

## Valorization of agro-industrial wastes using fungi for industrial enzymes production

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### Abstract

Huge quantities of the agricultural wastes are produced from practices related to industrial processing, which are mainly burned and thrown in landfills, becoming a threat to the human health and causing environmental pollution. Agro-industrial residues, such as sugarcane bagasse, orange peel, wheat bran, corn straw, barley, rice straw, corn cob, husk, soy bran, and coffee husk were considered as valuable raw materials that can be converted into products of biotechnological interest using microorganisms. Agro-industrial wastes rich in nutrients, such as proteins, minerals, and sugars that, used as possible low cost substrates, for the fungal cultivation, thus considered as an alternative eco-friendly tool for many biological products of economic value, such as enzymes. Fungi organisms are capable of synthesizing a wide range of relevant oxidative and hydrolytic extracellular enzymes, such as peroxidases, laccases, xylanases, and cellulases. The enzymes secreted fungi, are proteins that function as bio-catalysts, responsible for carrying out various biochemical reactions, which applied in food, detergent, cellulose, paper, cosmetics and textile industries, etc. Thus, the replacement of raw materials with lignocellulosic-sources can result in reducing environmental problems and resolving the pollution associated with their disposal, in addition a higher investment will be gained. Therefore, a review was carried out on the important value of agro-industrial wastes, and the cultivation of fungal isolates for economic production of the industrially important enzymes, based on submerged and solid-state fermentation processes, as well as the important application of these enzymes.

**Keywords:** Agro-industrial wastes; Fungi; Enzymes; Fermentation; Application

### 1 Introduction

Increasing industrialization of the agricultural sector, directly increase the waste generation which represents a significant environmental challenge. Agro-based food industries generated large amounts of wastes with high nutrient value and can form breeding medium for disease-causing microbes, if it inadequately treated and left unprocessed. Interestingly, the most of agro-industrial wastes are lignocellulosic in nature, containing high amounts of polysaccharides such as cellulose, hemicellulose and, lignin (an aromatic polymer) [1,2]. In addition, these wastes containing other nutrients such as lipids, proteins, polyphenol and pectin, therefore, these wastes can serve as raw materials for the production of value-added products or as a source of renewable energy. This can achieve by applying the fermentation strategies such as hydrolysis of wastes, followed by fermentation and simultaneous saccharification [3]. In this context, a new directive by the European Union demonstrated the concepts of the 'Biorefinery' and 'Bioeconomy', where the wastes of an industry can be used as the raw material for another industry [4].

Utilization of agro-industrial wastes as substrates, for fungal cultivation intended to produce cellular biologically important primary and secondary metabolites, like; enzymes, proteins, organic acids, prebiotic oligosaccharides, and using it as sources of fermentable sugars in the bio-ethanol production has been reported [5]. Such residues are superior substrates for the cultivation of filamentous fungi, and inducing the fungal cells to produce cellulolytic, hemicellulolytic and ligninolytic enzymes such as; laccase, amylase, glucose oxidase, glucosidase, protease, pectinase, invertase,

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ligninase, xylanase, lipase, cellulose, and chitinase to meet out their nutrient requirement by solid state fermentation (SSF) and submerged fermentation (SMF) techniques [6,7].

The filamentous *Aspergillus* and *Trichoderma* species, and white-rot fungi are the most appropriate for production of industrial enzymes by fermentation of agro-wastes [8, 9].

Fungal enzymes are large group of complex proteins, which potentially used in different fields, of pulp and paper industry, food industry, pharmaceutical industry, textile industry, bio-fuel production, agriculture field, and bio-remediation. These enzymatic processes have more advantages, over the conventional chemical methods, because enzymatic processes cause a gentle reaction, have advanced specificity and selectivity, usually produce lower amount of waste materials by products during the reaction and its superior characters [10]. Also applying fungal enzymes in the industrial field, contributes to the sustainable growth concept where it gives more efficient and clean industrial processes [11-13]. As a result, the production of cost effective enzymes using agricultural waste fermentation optimized, and the need of variable enzyme production from fungal sources has been increased nowadays [14-15].

In the current review, we will describe the potential application of agro-industrial wastes as substrates for the growth of fungi organism, which able to synthesis multitude of hydrolytic and oxidative enzymes, to breakdowns the complex organic waste materials into simple products, and fermentation characteristics. It will be emphasized the different sources of the industrially important fungal enzymes and highlighted with their production conditions, properties, and its various industrial applications.

## 2 Wastes of agricultural and food-industry

Agro-industry usually produced large amount of residues with different properties and characteristics. The researches indicated that, accumulation of agricultural waste is in the excess of 2-billion-tons worldwide [16]. These agro-based industries can be classified into field-residues, process-residues and nonfood-wastes. The field-residues are usually defined as the waste materials that remain in the field after harvesting the crops such as (leaves, stalks, seed pods, and stems). Where the processed-residues, on the other hand, are the wastes generated after processing the raw products, and it can be grouped into food-based agro-industries such as, the by-products of sugar industries, fermentation-based-industries, food and fruit processing and grain-mills. The nonfood-based agro-industries usually produce waste effluents which are highly bio-degradable in nature like paper and pulp industry. But the effluents released from textile industries are mainly toxic, and threat to animal and human health as well as environment **Table (1)**

**Table 1** Valuable wastes of agro-based industries as potent by-products

Category	Industry	Valuable wastes	References
<b>Food-based agro-industries</b>	Sugar industry	Molasses, Bagasse	[17]
	Edible oils	Crude olive pomace, Sunflower stalks, Oil cakes, Groundnut stalks/shells	[18-21]
	starch products, Grain and four mills	Barley straw, Rice straw, Rice bran, Corn stalks, Wheat straw, Oat straw , and cobs Soya stalks Soy meal	[22-29]
	Fruits and vegetables processing	Potato peel waste, Orange peel, artichoke leaf wastes, Banana peel, Cashew shells, Spent cofee waste	[17] [30-32]
<b>Non-food based agro-industries</b>	Paper and pulp industry	Solid cellulosic wastes, Effluent sludge	[33]
	Textile industry	Solid wastes, Effluent	[34]
	Jute/ coir retting units	Lignocellulose residues	[35]

### 3 Health and environmental problems of agro-industry wastes

Huge amounts of agro-industrial wastes are considered one of the major concern in all countries, and results in different problems, that become worse every year [18]

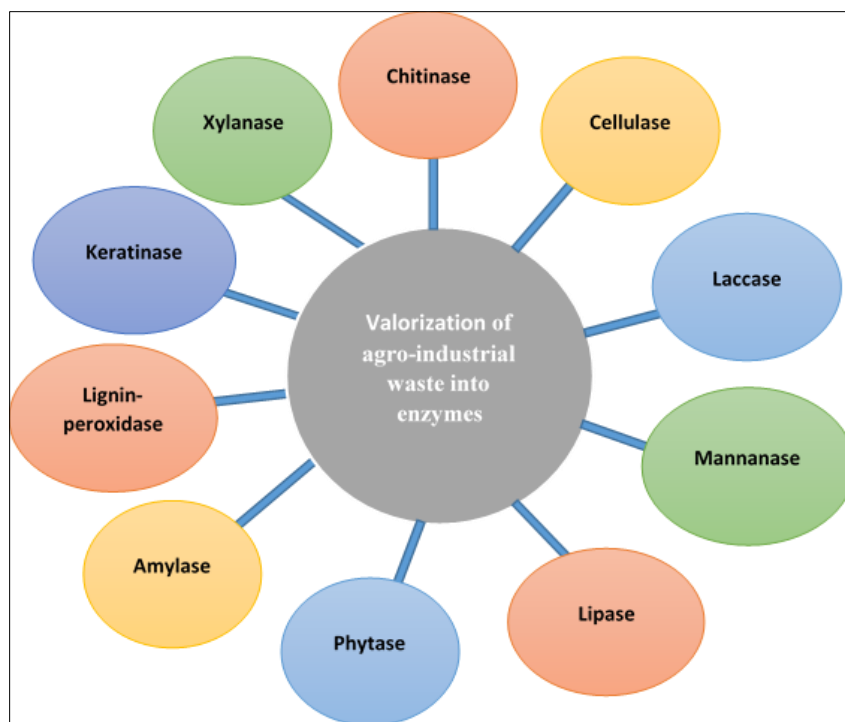
These agro-wastes and processing-effluents (contains variable chemical characteristics and metal contents) are usually discharged into water bodies or on land which directly harmful to environment, such as wastes from mills of pulp and paper, mills of textile, mainly contain hazardous that can pollute water, air, and soil [36]. In addition, the burning of agro-industrial waste under uncontrolled conditions, releases high amounts of toxic gases like; SO<sub>2</sub>, nitrogen oxides, dioxins, polycyclic aromatic hydrocarbons, and smoke, which directly contribute to excess haze and global warming, that harms the human health [37].

Agro-wastes are normally having high nutrient value, but they can become source of pathogenic diseases, if left without treatment [31]. Also disposal of such wastes on land without any treatment, increasing the concentrations of herbicide and pesticide residue in soil, which directly harm the beneficial soil microflora [38].

Throwing nutrient rich wastes in water surfaces, can enhance a massive algal-blooms and a disruption hazardous in the aquatic ecosystem [39], also the water soluble wastes sometimes end up in drinking water, causing severe problems in human health, as cancers, Alzheimer's disease, and birth defect problems [40]. Repurpose of agro-industrial wastes as raw materials for making valuable products is an excellent solution to avoid all previously mentioned health risks, and important for the environmental challenges [41].

### 4 Effective valorization of agro-industry wastes

Agro-industrial waste can be converted to valuable products majorly by biochemical, thermochemical and microbial biotechnology pathways. This section includes significant highlights for the role of microorganisms in using agro-wastes as raw materials for potential industrial enzymes production.



**Figure 1** Enzymes produced by degradation of agro-industrial waste

In order to use the plant-biomass as raw material for production of valuable products using microbial fermentation, the ligno-cellulose structure (the main component of plant-biomass which is mainly contains hemicellulose, cellulose, and lignin) must be pretreated for easier degradation and production of fermentable sugars, including arabinose, glucose, galacturonic acid, xylose, etc. [42]. Chemical methods can be applied for hydrolysis process using acids (sulphuric acid,

hydrochloric acid, etc.), but they show several drawbacks, including, the corrosion of the reactors, the production of inhibitory compounds for the subsequent fermentation step and the high temperatures needed. On the other hand, using enzymes like (cellulases, hemicellulases, pectinases, etc.), showing several save advantages but the cost of the enzymes is a limiting factor because it need high costs for the enzymes preparation [42].

The enzymes production cost is mainly affected by the used substrate of fermentation process, where the cost could be greatly decreased by using low-cost substrates such as the agro-food residues [43].

Many successful efforts have been made applying biotechnology as a tool to get valuable products, such as enzymes, from biodegradation of lingo-cellulosic materials for industrial uses at a low cost [44]. Enzymes including; phytase, amylase, chitinase, etc. are biocatalysts can be used in different industrial processes and produced from agro-industrial waste by microbial fermentation, hemicellulose-degrading enzymes such as xylanases, arabinanases, mannanases, and galactanases, also can be obtained from microbial biodegradation of the plant cell wall hemicelloses. In addition, lignin-degrading enzymes including lignin peroxidase, laccase, and manganese peroxidase, induced in microorganisms to decay lignin-based woody substrates into phenolic compounds, and enzyme keratinase produced by using keratinous wastes from sectors of meat production [44]. Fig. (1)

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## 5 Hydrolytic activity of fungal cultures

Fungi are heterotrophic-microorganisms which could obtain nutrients only through breakdown of various external organic matters [46]. Fungi able to breakdowns different complex organic materials into simple forms depending on the extracellular production of multitude of hydrolytic and oxidative enzymes such as; proteolytic, glycolytic, and lipolytic enzymes [47]. Fungal cultures able to secrete these enzymes extra-cellular (exoenzymes) and intracellular (endoenzymes).

Exo-enzymes diffused into the environment, can breakdown complex polymeric compounds and predigest the food prior to absorption, as the fungi lack digestive system within their bodies. Endo-enzymes in turn are retained within the cell wall after synthesis and help in further assimilation of absorbed nutrients which is vital for culture growth.

For carbon and nitrogen assimilation by fungal cultures, different genera of fungi synthesized multitudes of enzymes to meet out their nutrient requirements. In this context; the fungal cultures *Aspergillus*, *Penicillium* and *Trichoderma*, synthesize cellulose and hemicellulose-degrading enzymes such as cellulases, mannanases, xylanases and  $\beta$ -glucosidases (BGL) to hydrolyze and breakdown the hemicellulose and cellulytic content of plant cell wall, in plant residues into mono-sugars of xylose and glucose units [24, 48, 50]. Kantharaj et al. [51] indicated the ability of white rot fungi (*Phanerochaete*, *Phlebia*, *Ceriporiopsis*, etc.) and brown-rot fungi (*Fomitopsis*, *Laetiporus*, *Gloeophyllum*, etc.) to exudate adequate quantities of lignin-degrading enzymes including lignin peroxidase and laccase that showed high activity in degrading lignin-matter of woody substrates into phenolic compounds; also Abd El Aty et al. [52] enhancement the production of laccase enzyme from the marine-derived *Alternaria tenuissima* KM651985. Extracellular production of amylase enzyme from filamentous fungi like marine *Alternaria alternata* hydrolyzes starch (the plants storage material) into glucose [53, 54]. *Rhizopus* and *Trichoderma* secrete pectinase and polygalacturonase which potentially converts pectin in fruit-residues into galacturonic acid. The filamentous fungi; *Chaetomium globosum*, *Aspergillus griseoaurantiacus*, *Trichoderma longibrachiatum* potentially produced the industrially important chitinase enzyme using wastes [55-57]. Fungal chitinases, chitosinases and N-acetylglucosaminidases can breach out the chitin-rich cell wall of fungi to access the nutrients [58].

Fungal proteases showed great value in hydrolysis of plant and animal proteins from high-molecular weight form into simple amino-acids which can be easily assimilated by fungal cells [59]. Where other fungal species of *Rhizopus*, *Candida*, *Geotrichum*, *Mucor*, etc. were enhanced by wastes for lipase enzyme production to hydrolyze lipid molecules into fatty acids [60]. Other fungal enzymes like, Inulinase and naringinase were produced by the filamentous *Aspergillus terreus* and *Aspergillus niger* using low cost agricultural wastes [61, 62]. The enzymes such as urea-aminohydrolase, nitrate-reductase, and nitrite-reductase able to hydrolyze urea into ammonia which can be easily assimilated by fugal cells for nitrogen nourishment.

The important fungal enzymes obtained from fungal fermentation of agro-industry wastes are depicted in **Table (2)**.

**Table 2** Fungal fermentation of agro-wastes to produce variable enzymes

Enzyme	Fungal culture	Agro-industry wastes	References
Chitinase	<i>Aspergillus griseoaurantiacus</i>	potato shells	[57]
	<i>Trichoderma longibrachiatum</i>	wheat bran , rice straw , potato peels , artichoke leaves , saw dust , and licorice residue	[56]
Laccase	<i>Trametes versicolor</i>	Horticultural wastes	[63]
	<i>Pleurotus ostreatus</i>	Peanut shell	[64]
	<i>Alternaria alternata</i>	Wheat bran	[32]
	<i>Alternaria tenuissima</i>	Wheat bran	[52]
$\alpha$ -Amylase	<i>Aspergillus oryzae</i>	Coconut oil cake,	[65]
	<i>Aspergillus niger</i>	black gram bran, and soybean	[66]
	<i>Aspergillus niger</i>	Papaya waste	[67]
	<i>Alternaria alternata</i>	corn cobs, wheat bran, potato shells, wheat straw and rice straw	[53]
Xylanase	<i>Aspergillus flavus</i> <i>Cladosporium sphaerospermum</i> <i>Epicoccum purpurascens</i>	wheat bran - saw dust	[48]
Invertase	<i>Aspergillus niger</i>	Fruits peel waste	[68]
Lignin peroxidase	<i>Pleurotus ostreatus</i> , <i>Phanerochaete chrysosporium</i>	Sugarcane bagasse	[69]
Endoglucanase	<i>Trichoderma reesei</i> QM9414	Rice bran	[70]
$\beta$ -Glucosidase	<i>Aspergillus sydowii</i> BTMFS 55	Corn straw,wheat bran,	[71]
	<i>Thermoascus aurantiacus</i> CBMAI 456	soy bran, corncob soy peel	[72]
Manganese peroxidase	<i>P. chrysosporium</i>	Wheat straw	[73]

## 6 Fermentation strategy of agro-industry wastes for enzymes production

Fermentation can be defined as a technique used by microorganisms, such as fungi, for biological conversion of complex substrates into simple products [74].

There are two main fermentation techniques applied for fungal cultivation of agro-industry wastes to produce enzymes, submerged-fermentation (SMF) and solid state fermentation technologies (SSF).

### 6.1 Submerged fermentation, SMF

In submerged fermentation, known as (liquid fermentation), the nutrients are present in the form dissolved in a large volume of water. This method is useful in the production of some enzymes which suffer from the catabolic -repression caused by very low concentrations of residual substrates like; amino acids and glucose [75].

### 6.2 Solid state fermentation, SSF

SSF is a good technology for potential waste valorization, in which the insoluble matter serves as nutrients source and physical support in absence of free water. [76, 77].

Using solid state fermentation technology, for biodegradation of agro-industry wastes is preferred over conventional fermentation methods, because the similarity to natural ones and simplicity [78], also it has several advantages of, low energy and water consumption, high product yields and enzymatic extraction facilitated, solving disposal problems, reduction of medium contamination risk, therefore this technology has been proved to be both economical and eco-friendly [79]. Therefore, SSF is often indicated as a promising way to produce higher enzyme yields compared to SMF [80].

Agro-industry wastes are preferred substrates for SSF strategy, which are rich in polymeric sugars, such as hemicellulose, cellulose, and pectin, that can be converted into simple ones to be easily assimilated by fungal colonies [81]. In addition, filamentous fungi are usually preferred for SSF processes, due to their specific ability to colonize the inter-particle spaces of solid waste matrices, and to produce various enzymes that, hydrolyze the solid substrate into fermentable sugars, and valuable products [82]. *Aspergillus*, *Alternaria*, *Trichoderma* and *Pleurotus*, were the most genera employed in this technique [52, 83].

Various studies have been indicated, in applying SSF-strategy of the agro-wastes as a substrate for different fungal species, such as *Aspergillus awamori* produce amylase and glucoamylase, with SSF of field-residues such as rice bran and wheat bran as substrates [84]. *Aspergillus niger* and *Alternaria alternata* have been reported to produce  $\alpha$ -amylase employing SSF of agricultural wastes like wheat bran [53, 85]. Solid state fermentation of the agro-industrial waste, orange rind was used as a substrate containing naringin for naringinase enzyme production using *Aspergillus niger* [62]. Inulinase enzyme secreted by the fungus *Aspergillus terreus* under SSF of the low cost substrate (artichoke leaves) under solid state fermentation (SSF) [61]. Oil cakes, like; palm kernel oil cakes, were also applied as substrates for lipase enzyme production by *Aspergillus ibericus* [86]. SSF of different agro-industrial wastes, including corn cobs, sugarcane bagasse, and coconut husks, used as substrates for ellagitannase enzyme production, which used for biodegradation of ellagic acid [87]. Fermentation of peanut cake with *Aspergillus oryzae* significantly increase  $\alpha$ -amylase,  $\beta$ -glucosidase, lipases, and xylanase enzyme activities for industry [88].

Out of all previously reported results, we can conclude that, SSF-strategy of agro-industry wastes can produce variable industrial enzymes, such as; exo-polygalacturonase and xylanases [89], cellulases, ligninases and cinnamoyl esterases, lignocellulosic enzymes [90], pectin methylesterase and polygalacturonase [91], constitutive extracellular chitinases [56], in addition the enzymes, pectinases, xylanases, cellulases were obtained [92]. **Table (3)** shows an overview on production of enzymes by SMF and SSF of various agro-wastes.

**Table 3** Production of fungal enzymes by SSF and SMF strategy of various agro-wastes

Fermentation strategy	Enzyme produced	Agro-industry waste	Fungal strain	Conditions of cultivation	References
SSF	Cellulase	Green tea waste	<i>Aspergillus niger</i>	96h; 30 °C	[93]
	Cellulase	Rice straw	<i>Trichoderma reesei</i>	96h; 30 °C	[94]
	Chitinase - Chitosanase	potato shells	<i>Aspergillus griseoaurantiacus</i>	7 days ; 28 °C	[57]
	Amylase	Babassu cake	<i>Aspergillus awamori</i>	144h; 30 °C	[95]
	Laccase	Wheat bran	<i>Alternaria tenuissima</i>	14 day; 28 °C	[52]
	Chitinase	wheat bran, rice straw, potato peels, artichoke leaves,	<i>Trichoderma longibrachiatum</i>	7 days ; 28 °C	[56]
	Xylanase	Rice straw	<i>Trichoderma reesei</i>	96h; 30 °C	[94]
	Amylase	Soybean husk and flour mill waste	<i>Aspergillus oryzae</i>	144h; 30 °C	[96]
	Mannanase	Wheat straw	<i>Aspergillus niger</i>	96h; 32 °C	[97]
	Xylanase	Ragi husk	<i>Aspergillus fumigatus</i>	120h; 49,9 °C	[98]

	Pectinase	Citrus waste and sugarcane bagasse	<i>Aspergillus oryzae</i>	24h; 32 °C	[99]
	Mannanase	Açaí seed	<i>Penicillium citrinum</i>	144h; 30 °C	[100]
	Invertase	Wheat bran plus oat mea	<i>Aspergillus caespitosus</i>	72h; 40 °C	[101]
	Pectinase	Pomelo peels	<i>Aspergillus niger</i>	168h; 50 °C	[102]
	Protease	Wheat bran	<i>Aspergillus oryzae</i>	48h; 30 °C	[103]
	Lipase	Sugarcane bagasse	<i>Rhizopus oryzae</i>	72h; 45 °C	[104]
	Lipase	Malt bagasse	<i>Aspergillus brasiliensis</i>	96h; 32,7 °C	[105]
	Protease	Orange peel	<i>Aspergillus brasiliensis</i>	72h; 30 °C	[106]
	Laccase	Apple pomace	<i>Phanerochaete chrysosporium</i>	192h; 37 °C	[107]
SMF	<b>Enzyme produced</b>	<b>Agro-industry waste</b>	<b>Fungal strain</b>	<b>Conditions of cultivation</b>	<b>References</b>
	Cellulase	Coir waste	<i>Aspergillus niger</i>	72h; 30 °C; 120 rpm	[108]
	Xylanase	Sugarcane bagasse	<i>Trichoderma lanuginosu</i>	96h; 30 °C	[109]
	Amylase	Shorea robusta deoiled cake	<i>Aspergillus flavus</i>	72h; 30 °C; 200 rpm	[110]
	Mannanase	Banana stem	<i>Aspergillus sydowii</i>	168h; 30 °C; 120 rpm	[111]
	Invertase	Orange peel	<i>Penicillium sp.</i>	168d; 28 °C; 180 rpm	[112]
	Invertase	Pomegranate pee	<i>Cladosporium cladosporioides</i>	96h; 30 °C; 125 rpm	[113]
	Pectinase	Sawdust	<i>Aspergillus terreus</i>	96h; 30 °C; 100 rpm	[114]
	Protease	Peanut meal	<i>Aspergillus awamori</i>	96h; 30 °C; 220 rpm	[75]
	Laccase	Wheat straw	<i>Trametes versicolor</i>	72h; 30 °C; 150 rpm	[115]
	Lipase	Corn oil	<i>Trichoderma harzianum</i>	48h; 28 °C; 180 rpm	[116]

Production of fungal enzymes can be optimized by combination of different substrates from agro-industrial residues, where a single by-product may not provide all the sufficient essential nutrients for microbial growth. In this context, Filipe et al uses a mixture of winery residues and olive mill residues in SSF using *Aspergillus niger* and *A. ibericus*, that results in a production of xylanase (189,1U/g), cellulose (56,3 U/g), and Beta-glucosidase (10,9 U/g) enzymes. Studies of Mostafa et al. [48], also indicated that, the mixing of wheat bran and saw dust in different ratios improved xylanase enzyme production by *Epicoccum purpurascens*, *Cladosporium sphaerospermum* and *Aspergillus flavus* in the ratio of 22.09, 50.22 and 21.82 %, respectively.

In addition, Rayhane et al [117] showed the production of cellulose (19.36U/g), amylase (15.22 U/g) and lipases (38.73U/g), by *Trichoderma asperellum* using a mix of jatropha, vine shoots, olive oil and olive pomace as substrates.

## 7 Evaluation and application of the fungal-enzymes

In recent years, conversion of agricultural waste, as well as wastes of food- industries (as renewable resources), to the production of low-cost enzymes using microbial fermentation strategies has attracted the attention of researchers that work in such field [15].

Fungal cells usually produce and release the enzymes to breakdown and digest the high complex components of waste substrates for their own benefit, those synthesized fungal enzymes are exploited for various valuable applications for human. Previous studies conducted in this field, reported that more than 50% of industrially important enzymes are synthesized and extracted from fungal sources [118].

These fungi organism are able to synthesize a wide range of highly effective hydrolytic extracellular enzymes, and oxidative ones, like cellulase, xylanase, laccase, inulinase, mannanase, invertase, pectinase, amylase, protease and peroxidase. All fungal synthesized enzymes have potential application in industrial biotechnology, which directly encourages research study, for the suitable substrates and the best promising enzyme producers, to obtain large amounts of industrial enzymes at a low cost [119].

The enzyme-based economy showed a healthy-upward-trend, Therefore, Government policies now directed to the use of sustainable resources and focusing on Green-technology [15, 31].

### 7.1 Xylanase

Xylanase (E. C. 3.2.1.8, 1,4- $\beta$ -xylanxylanohydrolase) is the enzymes that hydrolyze xylan which is a complex polysaccharide of the plants. Xylan is the most abundant non-cellulosic polymer in plants and hard-woods, consists of a xylose-residue backbone, with each subunit linked by a  $\beta$ -1,4-glycosidic bond [120]. Xylanases work by cleaving the  $\beta$ -1,4 glycosidic bonds, and forming the usable products such as xylose [121].

Xylanases are produced by several bacteria, actinomycete and fungi. Where, several studies showed that, the filamentous fungi are of particular interest, because they secrete it into the media in large quantities in comparison to other microorganisms [122]. In this context, numerous research studies have been carried out on the production of xylanases from different species of fungi, such as; *Chaetomium thermophilum*, *Thielevia terrestris*, *Paecilomyces thermophile*, *Sporotrichum thermophile* and *Melanocarpus albomyces* [109]. Similarly, the type of lignocellulosic-wastes suitable as a nutrient source for a commercial production of xylanase has been studied by several researchers. Botella et al. [123] Investigated grape pomace as a viable carbon source for the production of a xylanase using *Aspergillus awamori*. In another study, Apple pomace, hazelnut shell, and melon peel were used by Seyis and Aksoz [124]. for xylanase production showing a maximum activity of 26.5 U/mg using *Trichoderma harzianum*. In recent years, *N. intermedia* considered as a potential candidate for production of xylanase through a wheat-based bio-refinery [125].

In addition to the great efficiency of xylanases in biomass degradation process, these enzymes have other commercial applications, where xylanases can be used in improving the quality of animal feed, in the textile and paper industry, as well as being used in food processing industries, and used in production of bioethanol, depending on the transformation of some agro-industrial wastes into fermentable sugars [126, 127]. xylanase has potential advantages over chemical treatments, which can be applied in bio-bleaching of kraft pulp and decreased chlorine requirement, that lower the overall production cost [128, 129]. In textile-industry, xylanase is used for processing of fibers, especially for linen making [130].

In bread-making industry, xylanases break down wheat flour hemicelluloses, results in improvement of dough volume, softness, quantity, and quality of bread [58, 128] [131]. In wine and juice industries, xylanolytic enzymes increased clearance of juices, improved liquefaction of fruit and vegetable juice, and reduce its viscosity [132]. Xylanases of *Fusarium sp.* showed potential application in clarification of orange fruit juice due to degradation of xylan reduces the viscosity [133].

### 7.2 Cellulase

Cellulolytic enzymes classified into, endoglucanases (E.C. 3.2.1.4), exoglucanases (E.C. 3.2.1.176), and  $\beta$ -glycosidases (E.C. 3.2.1.21) [134]. They are the main enzymes efficient in the hydrolysis of polysaccharide (cellulose), by degrading cellulosic chains found in biomass to its primary products such as glucose and cellobiose [135]. Cellulases can be produced by a wide range of fungal organisms, such as the *Aspergillus*, *Fusarium*, *Trichoderma*, *Penicillium*, *Alternaria* and *Schizophyllum* genera [32, 95]. Similarly, the fungal species *Myceliophthora thermophila*, *Rhizopus oryzae*, and *Thielavia terrestris* have been characterized to produce cellulases [136-138].



Cellulases are highly effective in many industrial applications, like; textiles, pulp and paper, detergent, food and agricultural, and biofuel production. In food processing, cellulases were applied for extracting and clarifying fruit and vegetable juices, that enhance the texture stability by decreasing its viscosity [139]. In paper and pulp industry, crude and pure cellulases applied for improving the fiber properties and reducing pulp viscosity, which directly deinking the paper wastes, in addition, improve the fiber strength and brightness properties [140]. In agricultural field, cellulase able to controls plant diseases by degrading the pathogens cell wall [141]. Further, cellulase increased soil fertility by accelerating the straw disintegration rate in field [142].

Cellulases are successfully applied in different steps of industry, i.e., wet processing steps of bio-polishing and bio-stoning of cellulosic fabrics and jeans. Also, acidic cellulases enhanced the softness of the fabric [143]. Results obtained by Imran et al. [144] indicated good application of fungal cellulases from *Penicillium echinulatum* and *Acrophialophora nainiana* in finishing process of knitted cotton fabrics.

Karmakar and Ray, [143] showed that, the addition of cellulases in liquid laundry detergent can remove dirt in the fabric spaces and maintain the clothes quality, also indicated the potential application of cellulases in pharmaceutical-industry, cellulases act as biocatalysts, help to release therapeutic compounds for treating metabolic disorders. In feed-industry, pretreating of agricultural silage and grain with cellulases increased the feed and energy values, also improved their digestibility. In wine industry, cellulases was applied during fermentation process to increase the wine viscosity and stability. The ability of cellulases to deconstruct lignocellulose, with releasing glucose units, which in turn can be converted into ethanol, has made these enzymes very important in biofuel research. The studies of Vázquez-Montoya [145] emphasized significant application of *Cladosporium cladosporioides*, *Fusarium verticillioides*, and *Penicillium funiculosum* in bioethanol production from moringa wastes, on the other hand, *Aspergillus niger* MK543209 produced cellulases from paper wastes which applied in biofuel production [146].

### 7.3 $\alpha$ -amylase

Alpha amylases (EC 3.2.1.1) are endoglycosidases, that randomly cleave  $\alpha$ -1, 4 linkages between adjacent glucose subunits in polysaccharides (amylose), resulting in the release of short chain oligomers and smaller dextrans [147].

*Aspergillus sp.* between fungi is considered important amylase producers, like *A. niger*, *A. oryzae*, *A. terreus*, *A. fumigatus*, *A. awamori*, *A. favus* [148]. In addition, amylases are produced from the fungi organisms, *Thermomyces lanuginosus*, *Penicillium brunneum*, *Paecilomyces variotii*, *P. chrysogenum*, *Trichoderma pseudokoningii*, *Mucor sp.*, and *Rhizopus oryzae* [149]. *Penicillium chrysogenum* HTF24 produce amylase with wide range of biotechnological application [150]. *Paecilomyces variotii* ATHUM 8891 synthesis  $\alpha$ -amylase with higher stability [151]. The potential function of amylase in the hydrolysis of the starch into units of simple sugars such as dextrans and oligosaccharides, are the main reason for effective amylases application in food, textile, detergent, paper, and pharmaceutical industries since nineteenth century [152,153]. In addition, amylase has several applications in fermentation, starch liquefaction, sugar syrups production (glucose/maltose), and saccharification process [154]. Further, in textile-industry application of amylase in desizing process breakdown the starch particles from the fabrics without attacking the fibers [155]. Additionally,  $\alpha$ -amylase obtained from *Aspergillus oryzae* is applied in digestive medicines which used for indigestion treatment with syrup and capsule formulation. Application of  $\alpha$ -amylases in detergent formulation indicated, potential application in washing, because its characteristics of alkalophilic, and oxidant in-sensitive characters [149].

### 7.4 Inulinase

Inulinases are classified into exo and endo-inulinases (EC 3.2.1.80) and (EC 3.2.1.7) respectively, depending upon their mode of activity [156]. Inulinase acts by converting the polyfructose, inulin (fructan), into fructose and fructo-oligosaccharides. The amounts of fructose syrup obtained by inulinase enzyme action is highly increased compared to the amount produced from starch hydrolysis by combined activities of  $\alpha$ -amylase, amyloglucosidase and pullulanase, [157].

Several fungal isolates such as *Chrysosporium pannorum*, *Penicillium sp.* and *Aspergillus niger* have been known to synthesize inulinase [158]. Inulinase can easily obtained by fermentation of lignocellulosic substrates, such as Abd El Aty et al. [61]. showed the ability of *Aspergillus terreus* to produce adequate amounts of inulinase enzyme using artichoke leaves as a solid substrate. Coconut oil-cake was also used in a study of [159], for production and optimization of inulinase using *Penicillium rugulosum* (MTCC-3487). Sugar cane baggase has also been used as substrate in different studies for inulinase production [160]. The importance of inulinase has arisen from their activity to complete conversion of the substrate to fructose, as consumer-preferred sweeteners, which is highly preferred for human in the food and pharmaceutical industry. Additionally, Inulinase finds application in the production of citric acid, bioethanol, and lactic acid.

## 7.5 Pectinase

Pectinases are a class of enzymes, which catalyze the decay of pectin-containing materials. Pectin is a major constituent of the plant primary cell wall and contains a variety of polysaccharides especially rich in galacturonic acid [161].

Pectinases hydrolyze the pectin present in varieties of agricultural residue using different microbial species. Among all pectinases, fungi organisms are preferred, with the strains of *Aspergillus spp.* dominating many industrial processes [114]. Recently, *Aspergillus niger*, *A. versicolor*, *A. oryzae*, *A. pulverulentus*, *Fusarium oxysporum*, *A. favus*, *Mucor racemosus*, *Rhizopus stolonifer*, *Mucor hiemalis*, *Penicillium Jenseni*, *Trichoderma viride* and *P. citrinum*, were explored to release pectinases, which is favorable for industrial pectinase applications [162].

Pectinases have vital roles in the extraction and manufacturing of fruit juices, vegetable oil, wine clarification, coffee and cocoa fermentation [163]. Additionally, potential application in retting of plant fibers, bleaching of paper, scouring of cotton, bio-energy production, and waste-water treatment [163]. In this context, Sudeep et al. [164] produced highly stable pectinase enzyme by *Aspergillus sp.*, which can be applied in juice clarification, and boosted the flavor and aroma of fruit and vegetable juices. In addition, pectinase from *A. niger* and *Mucor circinelloids* is used for coffee fermentation [165].

In poultry-feed industry, pectinase used as supplement to reduce the feed viscosity [166]. In the textile sectors, pectinases can be applied in the process of gum removal from plant fibers like, sunn hemp, jute and fax [164]. Furthermore, pectinases usually applied in pulp and paper industry, to improve the physical characters of paper, depending on depolymerization of the galacturonic acids polymers [167]. Also, in beverage industry, it is play important role in, improving stability, and aroma of red wines with increasing its clarity [164].

## 7.6 Invertase

Invertase enzyme, known as (beta-fructofuranosidase), which catalyze the hydrolysis of sucrose, by break down of  $\alpha$ -1,4- glycosidic linkage between D-fructose and D-glucose of sucrose [168].

Invertase synthesized by variety of fungal genera, i.e., *Aspergillus*, *Fusarium*, *Cladosporium*, *Aureobasidium*, *Eremothecium*, *Trichoderma*, *Ceratocystis*, *Sclerotinia*, *Metarhizium*, *Paecylomices* and *Penicillium*, *Rhizopus*.

Invertase has been applied in various industrial applications, i.e., beverages, food and bakery. additionally, invertase has a wide range of application in pharmaceutical industry, depending on its ability to synthesis fructo-oligosaccharides (FOS), an important non-digestible carbohydrate composed of fructose chains. The FOS used for diabetic patients due its characters of short-chain fatty acids and lower caloric value [169].

Invertase is one of the most used enzymes in the food industry, because it is sweeter and does not crystallize easily, especially in the preparation of jams and sweets. They produced non-crystallizable sugar which used in baking industry and preparation of chocolates, candies, jams, cookies and artificial honey for children for more iron absorption [170].

## 7.7 Mannanase

Mannanases are enzymes that degrade the hemicellulose mannan, which is an integral part of the plant cell wall [171]. Mannans form the largest hemicellulosic fraction of soft and hard wood, which are closely associated with cellulose and xylan as components of the cell wall, being organized in paracrystalline arrangements, adsorbed on the cellulose microfibrillar surface [172]. Therefore, mannanases are the second most important enzymes for the hydrolysis of hemicelluloses after xylanases.

Among fungal organisms, different *Aspergillus spp.* showed good ability to produce mannanase [173], similarly, a mixture of apple pomace and cottonseed powder used by *Aspergillus niger* SN-09 as a raw material for production of  $\beta$ -mannanase [174]. Furthermore, filamentous fungi, such as *Trichoderma reesei*, and *Pencillium spp.* are considered to have great potential for the industry in the production of  $\beta$ -mannanases [97].

Due to its ability to randomly hydrolyze the  $\beta$ D1,4 bonds of mannopyranose and effectively remove hemicellulose, mannanase have found applications in the paper, and textile industries [175]. Mannanases also applied in the food, oil, feed and pharmaceutical industries [176].

## 7.8 Laccase

Laccases are group of multi-copper proteins, which able to oxidize a variety of phenolic compounds and degrade lignin, received much attention in industrial applications [177].

Among microorganisms, white rot fungi are the best producer for laccase enzyme, like; *Phanerochaete chrysosporium Matei*, because of its capacity to produce adequate amounts of ligninolytic enzymes, grow fast, and easy handling during culture and fermentation [107, 178]. Additionally, the filamentous fungi *Aspergillus tubingensis*, *R. oryzae*, *Aspergillus favus*, *Mucor indicus*, *Fusarium udum*, *Mucor hiemalis*, *Trichoderma viride* and *Peniophora*, were confirmed for higher laccase production [179].

laccases have a broad range of substrate specificity, also able to degrade lignin and phenolic compounds, therefore, laccases have been evaluated in a large number of biotechnological applications, such as pulp biobleaching, biodegradation of toxic chemical residues, bioremediation of dyes, treatment of wastewater and soil. In addition, laccases industrially applied in pharmaceutical, paper and pulp manufacture, food processing, and, textile industries [180]. Lignolytic laccases industrially applied in food processing, for changing color appearance of beverage of food, and clarifying fruits and vegetables juices, wine and beer [181]. Furthermore, laccases play a vital role in the environment sector, depending on its ability in biodegradation of hazardous recalcitrant synthetic substances as, herbicides, fungicides, and synthetic dyes, (i.e. Reactive Blue 15, Congo Red and Remazol Brilliant Blue R) [182].

## 7.9 Protease

Proteases are proteolytic enzymes, which catalyze the hydrolysis of peptide bonds and mediated degradation of proteins into their constituent amino acids.

The fermentation of oriental foods by, *Rhizopus oligosporus*, *Aspergillus awamori* and *Rhizomucor miehei* mostly used for the production of proteases [183]. *Aspergillus fumigatus*, *Aspergillus oryzae*, *Aspergillus versicolor*, *Aspergillus terreus*, *Aspergillus niger*, and *Aspergillus favus* also favorable fungal source for protease enzyme production [184, 185].

Interestingly, lingo-cellulosic residues have been used as raw substrate for proteases production in several studies, such as, Chancharoonpong et al. [186] studied the growth of *A. oryzae* in solid-state culture (soybeans) for protease production. Other substrates such as jatropha seed cake and tomato pomace have been applied for protease secretion [187].

The hydrolytic characteristics of protease has great advantages in degradation of proteins in alcoholic liquors and juice, clarification in beer production, gelatin hydrolysis, whey and soy protein hydrolysis, and meat protein recovery. Nowadays, in cheese production, proteases popularly applied as milk-clotting agent, due to high stability and activity at acidic pH and flavors properties [11]. Similarly, protease of *Rhizomucor miehei* NRRL 2034, and *A. niger* FFB1, which were free from aflatoxins and ochratoxin were applied for white soft cheese production [188]. Organoleptic danbo cheese production was preferred using *Aspergillus oryzae* DRDFS 13 proteases than bacterial enzyme [11].

Also, fungal proteases are widely used in detergents, leather, pharmaceuticals and agricultural industries [189, 190]. In this context, alkaline protease from *Aspergillus flavus* and *Conidiobolus coronatus* were successfully replaces the hazardous chemicals involved in soaking and leather tanning process. In cosmetic industries, collagenolytic proteases have used in wound healing, also fungal proteases hydrolyzed the peptide-bonds of keratin [191].

## 7.10 Lipase

Lipases are a class of enzymes, which catalyzes the hydrolysis of the insoluble triglycerides, to glycerol and free fatty acids. In addition, lipases catalyze esterification, alcoholysis, and acidolysis [192].

Fungal lipases gained special industrial attention due to their broad substrate specificity, stability, and selectivity, [193]. *Rhizopus*, *Rhizomucor*, *Aspergillus*, *Geotrichum*, *Penicillium*, *Mucor* and *Geotrichum* species are good strains for commercial lipases secretion [194]. Lipases can easily have obtained by fungal fermentation of lignocellulose-waste. Similarly, a study of Salihu et al [195] investigated the fungus *A. niger* for lipase synthesis using peanut cake as substrate with addition of 1.0% (v/w) Tween-80. Furthermore, shea-nut cake used for lipase production, applied for the inherent tannin and saponin content reduction in the animal feed to be more suitable [196].

Lipases are highly applicable owing to the type of reactions they can catalyze. *Penicillium chrysogenum* producing active-lipase with several advantages in environmental and industrial sectors [150]. Furthermore, they are used in various

industries such as detergents (for fat hydrolysis), textiles, biofuels, pharmaceuticals, pulp and paper [197]. In food-processing, lipase of *Penicillium roquefortii* can be applied for synthesizing methyl ketones in order to improve blue cheese flavor [198]. High yield of fatty acid methyl esters was obtained by lipase transesterification, which is important for biodiesel production. The capability of lipase to produce biodiesel is a useful approach, depending on waste oils utilization. [199, 200]. Additionally, lipases are widely applied in treatment of fat-containing effluent released from food processing, paper, cellulose and tanneries, industries [201].

### 7.11 Chitinase

Chitinase enzyme hydrolyzes the polysaccharide chitin (the world's second most polymer after cellulose), and results in the production of N-acetyl d-glucosamines, chitooligo-saccharides [202].

The most known fungi organisms producing chitinase are *Trichoderma harzianum*, *Penicillium sp.* *Saccharomyces cerevisiae*, *Beauveria bassiana*, *Coprinopsis cinerea*, *Ustilago maydis*, and *Xenorhabdus nematophila* [203-206]. *Aspergillus flavus*, *Penicillium monovorticillium*, and *Fusarium oxysporum* were able to produce chitinase enzyme, which can be applied for preparation of certain oligomer chain length and also used to produce chito-oligosaccharides and N-acetylglucosamine from  $\alpha$ -chitin, that showed various healthcare benefits [207]. Fungal chitinases can act as biocatalyst for chitobiose, have several applications in various industries [208].

Chitin is the structural component of several organism's cell wall, such as fungi, mollusks, crustaceans, and algae, the chitinase antifungal activity has potential bio-control application [209]. Similarly, Deng et al. [210] obtained chitinase from *Trichoderma harzianum*, which efficiently inhibited the growth of (*Botrytis cinerea*) pathogen. Chitinase also applied in the postharvest biocontrol of *Verticillium dahlia*, as mentioned by Liu et al. [203] In therapeutic application, chitinase has been applied in the antifungal drugs, against various infectious diseases [211].

Shehata et al. [57] showed good production of chitinase and chitosanase enzymes from the fermentation of potato shells as a sole nutrient source, using the marine-derived fungus *Aspergillus griseoaurantiacus* KX010988. Obtained chitinase exhibited antifungal activity against pathogenic *Fusarium solani*, and the hydrolysis of moderate molecular weight chitosan by chitosanase, produced Chitosan-oligosaccharides with good antioxidant and antibacterial activities.

Low cost, non-chitin containing agricultural wastes (artichoke leaves, wheat bran, rice straw, and potato peels), were fermented by *Trichoderma longibrachiatum* KT693225 for exochitinase synthesis. Exochitinase showed highly effective antifungal effects against the pathogenic strains *Aspergillus niger*, *Fusarium oxysporium* and *Alternaria alternata* [56].

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## 8 Conclusion

Clean technologies are economically advantageous and has been encouraged for solving several environmental issues and obtaining products of industrial interests. Therefore, many different researches dealing with the use of agro-industrial wastes in bioprocesses to avoid the health and pollution problems related to disposal of these residues in the environment. The microbial fermentations of such residues, is a promising way to produce higher enzyme yields can be applied in different industrial sectors.

Fungi considered one of the most active microorganisms, which able to valorize different wastes, producing hydrolytic and oxidative enzymes, i.e., Xylanases, Cellulases, Pectinases, Laccases, Inulinases, Invertases,,,,,etc. for potential various biotechnological applications. Many other fungal species can still be discovered with better use of agro-industrial residues to obtain higher enzyme yields. Therefore, the future demand is working on more production of fungal enzymes, for new products with new features, functions, and applications.

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## Compliance with ethical standards

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### Disclosure of conflict of interest

The author declares that there is no conflict of interests regarding the publication of this review.

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