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METHODS FOR PRETREATMENT OF LIGNOCELLULOSIC BIOMASSAND BY-PRODUCTS FOR LIGNOCELLULOLYTIC ENZYMES MODIFICATION: A REVIEW

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ABSTRACT

Because of its year-round availability as a sustainable source for industrial use, lignocellulosic content has gained considerable interest in the scientific community.Various industries reassess processes that integrate derived compounds from these materials as an alternative to the economic substratum. These varieties of fungi and bacteria are capable of depolymerizing degradant enzymes by lignocellulose biomass synthesis.Lignocellulolytic enzymes from fungi currently have a high variety of industrial applications due to the stability of catalytic activities and their high conversion rates. These products, in addition, deliver low energy inputs as economical, environmentally sustainable and non-toxic. For existing enzyme production technologies, techno-economic research suggests that synthetic production is not commercially viable. Rather, the economic forecasts for the use of the ligninolytic enzymes generated naturally are encouraging.

Keywords: Lignocellulosic by-products; Fungi; Lignocellulolytic enzymes; Catalytic activity.

1. INTRODUCTION

Lignocellulose is a highly degenerative polymer substance. Its structure consists mainly of a network of 1) cellulose (40-50 percent): formed by glucose monomers and the main component of plant cells that gives them strength; 2) hemicellulose (20-30 percent): a hexose, pentose and sugar acid polymer; and 3) lignin (10-25 percent): made up of three aromatic alcohols that form a protective shield around the other two polymers (Fig-1) (Sun et al., 2011), (Iqbal et al., 2011), (Durães Sette & Costa Bonugli Santos, 2013), (Damm et al., 2016). Lignocellulose composition

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can vary depending on the source. Due to its design, however, lignocellulose saccharification is very difficult (Camacho-Morales et al., 2017). The conventional method for lignocellulosic waste degradation is acid hydrolysis and thermal shock, although it involves high energy cost procedures and unfavorable environment effects. In the case of acid hydrolysis, in particular, the most common method involves high acid concentrations and long retention periods. This not only means high costs, but also contributes to the production of toxic substances that can interfere with downstream fermentation. (V. K. Gupta et al., 2016), (Bak et al., 2009), (Damm et al., 2016), (P. Kumar et al., 2009). Intense energy costs, high costs and carbon pollution include physical processes such as mechanical extrusion and pyrolysis comminution; (Mohan et al., 2016), (P. Kumar et al., 2009).

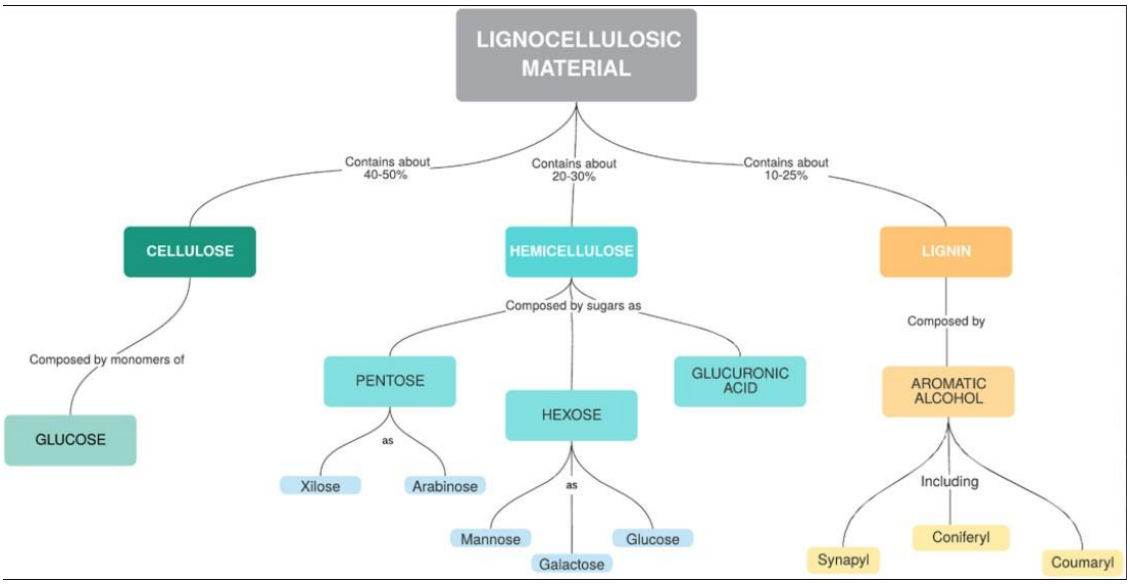

Fig. 1: Structural scheme of lignocellulosic material.

Cosy technology, high energy consumption is needed for physicochemical catalysis such as wet oxidation, carbon dioxide, plasma, and steam exploding. The conversion could also lead to the production of toxic substances, as most processes involve high pressure and temperatures. Pre-treatment of biomass may produce enzyme inhibitory compounds.(Haghighi Mood et al., 2013).

An additional technology is being developed to increase the rates of biomass transformation, as are the ionic liquid (ILs) and the super-critical fluid. (Gu et al., 2013). The most famous fluid in this technology is supercritical CO2. CO2 is cheap but demands high pressure and high temperatures which demand more energy and demand for manufacturing processes. (Gu et al., 2013). Biomass structure can be altered by disconnecting connections between cellulose, hemicellulose and lignin. The output for hemicellulose conversion is better than conventional

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biomass hydrolysis methods, reaching up to 99% in 4 hours at 160oC (Matsagar & Dhepe, 2017). The requires a significant amount of ILs are that with few exceptions they are costly (e.g. cholinium). Although many ILs are non-biodegradable and unsafe for proteolytic enzymes, plants and animals. (Frade & Afonso, 2010), (Shirkavand et al., 2016). In addition, ILs are heavier than water and become more viscous throughout the process, resulting in difficulties using them. (Haghighi Mood et al., 2013), (Asim et al., 2019).

On the other hand, enzymatic hydrolysis may be advantageous for the degradation of lignocellulose, allowing polymers with low energy consumption and high specificity to be effectively broken down without the creation of toxic by-products. (Fig-2) (Table-1) (V. K. Gupta et al., 2016), (Haghighi Mood et al., 2013), (Singh et al., 2016). The technology has shown the efficacy of lignocellulosic by-product hydrolyses with enzyme systems; some drawbacks still exist today, and they must be overcome if their use is to be extended. Firstly, the digestion time must be decreased so that hydrolysis conditions can be optimized. The second is that in few companies that sell the industrial enzymes, so the cost of the enzymes is very high as the low supply chain (Singh et al., 2016). This application of enzyme hydrolysis with other physical, chemical or physicochemical pretreatment methods has shown that the time taken for the conversion of biomass has been reduced and more cost-effective. However, further studies in combined processes are required to explore environmental effects and energy balance (Shirkavand et al., 2016).

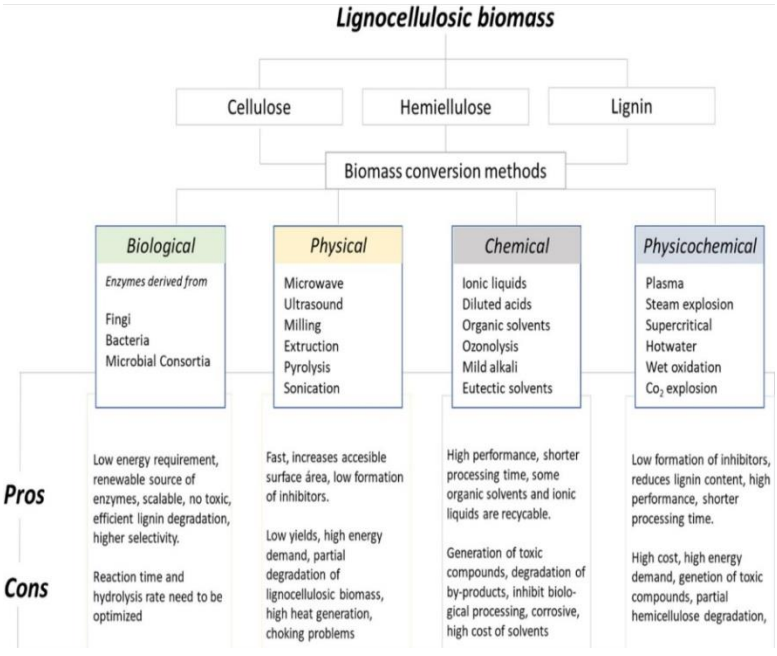

Fig. 2: Comparison between different biomass conversion methods

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Table 1: Advantages and drawbacks of enzyme-catalyzed biomassconversion.

Enzymatic hydrolysis		
Advantages	**Drawbacks**	**Reference**
Effective in degradation of lignin and hemicellulose at normal temperature. Low energy consumption. Environmentally friendly.	Low rate of hydrolysis	(Bhatt & Shilpa, 2014)
Organisms will degrade lignin. Low energy requirement. Mild reaction conditions.	Long reaction time 2 to 4 weeks	(Ussiri & Lal, 2014)
High selectivity toward desired product	Narrow operating conditions. High volume of waste water.	(Chatterjee et al., 2015)
Whole cell (e.g. fungi or bacteria) biocatalytic processes are scalable	High volume of waste water produced during product purification	
No inhibitors generation No chemical or harsh conditions required Low energy requirements	very low rate at experimental stage	(Saini et al., 2015)
Carry out at mild conditions at 40–50 °C	High cost of catalysis. Takes longer than other methods.	(Jahnavi et al., 2017)
High yields of sugars can be obtained No inhibitors formation	Product inhibition during hydrolysis	
Low energy requirements Delignification Reduction in degree of polymerization of cellulose Partial hydrolysis of hemicelluloses No chemical requirements	Slow process rate Very low treatment rate Not very effective for commercial application	(Ingle et al., 2019)

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Mild environmental conditions	
No protection/deprotection steps involvement	(Parra-Saldivar et al., 2020)
Economical	
Environmentally friendly	
Display regioselectivity, diastereoselectivity, enantioselectivity, and chemoselectivity	

Most of those fungi and bacteria have formed in a synergistic system through the development of lignocellulolytic enzymes which have the capacity to break down key LCB polymer. (2) cellulolytic enzymes like endoglucanase, exoglucanase, and ß-glucoside, and (3) hemicellulolytic enzymes like endoxylase, arabinofuranosidase, β-xylosidases and feruloyla esterase, and other cellulolytic enzymes able to deteriorate lignocellulotic biomass in the system of a synergistic system these enzymes are: (1) the laccae enzyme, lignin peroxidase and polymorphosa (Fig-3) (Jayasekara & Ratnayake, 2019). These groups are called lignocellulolytic enzymes that can degrave the main polymers of lignocellulosic materials.

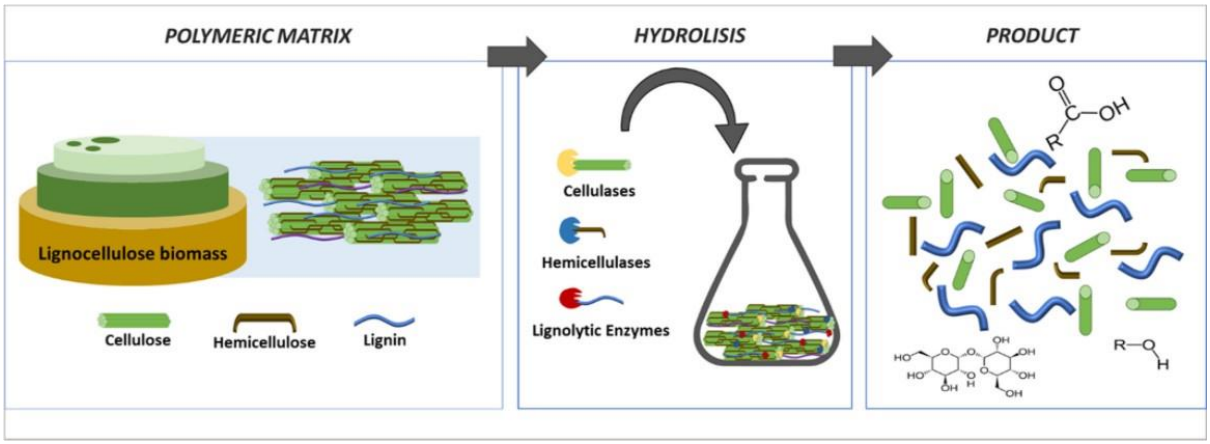

Fig. 3: Enzymatic hydrolysis of lignocellulosic material.

Interestingly, the lignine, including cellulose, can be degraded effectively by a number of selected groups of fungi such as white-red fungi, Phanerochaete chrysosporium, Ganoderma lucidum, Pleurotus dryinus, Pleurotus ostreatus, Tramethe versicolor and Trametes hirsuta (Anwar et al., 2014), (Mattila et al., 2017), (Millati et al., 2011), (Karimi & Zamani, 2013),(Manavalan et al., 2015). Filamentous fungi such as Aspergillus niger, Trichoderma viride and Penicillium nonetheless effectively decumb, but lignin can only partially degrade(Anwar et al., 2014), (Asgher et al., 2013).

There are currently several industrial applications using a variety of lignocellulolytic enzymes extracted from fungi. One of the best applications was the enzyme hydrolysis in the second

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generation bioethanol production systems. (Anwar et al., 2014), (P. Kumar et al., 2009), (Asgher et al., 2013). Other applications of notorious importance are also: 1) Development of the feed supplement to improve the digestibility of nutrients. 2) Paper as a sustainable pulp blanching alternative. 3) Flavor and aromatic characteristics of the wine/fruit juice food industry. 3) 4) Manipulation of bread dough texture. 5) Take out recycled paper ink. 6) Textile dyes for bleaching. 7) Biorestructuring of the soil. 8) Synthetic chemical oxidative deprotection (Khambhaty et al., 2018), (He et al., 2010), (Jordan & Wagschal, 2010) And further explained other more applications.

At economic level there is a great potential for producing enzymes involving the deterioration of lignocellulosic by-products, adding value to waste materials, thus reducing the environmental impact of waste materials. (Singh et al., 2016). Lignocelulosis biomass degradation systems, lignocellulolytic enzymes derived from fungi are discussed, their projected demand, challenges and emerging trends for industrial and commercial use of lignocellulolytic enzymes are discussed.

2. LIGNOCELLLOLYTIC ENZYME STATISTICS AND MARKET VALUE

Due to its broad industrial uses (e.g., chemicals development and degradation of recalcitrant contaminants, medicines, biofuels, textiles, foods and drinks), and others, the lignocellulolytic enzyme demand has increased over recent years(Frazer, 1999)**.** It makes them key enzymes for developing and developed countries' economic growth because of their effect on industrial processes relating to goods and services development. (Jayasekara & Ratnayake, 2019). The demand for industrial enzymes could increase from $5.5 billion in 2018 up to $7.0 billion by 2023 at a compound annual rate of growth (CAGR) of 4.9% (2018–2023) as stated by Business Communication Company (BCC) Study. (Dsm, 2019). Enzymes in the textile, leather, paper and bio-diesel industries are increasingly produced, especially enzymes. This leads to the approach of immobilized biocatalyst scaling processes (Chapman et al., 2018).

All of the industrial enzymes requested are primarily divided into three categories: manufacturing, agriculture, and the environment, with an annual growth rate of up to 8%. (Fig-5). Largely condensed in several developed countries, including the U.S., Germany, the Netherlands, Denmark and Suiza, North America represents the bigest market for industrial enzymes in the world (40 per cent), led by Europe (30 per cent), (Nunes, 2018),(Jaramillo et al., n.d.). Developing Asia-Pacific countries are expected to broaden their markets in order to meet nutrition demand. Brazil, a Latin American country, is a leader in bioethanol production and consumption worldwide. The increased interest in lignocellulolytic enzymes is related to their use of lignocellulosic content for the development of second-generation agroethanol. (Nunes, 2018), (Sarrouh, 2012).

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Against this backdrop, there will obviously continue to expand the industrial enzyme market, as biotechnology developments in development are currently very small. Thus, research should aim at discovering new sources in this sector such as fungal derived enzymes, cost-effective alternatives for scaling up production and optimizing the general conditions to meet the global demand for lignocellulosus products and services.

3. ENZYMESDEGRADATION OFLIGNOCELLULOSE

Lignocellulose is the world's most abundant polymer; lignocellulose content contains nearly half of the biomass. (Toushik et al., 2017). As major substrate, cellulose, hemicellulose and lignin polymers are found in lignocellulose degrading enzymes. These enzymes are important if they are used by various species for the development of many other added value chemical products and metabolites. very complex matrices in available sugars. (Abdel-Hamid et al., 2013). The classification of lignocellulolytic enzymes in general is described Fig-4

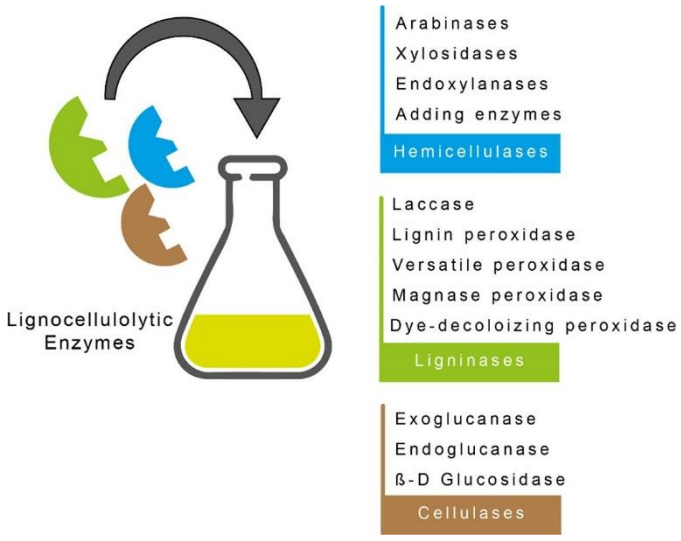

Fig. 4: Classification of lignocellulolytic enzymes

3.1. The Cellulose

Cellulose has been one of the world's most abundant organic polymers, provided mainly by plants (Srivastava et al., 2021). Cellulose applications are widely recognized among the most important industries that use it, including paper and pulp, textiles, detergents, food, animal industries, chemicals, fuel and pharmaceutical goods. (Srivastava et al., 2021), (Arrieta et al., 2016), (Molina et al., 2016). It is also the most abundant and essential structural components of

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the plant's primary cell wall, which is made up of many units of D-glucose connected by the glycosidic relations. (Garcia-Maraver et al., 2013), (Hosseini & Shah, 2011), (Jia et al., 2020).

Due to the strong hydrogen bonds and cyclic structure of the main polymer chain, the rigid cellulose structure (Poletto et al., 2014). There are, therefore, a high melting point and glass transition temperature for cellulose and some derived components. (Zhao et al., 2016). Additionally, the intramolecular hydrogen bonds between the hydroxyl groups are a function of an insoluble amount of cellulose. Such bonding produces an ordered crystalline zone blocks access to several organic solvents and water, so a strong alkaline such as caustic soda is important for breaking the main structure and making hydroxy groups accessible to reactive. (Garcia-Maraver et al., 2013).

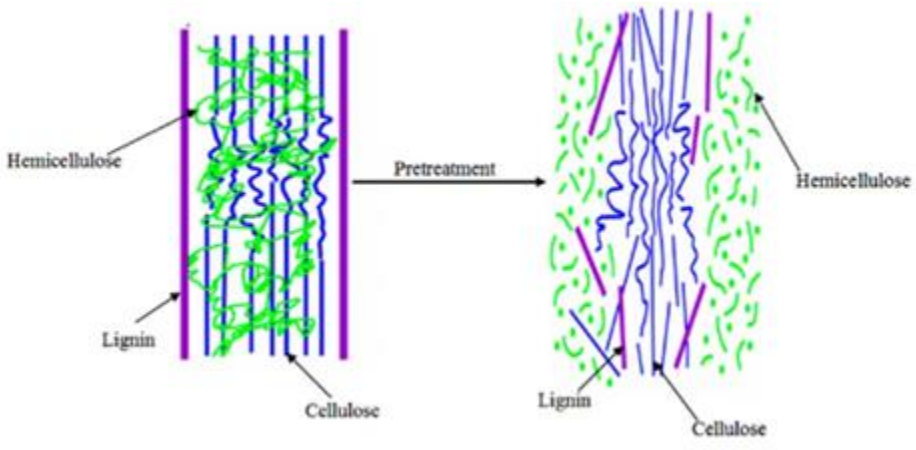

Fig. 5: Schematic of the role of pretreatment in the conversion of biomass to fuel. (Adapted from (P. Kumar et al., 2009)

Cellulases are a series, carried out by two stages of Hydrolysis, of enzymes responsible for the bioconversion of cellulose to soluble sugars and glucose. Endoglucanases and exoglucanases degrade the substratum in the first stage and release the complex organized sugars to the liquid process with a polymerization process of up to six degrees (Jayasekara & Ratnayake, 2019). β-glucosidase converts cellobioses into glucose in the second stage (Toor et al., 2020). Some microorganisms, such as bacteria, fungi and actinomycets, can generate three types of cellulase-containing components: exo-1,4-β-D-glucanase; endo-1,4-β-D-glucanase;(Horn et al., 2012)(M. Zhang et al., 2020), (Yao et al., 2016)In addition, fungi are known to create monooxygenasses of lytic polysaccharides, degrading cellulose of cellobiosis. The following are listed in greater detail in these five components.

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3.1.1. Exoglucanase

Exo-1,4-β-D-glucanases include the 1,4-β-D-glucan glucohydrolases (EC. 3.2.1.74) which help release D-glucose from 1,4-β-glucans and then hydrolyze D-cellobiose progressively and 1,4-β-D-glucan cellobiohydrolase (EC. 3.2.1.74), which is in charge of detaching D-cellubiose from 1,4-β-glucans (Zhao et al., 2016). As processive enzymes, exo (1,4) β-D-glucanases can separate from the cellulose polymer by the reducing or non-reducing end, and then start producing units of cellobiose (Langston et al., 2011) or glucose as major products (Brum et al., 2012). Like other cellulolytic enzymes, exoglucanases are widely used in the industry, as it will be detailed further on in this review.

3.1.2. Endoglucanase

Also known as 1,4-β-D-glucan–4–glucanohydrolase (EC 3.2.1.4) is endoglucanases. They are randomly cut in internal amorphous positions, which are β (1,4) glycosidic bonds, into oligosaccharides by cellulosic chains (Molina et al., 2016). These enzymes are present in many species, most commonly bacteria (anaerobic and aerobic), thermophilic and mesophilic microbes and fungi, but are also widespread in nematodes, protozoans, insects and molluscs which are complex to degrade cellulolytic materials in enzymatic batteries (Jordan & Wagschal, 2010), (Shah et al., 2019). Fungi are the most commonly used species for the development of lignocellulolytic enzymes at commercial levels (e.g., Trichoderma, Phanerochaete, Aspergillus, Trametes, Penicillium) (Karim et al., 2015). Endoglucanases have potential uses in the textile, paper and food industries (Brum et al., 2012).

3.1.3. β-D Glucosidase

The final stage of cellulose hydrolysis is induced by these enzymes (EC 3.2.1.21). They release cellulodextrin and cellobiosis from soluble D-glucose units (Yao et al., 2016). β-D Glucosidases are crucial for the cellulase degradation system, since they induce the cellulase enzyme system by synthesizing gentiobiose and sophorose (Naraian & Gautam, 2017). β-glucosidases are typically formed in an extracellular or intracellular manner by mammals, plants, fungi and bacteria. This enzyme has a high trade potential, as it has a broad range of applications in various industries such as textiles, animal feed, esthetics, natural polymers, organic chemicals, etc (Naraian & Gautam, 2017).

3.1.4. Lytic polysaccharide monooxygenases

These copper enzymes are connected to crystalline cellulose degradation (Karim et al., 2015). In order to activate the molecular oxygen at their copper active position, LPMO (EC 1.14.99.54) requires a reduction substrate (Frommhagen et al., 2017). Artificial reducers like ascorbic or

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gallic acid are usually employed for reducing Cu2+ to Cu+. The Cu+ ion is then reacted to a reactive copper-superoxide complex by converting O2. The monooxygenase activity and the oxidation of crystalline polysaccharide chains are accomplished by this reaction. In synergistic actions with CDH LPMO can work because these enzymes can also supply electrons as the reductants (Karim et al., 2015), (Frommhagen et al., 2017).

3.1.5. Cellobiose dehydrogenases

CDH produces white red fungi (EC 1.1.99.18). These extracellular enzymes have distinct domains of flavin and heme. (Karim et al., 2015), (Sulej et al., 2019). After the flavin domination is intra molecular electron transferred, the heme domination is able to transfer electrons from cellulose products to LPMO (Karim et al., 2015). CDH generates lactone and releases a reduced accepter using a cellobiosis and an acceptor like FAD, in this case a FADH2 (Sulej et al., 2019). CDH is also a catalytic LPMO electron donor. (Laurent et al., 2019).

3.2. Hemicellulose

In strengthening the cell walls of the plants Hemicellulose plays an important role by forming a system of hydrogen bonds that provide high stability and rigidity in cell walls, along with cellulose fibers and lignin residues. (Guerriero et al., 2016), (Ibn Yaich et al., 2017), (Kim et al., 2017). Hemicellulose is the second most common component of lignocellulose. (Jayasekara & Ratnayake, 2019). It contains most of pentose, as xyloosis and arabinose and is a complex and heterogeneous polymer. The pentose forms a β-(1 to 4) connection that conforms to the linear structure of the backbone, where its side groups are substituted in the majority of cases with hexosis such as galactose, glucose, manneses, and sugar acids. (X. Zhang et al., 2011). The variation in the composition of Hemicellulose is determined by plant species, type of tissue and stage of growth. Hemicellulose is primarily composed of xylans in angiosperms (monocots and dicots), while glucomannans, in addition to xylans, are main components of gymnosperms. (Eronen et al., 2011), (Tenhunen et al., 2014), (Naidu et al., 2018), (Penttilä et al., 2013). Because of their broad concentration in the base structure, hemicellulose in cereals is often referred to as arabinoxylans. On the other side, hardwoods have a high D-glucuronic acid concentration which is called glucuronoxylan. The other two classifications are homoxylene, where the xylose units are bound to β-1,4 bonds by the composition of the main chain of the hemicellulose structure, and glucuronoarabinoxylene which alternates between xylose, D-glucuronic acid and L-arabinosis monomers by its main chain (Polizeli et al., 2005), (Sun et al., 2011). It is a short strand arrangement of 100 to 200 residues, with ramifications which cause instability and make hydrolyzing much easier compared to homogeneous cellulose structures.

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However, some recurrent oligomeric structures, complex branching and acetylation are affecting depolymerization (Kameshwar & Qin, 2017).

It is a short strand arrangement of 100 to 200 residues, with ramifications which cause instability and make hydrolyzing much easier compared to homogeneous cellulose structures. However, some recurrent oligomeric structures, complex branching and acetylation are affecting depolymerization (Broeker et al., 2018). Without enzymes attacking its many implications, a complete xylanolytic system would not be like that. Xylanases are identified by genetic data and structural analysis (Bennett, 2003), (Motta et al., 2015).

3.2.1. Endoxylanases

Without enzymes attacking its many implications, a complete xylanolytic system would not be like that. Xylanases are identified by genetic data and structural analysis (EC 3.2.1.32). The same form of bond breaks down with carbon 1 and 3, resulting in xylobiosis, xylotriosis and xylotetraose formation. The non-reducing residue of the alpha-D-xylose terminal is hydrolysed and released from alpha-d-xylose (EC 3,2.1.177) (Luo et al., 2016), (Carli et al., 2016). As an animal feed supplement, endoxylanases were marketed. Endo-1,4-beta-xylanase (EC 3.2.1.8) is used to enhance digestibility of nutrients in weaned pork (He et al., 2010). Modification of textures, resistance to dough and crumb structures, and the application of these enzymes in the food industry may be manipulated. (Dornez et al., 2007), (McPhillips et al., 2014), (Wang et al., 2018). Some other test for endo-1,4-beta-xylanase is a sustainable alternative to pulp bleaching in the paper industry (Khambhaty et al., 2018) and the elimination of excess photocopying material (Pathak et al., 2014).

3.2.2. Arabinofuranosidase

In the GH43 band, the arabinose side-chains of the backbone of xylose residues include an α-L-arabinofuranosidases (EC 3.2.1.55) and endo-α-L-arabinanases (EC 3.2.1.19). These behaviors are unique to any organism that degrades the cell wall. The glycosidic bond between L-arabinofuranosides is broken by the enzymes of the GH51 family. One illustration is that alpa-L-arabinofuranosidase is not reduced at the end. (Gilbert et al., 2008) removing the side chains of Arabinose by hydrolyzing the bond between Arabinofuranose at the non-shrinking terminology of Arabian (Dornez et al., 2007),(McPhillips et al., 2014),(Wang et al., 2018). The potential food sweetener and inhibition of sucrose digestion characteristics of alpha-L-arabinofuranosidase have been assessed. Another use in the food field of aromatic and flavor-related properties in wine and fruit juices and manipulation of bread dough texture. Pumps in the paper industry and the manufacture of bioethanol are other industrial applications of alpha L-arabinofuranosideas to enhance digestion and the processing of pulp. (Broeker et al., 2018), (Gilbert et al., 2008).

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3.2.3. β-D-Xylosidases

The GH43 family is made up of β-D-Xylosidases. Xylan-1, 4-β-xylosidase (EC 3.2.1.37) removes residue of xylan from ends that do not have xylooligosaccharide reduction agents(Juturu & Wu, 2012), (Ghosh et al., 2019). Also capable of hydrolyzing xylobiosis is the development of monosaccharide xylose. β- D-xylopyranosidase extracts glycosyl residues from their substratum's non-reducing ends, according to CAZy. according to the GH3 family. In different industries, Xylan-1,4-β-xylosidase (EEC 3.2.1.37) plays a role in extracting ink from recycled paper, processing wood pulp, bread dough consistency and wine-producing aroma release (Jordan & Wagschal, 2010), (Gu et al., 2013).

3.2.4. Esterases

Acetyl-xyl esterase releases o-acetyl groups into acetyl-xylans with hydrolyzing acetyl ester bonds (Penttilä et al., 2013), (Motta et al., 2015). The enzyme (EC 3.1.1.72) Catalyzes the polymer xylon, xylatedacetylon, glucose acetylon, α-Napthyl acetate and p-nitrophenyl acetate hypersensitive and without effects on mannan or pectin acetylated. Catalyzes Feruloyl esterase (EC 3.1.1.73) is hydrolyzing feruloyl ester bonds to xylanes to remove ferulic acid from arabinoses.(Penttilä et al., 2013), (Motta et al., 2015). Esterases are also called accessory enzymes for hemicellulase as they hydrolyze hemicellulose from pectinases and xylanases. For the paper blanching procedures of the sulfite pulp, acetyl-xylan esterase (EC 3.1.1.72) was utilized. This enzyme has been identified as a potential medicine agent in hydrogel development by the pharmaceutical industry (Adesioye et al., 2016).

3.2.5. Aiding enzymes

Licheninase (EC 3.2.1.73) works on (1 x > 4)-beta-D-glycoside links in beta-D glycoside bonds (1 x 3) and (1 x 4) / ligaments. Unique to xyloglucano oligosaccharides, Xyloglucano-specific endo-beta-1,4-glucanase (3.2.1.15) has an effect not on other cell wall components yet. Xylose bonds with a xylooligosaccharide release exo—(1.5) Xilanase β-1.4 Xylose bonds xylooligosacchariids (EC 3.2.1.156) (Penttilä et al., 2013), (Motta et al., 2015). Alpha-glucuronidase (EC 3.2.1.139) (Gilbert et al., 2008) enables the hydrolyzing of alpha 1,2-glycosidic glucuronoxylan ties to eliminate side chains of glucuronic acid. (CAZy; ~www.cazy.org/). Because of its thermostable properties, licheninase has a possible use in the beer industry and feed industry. (Niu et al., 2016). As for alpha-glucuronidase (EC 3.2.1.139), bioethanol processing and wood pulp bleaching are industrial applications(Adebami & Adebayo-Tayo, 2020).

3.3. Lignin

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In all lignocelluloses, lignin is a complex biopolymer. It is formed during photosynthesis and often has a link in cell walls with cellulose. It has no "defined" structure because of its complexity and can differ from type to type of plant. There are 3 major monomers of p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. Polymerization once, The p-hydoxyphenyl units, guaiacyl unit, and syringeyl unit form three correspondence phenylpropanoic monomeric units (Davis et al., 2016), (Marcelo et al., 2013). They are all comprised of a phenolic community that constitutes the main substratum for enzyme deterioration.

Lignin is resistant to degradation because of its hetepolymeric, amorphous and complex composition. (Suárez Arango & Nieto, 2013), (M. Li et al., 2016). There are a variety of ways to depolymerize. The enzyme method is the course of white-red fungi. Because of lignin variation and size, enzyme systems are not unique and occur outside the cell (extracellular). (Asier et al., 2010). While this is a process involving a variety of enzymes, five are most effective: DYPs, flexible Peroxidase (VP), Manganese Peroxidase (MnP), Lignin Peroxidase (LiPs), and laccase. Therefore, there are five enzymes in the body: (Lacc) (Abdel-Hamid et al., 2013), (Camacho-Morales et al., 2017). This enzyme output is equalized with the production and degradation of lignin, which is why it is known as non-specific. For example, in Nitrogen-regulated organisms, LiP and MnP are regulated by nitrogen restriction (Manavalan et al., 2015), (Camacho-Morales et al., 2017). The following are further explained: priority enzymes, aryl-alcohol oxidases, glyoxidae, unspecific peroxygenases, aryl-alcohol dehydrogenases, and quinone reductases.

3.3.1. Dye-decolorizing peroxidases

DyPs will decarboxylate non-phenolic substrates associated with lignin as their key product p-anisaldehyde(Abdel-Hamid et al., 2013). You have a prothetichema group and you have a fold like ferredoxin(Camacho-Morales et al., 2017). By using phezolic lignin dimers, this enzyme indicates its potential for oxidase. A large range of DyP products include Vanillin, Guaiacol, Cresol and many other by-products(Abdel-Hamid et al., 2013). These enzymes, as the name implies, are used for decoloration and treatment of wastewater because they are used to dissolve high redox anthaquinone dyes, β-carotenes, aromatic sulfides, azo dyes and manganese. (Santos et al., 2014).

3.3.2. Versatile peroxidase

Via different oxidative sites and hemes, these enzymes (EC 1.11-1.16) are known (Abdel-Hamid et al., 2013). The catalytic activities of MnP and LiP can be performed with several peroxidases that can oxidize Mn2+ and high redox potential NPCs (Abdel-Hamid et al., 2013). Its catalytic diversity makes it possible to apply it in reactions mediated or independent of Mn3+ or Mn, resulting in convenient use of high and low redox potential aromatic substrates for such reactions

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(Abdel-Hamid et al., 2013). It is also considered a "hybrid" enzyme, called MnP-LiP (Camarero et al., 1999). VPs are used for decoloration of industrial dyes, toxins, industrial biocatalysts and bioremediation in textile industries (Camacho-Morales et al., 2017), (Tinoco et al., 2007), (Asgher et al., 2008), (Karigar & Rao, 2011).

3.3.3. Manganese peroxidase

The major distribution companies in MnP are fungi from white to red (EC 1.11.1.13). This glycosylated hemoprotein uses Mn2+ to bind Mn+3 ions to dicarboxy and alpha-hydroxy acids as oxidizing substrate.(Camacho-Morales et al., 2017). Almost every wood-colonizing fungus secretes these enzymes and is seen as the most frequent peroxidase for lignin transition. (Camacho-Morales et al., 2017). Phenolic substrates have an affinity, but MnP can also function on non-phenolic compounds(Asier et al., 2010). For processes such as bioblasting, pulping and purification of bleiching plant effluent pulp and paper industries, these enzymes are used (Winquist et al., 2008). Often used in the dairy, clothing, pharmaceutical and bioremediation industries are MnP enzymes. (Karigar & Rao, 2011).

3.3.4. Lignin-peroxidase

The phenol and non-phenolic substrate can be oxidized as a result of this enzyme (EC.1.11.1.14). LiP enzymes have distinctive redox ability, are also glycosylated hemoproteins and are highly dependent upon concentrations of hydrogen peroxide (Camacho-Morales et al., 2017). Although LiP is generally considered the primary catalyst in oxidative lignin depolymerisation, some white-red fungal substances do not generate these enzymes, laccases are therefore the solution. (Abdel-Hamid et al., 2013), (Eggert et al., 1996). LiP enzymes are applied in the fruit, paper and pulp, fiber, drug and bio-remedial industries (Karigar & Rao, 2011).

3.3.5. Laccases

The names of the largest blue multicopper oxidases (EC 1.10.3.2) are in Laccases. The copper redox power is used to catalyze a variety of aromatic substrates via an oxidation reaction (Tovar Herrera, 2013). Within the substrates which these enzymes can use are aromatic compounds, metal ions (such as Mn^{2+}) and organometallics(Abdel-Hamid et al., 2013). The catalysis resulting from laccases can be used with mediators on non-phenolic substrates. These mediators have a low molecular weight and an enzyme is oxidized. They are extremely active cation radicals that improve oxidation. (Mate & Alcalde, 2017). The most efficient mediators, such as hydroxybenzotriazole (HBT), are the N-heterocycles with N-OH ring, while 2,2′-azino-bis (3–ethylbenozthiazoline-6-sulfonic acid) is also a common alternative. (Abdel-Hamid et al., 2013). Laccases are multi-use enzymes and different enzymes are further described.

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3.3.6. Aryl-alcohol oxidases

In addition, lignin degradation for many white-rot basidiomycetes is involved in fungal aryl-alcohol oxidases (EC 1.1.3.7). This flavoenzyme is secreted by said species. Alcohols are mostly oxidized by AAO, both aromatically and predominantly, but in compounds such as furans and aldehyde groups with lower catalytic effectiveness, they can directly oxidize carbonyl groups. (Ferreira et al., 2009),(Carro et al., 2015). AAO reduces O2 to generate H2O2. This reduces O2. Two electros from alcohol substrates are extracted for that purpose and transferred to other oxidising substrates via the reduced AAO flavin to o2 forming H2O2 or O2. Substrate in lignin degradation, such as UPO, is then used by hydrogen peroxides as(Carro et al., 2015).

3.3.7. Glyoxal oxidase

The degradation is due to the copper-containing oxidoreductases, such as laccasses. Glyoxal oxidases are included (EC 1.2.3.15). Like AAO, they develop white red fungi with H_2O_2.(A. T. Martínez et al., 2017). GLOX enzymes are known to be present in wood-degrading molds, particularly in white-red degradation and symbiotic fungus as an extracellular enzyme. Fungal GLOX are activated in vitro by peroxidases, which seems to suggest that these enzymes have a synergistic and regulatory relationship. Methylglyoxal and glyoxal are the primary GLOX substracts, but lignin degradation products may also be treated. (Daou & Faulds, 2017).

3.3.8. Unspecific peroxygenases

These enzymes are co-substrate dependent on the existence of H2O2. For this reason, they are called "self-sufficient" (A. T. Martínez et al., 2017). UPO (EC 1.11.2.1) supports a wide variety of catalytic reactions such as hydroxy, epoxidation, O- and N-dealkylation, aromatization, sulfoxidation, N oxygenation, dichlorination, halide oxidation. (Carro et al., 2015), (Karich et al., 2017), (Hofrichter et al., 2015). UPO is using hydrogen peroxide to create alcohol and a water molecule by converting an H2O2 atom into a natural material such as aromatic products, alkanes, alkenes and fatty acids. (Karich et al., 2017), (Hofrichter et al., 2015). This enzyme has the capacity to conduct one-electrons oxidation, the integration of an oxygen atom into a sub-substratum and the use of H2O2 as an inter-substratum. (Karich et al., 2017).

3.3.9. Aryl-alcohol dehydrogenases

AAD (EC 1.1.1.90) works closely to generate H2O2 in accordance with the cyclical reddox reactions of the AAO.(Á. T. Martínez et al., 2005). The presence or exposure to aryl-alcohols is responsible for AAD development. (Feldman et al., 2017). AAD forms an aromatic aldehyde in the presence of an aromatic alcohol and a NAD+ receptor. This enzyme shows a high substratum characteristics caused by the catabolism (Yang et al., 2012)

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3.3.10. Quinone reductases

Lignin and other aromatic compounds are also reduced by quinone reducctases (EC 1,6.5.5). They aid redox cycling reactions to activate oxygen (Á. T. Martínez et al., 2005). As its name suggests QR, it decreases the presence of NADPH and a hydrogen ion of two quinone molecules creating two half-quinone and NADP+(Lee et al., 2007). Lee et al. (2007) NADH preferences have already been documented as an electron donor and several compounds including CuSO4, HgCl2, MgSO4, MnSO4, AgNO3, Dicumarol, KCN, NaN3, and EDTA can inhibit the intracellular enzyme as an electron donor. In both white and brown red fungi, this enzyme is found (P. C. Guo et al., 2011).

3.4. Lignocellulolytic enzyme-production by fungi

Taking advantage of the abundance of biomass and the pressing need for new fuels with less drawbacks than existing choices. As an alternative for its various available application including biofuel production, lignocellulolytic enzyme-producing fungi have been studied. Lignocellulose is an essential structural component in all plants, with cellulose, hemicellulose and lignin being the three main components(M. et al., 2009). Fungi are known to generate various lignocellulolytic enzymes that are also a costly and slow solution.

Although it is not a specific classification system, fungi are classified according to the type of red created. Ascomycets and Deuteromycets are soft-red fungi and occur when the process of white and brown rot has not begun. Soft-rot fungi, while distinguished by degrading timber in severe environment conditions, are the least destructive decomposers. To synthesize enzymes such as cellulases, that mainly break cellulose down from cell walls, they require fixed nitrogen to form microscopic cavities in the secondary cell wall. Withi this group of fungi are *Chaetomium globosum, Ceratocystis, Ustulinadeusta, Kretzschmariadeusta, Alternaria alternata, Thielaviaterrestres* and *Paecilomyces spp.* (Á. T. Martínez et al., 2005), (Liers et al., 2011), (Á. T. Martínez et al., 2009).

The three major polymers, lignin, hemicellulose and cellulose, are steadily breaking down white red fungi. White red basidiomycets secrete ligninolytic enzymes for the selective decomposition of lignin: peroxidase manganese, peroxidase lignin, flexible peroxidose and phenol oxidases containing copper (laccase).The most relevant white-rot basidiomycetes are: *Auricularia auricula-judae, Agrocybeaegerita, Bjerkanderaadusta, Ceriporiopsissubvermispora, Coprinellusradians, Irpexlacteus, Heterobasidiumannosum, Mycenahaematopus, Phanerochaetechrysosporium, Phlebia radiata, Pleurotuseryngii, Pleurotusostreatus, Pycnoporuscinnabarinus, Stropharia rugosoannulata, Trametes*

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versicolor and *Xylariahypoxylon* (A. T. Martínez et al., 2017), (Linde et al., 2015), (Fernández-Fueyo et al., 2014), (A. Kumar & Chandra, 2020) (Limaye et al., 2017).

Fungi are less likely to cause brown red than white red. Brown red basidiomycete fungi degrades the major polysaccharide, but first the lignin polymer has to be partly altered and converted into oxidized lignin. The key alteration of lignin due to the brown-rot decay is demethoxylation. The methanol creates peroxide with methanol oxidase overexpression for the treatment of cellulose using Fenton chemistry (Á. T. Martínez et al., 2009). Some of the brown red fungi found are *Coniophoraputeana, Gloeophyllumtrabeum, Laetiporussulphureus, Phaeolus schweinitzii Piptoporus betulinus, Postia placenta* and *Serpula lacrimans* (Á. T. Martínez et al., 2009), (Limaye et al., 2017).

Stinch fungi are a small group of Ascomycetes and Deuteromycetes that cause mild resin and parenchyme degradation of the cell wall, affecting extractives and water soluble components. Examples include Ophiostoma, Ceratocystis, Aureobasidium, Phialophora, and other examples. And trichoderma. And Trichoderma spp.(Á. T. Martínez et al., 2005).In addition, some fungal species, mostly in partially decomposed wood, do not form part of a community because of non-specific rot caused by them.

3.5. Lignocellulolytic enzyme-production by bacteria

Although the purpose of the article is not to generate enzymes from bacteria, some examples may be cited. In different organism types, such as plants, bacteria, insecticide and fungi, ligninolytic enzymes are present. The biocatalytical conditions are broader, tolerated by high temperature and pH concentrations, as well as by chloride; for Bacillus sp up to 600–800 mM. WT burden(Paz et al., 2020). Three classes are achieved: actinomycetes, α-proteobacteria, and μproteobacteria Lignin breaching (Plácido & Capareda, 2015). Actinomycetes are present for bio-mass degradation within soil or aquatic microflora (Limaye et al., 2017). Its morphology resembles fungi because of its elongated cells that form a structure of filaments or hyphae. Such structures help to degrade by enabling an effective spreading and penetration of organic substances(Limaye et al., 2017). Azospirillumlipoferum, Thermus thermophilus, Marinomonasmediterranea, Bacillus subtilis, and Streptumycescianeus developed Lacc enzymes as examples of actinomyces with Lip activitis enzymes, as well as those produced with Azospirillumlipoferum. (Plácido & Capareda, 2015). Bacterial laccases are normally intracellular; however, some strains were able to extracellularly express this (Paz et al., 2020). Examples of proteobacteria causing extracellular lip enzymes are Bacillus sp., Pseudomonas sp., Klebsiella pneumonia, Citrobacter sp., and Serratia marcescens (A. Kumar & Chandra, 2020).

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Acid to pH and temperatures below 50°C thrive most widely recognized cellulolytic enzymes. (Bala & Singh, 2019). However, aerobic, anaerobic, thermophilic and mesophilic bacteria develop cellulolytic enzymes (P. K. Gupta et al., 2019). The thermo- and mesophilic organism and its enzymes have limited phylogenetic diversity (Bala & Singh, 2019). Due to their potential industrial gain, thermostability, improved resistance to denaturating products and high pressure tolerance, and a rapid degradation of lignocellulosic substrates, many characteristic features emerged in heat cellulases. (Bala & Singh, 2019).

The synthesis of many cellulolytic enzymes can be achieved either by single enzymes or cellulosomes; (Husain, 2017). Popular cellulases consist of a catalytic field (P. K. Gupta et al., 2019). Genes coding for a cellulose-binding module are common in bacterial and viruses, but uncommon in eukaryotes, adding a second domain to their structure. (Horn et al., 2012); simplifying fungal cellulases as their bacterial equivalent (P. K. Gupta et al., 2019). Bacterial thermophiles including, *Clostridium thermocellum*, *Thermoanaerobacter* sp., *Clostridium straminisolvens*, *Thermoanaerobactercurvata* and *Thermotogamaritima* produced cellulases.

4. PROTEIN ENGINEERING TO IMPROVE ENZYME-BASED CONVERSION

Because of restrictions on adequate catalytic efficiency, selectivity and stability in pH and temperature, lignocellulolytic enzymes have struggled in the industry. Some of the above-noted disadvantages can nevertheless be overcome by novel advanced approaches such as enzyme stabilization, substratum technology, structural protein engineering, micro-environment engineering, and advanced computational modelling(Parra-Saldivar et al., 2020).

pH optimum conditions for most Laccfungalsoscilates between 3,0 and 5,5 and becomes basically inactive as neutral and alkaline pH values are approached. (Yin et al., 2019). The pH range is 2.5–3.5 with an activity of comparatively greater than 60% as pH rises to 4.0–6.0. activity falls to less than 40%. (J. Guo et al., 2019). The overall pH values for endoglucanases and β-glucosidases are 4,0–5,0. (de Souza Lima et al., 2019), (Vazquez-Ortega et al., 2018). Some methods have been tested to resolve pH stability, such as engineering Lacc modifications by site-directed mutagenesis, showing optimal pH results of 6.5 to 8.5 (Yin et al., 2019); LiP in Fe3O4, SiO2, PDA enzymes were also shown to have improved pH stability in nanoparticles. (J. Guo et al., 2019).

The immobilization of enzyme is also a crucial scientific step towards low thermostability and enzyme rehabilitation. Native enzymes cannot be recovered due to water solubility. (Parra-Saldivar et al., 2020). Due to bonds between enzyme and membrane, an increase of 10 percent at 40°C–60°C has been recorded. magnetic adsorption can be reused easily (J. Guo et al., 2019). Kaolin immobilised endoglucanases showed improved thermotolerance at 75°C, relative to free-

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enzyme activity at 45°, and retained maximum activity at 75°C (de Souza Lima et al., 2019). Some methods for immobilization by enzymes have been published, such as adsorption, connections, encapsulation, trapping etc. (Parra-Saldivar et al., 2020).

Substratum engineering is an instrument that improves bioconversion and specificity. Efficient promiscuity of substrates results in developing and refining current methods of biodiversisation using lignocellulolytic enzymes (Lairson et al., 2006). As a substrate enzymes can also be engineered, a design of standardized proteins can also be used to produce oligonucleotides, to alter the substrate recognition and selectivity at different functional sites. (Y. Li & Cirino, 2014).

In comparison, the design of new enzyme architectures is quickly developed and the goal is to improve the catalytic characteristics of enzymes such as activity, selectivity, stability and the specificity of substrates to broader applications. It consists of silicon simulations based on dynamics that accurately recognize the target catalytic regions for results estimation. The complex structural and functional characteristics of enzymes still pose significant unresolved challenges, but some models already have been documented to boost the specificities in part. (Khoury et al., 2009).

Generally, many enhancement techniques are being drawn up to boost the functionalities of enzymes, including inhibition resistance, reusability of the immobilized fractions, hyperactivity/stability, and improved turnover numbers. (Parra-Saldivar et al., 2020), (Bilal et al., 2019).

5. APPLICATIONS OF CELLULASE, XYLANASE AND LACCASE ENZYMES

Because of the functions they have, enzymes thrive in various industries. They are present in the textile, dairy, paper and pharmaceutical industries, in bioremediation processes, waste water and so on (Fig-6). They are therefore on company grounds. Cellulase, xylanase and laccape enzymes will be used in more detail because of the purpose of this work.

5.1. Cellulase enzymes

Enzymes in various fields of biotechnology are widely used. Because of their catalytic capabilities, many applications are available. Cellulases are used in a number of industrial processes due to their high secretion capability. The most popular applications are: waste, the pulp-and-paper industry, the pharmaceutical industry, emissions, detergents, juice colouring, etc. (Singh et al., 2016), (Fang & Qu, 2018).

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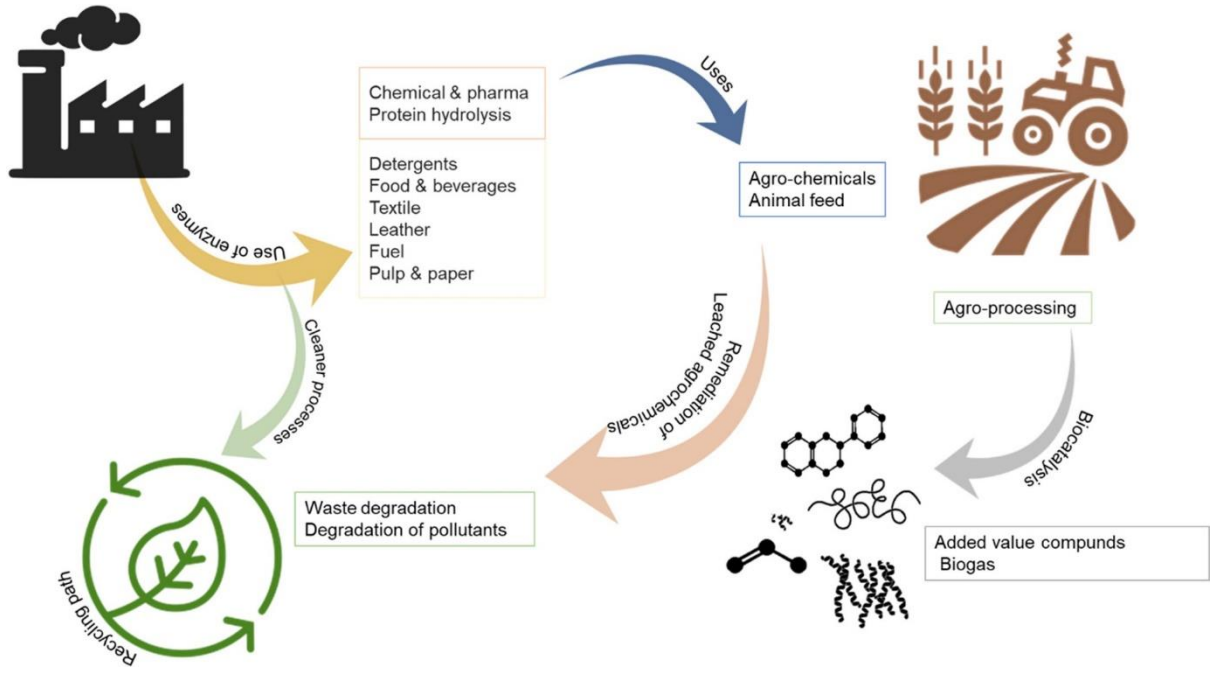

Fig. 6: Application of lignocellulolytic enzymes.

Cellulases are commonly used in the food industry to remove compounds of interest and value added, including fruit antioxidants, fruit pumace pigments, seed oil, polysaccharides and proteins of interest. They are also used to recover food additives for usable food and to remove enzymes from the cell wall of the plant biomass. Cellulases are known to promote industrial processes such as fruit juice extraction, clarification of solutions, improved cereal and oats soaking quality, emulsifying purposes, agar extraction and so on from algae. (Fang & Qu, 2018).

The fermentation, which is applied in the dairy, brewery and wine industries, is another related use of cellulases in fruit. Cellulase improves wine aroma, grape extraction, mashing, malting, and quality beer fermentation (Singh et al., 2016), (Phitsuwan et al., 2013), (Jordan & Wagschal, 2010).

The animal feed sector is used to supplement monogastric animals and ruminants in pretreatment of lignocellulosic material, i.e. the nutritional properties of animal feed by producing energy-intensive foods, by means of cellulases often enhance their digestion. Some innovators have undergone transgenic engineering to detach cellulases directly from their gastrointestinal tract to facilitate lignocellulosic digestion. (Chen et al., 2011), (Herna & Gutiérrez-soto, 2016), (Niño-Medina et al., 2017), (Iñiguez-Covarrubias et al., 2001).

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Excess teint and microfibrils, which remain attached to cotton textiles after a number of washing cycles, have been eliminated by cellulases in the textile industry. They are, however, used to restore fiber color and softness (Kuhad et al., 2011). The production of cellulase-based detergents, which have demonstrated better cleaning behavior compare to other products, while preventing damage to the fabric fibers, is a related application in the detergents industry. In total, cellulases improve color and luminosity and more effectively extract dirt (Fang & Qu, 2018). The mechanism of plant resistance, in particular the control of pests and diseases, includes cellulases in agriculture. The expression of various proteins, antibodies and enzymes is facilitated by enzymes such as exoglucanases in broad amountions. (Singh et al., 2016).

For cellulases or β-glucanases capable of degrading the cell walls of plant pathogens, certain cellulolytic microorganisms, such as Penicillium sp., Geocladium sp., Trichoderma sp., and Chaetomium sp. (Singh et al., 2016), (Bhat, 2000). Active interactions between species dominated by Trichoderma sp. Plants allow root condition improvements, seed growth to be enhanced, plant speed growth promoted and crop yields generally improved (Singh et al., 2016).

Cellulases engage in biomechanical pulping in the paper industry, which enables production of biodegradable carton, paper, hygienic paper, towels and other paper products (Singh et al., 2016). Pulp bleaching co-additives, pulping energy needs, improving drainability, reducing chlorine demand for bleaching, fiber brightness improving and enhancing drainage can be used in paper mills cellulase co-additives. (Pathak et al., 2014), (Kuhad et al., 2011), (Kuhad et al., 2016).

In the paper industry, cellulases are involved in biomechanical pulping, allowing the production of biodegradable cardboard, paper, sanitary paper, paper towels and other paper related amenities (Singh et al., 2016). Cellulases are co-additive in pulp bleaching, reduce energy requirements of pulping, improve draining, reduce chlorine requirement for bleaching, improve fiber brightness, can be used for enzymatic deinking and improve drainage in papers mills (Pathak et al., 2014), (Kuhad et al., 2011), (Kuhad et al., 2016).

To get added value compounds and resources, biorefineries are intended to take advantage of raw materials available. In the enzyme helped removal of bioactives as proteins, antioxidants, organic acids, lipids, polysaccharides, phenolic compounds, and even extracellular metabolites such as ethanol, organic solvents, among other products, cellulases have shown significant potential (Carrillo-Nieves et al., 2017), (Nieves et al., 2011), (Nieves et al., 2016). Reduce bioconversion risk of biomass waste. In order to release sugar free after fermentation, cellulases are used to treat second-generation substrates such as bioethanol waste by cellulose depolymerization (*An Overview of the Enzyme Potential in Bioenergy-Producing Biorefineries Carlos Escamilla-Alvarado*, n.d.).

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5.2. Xylanase enzymes

By extending its applications in various industries, Xylanases became slowly increasingly popular in biotechnology. In the paper industry, the xylanases make process-paste simpler, improves the efficiency of processes, promote deinking process, and are well known for reducing chlorine consumption by bioblinding kraft pulp. (Pathak et al., 2014), (Phitsuwan et al., 2013). Due to its position in bread quality, the importance of xyllenes has increased in the food industry, with particular emphasis on water absorption and contact with gluten. This helps Xylanase to bind the hemicellulose to a dough that improves durability and softens the dough to make the manipulation of the machinery simpler and the texture of crumbs thinder and uniform. A further use in the food sector is clarification of drinks, extraction of coffee, extraction of vegetable oil and starch and to enhance the dietary features of grain feeds and silages (Phitsuwan et al., 2013). There are few applications in the pharmaceutical industry for xylanases such as dietary use as digestive supplements in conjunction with complex enzymes (hemicellulases, proteases and others). A big current focus is on the development of biofuels such as ethanol, solvents and low-calorie artificial sweeteners, the transformation of xylans in simpler structures, such as β-D-xylopyranosyl residues. In the textiles industry, certain xylanolytic enzymes are used for the production of fabrics like hessian or linen. (Polizeli et al., 2005). Its main objective is the structural weakening of the cell walls of the plant. These systemic changes cause changes in the product characteristics of each industry. xylanases are used in the processing of lignocellulosic biomass ethanol, such as cellulases. Lignocellulosic biomass is first deligned with release of hemicelluloses and celluloses; then xylanases are depolymerised to get free sugars that are then fermented for bioethanol (Garg, 2015), (Alvira et al., 2010). The basic role information of xylanases was not specified in each industry.

5.3. Laccase enzymes

Laccases are very stable enzymes which can be used in biotechnological processes for a wide range of applications. In the environmental region laccases provide a viable alternative because of their capacity to oxidize phenolic and nontolic compounds and certain highly polluting compounds because of the constante need for oxidation and the undesirable side responses, or non-specific reactions resulting from the use of traditional oxidation technologies. (Rodríguez Couto & Toca Herrera, 2006). The laccases have relatively low redox potential in comparison to other ligninolytic enzymes (>1V) are important to remember. It is not possible to minimize redox. Its action is therefore limited to phenolic lignin oxidation, which constitutes < 20% of the polymer matrix. Laccase oxidation is not feasible directly from non-phénolic substrates with greater redox potential (e.g. 1.3 V). This inconvenience was overcome with redox mediator systems that increase the catalytic activity of non-phenolic lignin components like recalcitrant

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(Barreca et al., 2003). The first synthetic mediator to be identified was ABTS (2,2′-azino-bis, 3-ethylbenzothiazoline-6-sulfonic acid)(Abdel-Hamid et al., 2013). There have been examples of other organic, synthetic and natural compounds. However, the mediators that are most important are the ones that occur naturally because they are the most similar to those generated in the environment in the biomass biodegradation. Phenolic compound syringaldehyde, acetosyringon, acetovanillone, vanillin, méthylvanillate and p-coumaric acid are the most commonly studied natural mediators. (Cañas & Camarero, 2010). It is necessary to consider that laccases need a mediator in order to achieve high conversion rates by lacquer in various industries. Mediators are also inexpensive, eco-friendly and reusable. (Barreca et al., 2003), (Cañas & Camarero, 2010). It is necessary to consider that laccases need a mediator in order to achieve high conversion rates by lacquer in various industries. Mediators are also inexpensive, eco-friendly and reusable(Ire & Ahuekwe, 2016) . They are also used as food additives, in the manufacture of drinks, biofuels and are therefore known as environmentally friendly biocatalysts (Mate & Alcalde, 2017). Lacquers in food industries are used to manipulate the color of food by eliminating the unwanted browning of phenolic compound in drinks, including juices, beer and wine (Rodríguez Couto & Toca Herrera, 2006). Its ability to connect biopolymers is used to reduce the extensibility of wheat dough (Rodríguez Couto & Toca Herrera, 2006) . It was understood that the textile sector used dyes to be replaced by toxic compounds caused by their use and laccases provide a remedy for polluting effluents by bleaching textiles. (Rodríguez Couto & Toca Herrera, 2006) (Salazar-López et al., 2017), (Teerapatsakul et al., 2017). The laccases characteristic for polymerizing compound makes it a medium for the environmentally safe synthesization of organic compounds, e.g. aryl-sulfonyl thiazolidinediones, pyrimidobenzothiazoles, catechol thioeters and 2,6-dimethoxyphenol dimmers (Abdel-Hamid et al., 2013). The laccases characteristic for polymerizing compound makes it a medium for the environmentally safe synthesization of organic compounds, e.g. aryl-sulfonyl thiazolidinediones, pyrimidobenzothiazoles, catechol thioeters and 2,6-dimethoxyphenol dimers (Rodríguez Couto & Toca Herrera, 2006). The laccases have demonstrated their ability to combine a range of molecules to create new low molecular compounds, such as antibiotics, amino acids, antioxidants and high-render cytostatic(Mikolasch & Schauer, 2009). Lacases have been used in recent years as a method to biotransform or biodegrade emerging pollutants (Barrios-Estrada, de Jesús Rostro-Alanis, et al., 2018)For eg, nonyl phenol, triclosan, Bisphenol A, Ethynylestradiol, Diclofenac sodium, β-naphthol, 2,4-dichlorophenol, m-cresol and more DI-Diiodo-8-hydroxyquinoline (Ramírez-Cavazos et al., 2014), (M. Rodríguez-Delgado et al., 2016), (Gonzalez-Coronel et al., 2017), (Barrios-Estrada, Rostro-Alanis, et al., 2018). Recently the use of lacases as catechol determination biosensors, polyphenol identification in various samples, 17β-stradiol determination, 2,6-dimethoxyphenol measurement in wastewater and the determination of L-tyrosinases in aqueous solutions have been published, and more recently (Abdel-Hamid et al.,

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2013), (M. M. Rodríguez-Delgado et al., 2015) Laccases for the formation of bioelectric cells are also being studied (Abdel-Hamid et al., 2013).

6. CURRENT SEARCH IN MEXICO FOR MICROORGANISMS THAT EFFICIENTLY DEGRADE LIGNOCELLULOSIC MATERIALS

Many species are devoid of enzyme systems required because of their complexity to thoroughly degrade the lignocellulosic material. The individuals that can be found in the scientific community around the world (Dhevagi Periasamy, Sudhakarn Mani, 2019), (Niño-Medina et al., 2017). During this time, many microorganisms lack enzyme systems which are required for the successful degradation of lignocellulosic material due to the complex cell wall model of Lignocellulosic Biomass. While Mexico is one of the countries with the greatest diversity of fungi, with more than 140,000 species, it has been studied with a very restricted quest for certain producers of enzymes of biotechnological significance. Nuevo León's researchers are from Mexico (Herna & Gutiérrez-soto, 2016) (Niño-Medina et al., 2017), Hidalgo, Jalisco, Yucatán (Cruz-Ornelas et al., 2019), (Amaya-Delgado et al., 2010) and Veracruz (Dhevagi Periasamy, Sudhakarn Mani, 2019), (Cupul et al., 2014) The greatest number of described fungal species have been reported; however, the information is still profound.

Niño-Medina and others have measured the effect on the physicochemical properties of bread, extracted from Trametes maximaCU1 native fungus from Cerro La Silla, Nuevo León (Niño-Medina et al., 2017). 60 thermotolerant-isolated fungi from Hidalgo display a strong lacquer function, which according to Cruz Ramírez et al. has a biotechnological potential (Cruz Ramírez et al., 2012).

The expression of the enzyme lignin-cellulolytic *Pleurotusdjamor,* a white-red fungus widely spread in Mexico, was found. A recent application to extract diclofenac, naproxen and ketoprofen from aquatic cultures has been demonstrated by the degradations of enzymes such as laccases, manganese perojidases and lignin peroxidase. *(Cruz-Ornelas et al., 2019).*

Chan Cupul et al., 70 fungal species isolated, including Trametes maximum SM9, Pycnoporussanguineus ACT1 and Dedalea elegans PM7, from the state of Veracruz. Qualitative and quantitative assessments and liquid and solid fermentation in Agar plates were conducted respectively. Enzyme activity, particularly lacquer and MnP, showed an expression of results(Dhevagi Periasamy, Sudhakarn Mani, 2019). That is why T is concluded. Maximum SM9 and P. sanguineus ACT1 are successful productivity candidates.

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7. RESEARCH GAPS CHALLENGES AND RECOMMENDATIONS

For its full enzymatic hydrolysis, the heterogeneity of lignocellulosic biomass represents many challenges. The saccharging capability of the enzyme is impaired by factors such as thermal instability of protein, substrate excess inhibition, unspecific polymer chain binding and irreversible lignin adsorption. As a result, undegraded residues are important post-treatment and thus not cost-effective. The purification of enzymes has a huge impact on the increased cost of production and sale.

The ongoing studies to isolate higher-activity improved cellulases and stability in severe temperature environments, pH, etc. aim at enhancing the cost-effectiveness of the method as opposed to those that are currently usable. The main emphasis is on reducing manufacturing costs from 10 to 100 times. Due to the lack of all unique enzyme groups needed to efficient degradation in most microorganisms, several technologies have been combined to achieve the desired results. Genetic and metabolism strategies for the assembly of new enzymes capable of hydrolyzing a wide range of polysaccharide bonds, combined with a high activity dependent selection and combination of many enzymes, the complex structure of lignocellulosic biomass would be fully degraded to their monomeric forms. Several enzyme manufacturing multinationals are now creating cellulase cocktails to express desired mixtures.In other words, transformed microorganisms need genetic instruments to increase lignocellulolytic enzyme production. Genetic changes regulated will lead to a more cost effective and efficient manufacturing process. Mutagenesis on the site causes the specificity of the substrate to increase and the base properties to exceed. Transcriptomic technologies promote understanding of and impact on gene expression, regulation and networks on the genome's reaction to environmental disorders, which represents an advantage in enzyme expression that factors such as temperature and pH can easily alter.Therefore, a greater understanding of the expression and development of lignocellulolytic enzymes is made possible by the metabolic pathways and the genetic regulatory mechanism, which therefore contributes to optimization.

8. CONCLUSION

The world's abundant biodiversity of fungi providing a variety of enzymes with the catalytic capacity to carry out lignocellulosic biomass hydrolysis is an encouraged method and an environmental option for the revalorization of lignocellulolytic materials from agro-industrial waste. Because of the incorporation of autochthonous enzymes produced in a wide range of countries, research in this field would trigger favorable changes in the enzyme market with cost reductions.In order to boost the saccharification of lignocellulosic biomass by enzyme hydrolysis, new technologies need to be introduced, including genetic modification of microorganisms to increase the yield of enzyme output in a cost-effective manner, as well as

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predictions of genetic expression and transcriptomic metabolic responses. In the near future, the total degradation of lignocellulosic biomass by enzymes generated by fungi to create a wide variety of new products will be a reality. The ability of enzymes would pose new challenges in terms of commercial development, the cost of enzymes and the variety of enzyme cocktails appropriate for agro-industrial waste used as substrates and for new products in the light of the sustainable production of new products worldwide. The use of industrial enzymes for the hydrolysis of lignocellulosic substrates greatly improves the method of enzymatic application, as well as the manufacturing method of second-generation bioethanol. Thus, the discovery of new enzymes obtained from native Mexican fungal strains with high enzyme productivity and low production costs, using agricultural waste substrates, significantly stimulates the production of lignocellulolytic enzymes in situ. One of the major challenges in the industrial production of enzymes is the creation of the tailored, cost-effective and sequential action of a cocktail of enzymes with a high specific activity that allows the complete degradation of the complex structure of lignocellulosic biomass to its monomeric forms.

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