



Nature's Decomposers: How effectively Actinomycetes pioneering the path in efficient Biomass Degradation

Swati Priya¹, P. K. Roychoudhury² and Santosh Kumar¹

¹Department of Botany, B. R. A. Bihar University, Muzaffarpur (India)

²Dept. of Biochemical Eng and Biotechnology, Indian Institute of Technology, New Delhi

Abstract :

This review paper provides potential information of lignocellulosic biomass as an abundant, renewable, and cost effective for various industrial applications. It emphasizes the role of lignocellulolytic enzymes (cellulases, hemicellulases and lignolytic enzymes) in processing lignocelluloses which particular focus on diversity and effectiveness of actinomycetes. Potential of the pulp and paper industry to transform its processes and products on non-biodegradable based plastics and enhancing environmental sustainability. Application of lignocellulolytic enzymes, particularly from actinomycetes for the degradation of lignocellulosic biomass. Analysis of ecological and economic benefits of utilizing lignocellulosic waste generated from agricultural, forest and agroindustrial activities. This review critically evaluates the studies of environmental impact, biodegradability of lignocellulose-derived polymers and chemicals compared to their petrochemical. Primarily, it emphasizes that lignocellulosic biomass obtained from various sources like agriculture, forestry and agroindustrial activities is abundant and economically feasible as a feed stock. Actinomycetes are identified

as a enzyme production due to their diverse and effective lignocellulose-degrading capabilities. pulp and paper industry's proactive approach to sustainability challenges, exploring innovative processes and products derived from lignocellulosic biomass.

This review concludes that lignocellulosic biomass represents a significant opportunity to develop sustainable and economically viable alternatives to petroleum based products. Use of lignocellulolytic enzymes from actinomycetes for processing lignocellulosic materials can lead to the production of biodegradable and environment friendly chemical materials. in India, Pulp and paper industry is well positioned to lead adoption of lignocellulosic biomass in various applications beyond traditional uses, contributing to reduced carbon emissions and environmental pollution. Continued research and development are essential to optimize enzyme production, enhancing economic and environmental benefits of lignocellulosic biomass utilization.

Introduction :

Lignocellulosic biomass, derived from agricultural, forest, and agroindustrial activities is the most abundant form of biomass on Earth. Every year, these activities generate substantial amounts of lignocellulosic waste, offering a readily available,

Corresponding Author :

Swati Priya

Email: swathi.bio@gmail.com

Date of Acceptance : 13. 01. 2024

Date of Publication : 20. 04. 2024

cost-effective and renewable feed stock for various industrial applications (Pasti *et al.*, 1985; Shen and Patel, *et al.*, 2008). This biomass serves as a promising alternative to fossil fuels and petrochemical derived products, particularly in the realm of biofuel production and development of biodegradable materials. First generation biofuels, which utilize storage sugars from plants such as starch, pose a significant issue as they compete with global food resources (FAO, 2011). In second generation biofuels focus on structural polymers like cellulose. However, these polymers are challenging to process due to cellulose's crystallinity and presence of lignin, making pre-treatment processes costly (Himmel *et al.*, 2007; da Costa Souza *et al.*, 2009).

Significant research is being devoted to understanding the structure of lignocellulose to enhance efficiency and cost effectiveness. Wood is a primary source of lignocellulosic biomass, consists mainly of cellulose, hemicelluloses and lignin. The plant cell wall is composed of a primary wall, secondary wall, and middle lamella with cellulose primarily concentrated in the layer of secondary wall and lignin predominantly in the middle lamella (Sjostrom, 1993; Fengel and Wegener, 1989; Dix and Webster, 1995). Cellulose is most abundant natural polymer and composed of linear unbranched polysaccharide chains of α D-glucopyranose units linked by α (1-4) glucosidic bonds. These glucan chains form fibrils through hydrogen bonding which aggregate into fibers, giving wood its strength (Eaton and Hale, 1993; Kuhad *et al.*, 1997). Due to its high degree of polymerization and crystallinity, cellulose is used

in various materials (Heinze and Petzold, 2008). Hemicelluloses are composed of both linear and branched polysaccharides, including glucose, mannose, arabinose, xylose and galactose. They are covalently bonded with lignin, providing additional strength to wood (Fengel and Wegener, 1989; Kuhad *et al.*, 1997; Pelczar *et al.*, 1988; Priya *et al.*, 2015). Hemicelluloses are crucial for forming films and barrier materials (Spiridon and Popa, 2008). Mannans and xylans are most abundant hemicelluloses in wood's secondary cell walls with some monosaccharides being acetylated. The acetylation of hemicelluloses is thought to be essential for plant defense mechanisms, affecting enzymatic hydrolysis and providing resistance to fungal attacks by altering moisture sorption and polymer conformation (Teleman *et al.*, 2000; Gille and Pauly, 2012). Lignin is a complex and amorphous polymer with aromatic structures, significantly contributes to wood's hardness, strength and water impermeability, making plants resistant to biodegradation and environmental stresses (Eriksson *et al.*, 1990; Argyropoulos and Menachem, 1997). Understanding the intricate structures and interactions of these components is crucial for advancing lignocellulosic biomass processing technology which promise to reduce dependence on non-renewable resources and enhance environmental sustainability.

Bihar's agricultural land is rich in lignocellulosic substrates, offering a vast resource for biotechnological applications. Actinomycetes

biotechnological applications. Actinomycetes bacteria, isolated from the rhizosphere of litchi plants, can effectively degrade this biomass. These microorganisms produce lignocellulolytic enzymes, breaking down cellulose, hemicellulose and lignin. Utilizing these enzymes can enhance biofuel production and other industrial processes. This approach not only leverages local resources but also contributes to sustainable agricultural practices. Role of Actinomycetes in utilization of lignocellulose biomass.

A distinct taxonomic group domain Bacteria are members of the order Actinomycetales (Priya, S., *et al.*, 2016(a, b)). They are Gram-positive, primarily aerobic bacteria with high G+C content are known for their spore-forming capabilities (Zhao *et al.*, 2012 a; Shirling and Gottlieb, 1966). Actinomycetes exhibit filamentous growth similar to fungi, producing aerial and substrate mycelium and are responsible for the earthy smell of soil (Waksman, 1950). They are ubiquitous in nature, found in both terrestrial and aquatic habitats, including mangroves and sea sediments (Goodfellow and Williams, 1983; Zhu *et al.*, 2008; Maldonado *et al.*, 2009). Actinomycetes thrive in a wide range of environments, from mesophilic to thermophilic conditions, broadening their habitat range (Zhao *et al.*, 2012a; Lynd *et al.*, 2002; Kuhad *et al.*, 2011). Actinomycetes produce a diverse array of bioactive compounds, including lignocellulolytic enzymes, which are crucial for degrading lignocellulosic biomass. These enzymes including cellulases, hemicellulases and lignolytic enzymes facilitate the breakdown of plant materials,

making them valuable in various industries (Lynd *et al.*, 2002). Cellulases are used in bioethanol biomethane production, ligand binding, textile industry, pulp and paper manufacturing, detergents, animal feed and food processing ((Martinez *et al.*, 2005; Kuhad *et al.*, 1997). Hemicellulases find applications in biobleaching, paper waste deinking, fruit juice clarification, feed upgrading and saccharification of hemicelluloses to xylose sugars (Viikari *et al.*, 2007; Paterson *et al.*, 1984). Lignin degrading enzymes are employed in pretreatment of recalcitrant biomass for biofuel production, paper and textile industries, food industry, waste water treatment, bioremediation, organic synthesis, cosmetic and pharmaceutical industries (Martinez *et al.*, 2005).

Actinomycetes are an attractive group for the production of lignocellulolytic enzymes due to their diversity and efficiency in biomass degradation (Pan *et al.*, 2006; Ventura *et al.*, 2007). This review explores the diversity, applications of lignocellulolytic actinomycetes and describes their enzyme systems involved in biomass degradation, highlighting their potential for various industrial applications. Lignocellulolytic enzyme system in Actinomycetes. Cellulases enzymes, classified into different families based on sequence homologies, include exoglucanases (EC 3.2.1.74), endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) and glucosidases (EC 3.2.1.21). Exoglucanases release glucose units by acting on the reducing or non-reducing ends of cellulose chains, while endoglucanases hydrolyze α 1,4-glycosidic bonds

within the cellulose chains, producing dextrans of varying lengths. Cellobiohydrolases cleave glycosidic bonds at non-reducing ends, releasing cellobiose units and a glucosidases hydrolyze cellobiose into glucose monomers. The complete hydrolysis of cellulose requires synergistic action of all these enzymes, including synergy between endoglucanases and exoglucanases (endo-exo synergy). Microbial cellulase systems are either complexed like cellulosomes found in anaerobic bacteria or non-complexed (free) in aerobic bacteria including many actinomycetes. *Cellulomonas fimi*, *Microbispora bispora* and *Thermobifida fusca* are well-studied cellulase producers. *Thermobifida fusca*, thermophilic, spore-forming actinomycete have a genome encoding 36 glycoside hydrolases across 22 GH families. It has six extracellular cellulase system and one intracellular glucosidase, each with distinct catalytic and carbohydrate binding domains. The catalytic domains belong to different families: Cel5A and Cel5B (GH family 5), Cel6A and Cel6B (GH family 6), Cel9A and Cel9B (GH family 9) and Cel48A (family 48). Cel5A, Cel5B, Cel6A and Cel9B are endocellulases, whereas Cel6B and Cel48A are processive exocellulases acting on non-reducing and reducing ends respectively. Cel9A exhibits both exo and endocellulase activities. Structural studies show variations in the position of carbohydrate binding domains and significant sequence differences among cellulases from different microbes, suggesting horizontal gene transfer .

Hemicellulases often produced along side cellulases, primarily target xylan and mannan. Xylan hydrolysis involves a complex enzyme system including endo-

3,4- α -xylanases (EC 3.2.1.8), α -D-xylosidases (EC 3.2.1.37), α -L-arabinofuranosidases (EC 3.2.1.55), α -glucuronidases (EC 3.2.1.139), acetyl xylan esterases (EC 3.1.1.72) and ferulic/coumaric acid esterases (EC 3.1.1.73). Mannan is hydrolyzed by mannanases (EC 3.2.1.78), α -mannosidases (EC 3.2.1.25) and α -galactosidases (EC 3.2.1.22). These enzymes, often classified as glycosyl hydrolases, play crucial roles in hemicellulose degradation and consequently enhance cellulose hydrolysis. *Thermobifida fusca* and other actinomycetes produce multiple xylanases and mannan degrading enzymes, with their specific activities and classifications detailed in studies. Lignolytic Enzymes Lignin degradation involves a complex enzyme system with principal enzymes such as laccases (EC 1.10.3.2), manganese peroxidases (MnP, EC 1.11.1.13) and lignin peroxidases (LiP, EC 1.11.1.14). Laccases, extracellular inducible enzymes, degrade polyphenols using oxygen as an oxidizing agent and cofactor facilitated by four copper atoms in their active sites. Manganese and lignin peroxidases known as heme peroxidases, require H_2O_2 for their activity and can degrade high redox potential compounds, oxidizing both phenolic and non-phenolic aromatic rings in lignin. Laccases in actinomycetes often feature two Cu-binding domains and function as dimers or trimers with structural studies highlighting these unique characteristics.

Discussion :

The findings of this review highlight several critical aspects of lignocellulosic biomass utilization. First abundance and renewability of lignocellulosic

biomass make it a cost-effective feedstock for various industries. The use of actinomycetes derived enzymes offers a biological approach to processing this biomass which can be more sustainable and environmentally friendly compared to chemical methods. The diversity of lignocellulolytic enzymes produced by actinomycetes, including cellulases, hemicellulases, and lignolytic enzymes, allows for the complete breakdown of lignocellulosic materials into fermentable sugars and other valuable products. The pulp and paper industry's shift towards utilizing lignocellulosic biomass in biorefinery processes represents a significant step towards sustainability. By producing high-value by-products and reducing reliance on petroleum-based plastics, the industry can contribute to environmental conservation and the circular economy. However, challenges such as the high crystallinity of cellulose and the presence of lignin still pose obstacles to efficient biomass processing.

Future Prospects :

Future research should focus on enhancing the efficiency of lignocellulolytic enzymes through genetic engineering and optimization of fermentation conditions. Developing robust microbial strains with higher enzyme yields and improved substrate specificity can significantly advance the field. Additionally, exploring the potential of co-cultivation and enzyme cocktails could lead to more efficient biomass degradation. There is also a need for scalable and economically viable bioprocesses that can integrate into existing industrial frameworks, particularly in the pulp and paper industry.

Advancements in biotechnology and synthetic biology offer promising avenues for designing novel enzyme systems with tailored properties. Furthermore, research on the environmental impact and life cycle assessment of biomass-derived products compared to their petrochemical counterparts will provide valuable insights into the sustainability of these processes. The exploration of new lignocellulosic feedstocks and their suitability for enzyme production and biomass conversion will also be crucial in expanding the applications of lignocellulosic biomass.

Limitations :

The primary limitations identified in this review include the variability in the efficiency of lignocellulolytic enzymes across different microbial sources and substrates. The complexity of lignocellulosic biomass, with its heterogeneous composition, poses challenges to uniform degradation. Additionally, the economic feasibility of large scale enzyme production and biomass conversion processes remains a significant barrier. The high cost of enzyme production and the need for pretreatment processes to increase biomass accessibility can limit the commercial viability of these technologies.

Conclusion

Lignocellulosic biomass stands out as a highly promising resource for sustainable and eco-friendly industrial applications, including biofuels, biodegradable materials, and other high-value products. The utilization of lignocellulolytic enzymes, especially those derived from actinomycetes, plays

a crucial role in breaking down complex lignocellulosic materials, thereby enabling the efficient conversion of biomass into valuable products. Actinomycetes exhibit diverse and efficient lignocellulose degrading capabilities, making them excellent candidates for industrial enzyme production. Pulp and paper industry is making significant strides toward sustainability by incorporating lignocellulosic biomass into its processes, thus reducing dependence on non-renewable, petroleum based resources. Review need continued research and development to optimize enzyme production and application, which will further enhance economic and environmental benefits of lignocellulosic biomass utilization.

Acknowledgements:

I would like to sincerely thank my mentor, Dr. Santosh Kumar, for his valuable guidance and continuous support throughout the development of this review paper.

References:

1. Argyropoulos, D. S., & Menachem, S. B. (1997). Structure and properties of lignin. In A. R. Pizzi & K. L. Mittal (Eds.), *Handbook of Adhesive Technology* (207-248). Marcel Dekker, Inc.
2. Da Costa Souza, A. P., Grandis, A., Leite, D. C. C., & Buckeridge, M. S. (2009). Sugarcane as a bioenergy source: history, performance, and perspectives for second generation bioethanol. *Bio Energy Research*, 2(1-2), 175-196.
3. Dix, N. J., & Webster, J. (1995). *Fungal Ecology*. Springer Science & Business Media.
4. Eaton, R. A., & Hale, M. D. C. (1993). *Wood: Decay, Pests and Protection*. Chapman & Hall.
5. Eriksson, K. E. L., Blanchette, R. A., & Ander, P. (1990). *Microbial and Enzymatic Degradation of Wood and Wood Components*. Springer-Verlag.
6. FAO (2011). *The State of Food and Agriculture 2010-11*. Food and Agriculture Organization of the United Nations.
7. Fengel, D., & Wegener, G. (1989). *Wood: Chemistry, Ultrastructure, Reactions*. Walter de Gruyter.
8. Gille, S., & Pauly, M. (2012). O-acetylation of plant cell wall polysaccharides. *Frontiers in Plant Science*, 3, 12.
9. Goodfellow, M., & Williams, S. T. (1983). Ecology of actinomycetes. *Annual Review of Microbiology*, 37(1), 189-216.
10. Heinze, T., & Petzold, K. (2008). *Cellulose chemistry: Novel products and synthesis paths*. Springer.
11. Himmel, M. E., Ding, S. Y., Johnson, D. K., Adney, W. S., Nimlos, M. R., Brady, J. W., & Foust, T. D. (2007). Biomass recalcitrance: Engineering plants and enzymes for biofuels production. *Science*, 315(5813), 804-807.
12. Kuhad, R. C., Gupta, R., & Singh, A. (1997). Microbial cellulases and their industrial applications. *Enzyme Research*, 2011.
13. Kuhad, R. C., Gupta, R., & Singh, A. (2011). Microbial cellulases and their industrial applications. *Enzyme Research*, 2011, 1-10.

14. Lynd, L. R., Weimer, P. J., van Zyl, W. H., & Pretorius, I. S. (2002). Microbial cellulose utilization: Fundamentals and biotechnology. *Microbiology and Molecular Biology Reviews*, 66(3), 506-577.
15. Maldonado, L. A., Fenical, W., Jensen, P. R., Kauffman, C. A., Mincer, T. J., & Ward, A. C. (2009). *Salinispora arenicola* gen. nov., sp. nov. and *Salinispora tropica* sp. nov., obligate marine actinomycetes belonging to the family Micromonosporaceae. *International Journal of Systematic and Evolutionary Microbiology*, 55(5), 1759-1766.
16. Maldonado, L. A., Stach, J. E. M., Pathomaree, W., Ward, A. C., Bull, A. T., & Goodfellow, M. (2009). Diversity of cultivable actinobacteria in geographically widespread marine sediments. *Antonie van Leeuwenhoek*, 95(1), 43-57.
17. Martinez, Á. T., Ruiz-Dueñas, F. J., Camarero, S., Serrano, A., Linde, D., Lund, H., & Floudas, D. (2005). Oxidative enzymatic conversion of lignin into value-added products. *Biotechnology for Biofuels*, 10(1), 113.
18. Martinez, A. T., Ruiz-Dueñas, F. J., Martínez, M. J., Del Río, J. C., & Gutiérrez, A. (2005). Enzymatic delignification of plant cell wall: From nature to mill. *Current Opinion in Biotechnology*, 16(4), 393-400.
19. Pelczar, M. J., & Chan, E. C. S. (1988). *Microbiology: Concepts and Applications*. McGraw-Hill.
20. Priya, S., Roychoudhury, P. K., & Kumar, S. (2015). 16S rRNA phylogenetic analysis of actinomycetes isolated from fruit orchard associated with lignocellulose degradation activities. *IJBASR*, 2(2), 155-161.
21. Priya, S., Roychoudhury, P. K., & Kumar, S. (2016). Screening and Molecular Characterization of Rhizospheric Actinomycetes for Industrially Significant Cellulase Enzymes. *IJBASR*, 3(1), 192-200.
22. Priya, S., Roychoudhury, P. K., & Kumar, S. (2016). Unlocking the Biotechnological Potential of Actinomycetes for various industrial application: A Comprehensive Investigation. *IJBASR*, 3(2), 206 - 216.
21. Pan, X., Gilkes, N., & Saddler, J. N. (2006). Effect of acetyl groups on enzymatic hydrolysis of cellulosic substrates. *Holzforschung*, 60(4), 398-401.
22. Paterson, A., McCarthy, A.J., & Broda, P. (1984). The application of molecular biology to lignin degradation. In J.M. Grainger & J.M. Lynch (Eds.), *Microbiological Methods for Environmental Biotechnology* (pp. 33-68). Academic Press, London.
23. Pasti, M.B., & Belli, M.L. (1985). Cellulolytic activity of actinomycetes isolated from termites (Termitidae) gut. *FEMS Microbiology Letters*, 26(1), 107-112.
24. Shen, L., & Patel, M. K. (2008). Life cycle assessment of polysaccharide materials: A review. *Journal of Polymers and the Environment*, 16(2), 154-167.
25. Shirling, E. B., & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species.

- from birch and beech. *Carbohydrate Research*, 329(4), 807-815.
29. Ventura, M., Canchaya, C., Tauch, A., Chandra, G., Fitzgerald, G. F., Chater, K. F., & van Sinderen, D. (2007). Genomics of Actinobacteria: Tracing the evolutionary history of an ancient phylum. *Microbiology and Molecular Biology Reviews*, 71(3), 495-548.
30. Ventura, M., O'Flaherty, S., Claesson, M. J., Turrioni, F., Klaenhammer, T. R., van Sinderen, D., & O'Toole, P. W. (2007). Genome-scale analyses of health-promoting bacteria: Probiogenomics. *Nature Reviews Microbiology*, 7(1), 61-71.
31. Viikari, L., Alapuranen, M., Puranen, T., Vehmaanperä, J., & Siika-aho, M. (2007). Thermostable enzymes in lignocellulose hydrolysis. *Advances in Biochemical Engineering/Biotechnology*, 108, 121-145.
32. Waksman, S. A. (1950). *Soil Microbiology*. John Wiley & Sons, Inc.
33. Waksman, S. A. (1950). *The Actinomycetes*. *Chronica Botanica*.
34. Cross, T. (1981). *Thermophiles: General, Molecular and Applied Microbiology*. Wiley-Interscience.
35. Zhao, X., Zhang, L., & Liu, D. (2012a). Biomass recalcitrance. Part II: fundamentals of different pre-treatments to increase the enzymatic digestibility of lignocellulose. *Biofuels Bioproducts and Biorefining*, 6(5), 561–579.
36. Zhao, X., Zhang, L., & Liu, D. (2012b). Biomass recalcitrance. Part I: the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose. *Biofuels Bioproducts and Biorefining*, 6(5), 465–482.
37. Zhu, L., O'Dwyer, J. P., Chang, V. S., Granda, C. B., & Holtzapple, M. T. (2008). Structural features affecting biomass enzymatic digestibility. *Bioresource Technology*, 99(9), 3817–3828.
