49. ACTUAL TASKS ON AGRICULTURAL ENGINEERING

Review paper

ENZYMES FOR LIGNOCELLULOSIC BIOMASS DEGRADATION AS AN APPROACH TO GREEN TECHNOLOGY

Anamarija GUDELJ – VELAGA[1*], Nikola BILANDŽIJA[1], Mateja GRUBOR[1],
Ivana TOMIĆ[1], Zorana KOVAČEVIĆ[2], Tajana KRIČKA[1]
[*]E-mail corresponding author: agvelaga@agr.hr
[1]Faculty of Agriculture University of Zagreb, Svetošimunska cesta 25, Zagreb 10 000
[2]Faculty of Textile Technology University of Zagreb, Prilaz Baruna Filipovića 28a, Zagreb 10 000

ABSTRACT

Enzyme technology is an interdisciplinary field because its application varies from industrial purposes to pharmaceutical development, and its use is recognized as good sustainable development practice. Enzymes use in industry is preferred over other chemicals because of their selectivity, efficiency, mild conditions, renewable and sustainable practice and cost effectiveness in industry use. Enzyme use requires understanding of the structure of treated material, in this case - lignocellulosic biomass. Lignocellulosic biomass is renewable resource composed of cellulose, hemicellulose, and lignin with a diverse possibility of exploitation. These three components influence characteristics, strength, and stiffness of lignocellulosic biomas. Enzymes for cellulose degradation are: cellulases, hemicellulases, xylanases and pectinases. Enzymes for hemicellulose degradation are hemicellulases that can be xylanases or arabinofuranosidases. Enzymes for lignin degradation are lignin peroxidases, manganese peroxidases, and laccases. All these enzymes work by principle of breaking down the bonds between structural composition or other cell wall components. Use of enzymes is cost saving and an environmentally friendly alternative.

Keywords: *enzymes, lignocelulosic biomass, degradation, sustainable development*

INTRODUCTION

Plant biomass is an excellent source of energy, fibre and bio-chemicals. The use of biomass can significantly reduce dependence on fossil sources and represents a key resource for achieving sustainable development that replaces the oil-based production system and reduces greenhouse gas emissions (Xu et al., 2013; Gonzalo et al., 2016; Kaminura et al., 2019). The structure of biomass consists mostly of lignin, cellulose and hemicellulose and is called lignocellulosic biomass (de Gonzalo et al., 2016). Lignocellulose is a macromolecular complex consisting of cellulose, hemicellulose, and lignin (Feng et al., 2011). In order to extract fibers and residues for biofuel production, there are various pretreatment methods used for biomass processing. Pretreatments are divided into physical, chemical, physico-chemical and biological. Physical pretreatment includes grinding, microwaving, extrusion, and ultrasonication. Chemical pretreatments include alkaline and acid hydrolysis, liquid ion process, and deep eutectic solvents. Physico-chemical pretreatment processes rely on steam explosion, fiber explosion with ammonia, CO2 explosion, and liquid hot treatment. Biological pretreatments include the processing of whole cells and the most interesting is enzyme pretreatment (Baruah et al., 2018). The use of enzyme technologies is becoming more and more attractive for the processing of natural fibers. The main reason for the acceptance and use of enzyme technology is the fact that the application of enzymes is environmentally friendly and has a focused performance (Bledzki et al., 2010; Hanana et al., 2015). Enzymes were first used in the production of animal feed in the 1920s. In the 1970s and 1980s, researchers began to develop enzymes specifically for use in agriculture, and these enzymes were used to improve the efficiency of various agricultural processes, such as silage fermentation, the production of biofuels, and the breakdown of plant fibers (Hartmann, 2007; Pariza et al., 2010). The aim of the paper is to review the literature on the influence of enzymes on the degradation of lignocellulosic biomass and their application.

STRUCTURAL COMPOSITION OF LIGNOCELLULOSIC BIOMASS

Lignocellulosic biomass is an attractive feedstock because of its variety. Biomass is widely available and can be collected from a variety of sources, including agricultural residues, forestry residues, and energy crops (Easterly and Burnham, 1996). Lignocellulosic biomass is a renewable resource that can be replenished through sustainable land management practices, and because it is composed of cellulose, hemicellulose, and lignin, which are all rich in energy and can be converted into a variety of products (Banu et al., 2021). Therefore, biomass has high energy content and can be used to produce biofuels such as ethanol, biodiesel, and biogas. In addition to being used for biofuels, biomass can also be used for production of chemicals, fibres and all kind of different materials. With a diverse possibility of exploitation of lignocellulosic biomass can help to reduce greenhouse gas emissions and promote the transition to a more sustainable, low-carbon economy (Fatma et al., 2018; Mussatto et al., 2021; Zhu et al., 2022).

The use of enzymes requires knowledge of the structure of lignocellulosic biomass. The main component is the plant cell wall, which serves as protection against external influences and enables the establishment of turgor pressure. Plant cell wall consists of cellulose, hemicellulose and lignin. Cellulose and hemicellulose are polysaccharides, while lignin is a complex phenolic polymer. These three components form the structural matrix of lignocelluloses biomass, and influence its characteristics, strength and stiffness (Giddings et al., 1980; Hernandez-Blanco et al., 2007; Xu et al., 2013).

Cellulose is one of the most important components of the plant cell and its most common applications are in the textile and paper industry. The molecular formula of cellulose is $C_6H_{10}O_5$, and it consists of hundreds of glucose molecules connected by covalent bonds, hydrogen bonds and van der Waals forces. It is the most abundant organic polymer on Earth and major source of energy for many organisms. Four different types of cellulose I, II, III and IV can be distinguished (Kraessig, 1993; Gutiérrez et al., 2009; Xu et al., 2013; Grubor, 2021).

Hemicellulose is also a polysaccharide with a more complex structure and bonds, its molecular formula is $C_6H_{10}O_5$. Hemicellulose is connected by cellulose microfibrils without covalent bonds and consists of several monosaccharides (Somerville et al., 2004; Xu et al., 2013). Depending on the type of biomass, it may contain pentoses (xylose, arabinose), hexoses (mannose, glucose, galactose) and acetyl sugars (Saha, 2003; Agbor et al., 2011). The share of hemicellulose in most plant species is 25%. Its main role is to provide a link between cellulose and lignin (Thomsen et al., 2005; Jurišić, 2012; Grubor, 2021).

Lignin is a stiffening material (like a glue) usually found between cellulose microfibrils. Because lignin is a complex polymer the exact composition of lignin can vary depending on the source. It is a complex mixture of monomers that are chemically linked together in various ways, therefore molecular formula of lignin is not a single fixed formula (Dashtban et al., 2010; Chung and Washburn, 2013). Lignin is insoluble in most solvents due to its high molecular weight and therefore represents an obstacle in the use of lignocellulosic biomass (Agbor et al., 2011; Kaminura et al., 2019; Grubor, 2021). It is responsible for providing structural support and protection, and because it is difficult to break down it can interfere with the breakdown of cellulose and hemicellulose that are the main sources of sugar for biofuel production (Weng and Chapple, 2010).

ENZYMES

Enzymes are proteins that serve as catalysts for chemical reactions (van Beilen et al., 2002). Enzyme technology is an interdisciplinary field, recognized as an integral part of sustainable industrial development. Its applications span from straightforward industrial processes to pharmaceutical discovery and development, offering cost-effective, clean, enzymatic, or biological alternatives to traditional chemical procedures which can promote technological advancements (Godfrey and West, 1996; Kirst et al., 2001). With the potential to transform agricultural waste biomass into a valuable resource to produce chemicals and fuels, biobased renewables offer many advantages, such as reduced CO2 production, flexibility, and self-reliance. The Royal Dutch/Shell group predicted that by the year 2050, renewable resources could potentially supply up to 30% of the global chemical and fuel needs, resulting in a biomass market of $150 billion. This information is vital to scientists and other professionals in the field of enzyme technology, as it provides insight into the potential of biobased renewables and the possibilities for sustainable industrial development (OECD, 1998; Novozymes, 2002; van Beilen et al., 2002).

Enzymes for cellulose degradation

There are several types of enzymes that can be used to break down cellulose for different production purposes, including cellulases and hemicellulases. Cellulases are enzymes that break down cellulose into glucose, while hemicellulases are enzymes that break down hemicellulose, a type of polysaccharide found in plant cell walls (Horn et al., 2012). The use of the enzyme hemicellulase increases the availability of cellulase to cellulose and enables better enzymatic hydrolysis (Zhao et al., 2018). Both types of enzymes are produced by microorganisms and can be used to hydrolyze cellulose and hemicellulose into simpler sugars that can be fermented. Other enzymes that are used in the production of biofuels from cellulose include xylanases, which break down xylan (another type of polysaccharide found in plant cell walls), and pectinases, which break down pectin (a polysaccharide found in plant cell walls and fruit) (Dodd and Cann, 2009). These enzymes can also be used to improve the efficiency of cellulose conversion by breaking down the other plant cell wall components that may interfere with cellulose accessibility to the cellulases (Arantes and Saddler, 2010; Houfani et al., 2020).

Enzymes for hemicellulose degradation

Hemicellulose can be broken down into these simpler sugars which can then be fermented and converted into biofuels such as ethanol (Houfani et al., 2020). There are several types of hemicellulases that can be used to break down hemicellulose, including xylanases and arabinofuranosidases (which break down arabinose, sugar found in hemicellulose) (Perez et al., 2002; Saha, 2003). These enzymes work by breaking the bonds between the sugar monomers that make up hemicellulose, releasing the individual sugars and making them available for fermentation (Saha, 2003). Hemicellulases are produced by a variety of microorganisms, including bacteria and fungi, and can be used to improve the efficiency of biofuel production from plant feedstocks. In addition to their use in biofuel production, hemicellulases are also used in other industrial processes, such as the production of paper, food, and animal feed (Viikari et al., 1993; Saha 2003).

Enzymes for lignin degradation

There are several enzymes that have been identified as having the potential to break down lignin, including peroxidases (lignin peroxidases and manganese peroxidases), laccases, and proteases (Perez et al., 2002). These enzymes are produced by certain types of fungi and bacteria and are able to break down lignin by oxidizing it. Some proteases have been shown to be able to hydrolyse lignin, particularly in the presence of other enzymes such as laccases or peroxidases, but mainly protease is used to remove any residual cellulose and hemicellulose after treating lignin with other enzymes (Nakagame et al., 2011). These enzymes work together to break down the lignin polymer into smaller, more easily degradable fragments. It is important to note that the best enzymes for lignin degradation are peroxidases and laccases, and they are produced by a white rot fungi. These enzymes work by catalysing the oxidation of lignin molecules, which causes the polymer to break down into smaller, more manageable fragments (Perez et al., 2002). To make these enzymes more efficient for industrial application it is important to note that addition of activators, catalysts and optimal conditions are beneficiary to get the most out of enzymes.

THE INDUSTRIAL ADVANTAGE OF USING ENZYMES

Enzymes use in industry is preferred over other chemicals because enzymes are highly specific in their catalytic activity, meaning they can perform very specific chemical reactions without affecting other molecules (Bilal et al., 2019). This allows to produce very pure and high-quality products. Enzymes can perform reactions much faster than chemical catalysts, and they often require less energy to do so. Enzymes often work under mild conditions, such as at low temperatures and neutral pH (Zhao et al., 2017). This can be especially beneficial for sensitive compounds that can be damaged by harsher conditions. Enzymes are biocatalysts, meaning that they are derived from living organisms, usually microorganisms that can be grown and replenished (Robinson, 2015). They are not a finite resource like many chemical catalysts, making it a more sustainable option. Enzymes are often less expensive to produce than chemical catalysts, and they can be used in high concentrations. This can lead to significant cost savings for industrial processes (Perez et al., 2002).

CONCLUSION

Enzymes are a relatively new technology with diverse applications. The influence of enzymes in the utilization of lignocellulosic biomass has a positive effect on the concept of sustainability and circular economy. It is important to know which enzymes can be used depending on the structure for which they will be used, distinguishing between the use for cellulose, hemicellulose, and lignin degradation. Overall enzymes are valuable tools for many industrial processes as they can be very effective and environmentally friendly alternative to traditional chemical catalysts.

ACKNOWLEDGMENTS:

The research has received funding from the European Regional Development Fund via K.K.01.1.1.04.0091 project ‘’Design of Advanced Biocomposites from Renewable Energy Sources – BIOCOMPOSITES’’

REFERENCES

Agbor, V. B., Cicek, N., Sparling, R., Berlin, A., Levin, D. B. (2011). Biomass pretreatment: fundamentals toward application. Biotechnology advances, 29(6), 675-685.

Arantes, V., Saddler, J. N. (2010). Access to cellulose limits the efficiency of enzymatic hydrolysis: the role of amorphogenesis. Biotechnology for biofuels, 3(1), 1-11.

Banu, J. R., Kavitha, S., Tyagi, V. K., Gunasekaran, M., Karthikeyan, O. P., Kumar, G. (2021). Lignocellulosic biomass based biorefinery: A successful platform towards circular bioeconomy. Fuel, 302, 121086.

Baruah, J., Nath, B. K., Sharma, R., Kumar, S., Deka, R. C., Baruah, D. C., Kalita, E. (2018). Recent trends in the pretreatment of lignocellulosic biomass for value-added products. Frontiers in Energy Research, 6, 141.

Bilal, M., Zhao, Y., Noreen, S., Shah, S. Z. H., Bharagava, R. N., Iqbal, H. M. (2019). Modifying bio-catalytic properties of enzymes for efficient biocatalysis: A review from immobilization strategies viewpoint. Biocatalysis and Biotransformation, 37(3), 159-182.

Bledzki, A. K., Mamun, A. A., Jaszkiewicz, A., Erdmann, K., (2010). Polypropylene composites with enzyme modified abaca fibre. Composites Science and Technology. 70, 854–60.

Chung, H., Washburn, N. R. (2013). Chemistry of lignin-based materials. Green materials, 1(3), 137-160.

Dashtban, M., Schraft, H., Syed, T. A., Qin, W. (2010). Fungal biodegradation and enzymatic modification of lignin. International journal of biochemistry and molecular biology, 1(1), 36.

de Gonzalo, G., Colpa, D. I., Habib, M. H., Fraaije, M. W. (2016). Bacterial enzymes involved in lignin degradation. Journal of biotechnology, 236, 110-119.

Dodd, D., Cann, I. K. (2009). Enzymatic deconstruction of xylan for biofuel production. Gcb Bioenergy, 1(1), 2-17.

Easterly, J. L., Burnham, M. (1996). Overview of biomass and waste fuel resources for power production. Biomass and Bioenergy, 10(2-3), 79-92.

Fatma, S., Hameed, A., Noman, M., Ahmed, T., Shahid, M., Tariq, M., Sohail, I., Tabassum, R. (2018). Lignocellulosic biomass: a sustainable bioenergy source for the future. Protein and peptide letters, 25(2), 148-163.

Feng, C., Zeng, G., Huang, D., Hu, S., Zhao, M., Lai, C., Huang, C., Wei, Z., Li, N. (2011). Effect of ligninolytic enzymes on lignin degradation and carbon utilization during lignocellulosic waste composting. Process Biochemistry, 46(7), 1515-1520.

Giddings, T., Brower, D., Staehelin, L. (1980). Visualization of particle complexes in the plasma membrane of Micrasterias denticulata associated with the formation of cellulose fibrils in primary and secondary cell walls. The Journal of cell biology, 84(2), 327-339.

Godfrey, T., West, S. (1996). Industrial Enzymology. London: Macmillan Press Ltd.

Grubor, M. (2021). Utjecaj sastava i mehaničke pripreme biomase na kakvoću bioulja i biougljena dobivenog pirolizom (Doctoral dissertation, University of Zagreb. Faculty of Agriculture).

Gutiérrez, A., del Río, J. C., Martínez, A. T., (2009). Microbial and enzymatic control of pitch in the pulp and paper industry. Applied microbiology and biotechnology, 82(6), 1005-1018.

Hanana, S., Elloumi, A., Placet, V., Tounsi, H., Belghith, H., Bradai, C. (2015). An efficient enzymatic-based process for the extraction of high-mechanical properties alfa fibres. Industrial Crops and Products, 70, 190-200.

Hartmann, T. (2007). From waste products to ecochemicals: fifty years research of plant secondary metabolism. Phytochemistry, 68(22-24), 2831-2846.

Hernandez-Blanco, C., Feng, D., Hu, J., Sanchez-Vallet, A., Deslandes, L., Llorente, F., Berrocal-Lobo, M., Keller, H., Barlet, X., Sanchez-Rodriguez, C., Anderson, L. K., Somerville, S., Marco, Y., Molina, A. (2007). Impairment of cellulose synthases required for Arabidopsis secondary cell wall formation enhances disease resistance. The Plant Cell, 19(3), 890-903.

Horn, S. J., Vaaje-Kolstad, G., Westereng, B., Eijsink, V. (2012). Novel enzymes for the degradation of cellulose. Biotechnology for biofuels, 5(1), 1-13.

Houfani, A. A., Anders, N., Spiess, A. C., Baldrian, P., & Benallaoua, S. (2020). Insights from enzymatic degradation of cellulose and hemicellulose to fermentable sugars–a review. Biomass and Bioenergy, 134, 105481.

Jurišić, V. (2012). Optimizacija uvjeta visokosmicajne ekstruzije trave iz roda Miscanthus kao sirovine za proizvodnju bioetanola. Doktorski rad. Prehrambeno - biotehnološki fakultet Sveučilišta u Zagrebu, Zagreb, Hrvatska.

Kamimura, N., Sakamoto, S., Mitsuda, N., Masai, E., Kajita, S. (2019). Advances in microbial lignin degradation and its applications. Current Opinion in Biotechnology, 56, 179-186.

Kirst, A., Yeh, W. K., Zmijewski, M. J. J. (2001). Enzyme Technologies for Pharmaceutical and Biotechnological Applications. New York: Marcel Dekker, Inc.

Kraessig, H. (1993). Cellulose: structure, accessibility, and reactivity. CRC.

Mussatto, S. I., Yamakawa, C. K., van der Maas, L., Dragone, G. (2021). New trends in bioprocesses for lignocellulosic biomass and CO2 utilization. Renewable and Sustainable Energy Reviews, 152, 111620.

Nakagame, S., Chandra, R. P., Kadla, J. F., Saddler, J. N. (2011). Enhancing the enzymatic hydrolysis of lignocellulosic biomass by increasing the carboxylic acid content of the associated lignin. Biotechnology and bioengineering, 108(3), 538-548.

Novozymes AS: Annual Report 2001. Bagsvaerd, Denmark; 2002.

OECD (1998). Biotechnology for Clean Industrial Products and Processes. Paris, France: OECD.

Pariza, M. W., Cook, M. (2010). Determining the safety of enzymes used in animal feed. Regulatory Toxicology and Pharmacology, 56(3), 332-342.

Pérez, J., Munoz-Dorado, J., De la Rubia, T. D. L. R., & Martinez, J. (2002). Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. International microbiology, 5(2), 53-63.

Robinson, P. K. (2015). Enzymes: principles and biotechnological applications. Essays in biochemistry, 59, 1.

Saha, B. C. (2003). Hemicellulose bioconversion. Journal of industrial microbiology and biotechnology, 30(5), 279-291.

Somerville, C., Bauer, S., Brininstool, G., Facette, M., Hamann, T., Milne, J., Osborne, E., Paredez, A., Persson, S., Raab, T., Vorwerk, S., Youngs, H. (2004). Toward a systems approach to understanding plant cell walls. Science, 306:2206–2211.

Thomsen, A. B., Rasmussen, S., Bohn, V., Vad Nielsen, K., Thygesen, A. (2005). Hemp raw materials: The effect of cultivar, growth conditions and pretreatment on the chemical composition of the fibres. Risø-Report. Risø National Laboratory, Roskilde, Denmark.

van Beilen, J. B., Li, Z. (2002). Enzyme technology: an overview. Current opinion in biotechnology, 13(4), 338-344.

Viikari, L., Tenkanen, M., Buchert, J., Ratto, M., Bailey, M., Siikaaho, M., Linko, M. (1993). Hemicellulases for industrial applications. In: Saddler JN (ed) Bioconversion of forest and agricultural plant residues. CAB, Oxford, pp 131–182.

Weng, J. K., Chapple, C. (2010). The origin and evolution of lignin biosynthesis. New Phytologist, 187(2), 273-285.

Xu, F., Yu, J., Tesso, T., Dowell, F., Wang, D. (2013). Qualitative and quantitative analysis of lignocellulosic biomass using infrared techniques: a mini-review. Applied energy, 104, 801-809.

Zhao, J., Dong, Z., Li, J., Chen, L., Bai, Y., Jia, Y., Shao, T. (2018). Ensiling as pretreatment of rice straw: The effect of hemicellulase and Lactobacillus plantarum on hemicellulose degradation and cellulose conversion. Bioresource technology, 266, 158-165.

Zhao, X., Liu, W., Deng, Y., & Zhu, J. Y. (2017). Low-temperature microbial and direct conversion of lignocellulosic biomass to electricity: Advances and challenges. Renewable and Sustainable Energy Reviews, 71, 268-282.

Zhu, X., Liu, M., Sun, Q., Ma, J., Xia, A., Huang, Y., Zhu, X., Liao, Q. (2022). Elucidation of the interaction effects of cellulose, hemicellulose and lignin during degradative solvent extraction of lignocellulosic biomass. Fuel, 327, 125141.