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Review

Biotechnological Potential of Lignocellulosic Biomass as Substrates for Fungal Xylanases and Its Bioconversion into Useful Products: A Review

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Abstract: Lignocellulose, the most abundant and renewable plant resource, is a complex of polymers mainly composed of polysaccharides (cellulose and hemicelluloses) and an aromatic polymer (lignin). Utilisation of lignocellulosic biomass for biotechnological applications has increased over the past few years. Xylan is the second most abundant carbohydrate in plant cell walls, and structurally, it is a heteropolysaccharide with a backbone composed of β-1,4-d-xylopyranosyl units connected with glycosidic bonds. Xylanases degrade this complex structure of xylan and can be produced by various microorganisms, including fungi, bacteria, and yeasts. Lignocellulosic biomass is the most economical substrate for the production of fungal xylanases. The bioconversion of lignocellulosic biomass to industrially important products, i.e., xylooligosaccharides and biofuels, is possible via the application of xylanases. These enzymes also play a key role in enhancing the nutrition of food and feed and the bio-bleaching of paper and kraft pulp. However, the demand for more potent and efficient xylanases with high activity has increased, which is fulfilled by involving recombinant DNA technology. Hence, in this review, we thoroughly discussed the biotechnological potential of lignocellulosic biomass for the production of fungal xylanases, their purification, molecular strategies for improving their efficiency, and their utilisation for the production of valuable products and in other industrial processes.

Keywords: lignocellulosic biomass; xylan; fungal xylanases; biofuels; prebiotics; bio-bleaching

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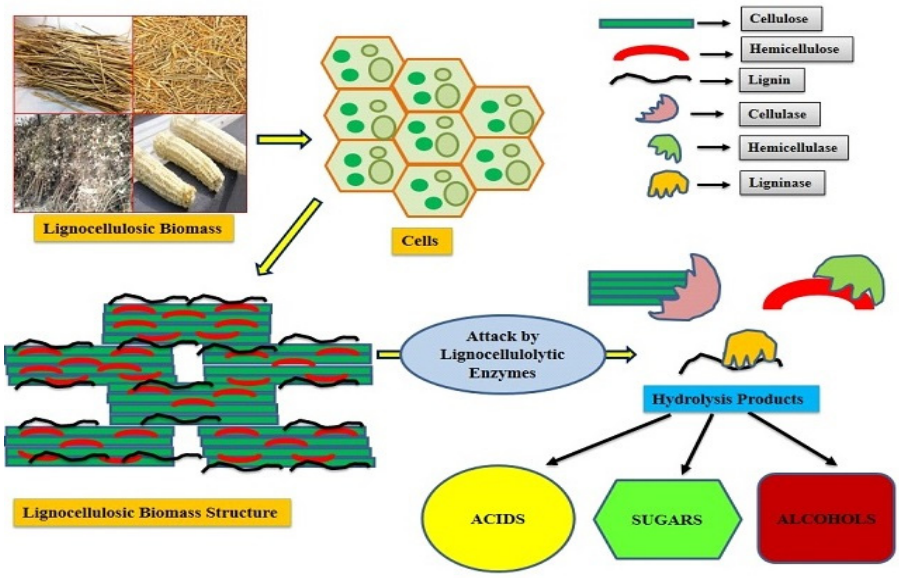
1. Introduction

Lignocellulosic residues/biomass are the most ample bio-material on earth and are globally believed to be a promising substitute feedstock for biotechnological processes. Lignocellulose consists of a complicated network of hemicelluloses, cellulose, and lignin with inconstant ratios relative to the category of biomass, together with growth conditions [1]. Cellulose and hemicelluloses are carbohydrate polymers of different nature and composition. Cellulose is made up of β-1,4-linked D-glucose units, creating a linear polymer that is assembled in a microfibril configuration. Hemicelluloses are a heteropolymer with multiple branches organised in random frames. Lignin is formed of phenyl-propane units with different methoxyl group displacements on the aromatic rings amassed in an irregular polymer. These constituents are intimately linked with each other through hydrogen bonds and ester-ether linkages, making the lignocellulose quite recalcitrant, i.e., difficult to decompose biologically and chemically. The structure of lignocellulosic biomass and the mode of action of lignocellulolytic enzymes for their degradation are illustrated in Figure 1a. Around 181.5 billion tons (approximately) of lignocellulosic biomass are annually produced globally, and out of this amount, only 8.2 billion tons are used in various application areas [2]. A recent study in India, sponsored by the Ministry of New and Renewable Energy,

stated that the present availability of biomass is estimated at about 750 MMT (million metric tons) per year. Roughly 800–1000 million tons per year of rice straw is produced globally, while only in Asia, production of rice straw is 600–800 million tons per year (https://www.irri.org/rice-straw-management, accessed on 1 December 2023). In India, rice is cultivated on 43.78 million hectares, and one ton of rice grain harvested produces around 1.4 tons of straw, equating to 165.8 million tons of rice straw in 2019–2020 [3]. Every year in India, about 80% of crop residues produced are used as fodder, fuel, and/or raw material for industrial applications; meanwhile, around 87 million tons of unused surplus crop residue are burned in fields [4]. Sugarcane bagasse is the major by-product of the sugar industry and an important substrate for the biofuel industry, producing about 513 million tons a year [5]. India produces 91 million tons of sugarcane bagasse a year from more than 500 sugar mills [6]. Another agricultural biomass that is abundantly available in India is wheat straw. The annual production of wheat straw in 2016–2017 was 747 million tons [7].



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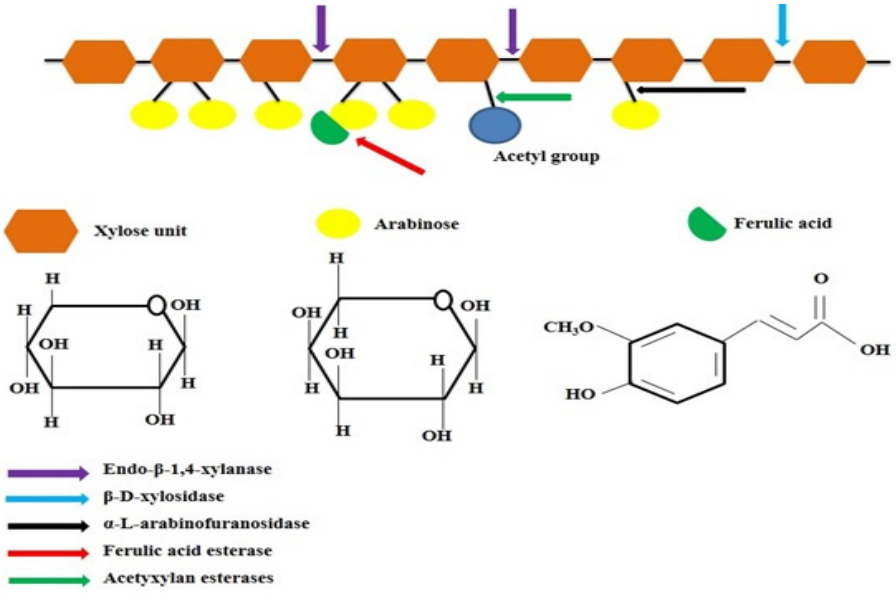

(**b**)

Figure 1. (**a**) Structural representation of lignocellulosic biomass and mode of action of lignocellulolytic enzymes, (**b**) Mode of action of different xylanolytic enzymes on xylan hydrolysis.

In order to obtain beneficial products out of these constituents, appropriate eco-friendly transformation technologies are needed for the effective implementation of bio-economy tactics [8]. Xylan is the major hemicellulosic component and the second most abundant renewable polysaccharide in nature. It has a composite polysaccharide structure with xylose residues as the backbone linked by a β-1,4-glycosidic bond, while the leading chain in xylan is made of -xylopyranose residues [9]. The cell wall of terrestrial plants also has xylan as the main and conventional hemicellulosic polysaccharide, showing a total dry weight of 30–35%. Hardwood from angiosperms is also composed of hemicelluloses (15–30% of total dry weight), whereas softwood from gymnosperms has a lower proportion of hemicelluloses (7–12%) [10]. Xylan, arabinan, mannan, and galactan are the main heteropolymeric components of hemicelluloses, and the categorisation of hemicelluloses depends on the type of sugar moiety found in it. Xylanases or endo-1,4-β-D-xylanohydrolases belong to a class of hydrolytic enzymes that break down plant cell wall components, i.e., hemicelluloses, resulting in the depolymerisation of 'xylan'. Currently, xylanases, alone and in synergism with other microbial enzymes like cellulase, phytase, amylase, lipase, and glucose oxidase, are used in the food and feed industries for upgrading animal feed stock digestibility [11], modification of baked products [12], enhancing the nutritional characteristics of agricultural grain feed and silage [13], and clarification of fruit juices [14]. Xylanases, individually or along with other lignocellulolytic enzymes, assist in the bio-conversion of lignocellulosic or agricultural biomass into useful products, e.g., bioethanol [15] and xylooligosaccharides [16]. Besides bio-conversion of lignocellulosic biomass, xylanases also participated in de-inking [17], bio-bleaching of paper pulp [18], and degumming of plant fibres (hemp, flax, ramie, and jute) [19]. An extensive application of microbial xylanases for the utilisation of lignocellulosic biomass has been assigned to their capability in bio-product and bio-energy development as a cost-effective measure. Production and applications of xylanases have been gaining attention all over the world because they are capable of degrading lignocellulosic biomass. These enzymes have been produced by bacteria [20], fungi [1], actinomycetes [21], and yeasts [22]. Conventionally, SmF and SSF techniques have been employed for xylanase production, but in the current scenario for large-scale production, advanced techniques, e.g., RDT, have also been used for the economical production of fungal xylanases for industrial applications.

Lignocellulose biomass is generated in huge amounts and lacks proper management. Major part of this biomass is burnt in fields by the farmers in order to sow the next crops. This burning is responsible for an increase in environmental pollution and human health hazards. Soil fertility is also negatively affected by this burning. Utilisation of plant biomass as substrate for the production of fungal xylanases will make the process economical at the industrial scale. Further, fungal xylanasses can hydrolyse this biomass for the production of value-added products like biofuels, prebiotics, and others. Therefore, the utilisation of lignocellulosic biomass as substrates for production of fungal xylanases and other value-added products will help in the sustainable and economic management of this huge amount of solid waste with a concomitant reduction in environmental pollution. This review focuses on the utilisation of lignocellulosic biomass for the production of fungal xylanases, followed by their biotechnological applications in different industries.

2. Categorisation and Action Mechanism of Xylanases

Xylanases are divided into 3 categories based on (1) molecular weight and isoelectric point, which are further divided into high molecular weight, having a low (acidic) isoelectric point, and low molecular weight, having a high (basic) isoelectric point; (2) kinetic or catalytic properties; and (3) crystal structure. Consequently, an adequate system involving primary crystal structure and comparison between catalytic properties, kinetic properties, and product categories has been adopted [23]. However, the structural, functional, and genomic data of xylanases are accessible inside glycoside hydrolase (GH) families, which are provided on the CAZy database. The principal GH families related to xylanases involve 5, 7, 8, 9, 10, 11, 12, 16, 26, 30, 43, 44, 51, and 62, and out of these GH families, 5, 7, 8,

10, 11, and 43 consist of a single specific catalytic domain, while GH families 16, 51, and 62 comprise two catalytic domains with bi-functional properties. Moreover, xylanases of the 9, 12, 26, 30, and 44 GH families exhibit secondary activity.

Xylanases employ two distinct mechanisms for xylan hydrolysis: (i) retention and (ii) inversion [24]. Xylanases of GH families 5, 7, 10, and 11 mostly follow retention mechanisms. This mechanism works on a double displacement mechanism that involves the formation of intermediates, e.g., α-glycosyl and oxo-carbonium, and their subsequent hydrolysis [Supplementary Figure S1a], and in this process, glutamate residues play a key role. On the other hand, xylanases that belong to GH families 8 and 43 mainly follow an inversion mechanism. Inversion of the anomeric center with aspartate and glutamate occurs as the prime catalytic residue. Inversion is a single displacement process that involves just a single carboxylate ion for the removal of the whole acid-catalysed group [Supplementary Figure S1b]. The enzyme, moreover, serves as a base for activation of a nucleophilic water molecule that further strikes on anomeric carbon, according to the distance between two molecules, cleaving the glycosidic bonds and causing an inversion of the anomeric carbon configuration [24].

3. Xylanolytic Enzymes

Xylanases perform a pivotal role in the disruption of the plant cell wall in combination with different enzymes that hydrolyse polysaccharides. Supplementary Table S1 indicates some of the commercially important xylanases with their brand names and applied fields. The mechanism of action of different xylanolytic enzymes on xylan is illustrated in Figure 1b.

3.1. Endo-1-4-β-Xylanases

Endo-1-4-β-xylanases or 1,4-β-D-xylan xylanohydrolase cuts the glycosidic bond in xylan backbone and decreased the degree of substrate polymerisation. Xylan is not attacked at random positions by enzymes; actually, the bond chosen for the hydrolysis depends on the properties (chain length, branching degree, and existence of substituents) of the substrate molecule [21]. These enzymes are categorised on the basis of formation of end products followed by xylan hydrolysis, like xylose, xylobiose, xylotriose, and arabinose. Consequently, xylanases are divided into arabinose liberating/de-branching enzymes and/or non-arabinose liberating/non-de-branching enzymes. Many microorganisms reported in the literature are capable of producing both types of xylanases and efficiently hydrolysed xylan. Usually, endoxylanases show peak activity at pH from 4.0 to 6.5 and temperature between 40–80 °C. However, optimal conditions different from this range of temperature and pH have also been detected. Specific bacteria and fungi can display heterogenecity of endoxylanases. Bacterial and fungal endoxylanase are nearly single sub-unit proteins with molecular mass (MM) and isoelectric point varying from 8.5 to 85 kDa and from 4.0 to 10.3, respectively, and most of them are glycosylated [21]. The physico-chemical characteristics of bacterial and fungal endoxylanases have a strong link between their molecular weight and isoelectric point (pI), and it is reported that with a few special cases, endoxylanases are divided into 2 main groups, one having a molecular mass lower than 30 kDa (basic proteins) and the other having a molecular mass greater than 30 kDa (acidic proteins) [22].

3.2. β-Xylosidases

β-D-Xylosidases or 1,4-β-D-xylan xylohydrolase can be categorised according to their relative affinity for larger xylooligosaccharides and xylobiose. They may be monomeric, dimeric and/or tetrameric having MM within range of 26–360 kDa. A variety of fungi and bacteria produced this enzyme [22]. Usually, purified β-xylosidases do not hydrolyse xylan, because for them xylobiose is an ideal substrate. Meanwhile, the affinity of β-xylosidase for XO is reciprocal to its degree of polymerisation. Unnatural substrates such as p- and o-nitrophenyl-β-D-xylopyranoside are also cleaved by β-D-xylosidases (Dhiman and Mukherjee, 2018). During xylan hydrolysis, β-xylosidases hydrolyse the short oligomers of

β-D-xylopyranosyl, which prevents the action of endoxylanase [22]. Optimum temperature for their activity may vary from 40 to 80 °C, whereas the majority of β-xylosidases are optimally active at 60 °C.

3.3. α-Arabinofuranosidases

L-arabinose residues substituted on β-D-xylopyranosyl at positions two and three are hydrolysed by arabinofurnosidases, which are divided into two groups depending on their mode of action. One is exo-α-L-arabinofuranosidase (EC 3.2.1.55), which breaks down p-nitrophenyl-α-L-arabinofuranosides and the branched arabinans. The second is endo-1,5-α-L-arabinase (EC 3.2.1.99), which degrades only linear arabinans [2]. Arabinose is produced by these enzymes, but there is no breakdown of the xylan backbone, and that is why no xylooligosaccharides formation.

3.4. Acetyl-Xylan Esterases

Acetyxylan esterases cut out o-acetyl substituents at the second and third positions on xylose residues in acetylated xylans [22]. Acetylxylan performs a major role in xylan hydrolysis, as the acetyl side-group can block the action of enzymes, which break down its backbone with steric hindrance. In this way, its removal promotes endoxylanase action [2].

3.5. α-Glucuronidases

α-glucuronidase breaks the α-1,2-bond that links β-D-xylopyranosyl backbone units and glucuronic acid residues present in glucuronoxylan [22].

4. Xylanases Production

Xylanases are very powerful enzymes from an environmental point of view, as they can help in reducing the environmental pollution in different ways. Numerous biotechnological strategies involving submerged (SMF) as well as solid-state fermentation (SSF) have been employed for xylanase production [25]. SMF is a universal process for the development of industrial enzymes, and in this process, microorganisms and solid substrates are grown in submerged conditions in salt solutions. Because of this, the modelling of the process is manageable, along with easy heat and oxygen transfer [25]. However, for increased production of xylanases in industries, the most significant strategy applied is SSF. In SSF, the cultivation of microbes is conducted in the absence of water or the near-absence of free-floating water under controlled conditions. Recently, this technique has gained much more attention as an acceptable strategy, as it reuses nutrient-rich agricultural residues with less energy input and also facilitates the bio-conversion of agricultural wastes into valuable products. Because of its lower operational cost and capital investment, this process is an attractive method for the economical production of industrial enzymes [25]. Varieties of fungal and bacterial species are well known to grow on minimally moist agro-residues in the absence of free water. In general, raw natural materials are used in the SSF process as sources of carbon and energy, which make this process cost-effective and economical [12]. Generally, SSF has advantages compared to SMF due to its cost effective and eco-friendly nature and it also produces high yields of enzymes. The chances of contamination are reduced due to low moisture content in SSF. Filamentous fungi are significantly attractive microbes for the production of xylanase for commercial utilisation because these fungi release their enzymes into the medium. Agro-industrial residues or agricultural residues involving soybean hulls, wheat and rice bran, rice straw, sugarcane bagasse, palm kernels, and wheat straw are commonly referred to as ideal substrates for the cultivation of microbes in SSF as they are accessible in abundance at a low cost. Supplementary Table S2 represent the optimised culture conditions for xylanase production by various microorganisms in SmF and SSF.

4.1. Culture Conditions for Xylanase Production

The production of xylanase is influenced by physical and chemical factors (Figure 2). The physio-chemical properties of substrates, like large surface area, crystallinity, and bed porosity, can influence the enzyme production by fungi. However, the generation of heat and the presence of oxygen in open space in between particles of substrate are the major challenges that come with SSF; therefore, optimisation of all physio-chemical parameters is performed to achieve higher production of enzymes [25]. Different physical and chemical parameters that affect xylanase production in SmF and SSF are given below in brief:

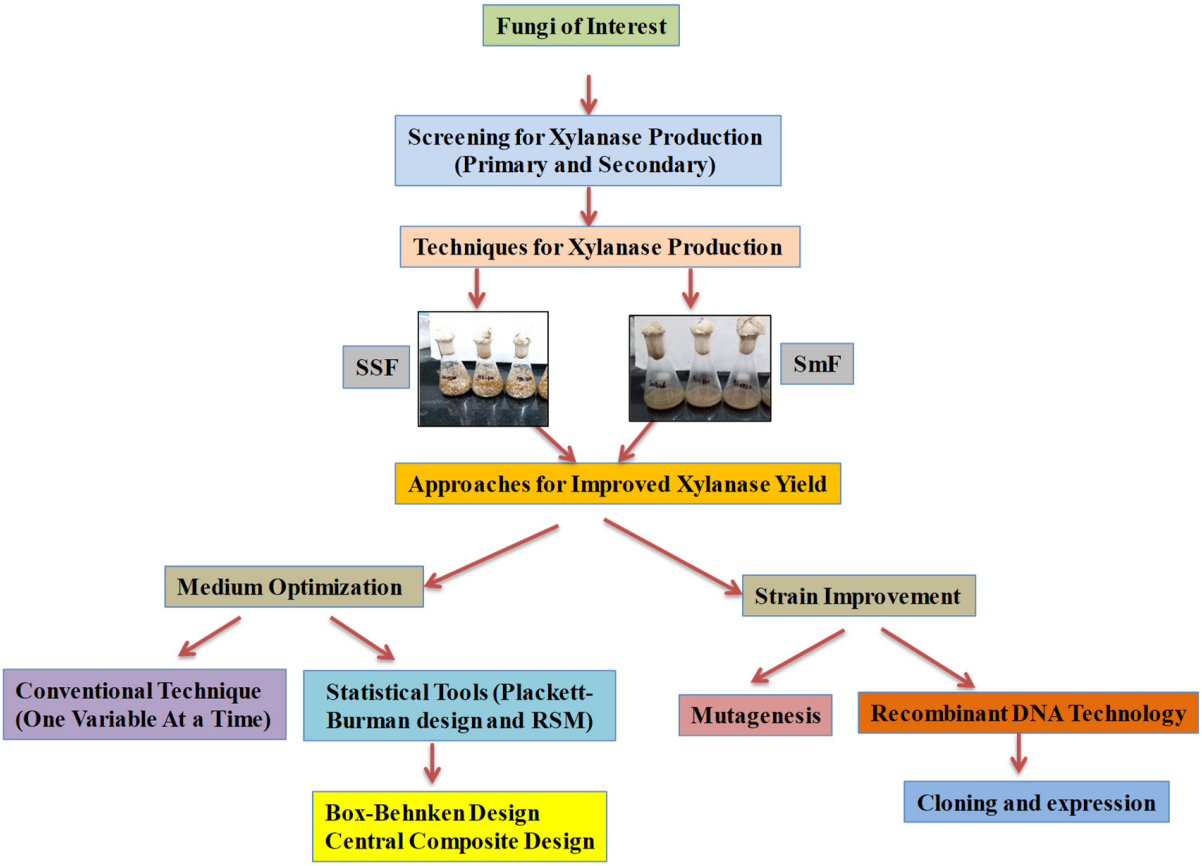

Figure 2. Schematic representation of methodology for fungal xylanase production.

Temperature: Cultivation temperature is among the key physical factors affecting the production of enzymes by microorganisms. At optimum temperatures, enzyme activity is high because of high metabolic activity, high protein content, and production of extracellular enzymes in culture filtrate, while at lower temperatures, membranes solidify, and increased temperature harms the microbes by denaturing their enzymes, transport carriers, and other proteins, thus reducing the production of enzymes [25].

pH: The extra-cellular pH has a powerful impact on the metabolic pathway as well as product formation by microorganisms. Variation in the surrounding pH reshapes the ionic pattern of nutrient molecules and decreases their accessibility to microbes, thereby reducing their overall metabolic activity [25].

Inoculum level: The enzyme activity is highest at optimal inoculum level, as in this condition equilibrium is set between the availability of substrates and inoculum size. However, the decrease in enzyme activity at higher inoculum levels might be due to the generation of viscous suspensions and insufficient blending of substrates in shaking conditions, and with successive increases in inoculum levels, competition for substrate is also increased between microbial cells, which causes fast depletion of nutrients that inhibit growth as well as enzyme production [25].

Agitation: In SmF, the production of enzymes is greatly affected by agitation. Low enzyme activity is recorded in non-agitated or static flasks, most likely because of oxygen or mass transfer limitations, whereas high enzyme activities are found in agitated flasks, probably because of a proper supply of oxygen.

Incubation time: The rate of enzyme production is significantly affected by incubation time. Due to the formation of unwanted and toxic products and the exhaustion of growth nutrients in the medium may cause decreased growth rate of microorganisms and enzyme titres may occur [1].

Moisture level: The substrate-to-moisture ratio, or moisture level, is an important factor affecting the production of enzymes in SSF. A moisture ratio above or below the required amount reduces the yield of enzymes [1].

Moistening medium: The type of moistening medium and its composition are considered a prime factor which influences the cultivation of microbes and, thus, enzyme yield. Supplementation of salts in the production medium improves enzyme production [25].

Carbon source: The addition of a freely accessible carbon source is helpful for increasing enzyme yield from filamentous fungi. External carbon sources enhance enzyme production, while many investigations have reported that lignocellulosic substrates are the ideal carbon source for xylanase production in SSF. In many studies, there was no need for an external carbon source for improved xylanase production as lignocellulosic substrates supported the maximum growth of microbes and, thus, xylanase production. Agricultural residues, including rice straw [25], corncob [22], and wheat bran [26], are used for cultivation of different fungi for xylanase production.

Nitrogen sources: Organic nitrogen sources are less preferred than inorganic ones for xylanase production. This may be due to the fact that mostly microorganisms have been isolated from soil samples, where the inorganic form of nitrogen is already present in the form of fertilisers. Therefore, microbes assimilate the inorganic form of nitrogen more efficiently than organic sources [25].

Surfactants: The primary function of a surfactant is to enhance the liberation of enzymes from solid substrates. Enzyme production is considerably increased by surfactants because they enhance water perforation into the surface of solid residues and thus increase the surface area for the cultivation of microorganisms [25].

Other salts: Beyond carbon and nitrogen sources, other salts are also needed for the growth of microorganisms in the production medium. Salts of calcium, phosphorous, magnesium, zinc, potassium, and others stimulate the growth and thus enhance the yield of enzymes [25].

4.2. Statistical Optimisation for Improving Production of Xylanases

Optimisation of medium employing the 'one variable at a time' (OVAT) strategy is traditionally applied in analytical chemistry, but that technique has major disadvantages. First, it does not count the mutual interaction between the factors studied. To overcome these problems, the use of statistical tools in biotechnological applications has gained immense emphasis for the optimisation of enzyme production [20]. Optimisation with statistical tools gives a superior formulation of cultivation medium in a minimum period of time with a fewer number of experiments; however, besides such benefits, it also analyses the interactions among the chosen variables [1]. Plackett–Burman design (PBD) and response surface methodology (RSM) design are both well-documented and universally applied tool designs and assessment methods to screen media elements for increasing enzyme production [20]. RSM is a group of developed design-of-experiments (DOE) methods that assist in better understanding and optimizing responses. Box–Behnken design (BBD) and central composite design (CCD) are the two prime types of RSM. These tools help in determining the experimental variables and their interactions that have a major effect on the production with the minimum possible runs and to resolve the variables that would affect the properties of the product. The critical factors affecting xylanase production in *T. orientalis* EU7-22 in SmF that were detected using PBD involved fermentation time,

incubation temperature, and $MgSO_4$ concentration [26]. Maltose, sodium nitrate, and KCl were reported as key factors for xylanase production by *A. fumigatus* var. *niveus* in SSF using a 2-level PBD [27]. A study repoted the significant influence of temperature, moisture level, and inoculum size on the production of xylanase using *A. niger* [28]. PBD analysis revealed wheat bran, pH, and fermentation time as the most effective process factors for xylanase yield by *A. terreus* S9 in SSF [18].

Optimisation of selected factors using the Box–Behnken design of RSM reported a 150% and 280% increase in xylanase production by *T. orientalis* EU7-22 [26] and *A. fumigatus* var. *niveus* [27] under optimised conditions, respectively. The use of Box–Behnken design of RSM resulted in a 1.39-fold higher xylanase production by *A. niger* than that of an unoptimised medium [28]. A 1.82-fold increment in xylanase yield by *A. terreus* was found in SSF after employing the RSM approach [18]. Similarly, a 4-fold increase in the yield of endoxylanase by *Sporotrichum thermophile* in SmF was obtained on employing CCD-RSM [29]. The production of endoxylanase from *M. thermophile* BJTLRMDU3 increased 2.53-fold after optimisation of critical factors using CCD-RSM [1].

4.3. Characteristics of Fungal Xylanases Useful for Bioconversion of Lignocellulosic Biomass

Purification and characterisation of crude xylanases can be carried out using various techniques. The concentration of enzyme can be enriched via salt/solvent fractional precipitation, such as ammonium sulphate precipitation and acetone precipitation followed by dialysis and lyophilisation [1]. A schematic representation of the purification of xylanases is shown in Supplementary Figure S2. Native-polyacrylamide gel electrophoresis and sodium dodecyl sulphate-polyacrylamide gel electrophoresis are used for the determination of MM and the activity of the enzymes. Ultrafiltration, ion exchange, and gel filtration chromatography are some other techniques used for the purification of enzymes [1]. Supplementary Table S3 presents the purification method and biochemical characterisation of fungal xylanases.

5. Molecular Strategies for Improving Efficiency of Fungal Xylanases for Lignocellulose Conversion

5.1. Expression of Fungal Xylanases in Different System for Improved Production

Microbial xylanases are usually applied in many industries, but the yield of microbial xylanases is low due to the degradation of the product, causing product inhibition and catabolic repression. RDT can be used more successfully to avoid these problems. Xylanase-coding genes have been cloned in homologous and heterologous hosts to achieve the target of higher production of enzymes and reconstruct their characteristics so that they become suitable for commercial application [30]. For sustainable and low environmental-impact industrial operations, it needs the utility of high-level production of enzymes at low cost. However, current enzymes are always restricted by their low catalytic capability and high production costs, and this gives rise to challenges in generating a feasible and economical hydrolysis process. Due to advancements in recombinant DNA technology, considerable research is being carried out for the large-scale production of enzymes in a heterologous host and the evolution of highly productive enzymes [27]. For the production of recombinant xylanases, *Pichia pastoris* and *E. coli* are well-explored expression systems that have been successfully exploited for the overexpression of recombinant proteins [27].

5.1.1. *Pichia pastoris*

A heterologous expression system using *P. pastoris* would be a potent method for improving the yield of xylanase. Improved expression of *T. reesei* xylanase (XynB) in *P. pastoris* via the codon optimisation method [30]. This optimised gene revealed 67% identity with the native gene, xylanase activity by *P. pastoris* pPICZaA-optiXynB was increased to 1299 U/mL after 4 days of induction, and its molecular weight was 24 kDa. The GH10 family XYL gene of *A. niger* BE-2, i.e., XynC, was optimised and primarily expressed in *Pichia pastoris*, and it was found that the double-plasmid technique significantly increased XynC production by

around 33% as compared to the traditional single-plasmid strategy. On further optimisation, the expression level of GH10 XynC was extended to 1650 U/mL in a 5 L bioreactor [31]. The heterologous expression of a new endoxylanase from *Pycnoporus sanguineus* BAFC 2126 was expressed in *P. pastoris*, which resulted in the robust activity of the enzyme over a wide pH and temperature range, with a 3 h half-life at 70 °C, and also that the enzyme showed stability at 60 °C for more than 48 h [32]. A novel xylanase (XynC) from *P. purpurogenum* heterologously expressed in *P. pastoris* and the purified xylanase exhibited wide range of temperature and pH optima, i.e., 45 °C and 3.0–5.0, respectively [33].

5.1.2. *Escherichia coli*

Aspergillus nidulans was modified by inserting the AFUMN-GH10 gene of *A. fumigatus* var. *niveus* for increased production of endoxylanase, and their modification gave high-yield secretion and recombinant protein accumulation [27]. Genetically modified *A. nidulans* A773, transformed by using the pUC19-based *E. coli* expression vector pEXPYR, was used for enhanced xylanase production using soybean fibre under SSF, and the maximum xylanase was 157.5 U/g after 4 days of incubation [34]. One more efficient approach for improving the yield of lignocellulolytic enzymes from fungi is the genetic engineering of transcription factors. Camel rumen was found to be a potent source for microbial xylanases revealed after a metagenomic study [35]. A new xylanase, PersiXyn1, with 1146 bp that encodes a 381 amino acid protein, was detected on the basis of metagenomic data using contigs for in silico identification. The genes of the enzyme were cloned and expressed in *E. coli* using a DNA template obtained from samples from a camel rumen metagenomic study. The recombinant PersiXyn1 showed activity at a broad range of temperatures, i.e., 25 to 90 °C, and a broad range of pH, i.e., 6.0 to 11.0; however, the pH and temperature optima of the enzyme were 40 °C and 8.0, respectively [35]. This enzyme showed effectiveness in fibre separation, which may decrease the cost of carton paper production. Cloning and overexpression of *Aspergillus oryzae* LC1 xylanase XynF1 was achieved in *E. coli* BL21 (DE3) for the production of recombinant xylanase, which gave an increased titre of specific activity, i.e., 1037.3 U/mg, and this was 9.3 times higher compared to the native enzyme [36].

5.1.3. Other Expression Systems

Taking into account the safety protocols in the food and feed industries, xylanase gene B from *A. niger* was cloned and expressed in *Komagataella phaffii* and recombinant xylanase showed improved activity, i.e., 1827.19 U/mL, in methanol-mixed medium, and the purified enzyme showed optimal activity at 50 °C and pH 5.5, with a molecular mass of 18 kDa [37]. *Kluyveromyces lactis* was used as an expression system for the xylanase gene xynZG from *Plectosphaerella cucumerina*, and the recombinant enzyme exhibited a maximum activity of 115 U/mL after 72 h of incubation and a MM of 19 kDa on SDS-PAGE [38]. The successful expression of the *P. citrinum* xylanase gene xynA was exhibited in *Yarrowia lipolytica*, a fungus that produces water-soluble polysaccharides called liposan. The MM of recombinant xylanase XynA was 20 kDa, while optimum activity was reported at a pH of 5.0 and 50 °C [39].

5.2. Genetic Engineering of Fungal Xylanases for Improved Catalysis

In the scientific world, biotechnology is an important field where a lot of development has happened in the last few years. Biotechnology is the union of biological, engineering, and physical sciences in order to attain technological advantages employing biological systems. However, rapid applications of genetic engineering have been reported in bioprocesses that lead to optimisation of the production of conventional or novel products, such as enzymes, of commercial importance. The prime concerns of engineered strategies involve the application of available recombinant DNA technology (RDT) tools for modification of desired enzymes that will produce high yield and robustness under industrial conditions [40]. Another important challenge for RDT is to upgrade the cultivatable properties

of genetically engineered/modified enzymes by inserting genes of interest. For industrial applications, the strength of engineered enzymes is much desired. There are many investigations that report that cloning of enzyme genes, site-specific mutagenesis, random mutagenesis, or the fusion of both techniques have been applied, usually to make stable genetically modified enzymes for commercial purposes [40]. Presently, large-scale xylanase production is possible because of the involvement of genetic engineering tools. It helps in the rapid identification of unique xylanase genes and the genetic alterations that make them ideal enzymes [40]. In various cases, xylanases should undergo some genetic modifications to fulfil the industrial prerequisites of activity, stability, tolerance to toxic substances and extreme conditions, substrate specificity, sterospecificity, and enantioselectivity.

A 200-fold increase in xylanase activity in gpdAp::xlnR strains was reported after 4 h of transfer into inducing conditions, and 75% xylanase activity was found in the recombinant strain within 4 h. Structure-based protein engineering is used to improve the stability of xylanase against heat and alkaline conditions [41]. Engineered cysteine pairs hve created exterior disulphide bonds in Xyn12.1 variants and also increased their melting points. Their catalytic efficiency increased 4.2-fold at 50 °C and pH 9.0 after 1 h and 3-fold at 60 °C. The stability and catalytic activity of *A. niger* ATCC1015 xylanase (AnXynB) were increased using a combination of experimental and computational techniques [42]. Molecular dynamics and virtual mutation simulations showed that the insertion of Glu and Asn changed the inter-working network at subsite three. The resultant double mutant, S41N/T43E, showed a 72% increment in catalytic activity compared to that of the wild type. In addition, selective site-directed mutagenesis at subsite 3 increased enzyme activity and thermostability [42]. Xylanase A of *Penicillium canescens* (PcXylA) was cloned and subsequently expressed in *P. verruculosum* B1-537 (niaD-) [43]. The nested PCR was used to perform site-directed mutagenesis and transformed the mutated xylA genes into *E.coli*. Among all mutants, the L18F mutant exhibited higher thermostability in contrast to the wild-type enzyme, whereas its t/2 was 2–2.5 times longer at 50–60 °C than the recombinant PcXylA-wt. The thermostability and catalytic efficiency of xylanase PjxA from *Penicillium janthinellum* MA21601 were increased via the introduction of two disulphide bonds at major regions of the recombinant xylanases [44]. The mutant genes were cloned using overlap extension PCR. Among all recombinant xylanases, DB-S1S3 showed an increased optimal temperature, i.e., 50 to 70 °C, and the k_{cat}/k_m value of DB-S1S3 was also increased by 2.14-fold. The protoplast fusion of *Trichoderma harzianum* and *Trichoderma virens* resulted in the formation of fusant X3, which has 4.7-fold enhanced enzyme activity compared to parental xylanases. Furthermore, it was also observed that expression of the gene was increased in recombinant X3 4.9-fold in contrast to its parents [45]. Protein engineering was carried out in the xylanase of *A. fumigatus* RT-1, belonging to the GH11 family, at the N-terminal region near the active site. An improvement was found in catalytic efficiency for hydrolysis of pretreated *Hibiscus cannabinus* or kenaf. The combined action of site-directed mutagenesis, site saturation, and error-prone PCR was used to construct five mutants. The most effective hydrolysis reaction was shown using the double mutant c168t/Q192H with a 13.9-fold increase in catalytic performance [46]. Enhanced activity of *Trichoderma reesei* ATCC66589 xylanase was reported via disparity mutagenesis arising from errors in replication by DNA polymerase, including a plasmid that kept inserting δ in proofreading-impaired DNA polymerase. Site-directed mutagenesis was used for the construction of plasmids with DNA polymerase δ with missing proofreading activity. The mutant XM1 showed a 15.8- and 11-fold increased production of xylanase and β-xylosidase in xylan medium, respectively [47].

6. Applications of Fungal Xylanases for Lignocellulose Bioconversion into Useful Products

Agricultural waste, or lignocellulosic biomass, has great potential to be transformed into numerous value-added products like organic acids, oligosaccharides, biofuels, and other chemicals [48]. Despite that, there is a lack of proper disposal of lignocellulosic biomass. Environmental pollution is a global problem that has severe detrimental effects on the climate and human health. In the national economy of a country, industries are the major players, despite the fact that they are also the major contributors to environmental pollution. Industries like bleaching of paper and kraft pulp and de-inking of paper use a variety of harsh chemicals in their operations in order to form better-quality products in a limited amount of time. After completion of their process, chemicals discharged into the environment are responsible for toxicity in living organisms. The highly toxic chemicals in waste waters are major causes of water and soil pollution. As a consequence, adequate treatment of these pollutants is required for environmental protection and public health safety. Microbial xylanases have been shown to play a multifarious role in environment management as well as in many industrial applications such as de-inking and bio-bleaching of various kinds of papers [48]. Generally, in these processes, harsh chemicals have been used that cause environmental pollution, but the use of xylanases in place of those harsh chemicals helps to reduce this pollution. Moreover, xylanases also help in the management of agro-industrial waste like crop residues, wheat and rice bran, corncob, sugarcane bagasse, etc., as a huge volume of agricultural residues are burned in fields every year, which releases massive amounts of smog into the environment, leading to air pollution. With the help of xylanases, this agro-industrial waste can be transformed into valuable products, such as biofuel, xylooligosaccharides, and prebiotics.

6.1. Bioconversion of Lignocellulosic Biomass to Biofuel

Xylanases alone and in combination with cellulases have been utilised for biofuel production from lignocellulosic biomass [49], and the steps involved in the bioconversion of biomass to biofuel are illustrated in Figure 3a. Primarily lignocellulosic biomasses are consumed for the formation of second-generation biofuels. Through the action of xylanases, biomass is delignified following depolymerisation and fermentation, which generates biofuels like xylitol and ethanol. First, lignocellulosic biomass is fractionated into cellulose and hemicelluloses through delignification, produced as free sugars by depolymerisation, and finally fermented to produce ethanol [49]. As reported in [50], the sugars (407 g/L) generated after saccharification of dilute acid pretreated sorghum stover with a mixture of xylanase and cellulase from *Rhizopus oryzae* SN5 resulted in 411 g/L of ethanol production using *Saccharomyces cerevisiae* NCIM 3288. A cellulase-xylanase concoction (19.33 IU/mL) from *Trichoderma reesei* Rut C30 yielded 508 g/L of reducing sugars after saccharification of enzymatically treated sugarcane tops at 50 °C and pH 5.0, and the hydrolysate gave 27.2 g/L of ethanol upon fermentation with *S. cerevisiae* [51]. An enzyme cocktail comprising xylanase (100 U/g), cellulase (100 U/g), and laccase (20 U/g) of *A. oryzae* ITCC-8571, *S. commune* NAIMCC-F-03379, and *Myrothecium verrucaria* ITCC-8447, respectively, produced 26.7 g/L reducing sugars upon saccharification of rice straw for 24 h at 50 °C and produced 7.34 g/L of ethanol [23]. Co-expression of xylanase and cellulase genes in *S. cerevisiae* increased production of bioethanol using corn stover. The maximum yield of ethanol from the two recombinant strains was 1.66 g/L and 1.90 g/L, respectively, after 120 h [52]. The hydrotropic pretreatment of wheat straw was carried out using sodium xylene sulphonate for efficient production of biobutanol [53]. A list of xylanases used, individually and in synergy with other enzymes, for the production of biofuels is given in Table 1.

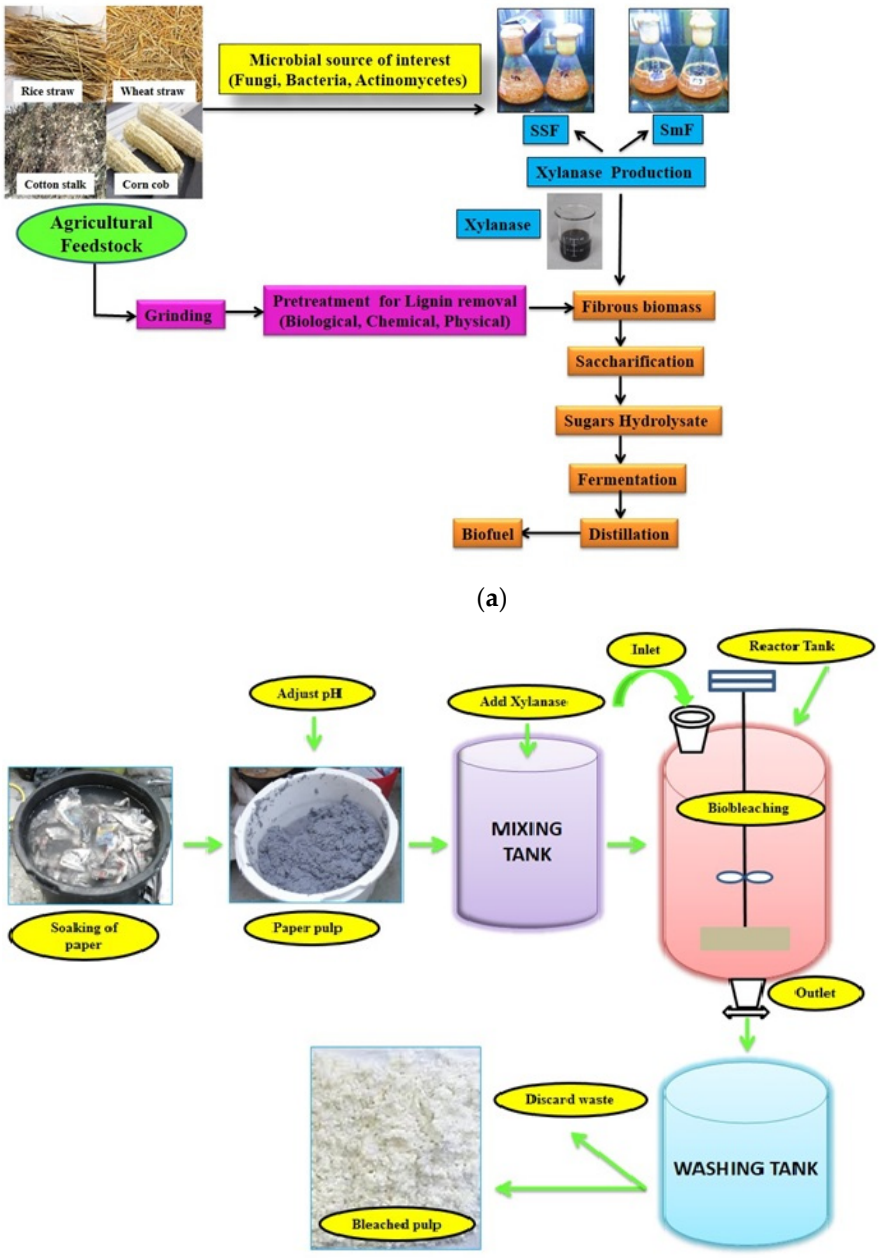

Figure 3. (**a**) Schematic representation of biofuel production from agricultural residues using fungal xylanases. (**b**) Schematic representation of different steps involved in bio-bleaching process.

6.2. Bioconversion of Ligocellulosic Biomass to Prebiotics

Xylan, the main constituent of hemicelluloses, is used for xylooligosaccharide (XO) production. XOs are non-digestible sugar oligomers composed of xylose monomers having a particular degree of polymerisation (DP), and the uses of XOs in food and non-food applications are described in a flowchart given as Supplementary Figure S3. The foremost application of XOs is their use as non-digestible food additives which exhibit prebiotic properties by supporting the multiplication of *Bifidobacterium* sp. and *B. adolescentis* in the gastrointestinal region [54]. Currently, XOs are already being utilised in foodstuffs in many countries. China and Japan are the commercial producers of XOs; moreover, in Japan, XOs are marked as Foods for Specified Health Use (FOSHUs). Besides this, XOs have various non-food benefits, such as feed additives for fish farming, growth accelerators

and stimulators, and ripening agents in the field of agriculture [55]. XOs also have different health benefits, such as reducing cholesterol and enhancing the biological availability of calcium to promote the proliferation of probiotics. They have some favourable properties, like being resistant to heat, stable at an acidic pH, and having adequate organoleptic properties without showing toxicity/negative consequences on individual health [37]. On the basis of previous literature on XOs, xylobiose (X2) and xylopentose (X5) have been recognised as good nutraceuticals having prebiotic properties. Among all these, X2 exhibited the maximum prebiotic ability for *Bifidobacteria* propagation in the human digestive tract [55]. Similarly, X5 also has significant prebiotic properties and exhibits anti-cancer activity. In this context, xylanases catalyse the endohydrolysis of xylosidic linkages in xylan and generate XO from diverse DPs. Undoubtedly, at present, xylanases having the unique hydrolytic properties that produce X2 and X5 XOs are strongly required for further applications [56]. According to reported investigations, most of the recorded xylanases hydrolyse xylan to yield XOs of low ($\leq$3) DP or a mixture of XOs with DP ranging from 2 to 7 [57]. The xylanases of *P. stipitis* with high activity can enhance the hydrolysis performance to yield XOs with required DP distributions [54]. The use of agricultural or lignocellulosic residues for xylanase production and then the use of these enzymes for XO production is one of the most cost-effective and easy methods for proper management of lignocellulosic residues. This whole process also helps in reducing the environmental pollution caused by the burning of agricultural residues in open fields. Worldwide, corncobs are among the most abundant renewable agri-waste, and most of them are burned off for energy, which has resulted in air pollution. Various techniques have evolved for the production of XOs, such as auto-hydrolysis, enzymatic hydrolysis, chemical methods, and fusion of different pretreatments with enzymatic hydrolysis [44]. Out of these methods, the last one is intended to be a most encouraging technique for XO production, as this approach is highly efficient and eco-friendly and has a low pollution rate. For XO production, an appropriate xylanase and pretreatment plan are the determining factors that influence the hydrolysis efficiency [57].

Purified xylanase of *Pichia stipitis,* produced using a mixture of corncob and wheat bran in SSF, effectively hydrolysed beechwood xylan and yielded XOs including xylotetroase (14%, DP 4), xylotriose (49%, DP 3), and xylobiose (29%, DP 2) at 50 °C [54]. *Aspergillus flavus* produced an endoxylanase in basal medium supplemented with corncob, and this purified endoxylanase hydrolysed beechwood xylan and resulted in XO (xylobiose and xylopentaose) production at 45 °C [16].

An endoxylanase from a pathogenic fungus, *Chrysoporthe cubensis*, was produced in SSF using wheat bran and degraded oat spelt xylan and yielded xylobiose and xylotriose as the main XOs at 50 °C [58]. Lower XOs are advantageous for utilisation in the prebiotics industry due to their beneficial effects on human health. The endoxylanase of *Talaromyces amestolkiae* produced XOs having prebiotic potential and resulted in production of organic acids with a reduction in pathogenic bacteria and an enhancement of bifidobacteria [59]. The xylanase of *Aspergillus brasiliensis* BLf1, has been produced using rice husk and is notable for the production of XOs, i.e., xylobiose, xylotetraose, and xylopentaose, along with xylose [60]. *Myceliophthora thermophila* BJTLRMDU3 was reported for the production of xylotiose, xylotetrose, and xylopantose by hydrolysing xylan at 60 °C [25]. Recombinant thermostable free xylanase from *T. thermophilus* hydrolysed pretreated corncobs and produced 26.4% XOs, and immobilised xylanase produced xylobiose and xylotriose, of lower DP, by hydrolysing Meranti wood sawdust xylan [61]. Xylanase of *A. fumigatus* hydrolysed arabinoxylan and yielded 3.3% (*m*/*v*) XOs, including xylobiose and xylotriose, chiefly with potential in food and feed applications [62]. *Aureobasidium pullulans* CCT xylanase hydrolysed xylan and resulted in improved total XO content (7.7 mg/mL and 7.9 mg/mL) and XO yield (25.7% and 26.5%) [63].

6.3. Bio-Bleaching of Paper and Kraft Pulp

The removal of lignin from pulps for producing paper with more brightness or for obtaining nearly white paper from that pulp is known as the bleaching process (Figure 3b). There are biological and chemical processes by which bleaching of pulp can be achieved. The application of chemicals such as alkaline for the elimination of lignin from pulp during the cooking stage of pulp at 179 °C is known as chemical bleaching. In this process, chlorine-based agents like hypochlorite, Cl_2, and ClO_2 are required, and these chemicals cause environmental pollution [64]. To minimise the environmental pollution burden, the application of bio-bleaching is an effective way. The use of microbial enzymes for bleaching of paper or kraft pulp at an industrial scale is referred to as bio-bleaching (BBL). In past years, the application of xylanases for bleaching of paper has gained attention from researchers and scientists worldwide [64]. Applications of various microbial xylanases for improving the properties of different kinds of pulp are given in Table 2. Xylanases improve water retention time and pulp fibrillation, increase freeness in recycled fibres, reduce beating time, restore bonding, etc. BBL minimises the use of chlorine for bleaching purposes because xylanases are used for the hydrolysis of xylan in paper and pulp and increase the removal of lignin from them via this process [65]. Commercially, xylanases are used for bleaching of kraft pulp, and they are gaining worldwide importance as eco-friendly alternatives to chlorine-containing chemicals, which are toxic to the environment. In 1988, at the global stage, Finnish forest companies were the first to initiate mill-scale experimentation. For enhancing the delignification of kraft pulp, endoxylanase was the chief enzyme, but the addition of hemicellulytic enzymes along with xylanase further improved the bio-bleaching process. Application of xylanase in the paper industry is generally called pre-bleaching because it promotes chemical bleaching instead of eliminating lignin directly. Xylanase does not strike lignin-based chromophores directly. That is why applications of xylanases for pre-bleaching of paper pulp were found to be a more suitable move for boosting bleaching of pulp.

The pretreatment of printed paper pulp with xylanase (60 U/g) of *A. niger* showed a decrease in kappa number with an increment in the brightness of the pulp. The results obtained from SEM and FTIR analysis revealed modifications in functional groups and surface morphology [66]. The potential of *Penicillum meleagrinum var. viridiflavum* xylanase alone and in combination with H_2O_2 was analysed for the bleaching of bamboo pulp [65]. A significant reduction in lignin concentration was observed from the action of xylanase alone, whereas addition of 2% H_2O_2 along with xylanase further reduced the lignin concentration (10.55 to 3.25%). The kappa number was decreased from 13.50 to 8.50 with improvements in brightness, viscosity, and crystallinity of pulp cellulose. Xylanase pretreatment also enhanced the physical properties of pulp, and changes in the surface properties and morphology of pulp fibres were analysed via SEM. BBL of crude kraft pulp using *Trichoderma reesei* QM9414 xylanase (30 U/g) showed successive reductions (10%) in final chlorine dioxide consumption and a decrease in kappa number to 12.5%, along with an increase in brightness [67].

6.4. Nutritional Enhancement in Plant-Based Diets

It is clear from recent studies that utilisation of xylanase in the food industry, like bread making, juice clarification, and other applications, has a very significant role [17,68,69]. Applications of xylanases, alone and in synergy with other enzymes to enhance the nutritional quality of food and feed, are given in Table 3. Xylanases in synergy with different enzymes, namely alpha- and malting amylase, glucose oxidase, and protease, have been applied in food industries. In many studies, it has been reported that the application of xylanases improved the qualities of bread by enhancing the volume of bread, and xylanases along with amylases also produced bread with improved quality. The hemicelluloses in wheat flour were broken down via the application of xylanase, which resulted in the re-distribution of water and increased the dough's softness [68]. During the bread making process, xylanases delay the crumb shaping and aid in the development of the dough. These enzymes are also

widely used in the wine and juice industries. For vegetable and fruit juice preparation, there are three main steps: extraction of juice, clearing, and stabilisation. In the early 19th century, when production of juices from citrus fruits started, the yield was low because of turbidity and improper filtration problems. However, the utilisation of microbial enzymes provides a solution to this problem.

Recently, xylanases, along with cellulases, pectinases, and amylases, have enhanced the properties and amount of vegetable and fruit juices and improved the recovery of key mineral salts, vitamins, aroma, etc. Along with endoglucanase, xylanase breaks down arabinoxylan and starch separations and isolates gluten from starch in wheat flour. Xylanases have been globally applied in the food industry because of their higher stability and maximum activity at an acidic pH. The application of *Sporotrichum thermophile* xylanase (0.35 U/g flour) in bread formulation is noted for increasing concentrations of soluble protein and amino acids by 11.23 and 10.43 mg/mL, respectively. Concentrations of Na^+, K^+, Ca^{2+}, and moisture (32.33%) have also been found to be increased in xylanase-treated bread [17]. Thermoresistant xylanase of *Trichoderma stromaticum* is used in bread making and for xylooligosaccharide production. Two xylanases, Xyl1 and Xyl2, effectively degrade beechwood xylan, while the addition of Xyl1 significantly increases the softness of whole grain and wheat bread and also improves the specific volume [70]. The first time the application of an extremophilic *Aureobasidum pullulans* NRRLY-2311-1 xylanase for bread preparation was analysed, it resulted in improvements in development time, water absorption, and dough stability, along with reductions in mixing tolerance and dough softening degree, by adding 100 U/100 g flour [71]. An improvement in dough performance was observed with the addition of *Kluyveromyces marxianus* xylanase in combination with endoglucanase, exoglucanase, β-glucosidase and ferulate esterases. This enzyme cocktail also affected the solubility and molecular weight distribution of dietary fibres and removed phenolic acid [72]. Wheat flour mixed with 15% wheat bran supplemented with commercial xylanase (120 ppm), α-amylase (6 ppm), and cellulase (60 ppm) resulted in increased extensibility of wheat flour dough (42%), along with an improvement in the rheological properties of the dough. This enzyme combination also increased the softness, stickiness, and mixing tolerance index of bread, while resistance to extension was found to be decreased [73].

Xylanases are applied in amalgamation with cellulase, pectinase, and amylase to upgrade juice extraction yield by liquefying the fruit, reducing the viscosity of juice, increasing the recovery of aromas, and stabilizing the fruit pulp. An endoxylanase from *Aspergillus japonicas* UFMS 48.136 was noted for having effectiveness in the clarification of various fruit juices, including tangerine (8.54%), mango (51.11%), and banana (9.99%), when added at a 1:1 (pulp and enzyme) ratio and incubated for 4 h at 55 °C [74]. Purified xylanase from *A. niger* was reported to increase the yield, reduce sugars, and improve the clarity of strawberry pulp with 10 U/g pulp and blueberry and raspberry pulp with 15 U/g pulp [28]. An increase (74.22%) in the clarity of orange juice was found compared to that of a control when treated with *A. flavus* L1 xylanase in a 9.5:0.5 mL juice to enzyme ratio after 24 h [75]. An increase, by 51.7 and 43.4%, in clarification of mango juice of two varieties, Baneshan and Totapuri was reported, respectively, after 56 h of incubation at 40 °C, on processing with 12.8 U of *Trichoderma koeningi* endoxylanase. The release of reducing sugars from Baneshan and Totapuri juices was 121.1 and 102.6 mg/mL, respectively [76]. A 1.17-, 1.27-, and 1.48-fold increase in clarification of orange juice was found as compared to control when treated with cloned xylanases, Xyn11A, Xyn11B, and Xyn11C, of *Fusarium* sp. strain 21, respectively [69]. A multi-enzyme (endoxylanase, β-xylosidase, β-mammanase, β-glucosidase, and α-galactosidase) system of *Aspergillus quadrilineatus* RSNK-1 increased the clarity of apple, orange, kiwi, and peach fruit juices by 23.3, 15.6, 11.05, and 10.40% at 1 g/20 mL concentration, respectively, as compared to control juices [14].

Xylanases are utilised for the pretreatment of forage crops to facilitate composition and enhance ruminant feed digestibility, and together with cellulases, phytases, amylases, glucanases, pectinases, galactosidases, lipases, and proteases, they are applied in animal

feed [77]. Arabinoxylans are converted into feed ingredients, and the viscosity of raw materials is decreased via the activity of these enzymes. The digestibility of feed having sorghum and maize (low-viscosity foods) in the initial part of the digestive tract when supplemented with xylanase may enhance the nutrients' digestibility at the initial stage of the digestive tract, resulting in improved utilisation of energy. Young swine and fowl, as compared to adults, produce smaller quantities of endogenous enzymes. Therefore, food additives having enzymes of exogenous origin should enhance their performance as livestock, and additionally, such a diet is reported to decrease the amount of undesirable residues (phosphorus, zinc, nitrogen, and copper) in animal excreta, and this effect could play an important part in decreasing environmental pollution. The efficacy of *A. niger* xylanase on guinea grass hay and rice husk have been examined. The results indicated a higher fibre digestibility in guinea grass and rice husk pretreated with xylanase (0.02 mL/g) than in the control. The degradability of the dry matter from rice husk and guinea grass was reduced to 82.1% from 87.1% and 62% from 88.5%, respectively. The degradation in neutral detergent fibre for rice husk (62.5% to 39.8%) was increased. The dry matter degradation of rice husk (from 87.09% to 81.89%) and guinea grass (88.45% to 62.12%) was also increased [78]. Extracts of xylanases from *Trichoderma piluliferum* and *Trichoderma viride* at a concentration of 6 U/l have been reported to increase the in vitro digestibility of sugarcane silage (12%), corn silage (9%), and Tifton 185 hay (7%) used for bovine diets following an incubation of 48 h at 39 °C [79]. The pretreatment of poultry feed with endoxylanase of *M. thermophile* resulted in 79.08 and 42.95% higher liberation of reducing sugars and soluble proteins, respectively, at 60°C and 48 h of incubation compared to untreated feed [1].

Multienzyme complexes from *Trichoderma harzianum* having xylanase, amylase, and cellulase showed synergistic effects on chick diets. A significant effect was found with increased intake, balance, and retention of nitrogen of 39.42 g, 28.30 g, and 45.95 g, respectively, in the pre-starter phase. An overall improvement in N_2 metabolism was achieved in the pre-starter phase [80]. The effect of xylanase (2 g/Kg dry matter) from *Penicillium chrosogenumin* in combination with commercial phytase (1 g/Kg dry matter) has been studied on goats' performance in early lactation. The results showed increased in vitro decomposition of dry matter (60.80%) and organic matter (66.91%) and the amount of ruminal ammonia and nitrogen (1.99%) and total volatile fatty acids (8.43%) without any effect on total gas production volume. Blood serum content and production of milk and its constituents also increased in goats fed diets supplemented with these enzymes [81].

Table 1. List of various xylanolytic enzymes used alone and with other enzymes for biofuel production.

Enzyme	Microorganism	Substrate	Biofuel	Temp. (°C)	Production Rate (g/L)	References
Xylanase	*Irpex lacteus*	Corn stover	Ethanol	50	13.5	[82]
Xylanase	*Irpex lacteus*	Wheat straw	Ethanol	50	12.5	[83]
Xylanase	*Irpex lacteus*	Barley straw	Ethanol	50	10.8	[83]
Xylanase	*Irpex lacteus*	Corncob	Ethanol	50	11.5	[83]
Xylanase	*Candida tropicalis* HNMA-1	Sugarcane bagasse	Ethanol	30	2.93	[84]
Xylanase Cellulse	*Penicillium oxalicum* RGXyl	NaOH-pretreated corn stover	Ethanol	50	14.9	[85]
Xylanase Cellulse	*Penicillium oxalicum* RGXyl	Ammonium sulphate-pretreated corn stover	Ethanol	50	16.95	[85]

Table 1. *Cont.*

Enzyme	Microorganism	Substrate	Biofuel	Temp. (°C)	Production Rate (g/L)	References
Xylanase	*Trichoderma koningiopsis* TM3	Oil palm trunk residues	Ethanol	50	4.15	[15]
Xylanase Cellulase	*Aspergillus tubingensis* NKBP-55	Sugarcane bagasse	Ethanol	45	41.5	[86]
Xylanase	*Aspergillus fumigatus* XC6	Rice straw	Ethanol	40	11.2	[87]
Xylanase	*Malbranchea flava*	Cotton stalk	Ethanol	50	46	[88]

Table 2. Application of fungal xylanases in improving properties of various kind of pulps.

Microorganism	Pulp Type	Dose of Xylanase (U/g)	Increase in Brightness (%)	Decrease in Kappa No. (%)	References
Penicillium crustosum FP 11	Eucalyptus kraft pulp	25	-	9.77	[89]
Trichoderma longibrachiatum MDU-6	Paper pulp	7.5	52	-	[90]
Aspergillus niger DX-23	Paper pulp	50	34.5	-	[91]
Aspergillus oryzae MDU-4	Newspaper pulp	500	57.9	66.7	[92]
Trichoderma asperellum	Paper pulp	20–40	43.2	4.0	[93]
Trichoderma viride	Newspaper pulp	30%	11	-	[94]
Penicillium sp.	Home-made paper pulp	-	30.6	-	[95]
Thermomyces lanuginosus	Mixed hardwood pulp	20	70.4	4.7	[96]
A. terreus S9	Paper pulp	-	-	11.8	[18]

Table 3. Application of fungal xylanase, alone and in synergism with other enzymes, in improving nutritional properties of food and feed.

Enzyme	Source	Flour Type	Enzyme Dose (U/Kg)	Improved Quality Parameter	References
Xylanase	*Penicillium citrinum* MTCC9620	Whole-wheat flour	59.9	Increase in specific volume (3.99 ± 0.035 mL/g), reduction in staling rate, along with softer crumb and brighter bread colour	[97]
Xylanase	*Penicillum citrinum*	Wheat flour	59.9	Reduction in water absorption, softer dough, greater extensibility, and less resistance to extension	[98]
Xylanase (XYL) Amylase (AML) Lipase (LIP)	Fungal alpha-amylase Fungamyl® 2500 SG (Fungamyl), Maltogenic alpha-amylase Novamyl® 10,000 BG (Novamyl), Xylanase Panzea® BG (Panzea), Lipase Lipopan® Xtra BG (Lipopan) and	Wheat flour and fermented cassava flour	LIP-25 * AML-10 *-75 * XYL-40 *	Increased loaf volume, softer texture and larger pore size correlated to higher loaf volume (1151 mL) and specific loaf volume (2.66 mL/g)	[99]

Table 3. *Cont.*

Enzyme	Source	Flour Type	Enzyme Dose (U/Kg)	Improved Quality Parameter	References
α-amylase (AML) Xylanase (XYL) Cellulase (CEL) Glucose oxidase (GOX)	α-amylase- (EC 3.2.1.1, Fungamyl FI 2500 BG), Xylanase-(Pentopan Mono BG), Cellulase- (EC 3.2.1.4, Celluclast BG), Glucose oxidase (Gluzyme Mono 10,000 BG), by Novozymes Investment Co. Ltd., Beijiing, China	Wheat flour	AML-30 XYL-150 CEL-42 GOX-100	Maximum height of frozen dough increased by 33.2, 19.7, and 7.4%, respectively, with xylanase, cellulase, and lipase; increased stability of the gluten network; dough with smoother surface, improved softness, and elasticity of the bread crumb; and increased dough volume	[100]
Xylanase, Endoglucanase, Exoglucanase, β-glucosidase	*Trametes trogii*	Corn stover	-	Improved corn stover fibre degradability and high release of reducing sugars	[13]
Xylanase Phytase	-	Pig feed	2500–5000 10,000	Increased crude protein digestibility, pig performance, enhancement in detectable total tract digestibility of calcium and phosphorus, increased digestibility of neutral detergent fibre, increased average daily gain, improved feed–gain ratio	[101]
Xylanase	-	Broiler feed	300	Significant increase in average daily gain and ileal digestibility of apparent metabolizable energy corrected to zero nitrogen retention, crude protein	[102]
Xylanase Mannanase	Hostazym X 100, Huvepharma USA, Peachtree City, GA, USA and CTCZYME, CTC Bio Inc., Seoul, Republic of Korea	Pigs feed	100 and 500	Increased expression of tight junction proteins (claudin and zona occludens), Decreased concentration of manolaldehyde indicating antioxidative capacity of pigs, increased digestibility of total non-starch polysaccharides and arabinoxylan	[103]
Xylanase	Kerry Ingredients and Flavours, Osberstown, Naas, Co., Kildare, Ireland	Broilers feed	-	35% higher ($p < 0.05$) N-corrected apparent metabolisable energy, higher ($p < 0.001$) coefficients of dry matter and nitrogen retention	[104]
Xylanase Phytase	*Trichoderma reesei* *Escherichia coli*	Turkeys's feed	16,000 500	Increased prececal phosphorus digestibility, lower proportions of InsP5 and higher proportions of InsP4, improved digestibility of feed	[11]

*—ppm/Kg of flour.

7. Conclusions and Future Development in the Field of Commercial Xylanase Production

Lignocellulosic residues are copious and the fastest-growing renewable organic carbon sources that offer opportunities for producing a considerable amount of biomass-derived products. Lignocellulosic residues are enclosed biochemical treasures and can be employed as raw materials in the progress of the circular economy. In this direction, utilisation of such lignocellulosic biomass for the production of xylanases and their subsequent conversion into beneficial products could be a solution for their proper management. Xylanases are the choice of many industries because of their multifarious applications, including food, biofuel, paper and pulp, and other industries. Lignocellulosic biomass is composed of cellulose and lignin, along with xylan, which have also been explored for different value-added products. Therefore, the synergistic use of all lignocellulolytic enzymes (cellulases, xylanases, and laccases) will be an eco-friendly and cost-effective biorefinery approach for the industrial generation of useful products. This review focused on the conversion of agricultural residues into different value-added products using microbial enzymes, which makes the bioprocess more economically sustainable. As a whole, advanced technologies involving RDT, protein engineering, synthetic biology, and bioinformatics are worthwhile for the efficient utilisation of lignocellulosic biomass for sustainable management of agricultural waste.

The hunt by researchers for superior xylanases is still ongoing; accordingly, the aim of searching for new microorganisms that can produce highly efficient and potent xylanases continues globally. The microorganisms that are isolated from superior environments have more potential for the degradation of lignocellulosic biomass and their transition into beneficial products for industrial applications, since such microorganisms already have the adeptness to bear stressed conditions such as elevated temperatures and alterations in pH. Progression in biotechnological techniques and tools, e.g., RDT, offers chances to choose a xylanase-coding gene and competently transfer it to a suitable expression system. Further, the expression system can be regulated for increased production of xylanase in conjugation with preferred properties for particular applications. The accessibility of huge data sets from genomics, metabolomics, and proteomics can be utilised through various bioinformatics tools that expand a variety of strategies for high yields of xylanase. On that account, RDT, synthetic biology, and conventional techniques collectively contribute to achieving the goal of potent xylanase production with the required industrial characteristics (Figure 4). Conventional approaches based on mutagenesis are used in enhancing yields of xylanases. These methods are time-consuming and non-specific in nature. Therefore, modern approaches including RDT and synthetic biology are highly useful for enhanced levels of xylanases with improved properties.

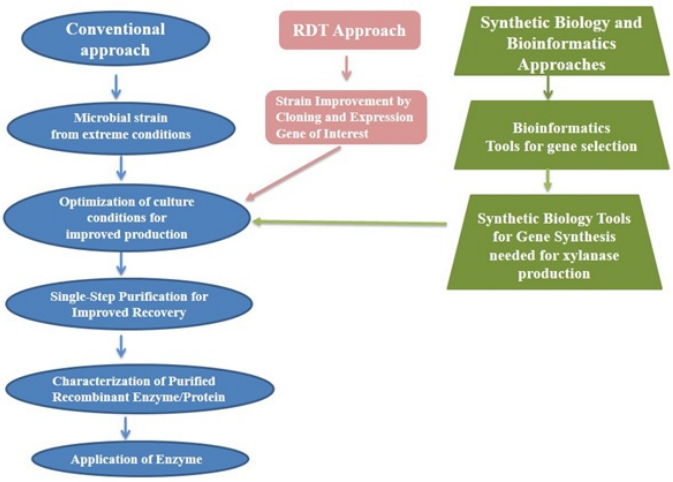

Figure 4. Future of xylanase production: Promising prospects through conventional and advanced approaches.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation10020082/s1, Figure S1: Mechanism of xylanase action-(a) Retention and (b) Inversion; Figure S2: Schematic representation for purification of xylanases; Figure S3: Flow chart showing uses of xylooligosaccarides in food and non-food applications; Table S1: Commercial xylanases with their market potential and applications; Table S2: Optimized culture conditions for fungal xylanases produced in submerged (SMF) and solid-state fermentation (SSF); Table S3: Purification scheme and biochemical characterization of fungal xylanases.

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