Hydrolytic Enzymes for Lignocellulose Materials and their Impacts on Food Additives and Health Promotion: A Review

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One of the most prevalent and renewable forms of biomass on Earth is lignocellulose, which has an enormous potential for bioconversion into valuable bioproducts. However, this resource is not fully exploited. This review considers the enzymatic hydrolyses of these materials and the impact of their bioproducts on the nutritional and health levels. Understanding lignocellulolytic enzymes and their uses in industry would aid in the development of innovative procedures that lower costs and increase the uptake of biomass, both of which are more beneficial. The conversion of lignocellulosic biomass is achieved by pre-treating biological process that considered inexpensive, feasible, and ecologically acceptable approach followed hydrolysis via enzymes. These enzymes can be applied in several industries, such as the textile, meals and beverages, personal hygiene, medicinal products, and in biofuel manufacturing sectors. Several products are based on lignocellulosic biomass conversion such as bioenergy compounds, organic acids, single cell protein, and Xylitol. Pretreatment and type of biological process of lignocellulosic biomass conversion plays a critical factor for quantitative and qualitative yields of bioproduct of lignocellulosic biomass conversion. Finally, the nutrition and health benefits of some end products of lignocellulosic biomass conversion are covered in this review.

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INTRODUCTION

One of the best solutions to address the issues of energy security, the environmental degradation, and the fossil fuels scarcity is the conversion of lignocellulosic material into renewable energy that is sustainable (Al-Rajhi *et al.* 2024a). However, there are certain scientific obstacles to the transformation of biomass into useful products for human and animals as food additives or health promotions, such as the high cost of cellulolytic enzyme combinations to break down lignocellulose into fermentable monosaccharides. To employ less cellulase, many studies have concentrated on increasing the rates of transformation in critical processes (Abdel-Ghany et al. 2018; Al Abboud *et al.* 2024). A variety of tactics have been used to get around this obstacle and improve the effectiveness of enzymatic hydrolysis, including screening microbes to identify auxiliary enzymes that work better, the addition of chemicals, and the improvement of the enzymatic hydrolysis procedure. However, the form of biomass, the enzyme cocktail utilized, the hydrolysis limitations, and the additive formulation all have a significant impact on the additives' effectiveness (Li *et*

al. 2016). Utilizing biomass has potential for ecological sustainability and is a renewable natural resource for energy production (Al Abboud et al. 20122). Biomass from food waste is widely accessible globally (Abdelghany et al. 2018). Its use won't strain current food sources because waste is often produced from anthropological pursuits and is considerably less expensive than crude oil. Due to its lower cost compared to other farming significant feed equities, it is being regarded as a viable option for raw materials to produce ordinary chemicals, biofuels, and polymers, which will ultimately result in substantial financial benefits and ecologic sustainability (Yasin et al. 2013). The biological conversion procedure has the ability to bio-refine woody biomass into products of great value (Tan et al. 2020). Since poplar is a woody plant that grows quickly and is found all over the globe, waste poplar can be utilized as an appropriate feedstock for bio-refineries.

Three main biopolymeric components that make up lignocellulosic biomass include cellulose (45% to 50%), hemicellulose (25% to 30%), and lignin (10% to 20%). Because of its intricate structure and substantial variance, woody biomass is more compressible than grassland biomass and agricultural straw (Ying et al. 2021). Achieving optimal enzymemediated hydrolysis of woody material is challenging because it requires a specific preparation (Wang et al. 2022). The most crucial stage in the transformation of ligninbased biomass to monosaccharides is the enzyme-driven hydrolysis pathway. On the other hand, a high quantity of lignin present in lignocelluloses hinders the procedure of enzymatic hydrolysis of cellulases and hemicellulases due to the nonproductive adhesion of the enzymes to lignin (Wen et al. 2022). The use of additives has drawn a lot of interest by preventing the non-productive adsorption of enzyme proteins. On the other hand, certain additives can improve the enzymatic hydrolysis action because they increase the enzymatic breakdown efficiency of lignocellulose (Wei et al. 2019). Investigators around the world are concentrating on developing technologies that use natural biomass to produce the same useful products (Fig. 1). Although lignocellulose has a bright future, its application around the world has been hampered by its physico-chemical resistance and high manufacturing expenses. Since lignocellulolytic enzymes would enable long-term hydrolysis of lignocellulosic materials, more research on them is therefore essential to solving this issue (Liang et al. 2020).

For decades, there has been a problem with trying to use more lignocellulosic waste. There are already several conventional uses across multiple sectors. Lignin-based health promoters for human as well as animal is a developing strategy and has gained extensive attention lately. The unique perspective of current review compared to other studies involves specific points related to bioproduct of lignocellulosic materials hydrolysis as food additives and health promising agent. This review considers the lignin-derived food additives and health promoters. In addition, the aim of this review is to highlight, the types, mechanisms, and the functions of hydrolytic enzymes for breaking down lignocellulosic components as additives to foods.

Kinds of Hydrolytic Enzymes for Lignocellulose

Enzymes that break down lignin and cellulose onto their constituent parts to undergo additional degradation into valuable compounds are known as lignocellulolytic enzymes (Gałązka *et al.* 2025). These hydrolytic enzymes, also known as lignocellulases, break down the resistant lignocellulose. According to research, lignocellulolytic enzymes are a broad category of extracellular proteins primarily consisting of hydrolytic enzymes (*e.g.*, cellulases, hemicellulases, pectinases, and mannanase) and ligninolytic enzymes (such as oxidases and peroxidases) (Raheja *et al.* 2025). Living things produce these

macromolecules, i.e. the enzymes, which can speed up biological and chemical processes when added to a system or reaction (Bakri et al. 2022; Al-Rajhi et al. 2024b). Because they provide the right conditions for processes, they facilitate the conversion of precursors into valuable final products (Karigar and Rao 2011). The microorganism must overcome several obstacles resulting from the chemical makeup of lignin in order to be regarded as a viable candidate for lignin decomposition. The lignin breakdown by microorganisms can be intricate and difficult. These include the following: (1) Its external enzymatic structure; (2) it is an oxidative process rather than hydrolysis owing to the lignin chemical structure that includes ether and carbon-carbon bonds; and (3) the disorganized stereochemistry of lignin which has less precision to hydrolytic enzymes. In summary, ligninolytic enzymes are outside the cell, reactive, and nonspecific enzymes that, due to their extreme instability, produce compounds that necessitate many oxidative reactions. Nonetheless, they are essential for starting the first stages of lignin depolymerization (De Souza 2011). Hydrolytic enzymes, also referred to as hydrolases, hydrolyze biomolecules like peptides, glycosides, protein, lipids, nucleic acids, carbohydrate, and fatty acids into their most basic units (Kucharska et al. 2018). Hydrolytic enzymes are easily accessible, do not have cofactor stereo specificity, and can withstand the incorporation of water-miscible solvents. They catalyze the hydrolytic cleavages of C-O, C-N, O-P, C-C bonds and other singlebonds in molecules (Chukwuma et al. 2020; Liang et al. 2022) (Table 1).

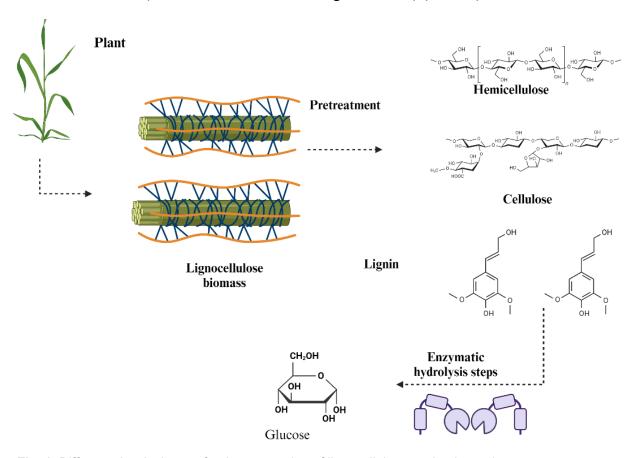


Fig. 1. Different classical steps for the processing of lignocellulose to simple products

Table 1. The Properties of Some Enzymes that Contribute to the Breakdown of Cellulose and Hemicelluloses

Enzyme Name	pH and	Producer	Process Name	Reference
	Temperature	Microorganism		
Cellulase	3.0–7.0 &	Aspergillus	SSF*	Chukwuma <i>et</i>
	30–60 °C	spp., <i>Pseudomonas</i>		al. 2020
		sp.		
Hemicellulase	5.5 & 55 °C	Aspergillus spp.,	SSF	Houfani <i>et al.</i>
		<i>Bacillus</i> spp.		2020
Protease	4.0-7.0& 28-	Aspergillus spp.,	SSF	De Souza 2013
	60°C	<i>Bacillus</i> spp.		
β-Mannanase	4.5 & 60°C	Aspergillus niger	SSF	Saleh <i>et al.</i>
				2024
Amylase	5.0-7.0 & 45-	Fusarium spp.,	SSF	De Souza 2013
	50 °C	Bacillus spp.		

*SSF: Solid state fermentation

Pretreatment and Enzymatic Hydrolysis of Lignocellulosic Materials

Two key unitary processes for a few valuable biochemical manufacturing procedures, such as the generation of bioethanol (Du et al. 2020), xylitol, aldehydes, acids, and saccharides (Chen et al. 2017) from lignocellulosic materials, are pretreatment and enzymatic hydrolysis. Since there is no need for an acid recovering stage, dilute acid processing seems to be a more advantageous approach for industrial uses. Sulphuric acid, which acts both as a dehydrating agent and catalyst, is the most often utilized acid. In contrast to other acids like hydrochloric and nitric acid, a greater monosaccharide transformation output has been achieved (Khattab and Watanabe 2019). The hemicelluloses mass could hydrolyze quickly into monosaccharides in an acidic environment, leaving less than 10% w/w of non-hydrolyzed solid behind. The lack of pentosan and xylan may be the reason why the cellulose and lignin portions are more durable and less vulnerable to comparable acidic environments. However, phenylpropanoid monomers in lignin can aid in preventing chemical and biologic attack (Lu et al. 2017). Moreover, cellulose's crystalline and amorphous, branch-free structures may provide some resilience against dilute acidic surroundings (Galbe and Wallberg 2019). Pretreatment procedures with a comparatively high yield of monosaccharide conversion can improve the access of cellulose and hemicelluloses to hydrolysis by enzymes (Kobkam et al. 2018). Furfural, 5-hydroxymethylfurfural (HMF), and acetic acid are among the several inhibitors that are generated in the hydrolysis product in comparatively significant amounts, 4% to 8% w/v in dilute sulphuric acid pretreatment phase (Wang et al. 2019). Acetic acid is mostly produced following the deacetylation phase of xylan side chains, whereas furfural and HMF are the final products of the dehydrating procedure for xylose or glucose, according to Davies et al. (2011). The use of active charcoal, ionic exchange resins, and over-liming, which has the adverse impact of causing sugar damages, could be used as part of a detoxification approach to lessen the harmful effects of these substances (Kaur et al. 2023).

Hydrolysis Mechanisms of Lignocellulosic Substrates

Lignin has been demonstrated to impede effective enzyme-mediated breakdown of lignocellulosic substrates. Previous work has shown that the lignin blocks hydrolysis by

two main processes. It limits substrate expanding, thus hindering cellulose availability. In addition, lignin can connect to the cellulolytic enzymes and thus inhibit their ability to function (Kumar *et al.* 2012). Despite the fact that lignin elimination has been demonstrated to be an effective way to improve enzyme-mediated cellulose breakdown, no economical delignification-based techniques have been commercialized to date, mainly due to the substantial cost related to the employing of chemicals (Takada *et al.* 2020). Because of this, a lot of research has been done on ways to lessen the hindering impact of lignin by altering its nature. For example, employing mild reaction circumstances that add acid groups to the lignin macromolecule can improve cellulose hydrolysis without requiring total delignification (Nakagame *et al.* 2011).

Released Bio-products of Lignocellulosic Substrates Hydrolysis

The lignocellulosic hydrolysis yields a variety of bio-products such as bioenergy products, organic acids, single cell proteins, and xylitol (Fig. 2).

Bioenergy products

There are several bioenergy products released from lignocellulosic hydrolysis such as bioethanol and bio-butanol. A significant alternative to fossil fuels, the manufacturing of bioethanol is growing quickly. Lignocellulosic biomass, including agricultural, industrial, and forest residues, has been recognized as a highly suitable, inexpensive, and easily accessible feedstock for the synthesis of bioethanol. As a chemical feedstock, gasoline additive, or primary fuel, ethanol now holds the biggest market share (Kumar *et al.* 2016).

Bio-butanol

One potential biofuel that could eventually replace petrol is biobutanol. Compared to ethanol, butanol has a higher energy density, is safer, and is compatible with engines. Butanol also serves as a crucial intermediate for paints and resins (Al-Shorgani *et al.* 2015; Al-Rajhi and Abdelghany 2023).

Organic acids

Citric acid, succinic acid, and acetic acid could be produced using different lignocellulosic agricultural biomass (Bao *et al.* 2014; Kumar *et al.* 2016). According to Ajala *et al.* (2020), lactic acid was produced from lignocellulose consumption by bacteria, numerous functions were attributed to lactic acid which utilized as acidulant, flavoring, and preservative. Lu *et al.* (2021) mentioned that there are limited strains of bacteria that can directly yield succinic acid via lignocellulose materials. *Via* biological or chemical procedures, several promising acids including fumaric, succinic, aspartic, itaconic, 2,5-furan dicarboxylic, levulinic, glucaric, 3-hydroxypropionic, malic, and glutamic were produced materials containing lignocellulosic (de Cárdenas and de Cárdenas 2020).

Single cell protein

Dry protein of microbial origin, which is referred to as single-cell protein and is grown on wastes of plants, has been counted as a powerful supply of protein which enhance the heath of animals and humans (Kumar *et al.* 2024). Affordable substrates for the synthesis of single-cell proteins are lignocellulosic wastes. Single-cell protein has been effectively produced using a variety of lignocellulosic wastes, including rice bran, cantaloupe skin, orange peel, banana, apple, and cucumber peels (Mondal *et al.* 2012). The

making of single-cell protein from lignocellulosic wastes was reported in numerous investigations which requests four phases starting from chemical and physical pretreatments followed by synthesis of cellulase, which is responsible for hydrolysis of wastes and ended by fermentation or assimilation of holocellulose (Reihani and Khosravi-Darani 2024).

Xylitol

A low in calories sweetness called xylitol is formed when xylose is reduced by xylose reductase, which requires either NADH⁺ H⁺ or NADPH⁺ H⁺ as co-factors. The subsequent transformation of xylitol to xylulose might then be catalyzed by a NAD+-dependent xylitol dehydrogenase. Xylulose may go through the pentose phosphate route to create ethanol following an additional phosphorylation process (Baptista *et al.* 2018).

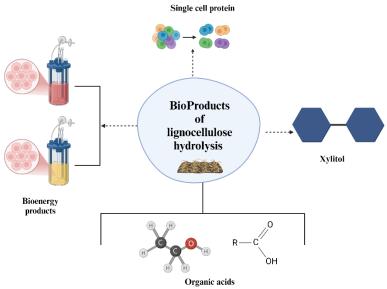


Fig. 2. Different groups of products released from hydrolysis of lignocellulose

Application of Released Products in Food

In several food processing sectors, lignocellulolytic enzymes are widely used to increase production rates and enhance product quality. By using lignocellulolytic enzymes in the relevant processing sectors to turn fruits and vegetables into food products, it is also feasible to promote their effective use on a world market scale. This will not only boost the fruits and vegetables' economic worth but also help to overcome nutritional problems internationally (Toushik *et al.* 2017; Al-Rajhi *et al.* 2024c). The fruit and vegetable juice sectors have made extensive use of mixtures of enzymes, including cellulases, hemicellulases, and pectinases, as steeping enzymes. The need for macerating enzymes has increased globally as a result of their perceived ability to enhance the manufacturing of juice from a variety of fruits and vegetables in various juice processing sectors (Zubaidi *et al.* 2025). The incorporation of pectinases in conjunction with hemicellulase and/or cellulases is advised not only for fruit processing but also for achieving cloud equilibrium, appearance, and the right amount of nectars and purees in the final product through decreasing viscosity. Tropical fruits have more cellulose and hemicellulose elements than other fruits (Kashyap *et al.* 2001).

On the other hand, the incorporation of cell-wall-degrading pectinolytic enzymes can enhance oil quality through boosting the extraction of phenolic compounds with strong

antioxidant properties and permits an aqueous procedure through compromising the structural elements and breaking down the cell walls in agricultural products containing oil (Sharma *et al.* 2013). Besides, enzyme technological advances have proven track record of effectiveness in wine production. Additionally, the lignin-degrading enzyme laccase has been utilized on occasion to prepare wine from grapes in order to prevent undesired polyphenols from changing the organoleptic qualities of wine. By oxidizing the cork stink and bitterness issues in vintage wine bottles, laccase is also utilized economically in the manufacturing of cork caps for bottles of wine (Osma *et al.* 2010).

Health Impacts of Food Containing Products

A few studies in the past 10 years have looked at the usage of nutritional properties of lignocelluloses for human and animals, indicating possible impacts on the physiological makeup and functioning of the digestive system (Slama *et al.* 2020). In terms of poultry nutrition, grill performance may be enhanced by feeding lignocellulose at incorporation levels less than 1% (Makivic *et al.* 2019). Other investigations, however, using comparable inclusion phases, demonstrated that feeding lignocellulose had either no effect (Zeitz *et al.* 2019) or a negative effect on chicken ratings (Rohe *et al.* 2019) when using greater inclusion rates. Studies examining how dietary lignocellulose affects nutritional digestibility are few. Broilers given 1% lignocellulose showed an improvement in the perceived total tract absorption of fat and fatty acids in Ross 308 broiler chicks (Bogusławska-Tryk *et al.* 2015); however, in another study, feeding 1% to 2% nutritional lignocellulose had no impact on nutritional digestibility in broiler chickens (Kheravii *et al.* 2017). These differences may depend on the kind of feeder animal and nutrient digestibility, either fats or proteins or carbohydrates.

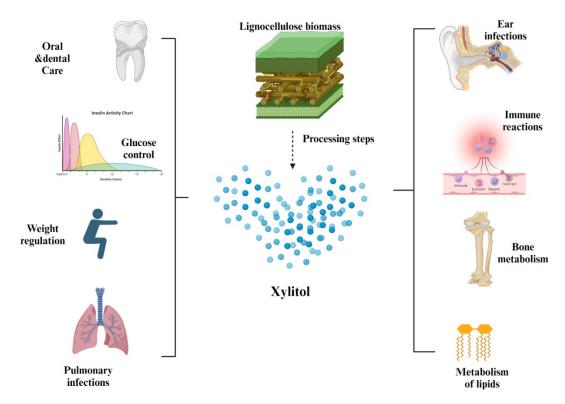


Fig. 3. Different applications of xylitol in human and animal health

Xylitol, a sugar alcohol and member of the carbohydrate family of biomolecules, is employed as a sweetness agent. It is present in extremely small amounts in nature. Xylitol is made economically by recovering waste components from chemically manufactured plants or by technological techniques. In animals, xylitol also causes the release of insulin into blood plasma; it can help lessen obesity and a few other metabolic disorders. Additionally, xylitol shows promise in many applications (Fig. 3), including preventing the formation of germs resistant to antibiotics. This sugar-based alcohol is useful for treating long term inflammatory disorders and has good anti-inflammatory properties. It can help alleviate respiratory conditions including pneumonia and middle ear diseases, among other conditions. Frequent use of xylitol may result in adverse consequences including stool loosening and diarrhea. Nonetheless, several studies have shown how helpful it is for the well-being of people (Gasmi Benahmed *et al.* 2020).

Enzyme Use in Animal Feed (Especially Ruminants)

The use of exogenous enzymes in animal feed has emerged as a promising strategy to enhance feed efficiency, nutrient utilization, and overall productivity in livestock. While enzyme supplementation is well-established in monogastric animals such as poultry and swine, its application in ruminants such as cattle and sheep has gained increasing attention in recent years (Velázquez-De et al. 2021). Ruminants present unique challenges due to their complex, multi-chambered stomach and highly developed microbial fermentation in the rumen. Therefore, enzyme effectiveness depends on its ability to function in synergy with ruminal microbes or to act pre-ruminally. Exogenous enzymes such as cellulases and xylanases have shown potential in improving fiber degradation in high-forage diets. For instance, research by Kamal et al. (2025) demonstrated that applying cellulase and xylanase enzymes to forage diets can enhance digestibility and milk production in dairy cows. Similarly, studies by Cappellozza et al. (2025) revealed improved feed efficiency and weight gain in beef cattle when enzymes were added to high-fiber rations. The mode of action of feed enzymes in ruminants varies. Some act directly on feed substrates before ingestion (pre-treatment), while others work synergistically with microbial enzymes within the rumen. OuYang et al. (2025) emphasized that enzyme stability under rumen conditions, along with enzyme-substrate specificity, are critical factors for success. Moreover, enzymes can enhance the release of fermentable sugars, reduce the lag time of microbial colonization and stimulate ruminal fermentation.

CONCLUSIONS AND FUTURE PROSPECTIVE

The characteristics of cellulose, hemicelluloses, and lignin—the chemical constituents of lignocellulosic biomass—as well as the conversion of lignin-based biomass into valuable products for industries have been briefly covered in the present review paper. Using industrial enzymes to bio-convert lignocellulosic biomass is a feasible method for forming bio-products with additional benefits. Burning is a common method of disposing of a significant portion of these lignocellulosic substances, and this practice is not limited to developing nations. Nonetheless, the vast quantity of leftover plant biomass that is seen as "waste" may be transformed into other products with additional value and other nutritious food items with valuable health benefits. The process of turning lignocellulosic biomass into a variety of industrial products through biotechnology is both cost-effective and green. However, new applications and additional development are required.

Furthermore, there should be a deeper comprehension of the structure and kinetic mechanisms of the lignocellulose-degrading enzymes, as well as better production methods, from a variety of reasonably priced sources. The inclusion of exogenous enzymes in ruminant nutrition holds considerable promise, especially in improving fiber utilization and reducing feed costs. However, more standardized trials and mechanistic studies are needed to fully realize their potential and tailor enzyme products to specific feeding systems and environments.

By using this data, outputs can be increased, and total expenditures can be reduced.

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