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Environment

Agro-industrial wastes for biotechnological production as potential substrates to obtain fungal enzymes

Resíduos agroindustriais para produção biotecnológica como potenciais substratos para obtenção de enzimas fúngicas

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ABSTRACT

Agro-industrial wastes contain high moisture content and are rich in nutrients, and can be used as useful substrates by microbes with the supplementation of nitrogen sources, thus providing an alternative tool for the industrial production of many products of economic value, such as enzymes for example. These are proteins that function as biological catalysts, responsible for carrying out various biochemical reactions, being applied in detergent, food, paper and cellulose, cosmetics, textile industries, etc. However, they are expensive raw materials, and it is worth noting that an important part of the cost of manufacturing enzymes is mainly due to the expense of means and fermentation processes. Thus, to minimize the cost of this production and in order to reduce the degradation of the environment due to agricultural waste, a variety of microorganisms and agro-industrial "by-products" can be used to facilitate the economic production of enzymes. In addition, the use of these materials as substrates for microbial cultivation is a factor that beef up the microbial enzymatic activity. Therefore, in this work, a review was carried out on agro-industrial residues and the main enzymes in the industrial market, as well as the use of these materials as sources to obtain enzymes produced by fungi.

Keywords: Bioconversion; Biotechnological Processes; Microorganisms

RESUMO

Resíduos agroindustriais contêm alto teor de umidade e são ricos em nutrientes, podendo ser usados como substratos úteis por micróbios com a suplementação de fontes de nitrogênio, proporcionando assim uma via alternativa para a produção industrial de muitos produtos de valor econômico, como as enzimas por exemplo. Estas são proteínas que funcionam como catalisadoras biológicas, responsáveis pela realização de diversas reações bioquímicas, sendo aplicadas em indústrias de detergentes, alimentos, papel e celulose, cosméticos, têxtil, etc. Contudo são matérias-primas caras, sendo válido ressaltar que uma parte importante do custo de fabricação das enzimas é principalmente em

consequência do gasto com meios e processos de fermentação. Desse modo, para minimizar o custo dessa produção e com a finalidade de reduzir a degradação do meio ambiente por conta dos resíduos agrícolas, uma variedade de microrganismos e "subprodutos" agroindustriais podem ser utilizados para facilitar a produção econômica das enzimas. Além disso, a utilização destes materiais como substratos para cultivo microbiano é um fator que potencializa a atividade enzimática microbiana. Sendo assim, nesse trabalho foi realizada uma revisão sobre os resíduos agroindustriais e as principais enzimas no mercado industrial, bem como a utilização destes materiais como fontes para obtenção de enzimas produzidas por fungos.

Palavras-chave: Bioconversão; Microrganismos; Processos Biotecnológicos

1 INTRODUCTION

Worldwide, huge amounts of agricultural waste are generated from practices related to industrial processing, which are simply burned or dumped in landfills, becoming a threat to the environment and human health (KAPOOR; PANWAR; KAIRA, 2016). A wide variety of agro-industrial residues, such as sugarcane bagasse, orange peel, rice husk, trail straw, beer grains, and olive bagasse can be used as raw materials in bioconversion into products of biotechnological interest with high added-value (PURI; ABRAHAM, 2016).

Agro-industrial waste contains sugars, minerals, and proteins, thus providing a low cost of possible raw material. Besides, recycling these wastes is important to ensure a balance between the environment and industry (ORLANDELLI *et al.*, 2017). Due to the high nutritional composition, these residues are not described as "residues", but considered as a raw material for the formation and development of other products. The availability of these nutrients in the residues provides appropriate environments for the growth of microorganisms, which can convert these substrates into several industrially important products (SADH; DUHAN; DUHAN, 2018).

Thus, in recent years, several eco-friendly and economical technologies have been developed with efforts at both the industrial and academic levels. These technologies aim to use agro-industrial waste for the development of value-added products, thus reducing environmental pollution and resolving the issues associated with their disposal. Therefore, the conversion of renewable resources

from agricultural waste, as well as from other industries, to the production of cost-effective enzymes using fermentation techniques has attracted the attention of researchers (PANESAR *et al.*, 2016; FERREIRA; AZZONI; FREITAS, 2020).

Enzymes for industrial applications are currently derived from fungi, especially from the *Trichoderma* and *Aspergillus* species (BANERJEE; SCOTT-CRAIG; WALTON, 2010; JIN *et al.*, 2021; SOUZA; BON; SILVA, 2021). In addition to these, white-rot fungi also appear as natural candidates to be used in fermentation processes. These microorganisms are capable of synthesizing a wide range of relevant hydrolytic and oxidative extracellular enzymes, such as cellulases, xylanases, laccases, and peroxidases. The potential application of these enzymes in biotechnology encourages studies that involve a selection of promising enzyme producers, as well as a search for suitable substrates to obtain large amounts of enzymes at a low cost (ZILLY *et al.*, 2012; BAJAR; SINGH; BISHNOI, 2020).

The world economy around enzymes is very dynamic due to different regulatory policies, as well as socio-geographical differences. However, there is a trend towards an increase in the use and consumption of biocatalytic enzymes in the daily routine of the people and the industrial sector. Thus, the slowdown in the global economy does not affect the growth of the enzyme industry. With government policies shifting towards the use of sustainable resources and focusing on green technologies, the enzyme-based economy showed a healthy upward trend (GOPALAN; NAMPOOTHIRI, 2016; BAJAR; SINGH; BISHNOI, 2020).

The global market for industrial enzymes is expected to increase at a compound annual growth rate of 4.7% between 2016 and 2021. Despite tangible demand, enzymes are relatively expensive reagents, and this increases the operating cost of the industrial processes that use them. Almost 50% of the cost of the enzyme production process is associated with capital investment, while the cost of raw materials is responsible for almost a third of these expenses. Thus, the replacement or complementation of raw materials with lignocellulosic sources can

result in a higher return on investment (RAVINDRAN *et al.*, 2018; FERREIRA; AZZONI; FREITAS, 2020).

Thus, the purpose of this review was to present the enzymes that can be produced using agro-industrial residues from microbial production, based on submerged and solid-state fermentation processes, as well as the importance of these substrates and the final product generated for the biotechnology sector and, mainly, for the environment.

2 MATERIAL AND METHODS

The choice of references that were used in this work was carried out by searching the scientific bases of Google academic and SciELO, using articles dated from 2009 to 2021 as the filter for selection. The search was carried out with some subject-specific keywords, both in Portuguese and in English: resíduos agroindustriais/agro-industria wastes, enzima/enzyme, bioconversão/bioconversion.

Furthermore, words that include some agro-industrial residues were searched for articles with a mention of such biomasses: bagaço de cana/sugarcane bagasse, palha de arroz/rice straw, palha de trigo/wheat straw, espiga de milho/corn cob. A search was also made for articles citing some specific enzymes using nomenclatures in English: amylase, cellulase, xylanase, mannanase, invertase, pectinase, protease, lipase, and laccase.

3 DEVELOPMENT

3.1 Agro-industrial wastes

Agricultural waste can be divided into fields and process waste. The field ones are those that consist of leaves, stems, seeds, and pods, which are present on the ground after the harvesting process. While the process residues are those

that remain after the culture is processed, consisting of molasses, bark, bagasse, seeds, leaves, stem, straw, pulp, roots, etc. (SADH; DUHAN; DUHAN, 2018).

Millions of tons of waste from agro-industrial activities are generated worldwide. Some of them are used as animal feed or are deposited in the field. However, a large part is still discarded without treatment, causing damage to the environment (MELO *et al.*, 2011). Sugar mills, for example, generate approximately 275 kg of bagasse per metric ton of sugarcane. The Brazilian production of this sugarcane residue was estimated at 186 million tons per year (SOCCOL *et al.*, 2010). BORGES *et al.* (2020) related that Brazil yields ~70 million tons straw annually after mechanized sugarcane harvesting, and this resource is a rich carbon and others nutrients source.

Concerning corn, currently, its use is very diverse, ranging from food to biofuels. According to data from the Food and Agriculture Organization (FAO), world corn production in 2019 was greater than 1 billion tons. However, it is important to mention that this fruit consists of 30% of residues from biomass in the form of ears of corn (MARDAWATI *et al.*, 2018).

Among the three most cultivated crops in the world, after sugar cane and corn, is wheat with a global production of approximately 762 million tons in 2017, according to the USDA. Considering a ratio of 1.5 straw per grain, more than one billion tons of wheat residues are produced annually (MANCINI et al., 2018). Fourth is rice, whose waste generation is equivalent to 290 kg of straw for 1 ton of grain produced (BISWAS *et al.*, 2018). In 2017, 495 million tons of this seed was produced globally (USDA, 2019), so a large amount of what was harvested from rice is wasted around the world each year.

Agro-industrial waste is lignocellulosic material, consisting of three main polymeric fractions: lignin, hemicellulose, and cellulose, which are joined together by covalent bonds. These major fractions are responsible for 97-99% of all dry mass of the materials and, just as the shape and size of the cell wall of these biomasses vary from species to species, their chemical composition is different between the

lignocellulosic representatives, as can be seen in Table 1. In general, cellulose is found in greater proportions, followed by hemicellulose and, finally, lignin (CASTRO; PEREIRA JR, 2010).

Table 1 – The average percentage of the lignocellulosic chemical composition of some Brazilian agro-industrial wastes

Agro-industrial Waste	% Cellulose	% Hemicellulose	% Lignin
Sugarcane bagasse	40	22	28
Corn cob	45	35	15
Wheat straw	30	50	15
Rice straw	43	26	16
Barley straw	38	32	17
Coconut fiber	40	0,2	43
Banana fiber	63	7	8
Cotton waste	95	2	0,3

Source: Adapted from Santos *et al.* (2012)

3.2 Enzymes

Enzymes are special proteins, which catalyze chemical reactions with high specificity. The Enzyme Commission, created by the International Union of Biochemists, published an enzyme classification system and more than 4000 were recognized. Nevertheless, 25000 natural enzymes have already been specified, which means that about 90% of the reservoir of these biocatalysts has yet to be discovered and characterized. Commercial exploitation of microbial enzymes began before their natures and properties were worked on. For a long time, plant extracts were used to cause the hydrolysis of polymeric materials. However, these

sources of enzymes were unreliable and expensive, hence the search for alternative sources. This was largely found in microbial cultures (VANDENBERGHE *et al.*, 2016).

Enzymes, which work under moderate conditions, have proven to be a viable option when compared to their chemical counterparts in various processes related to dairy, breweries, wines and juices, fats and oils, bakery, detergents, textiles, leather, cellulose and paper, and personal care industries (KAPOOR; PANWAR; KAIRA, 2016). The use of biocatalysts represents an important alternative to conventional chemical processes, since enzymes catalyze the reactions in a specific way, minimizing the generation of undesirable by-products, and act in mild temperatures, which reduces the energy cost of industrial processes (FLORENCIO; BADINO; FARINAS, 2017).

Almost 75% of the industrial enzymes used today have hydrolytic action, being used in the degradation of several natural substances. Approximately 200 original microbial types are used commercially. However, only about 20 enzymes are produced on a truly industrial scale (VANDENBERGHE *et al.*, 2016).

3.2.1. Amylase

The primary function of amylase is the hydrolysis of the starch molecule into units of glucose and oligosaccharides. There are two main forms of this enzyme which are alpha and beta-amylase. Alpha-amylases are endoglycosidases, which act at random sites by cleaving the internal α-1,4-D-glycosidic bonds between adjacent glucose units in a linear amylose chain, thereby producing smaller dextrins and oligosaccharides, with a C1-OH group in the α anomeric configuration. On the other hand, beta-amylases catalyze the hydrolysis of the second α-1,4-D-glycosidic bond, generating two units of glucose (maltose) at a time (SACHDEV; OJHA; MISHRA, 2016).

Aspergillus sp. between fungi and Bacillus sp. among bacteria, they are considered important amylase producers, being widely studied (SINGH; GUPTA, 2014). Amylases are one of the most important industrial enzymes with a wide variety of applications, from the conversion of starch to sugar syrups to the production of cyclodextrins for the pharmaceutical industry, in addition to their use in the food, textile, chemical, pharmaceutical, and detergent industries. It is worth mentioning that amylolytic enzymes are responsible for 25 to 33% of the world's production of enzymes (SILVA *et al.*, 2013).

3.2.2. Cellulase

Cellulase is a general term for cellulolytic enzymes, of which three classes are recognized based on the mode of enzymatic actions and the specificities of the substrate: endoglucanases, exoglucanases, and β-glycosidases (KUHAD *et al.*, 2016). They are the main enzymes responsible for the hydrolysis of cellulose, producing from its primary products such as glucose and cellobiose. Cellulolytic enzymes act synergistically in cellulose to break it down and are currently the subject of numerous studies due to its importance in the hydrolysis of biomasses (BUDIHAL; AGSAR; PATIL, 2016).

Cellulases can be produced by a wide variety of naturally occurring microorganisms, such as the *Aspergillus, Trichoderma, Penicillium*, and *Humicola* genera (CASTRO; PEREIRA JR, 2010). The ability to deconstruct lignocellulose, releasing glucose, which in turn can be converted into other valuable chemicals, such as ethanol, has made these enzymes very important in biofuel research. In addition, cellulases also have important applications in the sectors of food, detergents, and textile products (BUDIHAL; AGSAR; PATIL, 2016).

3.2.3. Xylanase

Xylanase is the enzyme that degrades xylan, the main polysaccharide present in hemicelluloses, cleaving the β-1,4 glycosidic bonds, thus forming usable products such as xylose. In plants and hardwoods, xylan is the most abundant non-cellulosic polymer, responsible for 20 to 35% of the total dry weight of biomass (IRFAN *et al*., 2016). Many studies have been carried out on the production of xylanases from thermophilic fungi as *Thielevia terrestris* and *Thermoascus crustaceus, Melanocarpus albomyces, Ceriporipsis subvermispora, Humicola grisea, Chaetomium thermophilum, Paecilomyces thermophila, Sporotrichum thermophile*, in addition to some species of the *Talaromyces* and *Therarascus* genera (ALI; IBRAHIM; ISAAC, 2013).

The application of xylanases in lignocellulosic materials provides greater availability of cellulose for the action of cellulases and contributes to an increase in the efficiency of the biomass degradation process. Besides, these enzymes have other commercial applications, which can be used in the paper industry, in improving the quality of animal feed, in the textile and food processing industries, as well as being used in the transformation of some agro-industrial wastes into fermentable sugars, for the production of second-generation ethanol (GOMES *et al.*, 2016).

3.2.4. Mannanase

Glucomannans form the largest hemicellulosic fraction of softwood and may represent up to 50% of hemicellulose in coniferous woods. They typically contain -1,4 bond linear chains of d-mannose and d-glucose residues in a 3/1 ratio. In contrast, in hardwood, this ratio is 1.5-2/1. These glucomannans are closely associated with cellulose and xylan as components of the cell wall, being organized in paracrystalline arrangements, adsorbed on the cellulose microfibrillar surface (VAN ZYL *et al.*, 2010).

After xylanases, mannanases are the second most important enzymes for the hydrolysis of hemicelluloses. These enzymes that randomly hydrolyze the βD-1,4 bonds of mannopyranose to β-1,4 of mannans have found applications in the paper and cellulose, pharmaceutical, food, oil, and textile industries (CHAUHAN *et al*., 2012). Filamentous fungi, such as *Aspergillus niger, Aspergillus nidulans*, and *Trichoderma reesei*, are considered to have great potential for the industry in the production of β-mannanases (WU *et al.*, 2011).

3.2.5. Invertase

The official name of invertase is beta-fructofuranosidase, which implies that the reaction catalyzed by the enzyme is the hydrolysis of the non-reducing terminal of β-fructofuranoside residues. This enzyme is a glycoprotein with an optimum pH of 4.5 and stability at 50°C (KULSHRESTHA *et al.*, 2013).

Invertase is then used for the inversion of sucrose in the preparation of inverted sugar and high fructose syrup. It is one of the most used enzymes in the food industry, where fructose is preferred over sucrose, especially in the preparation of jams and sweets, because it is sweeter and does not crystallize easily. The enzymatic activity of invertase has been characterized mainly in plants and microorganisms. Among the fungi, *Aspergillus niger, Thermomyces lanuginosus*, and *Penicillium chrisogenum* have been widely studied (UMA *et al.*, 2010).

3.2.6. Pectinase

Pectin is a major constituent of the primary cell wall of all terrestrial plants and involves a variety of polysaccharides rich in galacturonic acid. Pectinases hydrolyze the pectin present in the specific substrate and find its use and implementation in various industrial processes (SHAIKH *et al.*, 2018). They are enzymes applied especially in the juice and food preparation, paper, and cellulose industries (SETHI *et al.*, 2016).

Acid pectinases help reduce the cloudiness and bitterness of fruit juices, while alkaline pectinases are used in the textile industry for softening and degumming fiber crops, producing better quality paper, fermentation-based on substrates such as coffee and tea, extracting oil and pectic wastewater treatment (SHAIKH *et al.*, 2018).

Among all microbial pectinases, fungal sources are preferred, with strains of *Aspergillus* species dominating the industrial sector. Pectinases obtained through microorganisms account for 25% of global sales of food enzymes (SETHI *et al.*, 2016).

3.2.7. Protease

Proteases, also known as peptidases or proteolytic enzymes, constitute a large group of enzymes that catalyze the hydrolysis of peptide bonds in other proteins. This cleavage leads to the degradation of protein substrates into their constituent amino acids, or it can be specific, leading to selective protein cleavage for modification and post-translational processing. Proteases are classified as peptide hydrolases and constitute a large family of enzymes, divided into endopeptidases and exopeptidases, classified according to the position of the peptide bond to be cleaved (SOUZA *et al.*, 2015).

Alkaline proteases make up 60 to 65% of the global industrial market. Proteases catalyze or hydrolyze proteins and, therefore, play a vital role in various industrial applications. These proteases are the only class of enzymes widely used in detergents, pharmaceuticals, leather, and the food and agricultural industries (PANT *et al.*, 2015). Among the fungi most used for the production of proteases, in the fermentation of oriental foods are *Aspergillus awamori, Rhizopus oligosporus*, and *Rhizomucor miehei* (SINHA; SINHA, 2009).

3.2.8. Lipase

Lipases are a class of hydrolase, which catalyzes the hydrolysis of triglycerides to glycerol and free fatty acids, over an oil-water interface. In addition, these enzymes catalyze the hydrolysis and transesterification of other esters, as well as the synthesis of esters, exhibiting enantioselective properties. Microbial lipases gained special industrial attention due to their stability, selectivity, and broad substrate specificity (TREICHEL *et al.*, 2010). Several strains of fungi producing commercial lipases are quite dominant, including *Rhizopus, Rhizomucor, Aspergillus, Geotrichum*, and *Penicillium species* (VASEGHI *et al.*, 2012).

After proteases and amylases, lipases are considered the third class of enzymes in sales volume; moving billions of dollars, showing their versatility of application that makes them especially attractive for industrial uses. The potential for industrial-scale lipase applications includes the additive industry (flavor modification), fine chemicals (ester synthesis), detergents (fat hydrolysis), wastewater treatment (decomposition and removal of oil substances), leather (removal of fat from animal skin), pharmaceutical and medical (drugs, digestives, and enzymes for diagnosis) (RIGO *et al.*, 2010).

3.2.9. Laccase

Laccases are a diverse group of multi-copper proteins that oxidize a wide variety of organic and inorganic compounds, including diphenols, polyphenols, substituted phenols, diamines, and aromatic amines, with concomitant reduction of molecular oxygen in the water. Laccase is a dimeric or tetrameric glycoprotein and usually contains four copper atoms per monomer. To function, the laccase depends on the copper atoms distributed between the three binding sites (AFREEN *et al.*, 2016).

White rot fungi are the best laccase-producing organisms (BİRHANLI; YEŞİLADA, 2013). Among these, the basidiomycete *Phanerochaete chrysosporium*

has become the most used organism, due to its capacity to produce ligninolytic enzymes, rapid growth, and easy handling during culture techniques (GASSARA *et al.*, 2010).

The role of laccase in the degradation of lignin compounds and phenolic compounds has been evaluated in a large number of biotechnological applications, such as degradation of dyes, bioremediation of toxic chemical residues, wastewater and soil treatment and also biosensor developments (EL-BATAL *et al.*, 2015), as well as being widely used in biomass treatments for bioethanol production (MORENO *et al.*, 2012; ALVIRA *et al.*, 2013).

3.3. Cultivation Strategies for Fungal Enzyme Production

Previously, the methodologies used to produce enzymes were not well defined. The advent of fermentation technology made it possible to produce enzymes in large quantities and a well-characterized form. New developments, such as recombinant DNA technology, protein engineering, and directed evolution, have further revolutionized the development of industrial enzymes (KAPOOR; PANWAR; KAIRA, 2016).

Fermentation is the technique of biological conversion of complex substrates into simple compounds by various microorganisms, such as bacteria and fungi. During this metabolic decomposition, these organisms release several additional compounds in addition to the usual fermentation products, such as carbon dioxide and alcohol (SUBRAMANIYAM; VIMALA, 2012).

Solid-state fermentation (SSF) has been described as a process of transformation of insoluble matter that serves both as physical support and source of nutrients in the absence or almost absence of free water. However, the substrate must be moist enough to allow the growth and metabolism of microorganisms. Because this type of fermentation shows great potential in the production of

metabolites, this process is particularly useful in the production of microbial products, such as enzymes (SÁNCHEZ *et al.*, 2014).

In contrast, in submerged fermentation (SmF), also called liquid fermentation, nutrients are present in the form dissolved in a large amount of water. This method is useful in the production of enzymes that suffer from catabolic repression because residual substrates such as glucose and amino acids are kept in very low concentrations (SINHA; SINHA, 2009).

Table 2 shows some differences in parameters used in the fermentation processes, submerged, and in solid-state, for the production of enzymes using agro-industrial wastes.

Table 2 – Differences between the process parameters between solid-state fermentation and submerged fermentation for enzymatic production using agro-industrial wastes as substrates

Process Parameter	SSF	SmF
Water requirement for fermentation	Small	Large
Water requirement for "downstream"	Large	Small
Process control	No	Yes
Energy requirement	Low	High
Temperature maintenance	Poor	Good
Agitation (rpm)	3-150	100-500
Substrate particle size range	Broad (cm to inches)	Narrow (mm a cm)
Cultivation period	Long	Short
Product yield	High	Low

Source: Adapted from Kapoor; Panwar; Kaira (2016)

The main advantage of the solid-state fermentation technique is to use agro-industrial wastes, which are cheap alternatives, as the main substrate for the production of enzymes (VIJAYARAGHAVAN *et al.*, 2016). Other advantages are related to the simple culture medium and conditions close to the natural ones, reduction of the risk of contamination of the medium, decrease of liquid effluents to be treated, enzymatic extraction facilitated by the high concentration of products, low energy and water requirements (ORLANDELLI *et al.*, 2012).

Thus, although submerged fermentation is the most common method for commercial production of enzymes, the use of SSF is often indicated as a promising way to produce higher enzyme yields compared to SmF (Hansen *et al.*, 2015). In a study by Mohanasrinivasan *et al.* (2009), the comparison made between the two techniques showed that the yield in SSF was higher than that of SmF for two of the three strains of fungi tested for lipase production. The same was found by Okafor *et al.* (2010), whose research, involving 5 strains of different filamentous fungi and using wheat bran as a substrate, demonstrated that solid-state fermentation produced higher levels of pectinase activity than submerged fermentation.

3.4. Use of Agro-industrial Waste for Enzyme Production

In recent years, research on the selection of substrates for fermentation processes has been mainly on agro-industrial waste, due to its potential advantages. The use of agro-industrial waste provides alternative substrates, which can help to solve the pollution problems caused by their disposal. The nature of the substrate is the most important factor affecting fermentation processes. The choice depends on several factors, mainly related to cost and availability (TREICHEL *et al.*, 2010).

Fermentation techniques must be optimized for each material used. This is because an organism reacts differently to each substrate, as the utilization rates of various nutrients differ in each of these biomasses and, thus, affect productivity

(SUBRAMANIYAM; VIMALA, 2012). In addition, many studies have already demonstrated that pretreatment technologies for lignocellulosic substrates can increase enzyme yield several times (RAVINDRAN *et al.*, 2018).

The production of the most cited enzymes in the literature, being produced by different fungal species, exemplifying which of these are the most used in the process, and using different substrates, such as wheat bran and wheat straw, apple mulch, sawdust, rice straw, cane bagasse, etc., can be seen in Table 3.

Table 3 – Production of fungal enzymes using different agro-industrial wastes as substrates in solid-state and submerged fermentation processes

	Microorganism	**Agro-industrial Waste**	**Fermentation Process**	**Cultivation Conditions**	**Enzyme Activity**	**Reference**
Amylase	*Aspergillus awamori*	Babassu cake	SSF	144h; 30ºC	40,5 U/g	Castro *et al*., 2010
	Aspergillus flavus	*Shorea robusta* deoiled cake	SmF	72h; 30ºC; 200 rpm	26,4 U/ml	Singh; Gupta, 2014
	Aspergillus oryzae	Soybean husk and flour mill waste	SSF	144h; 30°C	47,000 U/g dry substrate	Melnichuk *et al*., 2020
Cellulase	*Trichoderma reesei*	Rice straw	SSF	96h; 30ºC	16,0 FPU/g 58,2 CMCU/g	Dhillon *et al*., 2011
	Aspergillus niger	Coir waste	SmF	72h; 30ºC; 120 rpm	2,3 FPU/ml 3,3 CMCU/ml	Mrudula; Murugammal, 2011
	Aspergillus niger	Green tea waste	SSF	96h; 30°C	2,5 U/g	Saldaña-Mendoza *et al*., 2021

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	Microorganism	Agro-industrial Waste	Fermentation Process	Cultivation Conditions	Enzyme Activity	Reference
Xylanase	*Trichoderma reesei*	Rice straw	SSF	96h; 30ºC	1298,7 U/g	Dhillon *et al*., 2011
	Trichoderma lanuginosus	Sugarcane bagasse	SmF	96h; 30ºC	411 U/ml	Ali; Ibrahim; Isaac, 2013
	Aspergillus fumigatus	Ragi husk	SSF	120h; 49,9°C	156,7 IU/ml	Saroj; Manasa; Narasimhulu, 2020
Mannanase	*Aspergillus niger*	Wheat straw	SSF	96h; 32ºC	36,7 U/g	Wu et al., 2011
	Aspergillus sydowii	Banana stem	SmF	168h; 30ºC; 120 rpm	1,2 U/ml	Siqueira *et al*., 2010
	Penicillium citrinum	Açaí seed	SSF	144h; 30°C	112 U/g	Lima et al., 2021
Invertase	*Aspergillus caespitosus*	Wheat bran plus oat meal	SSF	72h; 40ºC	181,8 U/g	Alegre *et al*., 2009
	Cladosporium cladosporioides	Pomegranate peel	SmF	96h; 30ºC; 125 rpm	31 U/ml	Uma *et al*., 2012
	Penicillium sp.	Orange peel	SmF	168d; 28 °C; 180 rpm	1,98 U/ml	Nehad; Atalla, 2020
Pectinase	*Aspergillus oryzae*	Citrus waste and sugarcane bagasse	SSF	24h; 32ºC	45U/g	Biz et al., 2016
	Aspergillus terreus	Sawdust	SmF	96h; 30ºC; 100 rpm	600 U/ml	Sethi *et al*., 2016
	Aspergillus niger	Pomelo peels	SSF	168h; 50°C	61,54 U/mg	Jalil; Ibrahim, 2021

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Conclusion

	Microorganism	Agro-industrial Waste	Fermentation Process	Cultivation Conditions	Enzyme Activity	Reference
Protease	*Aspergillus oryzae*	Wheat bran	SSF	48h; 30ºC	59,9 U/g	Castro; Sato, 2013
	Aspergillus awamori	Peanut meal	SmF	96h; 30ºC; 220 rpm	0,2 U/ml	Sinha; Sinha, 2009
	Aspergillus brasiliensis	Orange peel	SSF	72h; 30°C	1604 U/ml	Chimbekujwo; Ja'afaru; Adeyemo, 2020
Lipase	*Rhizopus oryzae*	Sugarcane bagasse	SSF	72h; 45ºC	215 U/g	Vaseghi *et al*., 2012
	Trichoderma harzianum	Corn oil	SmF	48h; 28ºC; 180 rpm	0,8 U/ml	Coradi *et al*., 2013
	Aspergillus brasiliensis	Malt bagasse	SSF	96h; 32,7°C	9,8 U/g	Eichler *et al*., 2020
Laccase	*Phanerochaete chrysosporium*	Apple pomace	SSF	192h; 37ºC	720 U/g	Gassara *et al*., 2010
	Trametes versicolor	Wheat straw	SmF	72h; 30ºC; 150 rpm	0,8 U/ml	Birhanli; Yeşilada, 2013
	Trichoderma viridae	Corn cob	SmF	216h; 25°C;	2,2 U/ml	Nuhu *et al*., 2020

Several enzymes of industrial importance were extracted from fungi belonging to the genus *Aspergillus*. The highlight of this genus is so great that it was studied as a model organism for the production of fungal enzymes. A. *niger* is the largest source of enzyme-producing fungi. As can be seen from Table 3, in addition to the enzymatic activity being affected by the substrates, the metabolic differences between SSF and SmF have a direct impact on productivity by the fungi of the genus (SUBRAMANIYAM; VIMALA, 2012).

In most cases, filamentous fungi are preferred for SSF processes due to their unique ability to colonize the porous spaces of solid matrices and to secrete various enzymes to hydrolyze the solid material. Agro-industrial wastes are relevant substrates for SSF processes since they are rich in polymeric sugars, such as cellulose and hemicellulose, which can be converted into simple sugars, thus being assimilated by fungi (MARZO *et al.*, 2019).

The fungal enzymes production can be improved using multivariate approaches such as a combination of different substrates from agro-industrial residues, incubation conditions (temperature, agitation, cell immobilization, etc.), types of bioreactors, nutrient concentration, and others. A single by-product may not provide all the essential nutrients for microbial growth (LEITE *et al.*, 2021). Filipe *et al* (2019) uses a mixture of olive mill wastes with winery wastes in SSF using *Aspergillus niger* and A. *ibericus*, that results in a production of 189,1±26,7, 56,3±2,1 and 10,9±0,8 U/g of xylanase, cellulose, and Beta-glucosidase enzymes, respectively. Rayhane *et al* (2019) evaluate the production of lytic enzymes by *Trichoderma asperellum* using a mix of vine shoots, jatropha, olive pomace and olive oil as substrates. The authors reached values to cellulose, amylase and lipases of 19.36 ± 0.19(44 h), 15.22 ± 0.13(72 h) and 38.73 ± 0.01 (48 h), respectively.

To optimization of microbial enzyme production the multi factors approaches is necessary. To do this, is frequently adopted the use of methodological strategies to investigated the effects of these factors as one-factor-at-a-time (OFAT) or design of experiments (DOE) as factorial design. In the first case, generally more experimental samples is necessary and has a difficult to determine the interaction of different factors tested (Kazemi *et al.*, 2016). Therefore, several works uses the DOE as a tool to reduce the experimental runs and the cost of experiments. Also, the DOE enables the researcher to a more comprehensive interpretation of interactions of your tested variables, probably let it a better choice of the ideal condition to produce your molecule (Kazemi *et al.*, 2016).

4 CONCLUSÃO

Enzymes can come from plants, animals, and microorganisms. The microorganisms are the main sources due to the economic and technical benefits (with higher enzyme yield in a shorter fermentation time). In addition, these organisms can naturally be subjected to genetic manipulation to improve productivity or a certain catalytic characteristic of the enzyme.

Although several microbial sources have been studied for the production of enzymes, many can still be discovered with better use of agro-industrial residues to obtain higher enzyme yields. The use of these materials for microbial fermentation, in addition to improving the economics of the process, can assist in the environmental problem related to the large generation and disposal of these residues in the environment.

The enzyme market does not stop, quite the contrary, it is on the rise. Thus, new products are emerging with new features and functions, resulting in new applications. For this, different areas of biotechnology such as genetic engineering of microorganisms and enzyme engineering work together and are of great importance soon, which will be even more technological, moving towards a more sustainable industrial development.

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