Microbial Xylanases: Recent Advances and Industrial Applications

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Abstract

Xylanase enzymes specifically target xylan, a complex polysaccharide found in the cell walls of plants, especially hemicellulose. These enzymes can break down the bonds present in xylan, resulting in the hydrolysis of the polysaccharide into smaller components such as xylose and xylooligosaccharides. Considering the economic value of xylose, the signicance of xylanase at the industrial level becomes more evident. Microbial xylanase plays a vital role in various sectors, contributing to improved processes, product quality, and sustainability in industries ranging from bioenergy and agriculture to food, paper, and textiles. They are employed in baking to enhance dough handling, increase bread volume, and prolong shelf life. Additionally, these enzymes aid in extracting fruit juices, wine, and vegetable oils, leading to higher yields and reduced waste. Microbial xylanases facilitate bio-bleaching processes in the pulp and paper industry. Their ability to reduce xylan and other impurities in wood pulp contributes to environment-friendly paper production and reduced chlorine-based bleaching chemicals. The present review summarises the different applications of microbial xylanase in various industries.

Keywords: Biofuel, Food industry, Juice saccharification, Microbial xylanase, Pharmaceuticals

Introduction

Xylanase is an important enzyme with many uses in modern times. It is a glycoside hydrolase, also known as endo-β-1,4-xylan-xylanohydrolase (EC - 3.2.1.8), that aids in breaking down xylan (Singh *et al*., 2019). Xylan, found in plant cell walls, is the Earth's second most abundant naturally occurring renewable polysaccharide. It has a complex structure, varying across several plant species, and consists of highly branched heteropolysaccharides (Curry *et al*., 2023). Xylan consists of a backbone chain of 1,4-linked β-D-xylopyranose units, unsubstituted or substituted with varying degrees of O-acetyl, α-L-arabinofuranosyl, α-1,2-linked glucuronic or 4-O-methyl glucuronic acid side-chain groups (Kulkarni *et al*., 1999). The heterogeneous nature of xylan restricts its breakdown, but the ability of xylanases to breakdown β-1,4-glycoside linkage can overcome this barrier (Bhardwaj *et al*., 2019). Xylanase breaks down xylan into xylobiose and xylotriose, with a small amount of xylooligosaccharide with a higher level of polymerisation (Mandal, 2015). Microbial xylanases are used as a catalyst for xylan hydrolysis because of their high specificity, gentle reaction conditions, minimal substrate losses, and limited side effects. In addition, using xylanase helps avoid the need for expensive high-grade chemicals that harm the environment (Chakdar *et al*., 2016). For example, in the

paper and pulp industry, xylanase is used to bleach the wood pulp organically through enzymatic actions. This process eliminates the use of chlorine, which helps reduce the formation of toxic dioxins and organic halogens. In addition, applying xylanase prevents damage to pulp fibres and improves the overall quality of paper (Kumar *et al*., 2018). Similarly, incorporating xylanase into bread-making processes enhances the bread's physical properties and nutritional benefits eliminating the use of potassium bromate (de Souza *et al*., 2022). Moreover, through the hydrolysis of xylan, xylanase generates xylooligosaccharides, which are sugar oligomers that promote the growth of beneficial prebiotic microorganisms in the lower gastrointestinal tract (de Freitas *et al*., 2019). In the animal feed industry, especially for poultry and livestock, nutritional additives are required to produce feed, a process that incurs high operational costs. Compared to other nutritional additives, xylanase enzyme is very cost effective as it enhances the digestibility in poultry animals (Nusairat and Wang, 2021). Beyond its use in paper, pulp, animal feed, and baking industries, xylanase is also extensively utilized for the clarification of fruit juices, helping to avoid the addition of chemical preservatives (Adiguzel *et al*., 2019). It also improves the shelf life of beverages, without the need of external preservatives (Xylanase market, 2022).

Future Market Insights has projected that the global xylanase market will experience a value CAGR of 5.4% between 2023 and 2033. The analysis indicates that the demand for xylanase in the market will be worth US\$ 19.5 billion by the end of 2023 and is predicted to reach US\$ 33 billion at the same growth rate by 2033 (Global Xylanase Market Outlook (2023 to 2033), 2023).

Classications of Xylanase

The Carbohydrate-Active enZymes Database (CAZy) classifies xylanase enzymes within the broader category of glycoside hydrolases (GHs) based on their structural folds, catalytic mechanisms, and sequence similarities. These enzymes primarily belong to families that include endo-acting enzymes (Lombard *et al*., 2014). The families of GH enzyme that are associated with xylanase include, GH 5, GH 7-12, GH 16, 26, 30, 43, 44, 51, and 62. The most prominent families for xylanases include GH 10, GH 11, and to a lesser extent, GH 5, GH 8, GH 30, and GH 43 (Verma and Satyanarayana, 2012). Each of these families encompasses enzymes with specific activities, substrate specificities, and modes of action. GH 10 and GH 11 are the primary families for endoxylanases, which cleave the internal βeta-1,4-glycosidic bonds of xylan. For example, the GH 10 family includes endoxylanases, such as endo-1,4-β-xylanases and endo-1,3-β-xylanases, and enzyme cellobiohydrolases. In contrast, xylanases under GH 11 are referred to as 'true xylanases' because they are work effectively on xylose substrates (Collins *et al.*, 2005). GH 5, GH 8, GH 30, and GH 43 also contain xylanases or enzymes with xylanase activity, but these tend to have varied specificities and roles in xylan breakdown (Bhardwaj*et al*., 2019).

Sources of Microbial Xylanases

Microbial xylanase production relies heavily on the selection of the appropriate microorganism. Research has indicated that various sources, such as bacteria, yeast, and other fungi, seeds, crustaceans, protozoans, marine algae, insects, and snails, are capable of producing xylanase (Polizeli *et al*., 2005). Among all the organisms studied, bacteria and fungi have demonstrated the most promising outcomes in the production of this enzyme. Bacteria like, *Micrococcus*, *Bacillus*, *Paenibacillus*, *Staphylococcus*, *Cellulomonas*, *Microbacterium*, *Arthrobacter*, *Rhodothermus*, and *Pseudoxanthomonas* have also displayed encouraging results in xylanase production (Chakdar *et al*., 2016). In addition to bacteria, some actinomycetes species like *Nonomuraea* sp*.*, *Streptomyces* sp*.*, and *Actinomadura* sp*.* have also been found to produce xylanase (Chakdar *et al*., 2016). Fungal species like *Aspergillus* spp., *Trichoderma*

spp., and *Penicillium* spp. are significant xylanase producers due to their elevated yields and extracellular release of enzymes (Nair *et al*., 2008). Fungal xylanases have higher activity compared to those derived from bacteria or yeast. However, some characteristics of xylanases from fungal sources make them unsuitable for certain industrial applications (Mandal, 2015).

Xylanase Production Using Microbial Sources

Xylanase production from microbial sources commences with the isolation and identification of potential microbial strains that may include fungi or bacteria. Samples are collected from diverse environments such as soil, decaying plant material, marine sediments etc. (Table 1) and screened for xylanase production on xylancontaining agar plates. The presence of a clear zone around microbial growth indicates xylan degradation, suggesting potential xylanase production and is the primary screening step (Shanthi and Roymon, 2018). For secondary screening, the potent strains are grown in liquid media containing xylan, and their xylanase activity is quantitatively measured using specific enzyme assays such as dinitrosalicylic (DNS) assay, Nelson-Samyogi assay, and others (Dhaver *et al*., 2022; Samantal *et al*., 2011). The promising strains are identified through microscopic examination and molecular techniques such as 16S rRNA sequencing for bacteria or ITS sequencing for fungi. After screening and identification, the xylanase-producing microbe is used for the production of the enzyme using fermentation. For optimal xylanase yield, culture conditions such as pH, temperature, aeration, and substrate concentration need to be meticulously optimized (Bhardwaj *et al*., 2019). Furthermore, the choice of carbon and nitrogen sources, metal ions, and other growth factors can significantly influence enzyme production (Kereh et al., 2018). The optimization can be done using OFAT (one factor at a time) approach, or RSM (Response Surface Methodology) or a combination of the two (Wu and Ahn, 2018). The fermentation process, which can either be solid-state or submerged depending on the microbial source, is then scaled up in a fermenter or bioreactor under these optimized conditions (Walia *et al*., 2017). Table 2 summarises some of the studies conducted on xylanase production from different microbial sources. Post-fermentation, the culture broth undergoes centrifugation to separate microbial cells and other particulate matter, leaving the supernatant rich in extracellular xylanase. This enzyme undergoes various purification techniques, such as ammonium sulphate precipitation, in which the enzyme xylanase is enriched by using $(NH_4)_2SO_4$ and left overnight; thereafter, the protein precipitates are separated using centrifugation. These precipitates are then dialyzed, and the resulting dialysed products are further purified through gel filtration chromatography. Upon purification, the enzyme's properties, including its optimal operational conditions and substrate specificity, are thoroughly characterized (Bhardwaj *et al*., 2019). Xylanase, after purification, is processed into a market-ready format, which could be either a powder or a liquid form (Fig.1). This product may also contain additional components such as stabilizing agents and preservatives to enhance shelf-life and efficacy (Yadav et al., 2018). Rigorous testing is conducted on the xylanase product to verify its enzymatic activity, purity, and shelf stability, confirming that the product conforms to the predetermined quality standards and complies with all regulatory guidelines. The final step involves the xylanase being packaged appropriately and then shipped out to the market or distribution points, making it available for various industrial or commercial applications**.**

Kumari et al., (2023) Kumari *et al.,* (2023)

Table 2. Xylanase production from different microorganisms under optimized conditions **Table 2. Xylanase production from different microorganisms under optimized conditions** BBD: Box Behnken; CCD: Central Composite Design; FCCCD: Face-Centered Central Composite Design; NM: Not mentioned; PBD: BBD: Box Behnken; CCD: Central Composite Design; FCCCD: Face-Centered Central Composite Design; NM: Not mentioned; PBD: Plackett-Burman design; SmF: Submerged fermentation; SSF: Solid state fermentation Plackett-Burman design; SmF: Submerged fermentation; SSF: Solid state fermentation

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Applications of Microbial Xylanases

Microbial xylanase have gained significant attention in recent years due to their various industrial applications, such as biofuel production; bio-bleaching of pulp and deinking of waste paper in the paper industry; improving the quality of dough and the final bread product in the baking industry; fruit softening and clarifying fruit juices and other beverages in the brewing industry; enhancing digestibility and nutritional value in the food and feed industries; acting as a detergent additive in the textile industry; and producing xylose and xylitol, which are used in the pharmaceutical industry (Fig. 2) (Mandal, 2015). Xylanases are now at the forefront of research, especially in areas like bioconversion of lignocellulosic materials and agrowaste fuel utilization (Baramee *et al*., 2020). Xylanases play a crucial role in the bioconversion of xylan into valuable products like xylitol, which finds applications in soft drinks, candies, ice cream, chewing gum, and pharmaceutical products. Xylitol serves as a natural sweetener in toothpaste and helps sweeten food products (Ahuja *et al*., 2020). The interest in microbial xylanases has increased markedly because of their wide range of potential biotechnological applications in different industries that have been discussed further in detail.

Pulp and paper industry

In pulp and paper industry, xylanases are utilized in two processes, bio-bleaching and deinking. In bio-bleaching, xylanases are typically used in conjunction with lignindegrading enzymes that make the fibres more permeable by degrading superficial xylan (Kumar and Shukla, 2018). Xylanase breaks down xylan into smaller fragments. This action exposes more of the lignin to the chemicals used in subsequent bleaching stages. With lignin more exposed, the subsequent bleaching stages become more effective. This lowers the quantities of harsh chemicals, like chlorine, needed to achieve the desired level of whiteness (Kumar *et al*., 2016). Xylanases in bio-bleaching decreases chlorine requirement from 10 to 50%, preventing pulp fibre damage, enhancing the paper quality, and lessening the overall paper production costs (Kumar *et al*., 2016; 2018). Overall, xylanase-aided bleaching process shows positive effects on pulp, paper, and effluent attributes to reduce bleaching chemical use, AOX formation, and energy use in the pulp refining process (Dukare *et al.*, 2023). Kaushal *et al*. (2022) used *Bacillus pumilus*, xylanase-producing bacteria, for bio-bleaching of soda pulp. This direct approach yielded significant results such as decreased kappa number by 26.4%. Additionally, the physical properties of the paper were highly improved where the

Fig.2. Applications of microbial xylanase in various industries

tear factor of the paper increased by 52.3%, and tensile strength increased by 5%. Similarly, Mhiri and coworkers (2020) also reported improved paper quality by using thermophilic and thermostable xylanase enzymes extracted from *Cladicoprobacter algeriensis* for kraft pulp bio-bleaching. The use of polluting chemicals was reduced as well. In another study, Sridevi *et al*. (2017) isolated xylanase enzymes from a fungal strain, *Trichoderma asperellum*. They observed that after pretreatment of paper pulp, the kappa number was reduced by 4.2 points, brightness was increased by 4.0 points and the fibres of the pulp were also loosened. Other than this, it was observed that by combining refining process and xylanase treatment for three hours, the xylan content of bamboo kraft pulp was reduced to 2.72% and produced a high-quality bamboo dissolving pulp (Zhao *et al*., 2017).

Xylanases also aids in deinking recycled paper. It works by loosening the structure of the fibers, facilitates the detachment and removal of ink particles, resulting in a cleaner pulp. Deinking with xylanase reduces the need for harmful chemicals, which can lead to less pollution and waste. The enzymatic process can often be done at lower temperatures and with less mechanical action, saving energy and operational costs. Singh *et al.* (2020) reported the use of xylano-pectinolytic enzymes, coproduced by a single microbial strain *Bacillus pumilus*for recycling of mixed office waste paper. The enzymes exhibited maximum deinking at pH 8.5, pulp consistency of 10%, xylanase-pectinase dose of 12 and 4 IU per gram pulp, respectively, after 120 min of deinking period, and temperature at 50 °C. Recently in 2023, Malhotra and Chapadgaonkar reported optimal deinking of copier paper using thermo-alkali bacterial xylanase $(20U/g \text{ of the dried pulp})$ at 60 °C for a treatment time of 1h. There was about 50% reduction in the usage of chemical bleach after xylanase pretreatment with negligible damage to the fiber strength as compared to the chemical bleach process.

Food and feed industry

Xylanases hold significant importance in the food industry due to its various applications that enhance food quality, processing efficiency, and nutritional value. Xylanase is widely used in bread-making. It breaks down the xylan in flour, improving dough handling and stability. During the bread-baking process, they delay crumb formation, allowing the dough to grow (Mandal, 2015). It also increases volume, reduces stickiness as well as staling, increases shelf life, and is even used as a substitute of emulsifiers and additives in bread production (Ahmed *et al*., 2016). Xylanases are also used as food additives in the baking industry because they improve the gluten network's elasticity in bakery dough. Al-Widyan and coworkers (2008), applied xylanases produced by rumen microorganism M6 and reported positive effects of the enzyme on loaf volume of bread as well as anti-firming potential. Beyond bread, the application of xylanase has also been studied in black gram papad making process. Xylanase has been reported to eased the rollability of papad (an Indian traditional food based on black gram) and also marginally decreased the oil uptake during the frying process (Awalgaonkar *et al*., 2015). Xylanase also aids in the extraction processes in the food industry, such as in oil extraction from plant sources. Marasabessy and coworkers (2011), reported extraction of Jatropha oil from kernels by degradation of hemicelluloses of cell wall using xylanase from bacteria isolated from paddy crab.

In poultry and animal feed, xylanase and other feed additives are increasingly utilized to improve their performance, reduce costs and environmental impacts. In a study of Nusairat and Wang (2021), xylanase was observed to enhance broiler performance, energy digestibility, and reduced intestinal lesion scores in broilers when included in reduced-energy-diet.

Also, treatment of poultry feed with xylanase enzyme (purified from *Pseudomonas nitroreducens* strain LLD06) increases their total reducing sugar content (Dhivahar *et al*., 2020). Whereas dietary supplementation of xylanase improves laying hen performance and digestibility. It also exerts a prebiotic effect by stimulating growth of beneficial gut bacteria and reducing pathogenic microorganisms (Van Hoeck *et al*., 2021). Similar stimbiotic and prebiotic effect of xylanase was also reported in pigs (Petry *et al*., 2021). Xylanase also results in better growth performance and corpse qualities of broilers with increased body weight gain, breast, and leg muscle weight (Hu *et al*., 2019). In addition to poultry feed, when xylanase was treated with cow feed, the dry matter consumption and milk production was increased in lactating cows (Romero *et al*., 2016).

Pharmaceutical industry

Recently, the interest in making xylo-oligosaccharides (XOS) using endoxylanases from xylan sources is increasing. XOS products are used in the pharmaceutical industry. Xylanases are biocatalysts that are highly specific and do not produce unwanted byproducts during oligomer production (Yegin, 2023). These enzymes catalyze the hydrolysis of xylan, which leads to the formation of XOS. XOS possess prebiotic properties

and can be used in functional foods. Thakur and coworkers (2022) isolated the bacteria *Paenibacillus* sp.PCH8 from Himalayan glacial soil. They found that it exhibited xylanolytic properties, which allowed it to hydrolyze lignocellulosic biomass and produce significant amounts of XOS. Production of this compound in enormous amounts using endoxylanase enzyme was also demonstrated by Nascimento *et al*. (2022). They produced a recombinant xylanase enzyme using plasmid pPIC9 of *Pichia pastoris* GS115 and the gene *xynA* of bacteria *Thermoascus aurantiacus* to hydrolyze the xylan extracted from sugarcane bagasse and for production of XOS. Also, it was observed that this product enhanced the growth of *Lactobacillus casei, L. rhamnosus, L. fermentum,* and *L. bulgaricus* strain that produces acetic acid and other organic acids. A similar study was conducted by Kallel and colleague (2015). They purified xylanase enzyme from bacteria *Bacillus mojavensis* UEB-FK to produce XOS from garlic straw and evaluated its effect on probiotic bacteria. Their findings concluded that this compound could serve as a specialized nutrient supporting the growth of lactic bacteria.

Production of biofuels

Due to the finite nature of fossil fuels, rising global energy demands, and climate change concerns, it has become crucial to seek sustainable, environmentally friendly, and economically feasible alternative energy sources. Recently, there has been an increased interest in second-generation biofuel production from non-food lignocellulosic biomass, specifically organic residues, driven by its abundant availability, renewable properties, and cost-effectiveness. Xylanases play a crucial role in producing various biofuels, such as ethanol, and biodiesel from different lignocellulosic wastes. The production of ethanol through biological processes necessitates the removal of lignin from lignocellulose, a step known as delignification. This process is crucial to free cellulose and hemicellulose from their complex integration with lignin. A combination of enzymes, including xylanase, mannase, ligninase, xylosidase, glucanase, and glucosidase, is employed to break down the carbohydrate polymers found in cellulose and hemicellulose. This enzymatic action results in the release of free sugars. Subsequently, the fermentation of these mixed pentose and hexose sugars leads to the production of ethanol (Mandal, 2015). In 2022, Danso *et al.* isolated xylanase and cellulase producing *Streptomyces* sp. MS-S2 from a wood-feeding termite (*Microcerotermes* sp.) using wheat straw as a carbon source. The purified enzymes were then

employed to hydrolyze wheat straw and convert it into reducing sugar, resulting in the production of 10.8 g/L of bioethanol. In another study, *Streptomyces flavogriseus* hydrolysed cellulose and xylan from lignocellulosic waste to produce sugars which were further fermented to produce high yields of succinic acid (Pennacchio *et al*., 2018).

Other than *Streptomyces*, several bacterial species were also used by researchers to produce bioethanol from lignocellulosic waste such as, *Geobacillus* sp. strain DUSELR13 isolated from deep biosphere of gold mine. When prairie cord grass and corn stover was treated with this strain, it transformed these compounds into bioethanol at concentration 3.53 and 3.72 g/L, respectively (Bibra *et al*., 2018). *Bacillus cereus* and *B. thuringenesis* also exhibited cellulolytic and xylanolytic properties*.* The bacteria were used individually to ferment sugarcane bagasse. *B. cereus* produced 18.40 g/L of bioethanol and 15.27 g/L bioethanol was produced using *B. thuringenesis.* In addition, a yield of 19.08 g/L bioethanol was obtained from a co-culture of the two *Bacillus* spp. (Ire *et al*., 2016).

Juice recovery and clarification

Xylanase in conjunction with a combination of other enzymes, finds applications within the juice industry due to its capability to stabilize fruit pulp, reduce viscosity, and hydrolyze disruptive food materials that impede juice clarity (de Souza *et al.*, 2022). Specifically in juices like pomegranate, orange, kiwi, apricot, apple, peach, and grape, xylanase from *Pediococcus acidilactici* GC25 has been proven effective in diminishing haze formation aftertreatment (Adiguzel *et al*., 2019). Patil *et al*. (2021) reported that the xylanase from *Aspergillus* spp. could achieve 85% clarification of orange juice at 60 °C. In another study, xylanase isolated from the fungal strain *Aspergillus niger* exhibited superior performance in enhancing yield and clarity of pineapple juice, achieving a 71.3% yield and 64.7% clarity in compare to enzyme pectinase (with a 68.2% yield and 63.1% clarity), and cellulase (with a 66.5% yield and 62.8% clarity) (Pal and Khanum, 2011).

Other than juice clarification, researchers also studied the increasing yield of fruit juices after treating them with xylanase enzyme. Kumar and group (2014), purified xylanase enzyme from bacteria *Bacillus pumilus*, which was immobilized on aluminium oxide pellets, as well as in soluble form. To recover the juices in higher amount, they treated both the form of enzyme with orange and grapes pulp and the result demonstrated a significant increase in orange and grapes juice yield by 25% and 19%, using soluble form, and 29% and 26% with immobilized form. Alongside the increased juice recovery, the juice clarity was also increased upto 27 and 30% of grape juice, and 24-29% of orange juice. In another study, the xylanase enzyme from *Bacillus pumilus* SV-85S exhibited an improved yield from other juices like, apple (23.53%), pineapple (10.78%), and tomato (20.78%). Notably the increased yield was accompanied by reduced turbidity and viscosity in the juices, with no effect on their acidity or neutrality (Nagar *et al*., 2013). Due to these properties, xylanase is emerging as the optional enzyme for both clarifying and signicantly enhancing the recovery of fruit juices.

Laundry industry

Stains obtained from vegetables, juices, wine, beer, etc., are not easily removed from cotton cloth even after being washed with detergents. Thus, for proper removal of these stains, enzymes are used because they have the ability to cleave polymeric compounds into smaller fragments, which increases the solubility of fiber mass bound with the pigment. Generally, these detergents contain bleaching agents that decolourise the stain but do not remove them efficiently and also, they harm the material to be cleaned as well as the environment. Therefore, the use of enzyme that have bio-bleaching ability is the best alternative to be used as detergent additives. Moid and coworkers (2021) purified xylanase enzyme from fungal strain *Aspergillus niger* and found that addition of xylanase enzyme with detergent results in sufficient removal of plant stain and can be used in laundry industry for better results.

Patents on Applications of Xylanase

The innovative application of xylanases across various industries has led to the development and granting of several patents, underscoring the enzyme's versatility and value. These patents span a range of applications, from enhancing animal feed efficiency to advancing ecofriendly pulping processes (Table 3).

Table 3. Recent Patents on Xylanase Applications

Conclusion

Xylanase, an enzyme capable of degrading xylan into several intermediates, shows significant potential in increasing the quality and properties of numerous products such as its role in improving paper, animal and poultry feed, juice clarification, detergent additives, and biofuel production by degrading lignocellulosic waste, is well documented. Furthermore, this enzyme also produces xylooligosaccharides (XOS) that exhibit prebiotic properties. Researchers have investigated the diverse sources for xylanase production, including microorganisms like bacteria, fungi, and actinomycetes, often optimizing their growth conditions to enhance enzyme yield and efficacy. Techniques such as recombinant DNA technology also help in development of specialized xylanases that offers great specificity and efficacy in several industrial processes. Overall, this review paper mainly focused on the versatility and promising applications of xylanase across multiple sectors, emphasizing its pivotal role in improving processes and product quality. Continued research and innovation in xylanase technology are expected to provide further opportunities for its applications and advancement in various sectors such as biotechnology, food processing and so on.

Conflict of Interest

The Authors declare no conflict of interest.

Reference

- Abdelaliem, Y.F., Mahmoud, M.H., Elkassem, N.A., Mansour, S.M., Ramadan, M.F., Mohdaly, A.A.A. 2023. Utilization of agro-industrial biowastes to produce xylanase using *Aspergillus niger* AUMC 14230: Optimization of production parameters. Rend. Fis. Acc. Lincei. 34(3), 941–951. doi: https://doi.org/10.1007/s12210-023-01180-2
- Adiguzel, G., Faiz, O., Sisecioglu, M., Sari, B., Baltaci, O., Akbulut, S., Genc, B., Adiguzel, A. 2019. A novel endo-β-1,4-xylanase from *Pediococcus acidilactici* GC25; Purification, characterization and application in clarification of fruit juices. Int. J. Biol. Macromol. 129, 571-578. doi: https://doi.org /10.1016/j.ijbiomac.2019.02.054
- Ahmed, S.A., Saleh, S.A.A., Mostafa, F.A., Abd El Aty, A.A., Ammar, H.A.M. 2016. Characterization and valuable applications of xylanase from endophytic fungus *Aspergillus terreus* KP900973 isolated from *Corchorus olitorius*. Biocatal. Agric. Biotechnol. 7, 134-144. doi: https://doi.org/ 10.1016/j.bcab.2016.05.015
- Ahuja, V., Macho, M., Ewe, D., Singh, M., Saha, S., Saurav, K. 2020. Biological and pharmacological potential of xylitol: A molecular insight of unique metabolism. Foods 9(11), 1592. doi: https://doi. org/10.3390/foods9111592
- Akinyele, H.A., Gabriel-Ajobiewe, R.A.O., Ukhureigbe, O.M., Adebesin, A.A., Omotayo, T.L. 2019. Xylanase-production potential of *Trichoderma asperellum* NG-T161 and NG-T163 isolated from banana farm soils in South Western Nigeria. Current Research in Environmental & Applied Mycology 9(1), 301-312.
- Al-Widyan, O., Khataibeh, M.H., Abu-Alruz, K. 2008. The use of xylanases from different microbial origin in bread baking and their effects on bread qualities. J. Appl. Sci. 8, 672-676. doi: https://doi.org/ 10.3923/jas.2008.672.676
- Awalgaonkar, G., Sarkar, S., Bankar, S., Singhal, R.S. 2015. Xylanase as a processing aid for papads, an Indian traditional food based on black gram. LWT - Food Sci. Technol. 62(2), 1148–1153. doi: https:// doi.org/10.1016/j.lwt.2015.02.034
- Azzouz, Z., Bettache, A., Djinni, I., Boucherba, N., Benallaoua, S. 2022. Biotechnological production and statistical optimization of fungal xylanase by bioconversion of the lignocellulosic biomass residues in solid-state fermentation. Biomass Conv. Bioref. 12, 5923-5935. doi: https:// doi.org/10.1007/s13399-020-01018-z
- Bajar, S., Singh, A., Bishnoi, N.R. 2020. Exploration of lowcost agro-industrial waste substrate for cellulase and xylanase production using *Aspergillus heteromorphus*. Applied Water Science 10(6), 153. doi: https://doi.org/ 10.1007/s13201-020-01236 w
- Baramee, S., Siriatcharanon, A., Ketbot, P., Teeravivattanakit, T., Waeonukul, R., Pason, P., Tachaapaikoon, C., Ratanakhanokchai, K., Phitsuwan, P. 2020. Biological pretreatment of rice straw with cellulase-free xylanolytic enzyme-producing Bacillus firmus K-1: Structural modification and biomass digestibility. Renew. Energy 160, 555- 563. doi: https://doi.org/ 10.1016/j.renene.2020. 06.061
- Bhardwaj, N., Kumar, B., Verma, P. 2019. A detailed overview of xylanases: An emerging biomolecule for current and future prospective. Bioresour. Bioprocess 6, 40. doi: https://doi.org/ 10.1186/ s40643-019-0276-2
- Bibra, M., Kunreddy, V.R., Sani, R.K. 2018. Thermostable xylanase production by *Geobacillus* sp. strain

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DUSELR13, and its application in ethanol production with lignocellulosic biomass. Microorganisms 6, 93. doi: https://doi.org/ 10.3390/microorganisms6030093

- Chakdar, H., Kumar, M., Pandiyan, K., Singh, A., Nanjappan, K., Kashyap, P.L., Srivastava, A.K. 2016. Bacterial xylanases: Biology to biotechnology. 3 Biotech 6(2), 150. doi: https:// doi.org/10.1007/s13205-016-0457-z
- Chaturvedi, S., Kohli, K.U., Rajni, S., Khurana, S.M.P. 2015. Statistical optimization of medium composition for xylanase production by solid state fermentation using agroresidues. Am. J. Microbiol. Res. 3(2), 85–92.
- Collins, T., Gerday, C., Feller, G. 2005. Xylanases, xylanase families and extremophilic xylanases. FEMS Microbiology Reviews 29(1), 3-23. doi: https://doi.org/10.1016/j.femsre.2004.06.005
- Curry, T.M., Peña, M.J., Urbanowicz, B.R. 2023. An update on xylan structure, biosynthesis, and potential commercial applications. Cell Surf. 28(9), 100101. doi: 10.1016/j.tcsw.2023.100101.
- Danso, B., Ali, S.S., Xie, R., Sun, J. 2022. Valorisation of wheat straw and bioethanol production by a novel xylanase- and cellulase-producing *Streptomyces* strain isolated from the woodfeeding termite, *Microcerotermes* species. Fuel 310(A), 122333. doi: https://doi.org/ 10.1016/ j.fuel.2021.122333
- de Freitas, C., Carmona, E., Brienzo, M. 2019. Xylooligosaccharides production process from lignocellulosic biomass and bioactive effects. Bioact. Carbohydr. Diet Fibre 18, 100184. doi: https:// doi.org/10.1016/j.bcdf.2019.100184
- de Souza, H.F., Borges, L.A., Gonçalves, V.D.D.P, dos Santos, J.V., Bessa, M.S., Carosia, M.F., de Carvalho, M.V., Brandi, I.V., Kamimura, E.S. 2022. Recent advances in the application of xylanases in the food industry and production by actinobacteria: A review. Food Res. Int. 162(B), 112103. doi: https://doi.org/ 10.1016/j.foodres. 2022.112103.
- Devi, S., Dwivedi, D., Bhatt, A.K. 2022. Utilization of agroresidues for the production of xylanase by *Bacillus safensis* XPS7 and optimization of production parameters. *Fermentation 8,* 221. doi: https://doi.org/10.3390/fermentation8050221
- Dhaver, P., Pletschke, B., Sithole, B., Govinden, R. 2022. Isolation, screening, preliminary optimisation and characterisation of thermostable xylanase

production under submerged fermentation by fungi in Durban, South Africa. Mycology 13(4), 271-292. doi: https ://doi.org/10.1080/ 21501203.2022.2079745

- Dhivahar, J., Khusro, A., Paray, B.A., Rehman, M.U., Agastian, P. 2020. Production and partial purification of extracellular xylanase from *Pseudomonas nitroreducens* using frugivorous bat (*Pteropus giganteus*) faeces as ideal substrate and its role in poultry feed digestion. J. King Saud. Univ. – Sci. 32(4), 2474-2479. doi: https://doi.org/ 10.1016/j.jksus.2020.03.046
- Dukare, A., Sharma, K., Kautkar, S., Dhakane-Lad, J., Yadav, R., Nadanathangam, V., Saxena, S. 2023. Microbial xylanase aided biobleaching effect on multiple components of lignocelluloses biomass based pulp and paper: A review. Nord. Pulp Pap. Res. J. 38(3), 459-480. doi: https://doi.org/ 10.1515/npprj-2023-0005
- Global xylanase market outlook (2023 to 2033), 2023. https://www.futuremarketinsights.com/report s/xylanase-market accessed on 24 July 2023.
- Guo, H., Hong, C., Zhang, C., Zheng, B., Jiang, D., Qin, W. 2018. Bioflocculants' production from a cellulasefree xylanase-producing *Pseudomonas boreopolis* G22 by degrading biomass and its application in cost-effective harvest of microalgae. Bioresour. Technol. 255, 171-179. doi: https://doi.org/10. 1016/j.biortech. 2018.01.082
- Hu, H., Dai, S., Wen, A., Bai, X. 2019. Efficient expression of xylanase by codon optimization and its effects on the growth performance and carcass characteristics of broiler. Anim. 9(2), 65. doi: https://doi. org/10.3390/ani9020065
- Ire, F.S., Ezebuiro, V., Ogugbue, C.J. 2016. Production of bioethanol by bacterial co-culture from agrowaste-impacted soil through simultaneous saccharification and co-fermentation of steamexploded bagasse. Bioresour. Bioprocess. 3, 26. doi: https://doi.org/10.1186/s40643-016-0104-x
- Istiqomah, L., Cahyanto, M. N., Zuprizal, Z. 2022. Xylanase production by *Trichoderma virens* MLT2J2 under solid-state fermentation using corn cob as a substrate. Biodiversitas Journal of Biological Diversity 23(12), 6530-6538. doi: https://doi.org/10.13057/biodiv/d231251
- JI, X., Chen, J., TIAN, Z., WANG, R., Wang, D., Liu, Y. 2022. *Method for preparing unbleached biomechanical pulp and fully utilizing by-products by treating straws with heat steam in synergy with biological enzyme* (United States Patent US20220205178A1).https://patents.

google.com/patent/US20220205178A1/en?q=(a pplications+of+xylanase)&before=priority:20241 231&after=priority:20180101&oq=applications+ of+xylanase+before:priority:20241231+after:prio rity:20180101&page=4

- Kallel, F., Driss, D., Bouaziz, F., Neifer, M., Ghorbel, R., Ellouz Chaabouni, S. 2015. Production of xylooligosaccharides from garlic straw xylan by purified xylanase from *Bacillus mojavensis* UEB-FK and their in vitro evaluation as prebiotics. Food Bioprod. Process 94, 536-546. doi: https://doi.org/10.1016/j.fbp.2014.07.012
- Kaushal, J., Raina, A., Singh, G., Khatri, M., Arya, S.K., Karmegam, N., Ravindran, B., Chang, S.W., Mani, R., Awasthi, M.K. (2022) Methodical study implicating the effectiveness of microbial treatment over xylanase enzymatic treatment for pulp bio-bleaching. Environ. Technol. Innov. 28:102731. doi: https://doi.org/10.1016/j.eti. 2022.102731
- Kereh, H., Mubarik, N., Palar, R., Santoso, R., Yopi 2018. Optimization of process parameters and scale-up of xylanase production using corn cob raw biomass by marine bacteria *Bacillus subtilis* LBF M8 in stirred tank bioreactor. Pak. J. Biotechnol 15(3), 707-714.
- Khandeparker, R., Parab, P., Amberkar, U. 2017. Recombinant xylanase from *Bacillus tequilensis* BT21: Biochemical characterisation and its application in the production of xylobiose from agricultural residues. Food Technol. Biotechnol. 55(2), 164-172. doi: https://doi.org/10.17113/ ftb.55.02.17.4896
- Kulkarni, N., Shendye, A., Rao, M. 1999. Molecular and biotechnological aspects of xylanases. FEMS Microbiol. Rev. 23, 411-456. doi: https://doi.org/ 10.1111/j.1574-6976.1999. tb00407.x
- Kumar, L., Nagar, S., Mittal, A., Garg, N., Gupta, V.K. 2014. Immobilization of xylanase purified from *Bacillus pumilus* VLK-1 and its application in enrichment of orange and grape juices. J. Food Sci. Technol. 51, 1737-1749. doi: https://doi.org/10.1007/ s13197-014-1268-z
- Kumar, V., Dangi, A.K., Shukla, P. 2018. Engineering thermostable microbial xylanases toward its industrial applications. Mol. Biotechnol. 60, 226- 235. doi: https://doi.org/10.1007/s12033-018- 0059-6
- Kumar, V., Marín-Navarro, J., Shukla, P. 2016. Thermostable microbial xylanases for pulp and

paper industries: Trends, applications and further perspectives. World J. Microbiol. Biotechnol. 32, 34. doi: https://doi.org/ 10.1007/s11274-015- 2005-0

- Kumar, V., Shukla, P. 2018. Extracellular xylanase production from *T. lanuginosus* VAPS24 at pilot scale and thermostability enhancement by immobilization. Process Biochem. 71, 53-60. doi: https://doi.org/10.1016/j.procbio.2018.05.019
- Liu, K., Ji, X., Li, P., Wei, S., Wang, R. 2022. *Method for pulping wheat straw by using xylanase and pectinase* (Patent LU500846B1). https://patents.google. com/patent/LU500846B1/en?q=(applications+o f+xylanase)&before=priority:20241231&after=pr iority:20180101&oq=applications+of+xylanase+ before:priority:20241231+after:priority:20180101 &page=6
- Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P.M., Henrissat, B. 2014. The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res. 42, 490-495. doi: https://doi.org/10.1093/ nar/gkt1178
- Lund, S. A., Bernardeau, M., Yu, Z., Qian, Z. 2021. *Xylanase-containing feed additives for cereal-based animal feed* (United States Patent US2021027 7374A1). https://patents.google.com/patent/ US20210277374A1/en?q=(applications+of+xyla nase)&before=priority:20241231&after=priority: 20180101&oq=applications+of+xylanase+before: priority:20241231+after:priority:20180101
- Malhotra, G., Chapadgaonkar, S.S. 2023. Thermo-alkali stable bacterial xylanase for deinking of copier paper. J. Genet. Eng. Biotechnol. 21(1), 107. doi: https://doi.org/10.1186/s43141-023-00563-0.
- Mandal, A. 2015. Review on microbial xylanases and their applications. Int. J. Life Sci. 4(3), 178-187.
- Marasabessy, A., Moeis, M.R., Sanders, J.P., Weusthuis, R.A. 2011. Enhancing Jatropha oil extraction yield from the kernels assisted by a xylan-degrading bacterium to preserve protein structure. Appl. Microbiol. Biotechnol. 90(6), 2027-2036.
- Marimuthu, M., Sorimuthu, A., Muruganantham, S. 2019. Production and optimization of xylanase enzyme from *Bacillus subtilis* using agricultural wastes by solid state fermentation. Int. J. Pharm. Investig. 9(4), 169-173. doi: https://doi.org/10.5530/ ijpi.2019.4.32
- Mhiri, S., Bouanane-Darenfed, A., Jemli, S., Neifar, S., Ameri, R., Mezghani, M., Bouacem, K., Jaouadi,

B., Bejar, S. 2020. A thermophilic and thermostable xylanase from *Caldicoprobacter a l g erie n sis*: Re c o m b i n a n t e x p r e s s i o n , characterization and application in paper biobleaching. Int. J. Biol. Macromol. 164, 808-817. doi: https://doi.org/10.1016/j.ijbiomac. 2020.07.162

- Moid, M.M., Idris, N., Othman, R., Wahid, D.A. 2021. Development of xylanase as detergent additive to improve laundry application. IOP Conf. Ser. Mater. Sci. Eng. 1092, 012053. doi: https://doi. org/10.1088/1757-899X/1092/ 1/012053
- Nagar, S., Mittal, A., Gupta, V.K. 2013. Enzymatic clarification of fruit juices (apple, pineapple, and tomato) using purified *Bacillus pumilus* SV-85S xylanase. Biotechnol. Bioprocess Eng. 17. doi: https://doi.org/10.1007/s12257-012-0375-9
- Nair, S.G., Sindhu, R., Shankar, S. 2008. Fungal xylanase production under solid state and submerged fermentation conditions. Afr. J. Microbiol. Res. 2, 82-86.
- Nascimento, C.E. de O., Simões, L.C. de O., Pereira, J. de C., da Silva, R.R., de Lima, E.A., de Almeida, G.C., Penna, A.L.B., Boscolo, M., Gomes, E., da Silva, R. 2022. Application of a recombinant GH10 endoxylanase from *Thermoascus aurantiacus* for xylooligosaccharide production from sugarcane bagasse and probiotic bacterial growth. J. Biotechnol. 347, 1-8. doi: https://doi.org/10. 1016/j.jbiotec.2022.02.003
- Núñez-Serrano, A., García-Reyes, R.B, García-González, A. 2024. Optimization of hydrolases production by *Peni c illium c rust o sum* in submerged fermentation using agro-waste residues as cosubstrate, Biocatalysis and Agricultural Biotechnology 57, 103116, https://doi.org/ 10.1016/j.bcab.2024.103116.
- Nusairat, B., Wang, J.-J. 2021. The effect of a modified GH11 xylanase on live performance, gut health, and *Clostridium perfringens* excretion of broilers fed corn-soy diets. Front. Vet. Sci. 8, 678536. doi: https://doi.org/10.3389/fvets.2021.678536
- Pal, A., Khanum, F. 2011. Efficacy of xylanase purified from *Aspergillus niger* DFR-5 alone and in combination with pectinase and cellulase to improve yield and clarity of pineapple juice. J. Food Sci. Technol. 48, 560-568. doi: https://doi.org/10.1007/s13197-010-0175-1
- Pasalari, A., Homaei, A. 2022. Isolation and molecular identification of xylanase-producing bacteria

from *Ulva flexuosa* of the Persian Gulf. Processes 10(9), 1834. doi: https://doi.org/10.3390/ pr10091834

- Patel, K., Dudhagara, P. 2020. Optimization of xylanase production by *Bacillus tequilensis* strain UD-3 using economical agricultural substrate and its application in rice straw pulp bleaching. Biocatalysis and Agricultural Biotechnology 30, 101846. doi: https://doi.org/10.1016/j.bcab. 2020.101846
- Patil, L.R., Shet, A.R., Achappa, S., Desai, S.V., Hombalimath, V.S., Kallur, M.M. 2021. Statistical optimization of media components for xylanase production by *Aspergillus* spp. using solid state fermentation and its application in fruit juice clarification. J. Pharm. Res. Int. 33(54A), 151-166.
- Pennacchio, A., Ventorino, V., Cimini, D., Pepe, O., Schiraldi, C., Inverso, M., Faraco, V. 2018. Isolation of new cellulase and xylanase producing strains and application to lignocellulosic biomasses hydrolysis and succinic acid production. Bioresour. Technol. 259, 325-333. doi: https://doi.org/10.1016/ j.biortech.2018.03.027
- Petry, A.L., Patience, J.F., Koester, L.R., Huntley, N.F., Bedford, M.R., Schmitz-Esser, S. 2021. Xylanase modulates the microbiota of ileal mucosa and digesta of pigs fed corn-based arabinoxylans likely through both a stimbiotic and prebiotic mechanism. PLoS ONE 16(1), e0246144. doi: https://doi.org/10.1371/journal.pone.0246144
- Pia, E.A.D. 2021. *Animal feed compositions and uses thereof* (United States Patent US20210227853A1). https://patents.google.com/patent/US2021022 7853A1/en?q=(applications+of+xylanase)&befo re=priority:20241231&after=priority:20180101&o q=applications+of+xylanase+before:priority:202 41231+after:priority:20180101&page=4
- Polizeli, M.L.T.M., Rizzatti, A.C.S., Monti, R., Terenzi, H.F., Jorge, J.A., Amorim, D.S. 2005. Xylanases from fungi: Properties and industrial applications. Appl. Microbiol. Biotechnol. 67, 577- 591. doi: https://doi.org/10.1007/s00253-005- 1904-7
- Ramanjaneyulu, G., Rajasekhar Reddy, B. 2016. Optimization of xylanase production through response surface methodology by *Fusarium* sp. BVKT R2 isolated from forest soil and its application in saccharification. Front. Microbiol. 7, 1450. doi: https ://doi.org/10.3389/ fmicb.2016.01450
- Reis, N. dos S., Lessa, O.A., Pacheco, C.S.V., Pereira, N.E., Soares, G.A., Silva, E.G.P., Oliveira, J.R., Franco, M. 2020. Cocoa shell as a substrate for obtaining endoglucanase and xylanase from *Aspergillus oryzae* ATCC 10124. Acta Scientiarum Technology 42. doi: https://www.redalyc.org/ journal/ 3032/303265671058/html/
- Romero, J.J., Macias, E.G., Ma, Z.X., Martins, R.M., Staples, C.R., Beauchemin, K.A., Adesogan, A.T. 2016. Improving the performance of dairy cattle with a xylanase-rich exogenous enzyme preparation. J. Dairy Sci. 99(5), 3486-3496. doi: https://doi.org /10.3168/jds.2015-10082
- Samantal, A.K., Kolte, A.P., Senani, S., Sridhar, M., Jayapal, N. 2011. A simple and efficient diffusion technique for assay of endo β-1,4-xylanase activity. Braz. J. Microbiol. 42(4), 1349-1353.
- Shanthi, V., Roymon, M.G. 2018. Isolation, identification and partial optimization of novel xylanolytic bacterial isolates from Bhilai-Durg region, Chhattisgarh, India. Iran J. Biotechnol. 16(3), e1333.
- Singh, A., Varghese, L.M., Yadav, R.D., Mahajan, R. 2020. A pollution reducing enzymatic deinking approach for recycling of mixed office waste paper. Environ. Sci. Pollut. Res. 27, 45814-45823. doi: https://doi.org/10.1007/s11356-020-10440- 9
- Singh, R.S., Singh, T., Pandey, A. 2019. Chapter 1 Microbial Enzymes—An Overview, in: Singh, R.S., Singhania, R.R., Pandey, A., Larroche, C. (Eds.), Biomass, Biofuels, Biochemicals: Advances in Enzyme Technology. Elsevier, pp. 1–40. doi: https://doi.org/10.1016/B978-0-444-64114- 4.00001-7
- Siwach, R., Sharma, S., Khan, A.A., Kumar, A., Agrawal, S. 2024. Optimization of xylanase production by *Bacillus* sp. MCC2212 under solid-state fermentation using response surface methodology. Biocatalysis and Agricultural Biotechnology 57, 103085. doi: https://doi.org/ 10.1016/j.bcab. 2024.103085.
- Sridevi, A., Ramanjaneyulu, G., Suvarnalatha Devi, P. 2017. Biobleaching of paper pulp with xylanase produced by *Trichoderma asperellum*. 3 Biotech 7, 266. doi: https://doi.org/10.1007/s13205-017- 0898-z
- Thakur, V., Kumar, V., Kumar, V., Singh, D. 2022. Xylooligosaccharides production using multisubstrate specific xylanases secreted by a psychrotolerant *Paenibacillus* sp. PCH8. Carbohydr. Polym. Technol. Appl. 3, 100215. doi: https://doi.org/10.1016/j.carpta. 2022.100215
- Van Hoeck, V., Somers, I., Abdelqader, A., Wealleans, A.L., Van de Craen, S., Morisset, D. 2021. Xylanase impact beyond performance: A microbiome approach in laying hens. PLoS One 16(9), e0257681. doi: https://doi.org/10.1371/ journal.pone.0257681.
- Verma, D., Satyanarayana, T. 2012. Molecular approaches for ameliorating microbial xylanases. Bioresour. Technol. 117, 360-367. doi: https://doi.org/ 10.1016/j.biortech.2012.04.034
- Walia, A., Guleria, S., Mehta, P., Chauhan, A., Parkash, J. 2017. Microbial xylanases and their industrial application in pulp and paper biobleaching: A review. 3 Biotech 7, 11. doi: https://doi.org/ 10.1007/s13205-016-0584-6
- Wu, W.-J., Ahn, B.-Y. 2018. Statistical optimization of medium components by response surface methodology to enhance menaquinone-7 (Vitamin K) production by *Bacillus subtilis*. J. Microbiol. Biotechnol. 28(6), 902-908. doi: https://doi.org/10.4014/jmb.1801.01042
- Xylanase market 2022. https://www. reportsanddata. com/report-detail/xylanase-market accessed on 24 July 2023.
- Yadav, P., Maharjan, J., Korpole, S., Prasad, G.S., Sahni, G., Bhattarai, T., Sreerama, L. 2018. Production, purification, and characterization of thermostable alkaline xylanase from Anoxybacillus *kamchatkensis* NASTPD13. Front. Bioeng. Biotechnol. 6, 65. doi: https://doi.org/10.3389 /fbioe.2018.00065
- Yegin, S. 2023. Microbial xylanases in xylooligosa ccharide production from lignocellulosic feedstocks. Biomass Conv. Bioref. 13, 3619-3658. doi: https://doi.org/10.1007/s13399-022-03190-w
- Zhao, L., Yuan, Z., Kapu, N.S., Chang, X.F., Beatson, R., Trajano, H.L., Martinez, D.M. 2017. Increasing efficiency of enzymatic hemicellulose removal from bamboo for production of high-grade dissolving pulp. Bioresour. Technol. 223, 40-46. doi: https://doi.org/10.1016/j.biortech. 2016.10.034

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