering Journal* 446(32), article ID 137073. DOI: 10.1016/J.CEJ.2022.137073

Zhu, Q.-Q., Dong, H.-X., Yan, D.-X., Gao, D., Xu, K.-X., Cheng, X.-J., Xin, J.-Y., and Lu, X-M. (2024). "High concentration bioethanol production from corn stalk *via* enhanced pretreatment with ionic liquids," *Chemical Engineering Science* 283(1), article ID 119375. DOI: 10.1016/J.CES.2023.119375

Article submitted: January 3, 2025; Peer review completed: May 2, 2025; Revised version received: June 1, 2025; Accepted: June 17, 2025; Published: August 26, 2025.
DOI: 10.15376/biores.20.4.Dong