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Emerging Trends in Xylanase Biotechnology: From Lignocellulose Degradation to Industrial Applications

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Abstract

Hemicellulose, a complex and heterogeneous polysaccharide, is one of the most abundant biopolymers found in nature. It plays a vital role in plant structure, acting as a key component of the plant cell wall matrix alongside cellulose and lignin. Microorganisms have evolved mechanisms to harness energy from lignocellulosic biomass by synthesizing and secreting a wide range of plant cell wall-degrading enzymes. Among these enzymes, xylanase stands out due to its ability to hydrolyze xylan, the main component of hemicellulose. The enzymatic breakdown of xylan releases simple sugars and oligomers, which are readily assimilated by microorganisms as substrates for their metabolic activities. Xylanase has garnered significant attention owing to its broad spectrum of industrial applications. In the food and beverage industry, it enhances the quality and shelf-life of baked goods and improves the clarity of juices and wines. In the paper and pulp industry, xylanase contributes to environmentally friendly processes by reducing the need for harsh chemical treatments during pulp bleaching. Additionally, xylanase plays a crucial role in the sustainable production of biofuels, biochemicals, and other value-added products from renewable resources, thus supporting green technologies. While a variety of microorganisms, including bacteria and fungi, are well-documented producers of xylanase, its production by the yeast *Saccharomyces cerevisiae* remains rare and underexplored. Advancements in metabolic engineering, synthetic biology, and fermentation technology hold the potential to enhance xylanase production in *S. cerevisiae,* thereby addressing existing challenges and expanding its industrial utility.

Key words: Microorgansims, *Saccharomyces cerevisiae*, Xylan, Xylanase, Yeast

1. INTRODUCTION

Generally, enzymes are deemed most important for humans as they are the biological catalysts that mediate biochemical reactions in the body, but the industrial use of enzymes has become important due to its increased demand (Manisha & Yadav, 2017). Xylanase enzyme is most commonly present in fungi and other microbes (Bhardwaj et al., 2019). Xylanase is pentosanases that is a class of enzyme that hydrolyses plants cell wall (Collins et al., 2005). Xylanase does hydrolysis of xylan into xylose that is a reducing sugar (Maulana Hidayatullah et al., 2020). Due to this reason, xylanase is considered as one of the most industrially important enzyme. It is a cell wall degrading enzyme that breaks down hemicellulose that is one of the chief constituents of plant cell wall (King et al., 2011). Xylanase is thus defined as a hydrolytic enzyme that arbitrarily cleaves the β 1,4 backbone of the plant cell wall (xylan). Various forms of this enzyme is found that displays different folds, substrate specificities, hydrolytic actions, physical and chemical features with the different mechanisms of action (Joshi et al., 2022). Xylanases can be industrially formed in submerged liquid culture as well as on a solid substrate medium, but mostly this enzyme is produced by using submerged fermentation method (Abena & Simachew, 2024a). The major function of the xylanase is hydrolysing D-xylan into D-xylose. Xylanase hydrolyses the plant cell wall component called the hemicellulose.

1.1 STRUCTURE OF XYLANASE

The xylanase structure has folds in an alpha/beta 8 barrel (TIM-barrel) that consists of additional helices and loops organized at the top which creates the active site cleft (Fredj Ben et al., 2018; Y. Zhang et al., 2016). Xylanases principally fits into hydrolase group of enzymes that is glycoside hydrolases. Sequence-based glycoside hydrolase arrangement places xylanase in two families GH10 and GH11 (Kojima et al., 2022). On the other hand, xylanase is also present in other glycoside hydrolase families, that are GH5, 7, 8, and 43 (Bhardwaj et al., 2019). Plant, fungal, and bacterial enzymes are included in GH10 family, while GH11 family is anatomically dissimilar includes only fungal and bacterial enzymes (Lafond et al., 2011).

1.2 SUBSTRATE OF XYLANASE (XYLAN)

Xylan is a major polysaccharide present in plant cells (Zhang et al., 2024). Moreover, xylan is the second most abundant polysaccharide in nature (Curry et al., 2023). It is a polysaccharide

comprising of xylose a reducing sugar. It forms the main component of hemicellulose as it consists of a S-1,4-linked xylose backbone, that retain branches comprising of xylose and pentose (Huang et al., 2021). Though, xylan structure is variable that is linear 1,4-ß-linked poly xylose sugars. Xylan molecules resides D-xylose as the monomeric component (Smith et al., 2022). There are massive amounts of xylan present in hemicellulosic materials as wastes, hence, xylanase can be useful in degrading such wastes into xylose (El-Gendi et al., 2023; Patipong et al., 2019).

1.3 APPLICATIONS OF XYLANASE

Generally, enzymes are deemed most important for humans as they are the biological catalysts that mediate biochemical reactions in the body, but the industrial use of enzymes has become as important due to its increased demand (Robinson, 2015). Since, the methods for the production of industrial enzymes i.e. of non-microbial origin, costs an extortionate amount, the demand of industrial enzymes of microbial origin is increasing rapidly due to its cost effective production (Sadh et al., 2018). Furthermore, owing to microbial enzymes' great diversity, they possess a variety of characteristics that make them efficient for different types of industrial applications (Gurung et al., 2013). Many enzymes such as protease, lipase, amylase, tannase, xylanase etc. are widely used in food, pharmaceutical, leather processing, cosmetic industries etc (Kuo et al., 2024; Kumar et al., 2018). Among the industrially important enzymes, xylanase, which is usually produced by bacteria and fungi, is responsible for the breakdown of xylan (Yadav et al., 2018). The demand of xylanase has increased worldwide, over the past few years (Burlacu et al., 2016). Xylanase is a group of enzyme that is used in various industries, from the food industry to biofuel making, paper and pulp making industry, animal feed processing, baking and brewing industries uses xylanase for the different purposes (Burlacu et al., 2016; Hazra et al., 2023). Xylanase is also used in health products (Ali et al., 2025). In chemical industries and textile industries, xylanase holds great importance (Kumar et al., 2017). Xylanase is also beneficial for microorganisms as it consents them to take the nutrients from plants that contain high fibre content (Mohammadi et al., 2024). Moreover, it is frequently used to advance cereal processing with good end product quality (Rakha et al., 2024). The major function of xylanase in the baking industry is that it helps in the transformation of insoluble hemicellulose into soluble sugars that in return provides a fluffy and voluminous loaves of bread which is soft and has more elasticity (Butt et al., 2008).

1.3.1 JUICE AND BEVERAGE INDUSTRIES

In the 1930s, the yields of citrus juice were low and problems were encountered in the filtration of the juice (Kumar, 2015; Sharma et al., 2017). However, with the speedy discovery and knowledge of enzymes and several chemical constituents, these problems came to rest. Xylanases along with cellulases and pectinases play a great role in the increased demand of enzymes in juice industries (Sosa-Martínez et al., 2024). They play a major role in improving the yield of juices and use in the production of fruit and vegetable juices which include the extraction, clarification of juices, liquification of fruits and vegetables, stabilization of pulp; increased recovery of aromas, essential oils, vitamins, mineral salts, edible dyes and pigments, reduction of viscosity, hydrolysis of substances that hinder the physical or chemical clearing of the juice, or that may cause cloudiness in the concentrate (Pal & Khanum, 2011). The main desirable properties for xylanases for use in the food industry are high stability and optimum activity at an acidic pH (Abena & Simachew, 2024b; Fernandes de Souza et al., 2022).

1.3.2 PAPER AND PULP INDUSTRIES

The use of xylanase in paper and pulp treatment was first reported by Viikari et al. (1990). Apart from the use of enzymes in paper and pulp industries, chemical bleaching is also used in increasing the brightness of papers. However, this causes harm to the components of cellulose as well as reduces the viscosity and yield of the pulp (Immerzeel & Fiskari, 2023; Li et al., 2023). Using xylanase in paper and pulp industries prove beneficial as its usage not only is harmless to cellulose but also reduces pollution caused by organochlorine such as dioxin (Malhotra & Chapadgaonkar, 2023; Sridevi et al., 2017). One of the most common processes of pulping is the Kraft process or the sulfate process in which the wood chips are cooked in a solution of Na_2S/NaOH at 170°C for about 2 h in order to solubilize and degrade lignin. The outcome is a brown colored pulp that is caused by the remnants of lignin and its derivatives present in it (Ejaz & Sohail, 2021a). The bleaching of the pulp can be regarded as a clearing process involving the destruction, alteration or solubilization of the lignin, colored organic matters, and other undesirable residues. After the pulping process, a considerable amount of xylan still remains which is then hydrolyzed by xylanase. This allows higher amounts of leaching of the lignin-carbohydrate molecules (Gangwar et al., 2014).

1.3.3 FOOD INDUSTRIES

Combination of xylanase with amylase increases the bread volume along with the quality of the bread. Xylanase makes the dough soft and slack by breaking down the hemicellulose in wheat flour (Kim & Yoo, 2020). Furthermore, xylanase delays the crumb formation which allows the dough to rise during bread-baking (Al-Widyan et al., 2008). A huge amount of arabinoxylo-oligosaccharides in bread might prove to be beneficial for health (Damen et al., 2012). Along with cellulase and pectinase, xylanase can be used for the preparation of dextrans for use as food thickeners (Mandal, 2015). In biscuit making, xylanase makes the cream crackers lighter in texture while enhancing its palatability (Awalgaonkar et al., 2015). Furthermore, xylanase and amylase altogether participate in the hydrolysis of arabinoxylans and starch which help in the separation of gluten from starch present in wheat flour (Ha & Kweon, 2024). Xylanase is also used in the preparation of coffee-bean mucilage (Richhariya et al., 2022).

1.3.4 ANIMAL FEED IMPROVEMENT

Xylanases used as pretreatment of forage crops to improve the nutritional properties (Liu et al., 2023). Animal feed contains many grains like rye and wheat which are partially digestible by animals due to their hard covering of seed coat. These types of seeds pose harm to the guts of chicken (Suleman et al., 2022). Incorporation of xylanase in animal feed is reported to not only improve gut health but also causes weight gain in animals because it increases digestibility by partially digesting these feed grains (Zhang et al., 2014).

1.3.5 BIOFUELS

Nowadays, production of biofuels is important since the natural resources of the world are constantly being depleted (Ejaz et al., 2024). Therefore, xylanase can be used for the generation of biofuels (Chaudhary et al., 2023). A combination of enzymes like mannanase, ligninase, xylanase, xylosidase, glucosidase and glucanase are used to depolymerize the polymers of carbohydrates which produces free sugars and hence, the fermentation of mixed sugars of hexose and pentose results in the production of ethanol (Kaur et al., 2021).

1.3.6 FLAX FIBER RETTING IMPROVEMENT

The process of retting is essential to perform before fibers are converted to linen (Ahmed et al., 2023). Pectinases are primarily used in the retting process but the incorporation of xylanase improves the process. A system including a combination of xylanase and pectinase is used in the first step of wood processing known as the debarking process. Other applications of this combined system are used in the degumming of fibers such as flax, hemp, jute and ramie or the fiber liberation from plant instead of retting (Dhiman et al., 2009).

1.3.7 SURFACTANTS

Alkyl glycosides are surfactants widely used in industrial applications being produced commercially from monomeric sugars (Ji et al., 2019). For industrial production of surfactants, the use of polysaccharides is more practical because various production processes can be eliminated. Moreover, purified alkalophilic xylanase can improve the cleaning ability of detergents (Mesbah, 2022).

1.3.8 BIO-PROCESSING OF FABRICS

Treating fabrics with xylanase can improve the water absorption properties of fibers by removing lignocellulosic impurities such that its strength stays unaffected during the spinning process. In the end, fiber becomes softer and smoother after desizing (Battan et al., 2012; Yilmaz et al., 2014).

1.4 XYLANASE PRODUCING MICROORGANISMS

Current studies on microbial xylanolytic systems are directed on generation of enzyme production (Kashyap et al., 2021). Microorganisms that produce the xylanase enzyme are bacteria, fungi, yeast. However, microorganisms producing xylanase possess different characteristics that act at their respective range of pH and temperatures making them useful for different types of applications (Beg et al., 2001). Previously, various microbial strains, including *Aspergillus oryzae*, *Penicillium* sp., *Aspergillus niger*, *Thermobacillus xylanilyticus*, *Bacillus* sp., and *Aspergillus foetidus*, have been reported to produce xylanase (Rashid et al., 2021).

A suitable source of microorganisms is necessary for the production of microbial enzymes. Each microorganism has specific growth factors hence, their optimization is vital for a large-

scale production of the microbial enzymes (Rashid et al., 2020). In solid state fermentation, controlling growth parameters like pH, temperature is difficult and agitation and aeration of the media is difficult to evenly disperse the nutrients for optimal growth of the microorganisms. Moreover, high impurity products require the need for recovery of products thus increasing the overall cost substantially (Ejaz & Sohail, 2021b).

1.4.1 *Saccharomyces cerevisiae*

S. cerevisiae is a unicellular fungus, a specie of yeast. This species of yeast has been used in baking and brewing purposes (Schuller & Casal, 2005). Yeast has differentiated functions in the preparation and processing of foodstuff. Saccharomyces cerevisiae is used in fruit juice clarification. *S. cerevisiae* helps in alcohol production and in bakery items by a biological process fermentation. The cell cycle for the yeast cells in ideal environment is 90 minutes that indicates an appropriate quantity of yeast cells grows within hours (Parapouli et al., 2020). The yeast cells have growth time that is less then fungi. Moreover, yeast cells have a unique characteristic that it can produce homogenous enzyme as compared to other microorganisms (Ejaz et al., 2018). Furthermore, yeast can be easily cultured in acidic, high sugar environment. The usage of this yeast in ranges in various fields of biotechnology, as it is essential for the production of insulin as well as for the formation of antibodies and other proteins that is used in some medications (Tullio, 2022). The study of strain, MK-157 has revealed its biotechnological uses and applications that includes ethanol (Hafeez et al., 2023). *S. cerevisiae* MK-157 has been previously reported for many industrially important enzymes such as pectinase (Qadir et al., 2020) and cellulase (Qadir et al., 2018). Aftab et al. (2023) reported the production of xylanase from the immobilized strain of *S. cerevisiae* MK-157 for the first time.

1.6 Conclusion

The literature emphasizes that enzyme-based processes, particularly xylanase production, are gaining traction over traditional chemical-based methods due to their process efficiency, cost-effectiveness, and environmentally friendly nature. Xylanase, a key enzyme in the degradation of hemicellulose, plays an essential role in various industrial applications, including food, beverage, paper, and pulp industries. Its ability to release fermentable sugars and oligomers enhances the metabolic processes of microorganisms. The potential for xylanase production by *Saccharomyces cerevisiae* presents an exciting opportunity to expand its industrial use, contributing to greener, more efficient processes across diverse sectors.

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