# Plant Cell Wall as a Substrate for the Production of Enzymes with Industrial Applications

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**Abstract:** The plant cell wall represents a vast carbon source for the induction of carbohydrate-degrading enzymes. The matrix of polysaccharides presents a great structural diversity, containing different sugar residues with the same or different bonds, branched to varying degrees and whose conformation may be like a straight ribbon, a twisted ribbon, an open helix or completely disordered. Cellulose and hemicellulose are the most abundant polysaccharides, accounting for as much as 35-50% and 25-30% of the dry weight of plant cell wall, respectively. The exploitation of plant cell wall polysaccharides requires an arsenal of enzymes with different mode of action. Enzymatic saccharification of plant cell wall components has potential applications in different fields, including fuel, solid waste disposal, animal feed, and paper/textile industry. The present review covers some aspects of plant cell structure and function, having in mind its potential as an inductor of enzyme systems with biotechnology applications.

**Keywords:** Cell wall, holocellulose, glycosidase, holocellulase, enzyme specificity.

## I. INTRODUCTION

The plant cell wall as a substrate for enzyme action must be considered in a different context, taking into account its complexity and nature (Fig. **1**). A kinetic model for the interaction between cell wall components and a consortium of enzyme systems requires the analysis of several factors, including the involvement of different types of chemical linkages and the environment that surrounds the cell wall structure (Fig. **2**). Recalcitrance to saccharification is a major limitation for the conversion of lignocellulosic biomass to valuable end products [1]. An intricate arrangement between polysaccharides of the cell wall matrix, hereafter called holocellulose, proteins and lignin makes the cell wall structure a challenge for carbohydrase and ligninase enzyme systems from different sources. Within the groups of carbohydrases, glycosidases have an important role in the hydrolysis of the glycosidic bonds in oligo- and polysaccharides [2]. The strategy adopted by some enzyme sources, like fungi and bacteria, is based on the use of glycosidases with varying substrate specificity, suggesting the role of some enzyme systems as promiscuous agents [2, 3]. Enzyme promiscuity has an important functional role in cell wall deconstruction, involving substrate and catalytic specificities. As a matter of fact, the type of chemical linkage, more than any other factor, determines the enzyme action in a certain kind of substrate. The plant cell wall structure is a good environment to induce substrate promiscuity. In this case, enzyme systems with high substrate promiscuity act in synergism with enzymes with strict substrate specificity, leading to a more efficient catalytic process. Within this context, enzymes that cut specific sites in the plant cell wall are also important tools for understanding the structure and function of cell wall.

## II. THE PLANT CELL WALL STRUCTURE

The plant cell wall is a strong fibrillar network that gives each cell its stable shape [4]. It is composed of cellulose, a linear polymer of β-1,4-linked glucose units; hemicelluloses which include a variety of polysaccharides with linear or branched polymers derived from sugars such as D-xylose, D-galactose, D-mannose, D-glucose and L-arabinose; pectin, a linear polymer of α-1,4 galacturonic acid units, some of which are methylated at C-6, some acetylated at C-2 and some with more extensive substitution; starch, composed of two polymers, a linear α-D-glucan (amylose) and a branched glucan (amylopectin); structural proteins, including glycoproteins, expansin and extensin; and lignin, a three-dimensional network of p-hydroxyphenylpropane units [5-7]. The composition and percentages of these cell wall components vary from one plant species to another [8].

The plant cell wall is a complex structure consisting of three layers (Fig. **1**). The outer layer is called middle lamella, being the first layer formed during cell division. It is shared by adjacent cells and consists mainly of pectic compounds and proteins [8, 9]. It is the part of the cell wall that is laid down between two daughter cells as they are separated during division and makes up the outer wall of the cell [10]. The pectic compounds form plastic, hydrophilic gels that cement the cells to one another and provide coherent tissues [9]. The packing of pectic compounds into the wall alters the texture and mechanical properties of the wall due to the fact that the hydrogen bonds between the polysaccharides and the microfibrils are weakened, becoming less rigid [11].

The primary wall is a highly hydrated structure having a relatively sparse distribution of cellulosic microfibrils embedded in a gelatinous matrix composed of pectic compounds, hemicelluloses, glycoproteins (hydroxyproline-rich extensions), extension, and expansin [12]. The primary cell wall is formed during the birth of cellulose fiber. The chemical components of plant cell have content and bonding network determined by the origin, age and the treatment of fiber. It defines not only the mode of growth of plant cells, but also their size and shape [9]. The primary cell wall presents some other functions, including structural and mechanical support, protection against pathogens and dehydration. Besides, the plant cell wall is also responsible for cell-cell interactions and carbohydrate storage. The primary walls are dynamic structures, whose composition and architecture changes during plant growth and development (http:www.ccrc.uga.edu/~mao/ouline.htm).

The pectic compounds present in the primary wall include pectins and protopectins (water-insoluble parent pectic substance) and have an essential role in the distribution of water within the wall, in the interaction between the water and the polysaccharides of matrix and between the matrix and microfibrils [11], while the hemicellulosic components include a variety of polysaccharides with linear or branched molecules [13, 14]. During cell wall growth or cell elongation the primary cell wall presents as membranes with arrangements of cellulosic microfibrils embedded in the gel-like matrix [12]. Cellulose from primary wall has a lower and more disperse degree of polymerization than from secondary wall and presents a biphasic distribution in the degree of polymerization range [9]. Pectin, cellulose and hemicellulose of the matrix are formed within the membrane of the Golgi body and their associated vesicles [11]. This material is released from the vesicle through the cytoplasm by the process called reverse pinocytosis and the polysaccharides are

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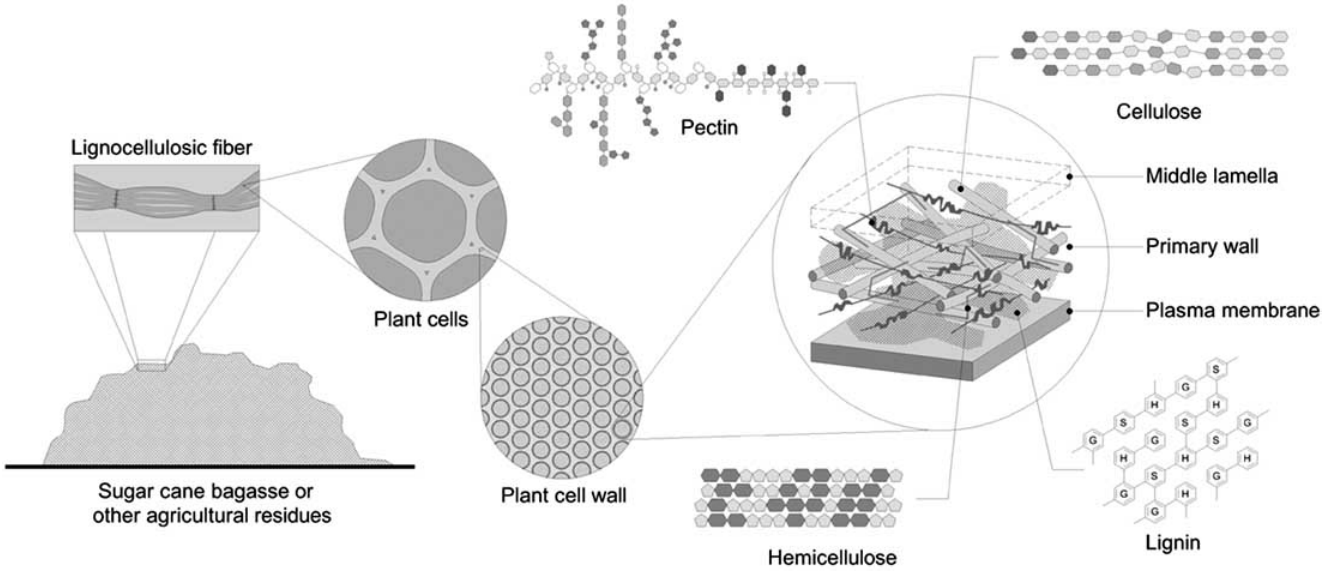


**Fig. (1).** Cell wall structure of agricultural residues.

packed into the wall. The model proposed by Keegstra *et al*. [15] suggests the binding of wall matrix polymers, including xyloglucan, pectin and glycoproteins, by covalent linkage. In this model cellulose is bonded to the wall matrix by H-bonding to xyloglucans, resulting in a non-covalently cross-linked cellulose-hemicellulose network, which is responsible for the wall tensile strength [4]. However, this model has been questioned because of the lack of evidence for the existence of covalent linkages between xyloglucan, pectin and glycoprotein [4]. Some other models were introduced, in order to give a re-evaluation of the control of wall enlargement. In the tethered network [16, 17], cellulose microfibrils are cross-linked and hydrogen bonded to xyloglucan. In this case, cellulose microfibrils may be tethered together directly via long xyloglucan chains [4]. In addition, the cellulose-xyloglucan network is physically entangled in a non-covalently cross-linked pectic network. Other variations of the tether model include the diffuse and the stratified layer models. In the diffuse model, xyloglucan is hydrogen bonded the surface of cellulose microfibrils without cross-link them directly. On the other hand, the stratified model has to do with the fact that xyloglucan are hydrogen bonded to and cross-link cellulose microfibrils. In this particular case, pectic layers act as spacers between xyloglucan-cellulose lamellae [18]. All the above models have in common the concept that cellulose microfibrils are coated with xyloglucan.

The secondary cell wall is formed inside the primary wall, when the cell enlargement is complete. The wall may become thickened and stronger and take on a distinctive shape and specialized properties [19]. Each type of mature plant cell has a characteristic secondary wall adapted to the particular function of the cell. The secondary wall is much denser and less hydrated than the primary wall and is laid down as three successive layers of cellulose, each of which is adjacent to the plasma membrane. During the cell wall thickening, cellulose is deposited in the secondary wall. Secondary cellulose deposition occurs after the cessation of expansion of the primary wall. Walls layers display a very orderly and parallel arrangement of the microfibrils [19]. In addition to cellulose, hemicelluloses are also laid down during secondary thickening. In angiosperms, these hemicelluloses are also laid down during secondary thickening and are preponderantly xylans, while in gymnosperms they are mainly glucomannans and galactoglucomannans. At the end of this period, lignin begins to form, mostly in the middle lamella, and serves to cement together the fibres, thereby strengthening the tissue and increasing the rigidity of cell wall. Lignin has an important function in restricting the breakdown of holocellulose structure by hydrolases. The contact between the microfibrils and the matrix in the lignified wall ensures a stress transfer between the components and also avoids that the layers and components of the wall will slip with respect to one another [11]. Thus, the cellulose fibrils are embedded in a network of hemicelluloses and lignin. Cellulose from secondary wall has a rather high degree of crystallinity, with all the glucan chains running in the same direction, and the individual chains being cross-linked to form microfibrils [9]. The network structure is responsible for the elimination of water from the wall and the formation of a hydrophobic composite that contributes to the recalcitrance of the secondary wall to enzyme action. In this context, ferulic acid plays an important function as the component that links hemicelluloses and pectin to each other as well as to lignin [13]. Xylans from monocots contain ferulolyl esters on the side chain of arabinofuranosyl residues [9]. It has been hypothesized that these esters are subject to a coupling reaction catalyzed by peroxidase [20]. The release of diferuloyl groups can cause the cross-linking of xylans, influencing the physical properties of the cell wall and its ability to grow and to resist enzymic attack. These coupling reactions may result in a binding together of the phenol-bearing polysaccharides within the cell wall, preventing the biodegradability of the plant cell wall by microorganisms [13]. The secondary wall presents most of the carbohydrate in biomass and shows much wider range of variability than of the primary cell wall. Because of that, it may play a key role as a source of renewable biomass that can be converted to in food and biofuel [21, 22].

The role of plant cell wall polysaccharides is a matter of intense discussion in several scientific reports [23]. The composition of plant cell wall polysaccharides varies from one cell type to another and one species to another [23, 24]. There is a great variety of linkages and branching types. The presence of branch points determines their solubility, viscosity and other physicochemical properties. These complex structures are cross-linked by ionic and covalent bonds that provide a barrier to physical penetration from microbial and mechanical forces [24]. A model of polysaccharide organization derived from a model of pectin structure was proposed by Vorwerk *et al*. [23]. In this model, the cellulose microfibrils are cross-linked by hemicellulose (xyloglucan) and loosely aligned with the highly conserved structure of rhamnogalacturonan I. Ho-

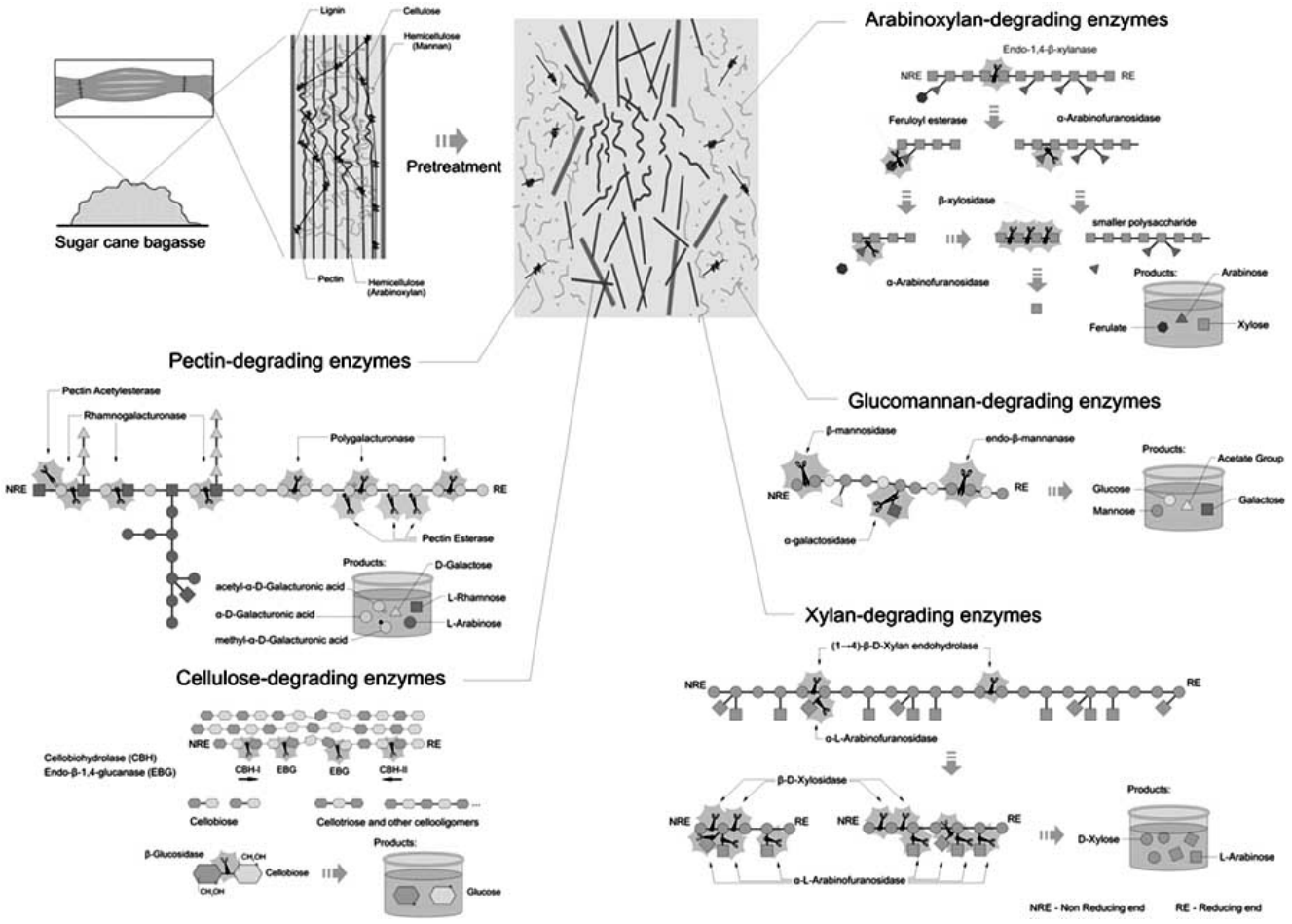


**Fig. (2).** Enzymatic attack on holocellulose structure.

mogalacturonan, arabinans, galactans and rhamnogalacturonan II are attached to rhamnogalacturonan I as side chains. It is worth to mention that cross-links between homogalacturonan and other pectins are formed through borate diester links between rhamnogalacturonan II, and by calcium molecule bridge between non-esterified domains on homogalacturonan.

Although extensive studies have been carried out, new insights into the structural complexity and heterogeneity of cell wall components such as holocellulose require the development of new techniques for imaging and characterizing the chemical topography of the cell wall at nanometre scale [1].

## III. ENZYMATIC BREAKDOWN OF HOLOCELLULOSE

Microorganisms are a rich source of enzyme systems displaying glycosyl hydrolase activities and involved in the breakdown of plant cell wall polysaccharides. The efficiency of bioconversion of these polymers to fermentable sugars depends upon an intricate mechanism of enzyme systems that includes a widespread group of glycosidases [2, 25, 26]. The breakdown of holocellulose is carried out by an ensemble of enzymes which hydrolyse glycosidic bonds in oligo- and polysaccharides (Fig. **2**). In some cases, a pretreatment method, such as steam explosion, is also required to increase holocellulose accessibility. The exo-holocellulases act on terminal glycosidic linkages and liberate monosaccharide units, while endo-holocellulases hydrolyse internal glycosidic bonds at random or at specific positions [27, 28]. In addition, enzymes that cleave various branch points are essential for complete hydrolysis of holocellulose. According to McCann and Carpita [24], the efficiency of holocellulose breakdown to fermentable sugars depends upon macroscopic and molecular features of cell wall polysaccharides. At macroscopic level, it must be considered as the spatial organization of different cell types, the strength and extent of cell-cell adhesion, and the spatial distribution of lignin. At the molecular level, the composition, structural heterogeneity and complexity of cell wall components of different cell types contribute to the recalcitrance of holocellulose to enzymatic attack [1]. This recalcitrance is also due to the strong interchain hydrogen-bonding network present in crystalline cellulose core.

Cellulases, hemicellulases and pectinases belong to a group of enzymes called holocellulase that shows two conserved mechanisms of acid/base hydrolysis of the glycosidic bonds with retention or inversion of the anomeric configuration at the cleavage point [2, 29, 30]. Retention occurs by way of double displacement and inversion via a single displacement reaction [25, 30, 31]. Both mechanisms involve stabilization of an oxacarbonium ion by electrostatic interaction and a pair of carboxylic acids at the active site [30]. Some xylanases and cellulases work via two consecutive single displacements in which anomeric configuration is retained, while others catalyze single displacement reactions with inversion of configuration [28]. However, the physiological role of these mechanisms of reaction remains to be established.

Holocellulose is an insoluble structure with a size of many thousands of carbohydrate residue units. Because of the heterogenous nature of the holocellulose structure, the synergistic associa-

tion between cellulase and other holocellulose-degrading enzymes is responsible for an efficient and extensive degradation of these carbohydrate structures. Holocellulases are involved in holocellulose breakdown at polymeric and oligomeric levels. It has been said that endo-holocellulases do not readily attack holocellulose because such complex polysaccharides lack unsubstituted regions of similar sugar residues and linkages. In this particular case, it is relevant to mention the action of enzymes that liberate substituents from the main chain structure of holocellulose. In contrast to those endo-holocellulases, the hydrolytic ability in the immediate vicinity of substituted regions have been reported [31]. Holocellulases are grouped in many families of glycosyl hydrolases and may contain non catalytic substrate binding domains in their structure, as well as linker sequences. Two of these families, named 10/F and G/11, present a variety of enzyme with narrow and absolute specificity towards the type of glycosidic bond, respectively [32]. The substrate cross-specificity is a characteristic of many holocellulases [20]. In this case, some holocellulases have a broad specificity whereas some are restricted to a specific substrate [33]. As mentioned before, the hydrolysis of holocellulose by glycosyl hydrolases is linked with plant cell wall structural characteristics as, for example, the nature and extent of the cross-links between different polysaccharides, the interactions between lignin and carbohydrates, the nature and extent of protein cross-linking, cellulose crystallinity and microfibril size [24].

Within the above context, it would be relevant to discuss some aspects of enzyme specificity with emphasis to promiscuity behavior. The nomenclature for glycosyl hydrolases based on reaction catalyzed and substrate specificity has to take into account some aspects related to evolutionary divergence or convergence [34]. Evolutionary divergence has to do with changes in specificity and reaction type, while convergence evolution implicates in enzymes with different folds to catalyze the same reaction on a given substrate.

The glossary below for divergent evolution gives some definitions to describe relationships in sequence, structure and function [35, 36]. Homologs are enzymes that derive from a common ancestor and are structurally related. This group of enzymes shows a high degree of sequence similarity and can also be highly divergent, being thus not specific to a determined chemical reaction. In addition, they can be classified into three categories [35]: family (group of enzymes that catalyze the same reaction mechanism and substrate specificity), superfamily (group of enzymes that catalyze either the same chemical reaction with different substrate specificities or different overall reactions that share a common mechanistic attribute, including partial reaction, intermediate, or transition state, enabled by conserved active site residues that perform the same function), and suprafamily (group of enzymes that catalyze different overall reactions which do not share mechanistic functions, performing different attributes in the members of the superfamily). Orthologs is another term to describe homologous enzymes in different species that catalyze the same reaction. On the other hand, paralogs are homologous enzymes in the same species that diverged from one to another by gene duplication after speciation. Analogs refer to enzymes that catalyze the same reaction but are not structurally related.

The above concepts can be useful to address fundamental questions about the behavior of glycosyl hydrolases in the hydrolysis of holocellulose, having in mind the ability of these enzyme systems to adapt under different structural conditions. Morevover, microbial strategies to overcome the natural resistance of plant cell wall to enzyme attack are concentrated in some parameters of enzyme and substrate specificity. These parameters have interesting implications on our understanding of how the holocellulose structures are enzymatically degraded. Many holocellulases act in a range of structurally similar substrates, while others show ability to catalyze alternative reactions with a range of substrates. Hult and Berglund [3] define promiscuous enzyme as one performs the action. Enzyme

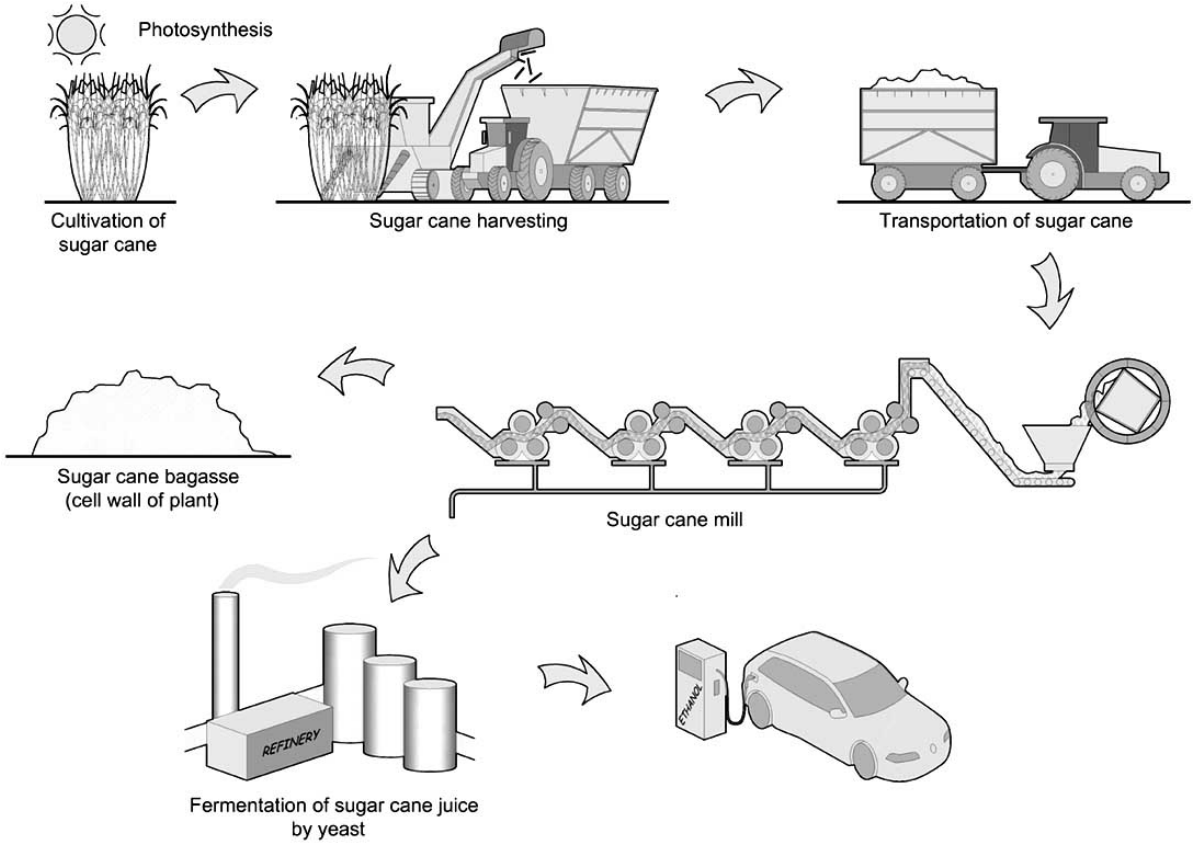


**Fig. (3).** Overview of sugar cane ethanol production.

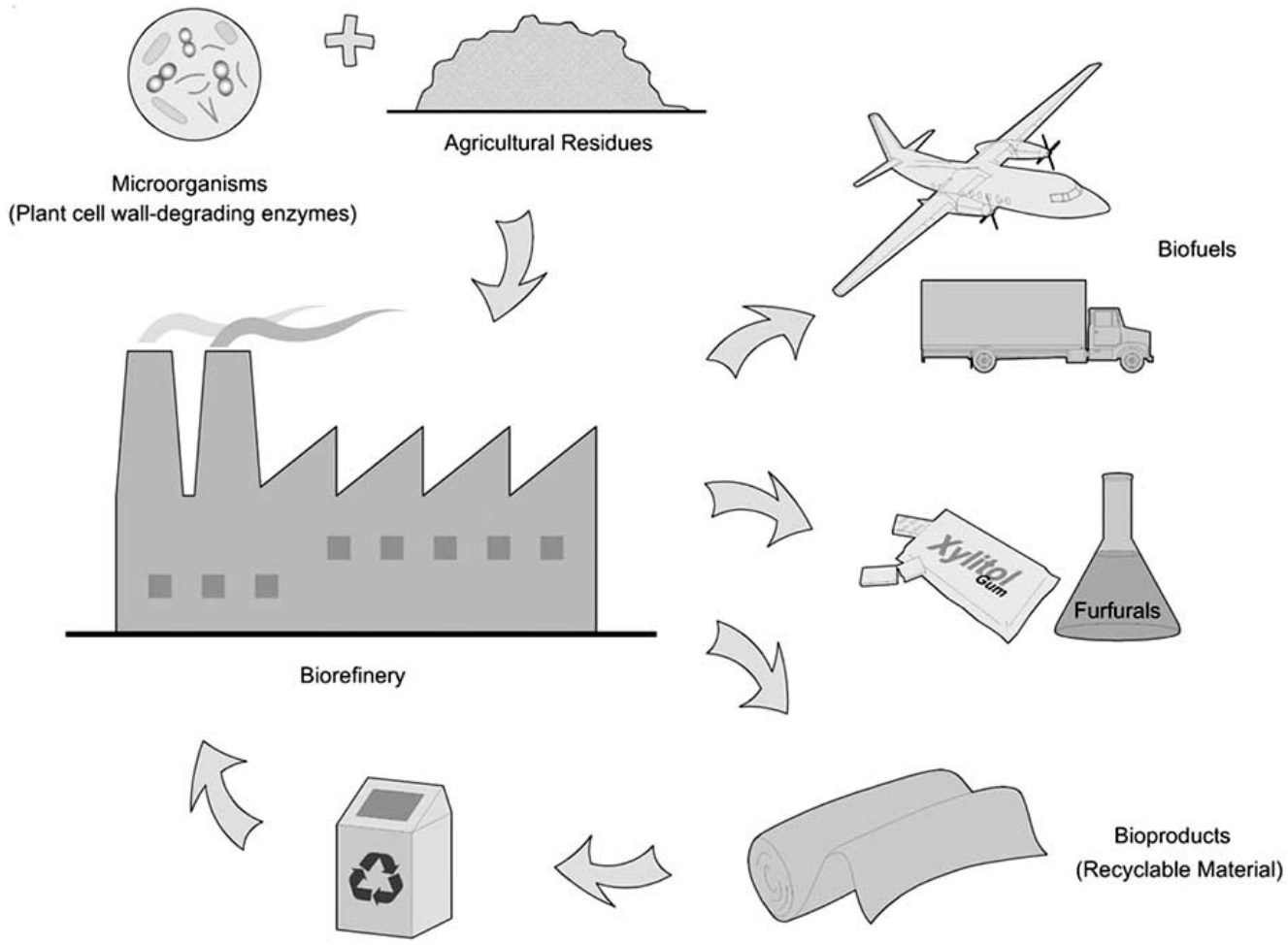


**Fig. (4).** Simplified model of biorefinery.

promiscuity can be classified in terms of reaction conditions, substrate with relaxed or broad specificity and catalytic properties through different chemical transformations with different transition states. According to Khersonsky *et al.* [37], when a need for new enzymatic functions arise, nature recruits existing enzymes that promiscuously bind the new substrate, or catalyze the new reaction, and then tinkers with their active sites to fit the new substrate and reaction. From the above concepts, it is possible to consider new families of holocellulases presenting a relaxed behavior against different types of substrates and ability to survive in a complex environment and as result of these enzymes have diverged from existing ones. One interesting question was proposed by Glasner *et al.* [36]: how was evolution produced an incredible variety of enzymatic activities from a limited number of protein folds? Back to the cell wall environment, we may consider a consortium of enzyme systems facing the cell wall matrix structure that includes different types of connections and a crystalline substrate like cellulose. This would require from these enzymes conformation states in order to adapt to change in reactions conditions.

## IV. ENZYME APPLICATIONS

Holocellulose represents a major reserve of reduced carbon in the environment. Large amounts of holocellulose are present in urban and agro-industrial residues in a form that cannot readily be buried and which has to be disposed off at considerable costs. Therefore, there is a great interest in holocellulose breakdown because of the possible applications in ruminal digestion, waste treatment, fuel chemical production, and paper manufacture [7, 25]. This may lead to an increased interest in the use of holocellulases, in order to reduce the costs. The exploitation of such materials would require the holocellulose components be used directly or degraded into their respective monomers and then to desirable end products (Fig. **4**). Moreover, holocelluloses may be used as a high-grade raw material to produce monomers as glucose and xylose which can then be used as a feedstock for single-cell protein production or in fermentation to ethanol [38-40]. Different regions of the world have used energy crops as feedstock for the production of fuel ethanol. Fig. (**3**) shows an example of ethanol production by using sugarcane having in mind the Brazilian model. The bioconversion of holocellulose into ethanol reduces processing costs in the overall process and hence makes the process economically viable [41-43]. Sugarcane bagasse is a fibrous organic material that remains after sugar liquor has been removed from the sugar cane and is considered as potential source of ethanol in some developing countries, including Brazil and India [44-46]. It is a lignocellulosic substrate, composed of 42% cellulose, 22% lignin, 28% hemicelluloses and 8% of cane wax and organic acid [44]. Thus, the enzymatic hydrolysis of this residue, aiming commercial and industrial applications, requires the synergistic associations between lignases and holocellulases. Holocellulases are also important for the efficient degradation of plant materials in animal feed [47]. The accessibility of cellulose to ruminal digestion can be improved by partial enzymatic hydrolysis of holocellulose in animal feed with

consequent improvement of the nutritional value of the feed. Holocellulases can also be used in the bleaching of Kraft pulps or to improve fibre properties [27, 45]. There are applications of holocellulases in clarification of juices, preparations of dextrans for use as food thickeners, production of fluids and juices from plant materials, and in processes for the manufacture of liquid coffee and adjustment of wine characteristics [39, 47]. The hydrolases are the majority of currently used industrial enzymes which have carbohydrate-degrading enzymes as the second largest group. The cost of these enzymes has been identified as an economic barrier for their use in biorefineries. Over the years much efforts has been employed to reduce the cost of producing holocellulases [48-50]. For example, cellulase production costs have reached the range of 10-20 cents per gallon of ethanol produced [48]. A major challenge is the improvement of strategies that includes enzyme engineering based on directed evolution and rational design [50, 51].

## V. CONCLUSIONS

Therefore, having in mind the obvious importance of holocellulases in the degradation of different types of polysaccharide structures, including agricultural residues, it would be relevant to address the following hypothesis adapted from Hult and Berglund [3]: holocellulases can be exposed to reaction conditions and substrates in the plant cell wall environment that will challenge their specificity and might force them to handle substrates and catalyze reactions they were not designed for. It is also interesting to mention the hypothesis that the above relaxed specificity can result from different conformations in the ensemble catalyzing different reactions, with the native activity catalyzed by the most stable (ground-state) conformation. According to Wroe *et al.* [52], a mutation that increases the stability of a nonnative conformation increases its occupancy in the ensemble and the activity corresponding to this conformation. Thus, holocellulose structure would be a source of nonnative substrates being catalyzed by a spectrum of enzymes showing varying efficiency. In addition, conformational changes enable holocellulases to accommodate different substrates and show relaxed substrate specificity. Another point to be considered has to do with a number of structurally unrelated holocellulases catalyzing the same biochemical reactions. Analogous enzymes (without detectable sequence similarity) are reported as performing functions related to adaptation to new environments and life styles and usually have a limited phylogenetic distribution [53]. Therefore, the scenario of plant cell wall degradation would also include the recruitment of existing holocellulases that take over new functions related to changes in holocellulose specificity or a modified catalytic mechanism.

Because of the structural complexity of plant cell wall, a wide variety of enzyme systems have been developed by different sources, including bacteria and fungi, as strategy to overcome the matrix components. Finally, the large market commercial appeal of holocellulases encourages the development of enzyme preparations able to carry out an efficient hydrolysis of plant cell wall structures.

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