Value added products from hemicellulose: Biotechnological perspective

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Abstract

Lignocellulosic biomass represents a renewable, widespread and low cost source of sugars which can be used as substrate for the production of specialty chemicals. Various agricultural residues contain about 20-30% hemicellulose, the second most abundant biopolymer found in nature. They are heterogeneous polymer of pentoses (xylose and arabinose) hexoses (mannose, glucose and galactose) and sugar acids. Xylans is the major component of hemicellulose and are heteropolysaccharides with homopolymeric backbone chains of 1, 4 linked β-D-xylopyranose units. The conversion of hemicellulose to bioethanol and other value added products is a challenging problem and is foreseen to be essential for the economic success of lignocellulosic ethanol. The xylose from the hemicellulose hydrolysate can be fermented to ethanol/ xylitol/lactic acid and other valuable products by pentose fermenting microbes. Xylitol a non caloric sugar has important applications in pharmaceuticals and food industries due to high sweetening power, non- and anticariogenicity property. The acid or enzymatic hydrolysis of hemicellulose to fermentable sugars will be outlined. The enzymatic and fermentation routes for the conversion of hemicellulose into useful products will be described.

Keywords

Hemicellulose, Hemicellulases, Hydrolysis, Fermentation route, Biochemicals, Recombinant approaches
1 Introduction

In collation to first generation bioethanol from starch and molasses, the development of second generation bioethanol from lignocellulosic biomass serves many advantages from both energy and environmental concerns. Efficient conversion of lignocellulosic materials is a global challenge for eco-friendly value addition. Cellulose is the primary constituent of lignocellulosic biomass and is the most abundant organic compound available. The estimated global annual production of biomass is $1 \times 10^{11}$ tons [1] and is considered to be one of the significant renewable resources for the production of biofuel and value added products. Unlike fossil fuels, cellulosic ethanol produced through fermentation of sugars is a renewable energy source. Biomass in general consists of 40-50% cellulose, 25 to 30% hemicellulose and 15 to 20% lignin. The effective utilization of all the three components would play a significant role in economic viability of the cellulose to ethanol process [2].

In Indian scenario, bagasse and starchy biomass are expected to be primary feedstocks in preference to other materials such as lipids. The setting up of biorefinery requires that technologies be developed for biomass pretreatment, hydrolysis of cellulose and hemicellulose and creation of microbes by recombinant DNA techniques for the production of desired biochemicals and further their strain improvement. The biorefinery concept is being built on two different platforms by National Renewable Energy Lab (NREL) to promote different product areas. The sugar platform based on biochemical conversion process focuses on the fermentation of sugar extracted from biomass feedstocks and the other platform is based on thermo chemical conversion processes and focuses on the gasification of biomass feedstocks and byproducts from the conversion process (www.nrel.gov/biomass/biorefinery.html). Biorefineries using fibrous lignocellulosic biomass as feedstock will produce C6 and C5 sugars (predominantly xylose and arabinose) and lignin.

Various agricultural residues contain about 20-30% hemicellulose, the second most abundant biopolymer found in nature. They are heterogeneous polymer of pentoses, hexoses and sugar acids. In recent years, bioconversion of hemicellulose has received
much attention because of its practical applications in various agro-industrial processes, for the production of fuels and chemicals, delignification of paper pulp, digestibility enhancement of animal feedstock, clarification of juices, and improvement in the consistency of beer [3]. The present review is a comprehensive state-of-the-art describing the acid and enzymatic hydrolysis of hemicellulose to fermentable sugars. The article also covers the recent developments in the biotechnological and recombinant aspects for the fermentation of hemicellulosic hydrolysates to value added products.

2 Structures of Hemicelluloses

Hemicelluloses are heterogeneous polysaccharides, which are located between the lignin and cellulose fibres and, depending on wood species, constitute about 20-30% of the naturally occurring lignocellulosic plant biomass [4]. They are composed of both linear and branched heteropolymers of D-xylose, L-arabinose, D-mannose, D-glucose, D-galactose, L-rhamnose, L-fucose and D-glucuronic acid, which may be acetylated or methylated. Most hemicelluloses contain two to six of these sugars [5]. They are usually classified according to the main sugar residues in the backbone. Homopolymers of xylose, so-called homoxyxylans only occur in seaweeds (red and green algae). The two main hemicelluloses in wood are xylans and glucomannans both of which are present in softwood, whereas in hardwood, xylan is the major component. Xylan is the second most abundant polysaccharide, comprising up to 30% of the cell wall material of annual plants, 15-30% of hardwoods and 7-10% of softwoods [6]. Xylan occurs as a heteropolysaccharide with a homopolymeric backbone chain of 1,4-linked β-D-xylopyranose units, which consists of O-acetyl, α-L-arabinofuranosyl, α-1, 2-linked glucuronic or 4-O-methylglucuronic acid substituents. In hardwoods, xylan exists as O-acetyl- 4-O-methylglucuronoxylan with a higher degree of polymerization (150-200) than softwoods (70-130), which occurs, as arabino-4-O-methylglucuronoxylan. Approximately one in ten of the β-D-xylopyranose backbone units of hardwood xylan are substituted at C-2 position with 1,2-linked 4-O-methyl-α-D-glucuronic acid residue, whereas 70% are acetylated at C-2 or C-3 positions or both [4]. Softwood xylans are not acetylated but the 4-O-methylglucuronic acid and the β-arabinofuranose residues are attached to the C-2 and C-3 positions, respectively of the relevant xylopyranose backbone.
units [7]. Birch wood (Roth) xylan contains 89.3% xylose, 1% arabinose, 1.4% glucose, and 8.3% anhydrouronic acid [8]. Rice bran neutral xylan contains 46% xylose, 44.9% arabinose, 6.1% galactose, 1.9% glucose, and 1.1% anhydrouronic acid [9]. Wheat arabinoxyylan contains 65.8% xylose, 33.5% arabinose, 0.1% mannose, 0.1% galactose, and 0.3% glucose [10]. Corn fiber xylan is one of the complex heteroxylans containing β-(1,4)-linked xylose residues [11]. It contains 48–54% xylose, 33–35% arabinose, 5–11% galactose and 3–6% glucuronic acid [12]. The xylan of grasses occurs as arabino-4-O-methylglucuronoxylan with a degree of polymerisation of 70 and contains β-arabinofuranosyl side chains linked to C-2 or C-3 positions, or both, of the β-D-xylopyranose main chain residues. The 1,2-linked 4-O-methyl-α-D-glucuronic acid is found less frequently in grasses than in hardwood xylan. Hardwood xylan contains 2-5% by weight of O-acetyl groups linked to C-2 or C-3 positions of the xylopyranose units, and 6% of the arabinosyl side chains are themselves substituted at position 5 with feruloyl groups, whereas 3% are substituted with p-coumaroyl residues [13]. The relative proportions of the various components of grass arabinoxylan vary from species to species and from tissue to tissue within a single species. The main mannan group in the cell walls of higher plants is galacto-glucomannan with the backbone composed of 1, 4-β-linked D-glucose and D-mannose units that are distributed randomly within the molecule. Softwood glucomannan has a backbone composed of β-1, 4- linked D-glucopyranose and D-mannopyranose units, which are partially substituted by α-galactose units and acetyl groups. Hardwoods contain a small amount of glucomannan (2-5%) that has no galactose or acetic acid side groups present [14]. Table 1 describes the composition of representative hemicellulosic feedstocks. Xylans are usually available in large amounts as by-products of forest, agriculture, agro-industries, wood and pulp and paper industries.

### 2.1 Arabinoxylans

Arabinoxylans represent the major hemicellulose structures of the cereal grain cell walls and are similar to hardwood xylan but the amount of L-arabinose is higher. The linear β-(1,4)-D-xylopyranose backbone is substituted by α-L-arabinofuranosyl units in the positions 2-O and/or 3-O and by α-D-glucopyranosyl uronic unit or its 4-O-methyl derivative in the position 2-O [40, 41]. O-acetyl substituents may also occur [42, 43].
Arabinofuranosyl residues of arabinoxylan may also be esterified with hydroxycinnamic acids residues, e.g., ferulic and p-coumaric acids [43]. Dimerization of esterified phenolic compounds may also lead to inter- and intra-molecular cross-links of xylan. The physical and/or covalent interactions with other cell-wall constituents, restricts the extractability of xylan. In lignified tissues for example, xylan is ester linked through its uronic acid side chains to lignin [44].

2.2 Glucuronoxylans

Glucuronoxylans (O-acetyl-4-O-methylglucuronoxylan) are the main hemicellulose of hardwoods, which may also contain small amounts of glucomannans (GM). In hardwoods, it represent 15–30% of their dry mass and consist of a linear backbone of β-D-xylopyranosyl units (xylp) linked by β-(1,4) glycosidic bonds. Some xylose units are acetylated at C2 and C3 and one in ten molecules has an uronic acid group (4-O-methylglucuronic acid) attached by α-(1,2) linkages. The percentage of acetyl groups ranges between 8% and 17% of total xylan, corresponding, in average, to 3.5–7 acetyl groups per 10 xylose units [45]. The 4- O-methylglucuronic side groups are more resistant to acids than the Xylp and acetyl groups. The average degree of polymerization (DP) of glucuronoxylan is in the range of 100–200 [46]. Besides these main structural units, it may also contain small amounts of L-rhamnose and galacturonic acid. The later increases the polymer resistance to alkaline agents.

2.3 Arabinoglucuronoxylans

Arabinoglucuronoxylans (arabino-4-O-methylglucuronoxylans) are the major components of non-woody materials (e.g., agricultural crops) and a minor component for softwoods (5–10% of dry mass). They also consist of a linear β-(1,4)-D-xylopyranose backbone containing 4-O-metil-α-D-glucopiranosyl uronic acid and α- L-arabinofuranosyl linked by α-(1,2) and α-(1,3) glycosidic bonds [47,48]. The typical ratio arabinose: glucuronic acid:xylose is 1:2:8 [45]. Conversely to hardwood xylan, arabinoglucuronoxylan might be less acetylated, but may contain low amounts of galacturonic acid and rhamnose. The average DP of AGX ranges between 50 and 185 [46].
2.4 Xyloglucans

Xyloglucan is a predominant hemicellulose found in the primary cell wall of dicots and several monocots ([49], consists of a β-(1→ 4) - linked β-D-Glcp (β-D-glucopyranose) backbone having up to 75% of the β-D-Glcp residues covalently linked to α-D-Xylp (α-D-xylopyranose) at the O-6 position. While the extent of xylose substitution varies considerably, the glucose backbone structure is typically substituted by 50% or 75% xylose [50]. Xylose can be further substituted by galactose or arabinose and the galactose may be fucosylated. The glucose, xylose and galactose residues are present in the ratio of 2.8: 2.25:1.0. In addition, xyloglucans can contain O-linked acetyl groups [51, 52]. Xyloglucans interact with cellulose microfibrils by the formation of hydrogen bonds, thus contributing to the structural integrity of the cellulose network [49, 53]. XG is also a storage polymer in the seed endosperm of many plants. Tamarind kernel powder (TKP) a rich source of hemicellulose, galactoxyloglucan (GXG) is a crude extract derived from the seeds of Tamarindus indica Linn., an abundantly grown tree of Southeast Asia, India, Mexico, and Costa Rica [54].

2.5 Galactoglucomannans

Galactoglucomannans (O-acetyl-galactoglucomannans) are the main hemicelluloses of softwoods, accounting up to 20–25% of their dry mass [46]. It consist of a linear backbone of β-D-glucopyranosyl and β-D-mannopyranosyl units, linked by β-(1,4) glycosidic bonds, partially acetylated at C2 or C3 and substituted by α-D-galactopyranosyl units attached to glucose and mannose by α-(1,6) bonds. Some are water soluble, presenting in that case higher galactose content than the insolubles [55]. Acetyl group’s content of galactoglucomannan is around 6%, corresponding, to 1 acetyl group per 3–4 hexoses units. Glucomannans occur in minor amounts in the secondary wall of hardwoods (<5% of the dry wood mass) [45]. They have linear backbone of β-D-glucopyranosyl (Glcp) and β-D-mannopyranosyl (Manp) units but the ratio Glcp:Manp is lower. The extent of galactosylation governs the association of galactoglucomannan and glucomannans to the cellulose microfibrils and hence, their extractability from the cell
wall matrix [44]. The average DP of galactoglucomannans ranges between 40 and 100 [46].

2.6 Complex heteroxylans

Complex heteroxylans are present in cereals, seeds, gum exudates and lucilages and they are structurally more complex [56]. The β-(1,4)-D-xylopyranose backbone is substituted with single uronic acid and arabinosyl residues and also with various mono- and oligoglycosyl side chains.

3 Separation and saccharification of hemicellulose

Without a profitable use of the hemicellulose fraction, bioethanol is too expensive to compete in commercial markets [57]. Therefore to foster the commercial production of lignocellulosic ethanol, bioconversion of the hemicelluloses into fermentable sugars is essential. The initial step for the production of bioethanol is pre-treatment. The hemicelluloses are commonly removed during the initial stage of biomass processing aiming to reduce the structural complexity for further enzymatic cellulose hydrolysis. Various pretreatment processes are available to fractionate, solubilize, hydrolyse and separate cellulose, hemicellulose and lignin. These include concentrated acid [58], dilute acid [11], alkaline [59], SO2 [60], hydrogen peroxide [61], steam explosion (autohydrolysis) [62], ammonia fiber explosion (AFEX) [63], wet-oxidation [64], lime [65], liquid hot water [66], CO2 explosion [67], organic solvent treatments [68], ionic liquids [69, 70] and supercritical fluids [71]. The main processes for the selective separation of hemicelluloses from biomass include the use of acids, water (liquid or steam), organic solvents and alkaline agents. The later two are not selective towards hemicellulose as they also remove lignin, which in turn can hinder the valorisation process, e.g., fermentation or bioconversion, as the lignin-derived compounds are usually microbial growth inhibitors. Therefore, acid/water/steam pretreatments are the most commonly applied technologies yielding a selectively solubilisation of hemicelluloses and producing hemicellulose-rich liquids totally or partially hydrolysed to oligomeric and monomeric sugars and cellulose-enriched solids for further bioprocessing [72]. Depending on the operational conditions degradation products are also formed, both from
sugars (furan and its derivates and weak acids) and, to a less extent, from lignin (phenolics) [73]. These compounds may also inhibit the fermentation processes, leading to lower ethanol yields and productivities; however a prior detoxification step is essential. The following section describes few selected pretreatments wherein most of the hemicellulose is obtained in the form of oligosaccharides and monomeric sugars.

3.1 Acid hydrolysis

3.1.1 Dilute acid hydrolysis

The dilute acid hydrolysis of the biomass is, by far, the oldest technology for converting the biomass to ethanol. The first attempt at commercializing a process for the ethanol from the wood was carried out in Germany in 1898. It involved the use of dilute acid to hydrolyze the cellulose to glucose, and was able to produce 7.6 liters of ethanol per 100 kg of wood waste (18 gal per ton). The hydrolysis occurs in two stages to accommodate the differences between the hemicellulose and the cellulose [74] and to maximize the sugar yields from the hemicellulose and cellulose fractions of the biomass. The first stage is operated under milder conditions to hydrolyze the hemicellulose, while the second stage is optimized to hydrolyze the more resistant cellulose fraction. The liquid hydrolyzates are recovered from each stage, neutralized, and fermented to ethanol.

National Renewable Energy Laboratory (NREL) a facility of US Department of energy (DOE) operated by Midwest Research institute, Bettelle, NREL outlined a process where the hydrolysis is carried out in two stages to accommodate the differences between hemicellulose and cellulose. NREL has reported the results for a dilute acid hydrolysis of softwoods in which the conditions of the reactors were as follows: Stage 1: 0.7% sulfuric acid, 190°C, and a 3-minute residence time; Stage 2: 0.4% sulfuric acid, 215°C, and a 3-minute residence time. The liquid hydrolyzates are recovered from each stage and fermented to alcohol. Residual cellulose and lignin left over in the solids from the hydrolysis reactors serve as boiler fuel for electricity or steam production. These bench-scale tests confirmed the potential to achieve yields of 89% for mannose, 82% for
galactose and 50% for glucose. The fermentation with \textit{Saccharomyces cerevisiae} achieved ethanol conversion of 90% of the theoretical yield [75].

Typical sulphuric acid concentrations for hemicellulose hydrolysis are in the range 0.5–1.5% and temperatures above 121–160°C. From hemicelluloses, dilute-acid processes yield sugar recoveries from 70% up to >95% [76-79]. The advantages of dilute-acid hydrolysis are the relatively low acid consumption, limited problem associated with equipment corrosion and less energy demanding for acid recovery. Under controlled conditions, the levels of the degradation compounds generated can also be reduced [72].

\subsection*{3.1.2 Concentrated Acid Hydrolysis}

This process is based on concentrated acid decrystallization of the cellulose followed by the dilute acid hydrolysis to sugars at near theoretical yields. The separation of acid from the sugars, acid recovery, and acid re-concentration are critical unit operations. The fermentation converts sugars to ethanol. A process was developed in Japan in which the concentrated sulfuric acid was used for the hydrolysis and the process was commercialized in 1948. The remarkable feature of their process was the use of membranes to separate the sugar and acid in the product stream. The membrane separation, a technology that was way ahead of its time, achieved 80% recovery of acid [80]. The concentrated sulfuric acid process has also been commercialized in the former Soviet Union. However, these processes were only successful during the times of the national crisis, when economic competitiveness of the ethanol production could be ignored. Concentrated hydrochloric acid has also been utilized and in this case, the prehydrolysis and hydrolysis are carried out in one step. Generally, acid hydrolysis procedures give rise to a broad range of compounds in the resulting hydrolysate, some of which might negatively influence the subsequent steps in the process. A weak acid hydrolysis process is often combined with a weak acid prehydrolysis.

In 1937, the Germans built and operated commercial concentrated acid hydrolysis plants based on the use and recovery of hydrochloric acid. Several such facilities were successfully operated. During World War II, researchers at USDA's Northern Regional
Research Laboratory in Peoria, Illinois further refined the concentrated sulfuric acid process for corncobs. They conducted process development studies on a continuous process that produced a 15%-20% xylose sugar stream and a 10%-12% glucose sugar stream, with the lignin residue remaining as a byproduct. The glucose was readily fermented to ethanol at 85%-90% of theoretical yield. The Japanese developed a concentrated sulfuric acid process that was commercialized in 1948. The remarkable feature of their process was the use of membranes to separate the sugar and acid in the product stream. The membrane separation, a technology that was way ahead of its time, achieved 80% recovery of acid [80]. R&D based on the concentrated sulfuric acid process studied by USDA (and which came to be known as the "Peoria Process") picked up again in the United States in the 1980s, particularly at Purdue University and at Tennessee Valley Authority (TVA) [81]. Among the improvements added by these researchers were 1) recycling of dilute acid from the hydrolysis step for pretreatment, and 2) improved recycling of sulfuric acid. Minimizing the use of sulfuric acid and recycling the acid cost-effectively are critical factors in the economic feasibility. (http://www1.eere.energy.gov/biomass/printable_versions/concentrated_acid.html). The conventional wisdom in the literature suggests that the Peoria and TVA processes cannot be economical because of the high volumes of acid required [82]. The improvements in the acid sugar separation and recovery have opened the door for the commercial application. Two companies namely Arkenol and Masada in the United States are currently working with DOE and NREL to commercialize this technology by taking advantage of niche opportunities involving the use of biomass as a means of mitigating waste disposal or other environmental problems (http://www1.eere.energy.gov/biomass/concentrated_acid.html). Minimizing the use of the sulfuric acid and recycling the acid cost-effectively are the critical factors in the economic feasibility of the process. U.S.Pat. No. 5,366,558 [83] uses two "stages" to hydrolyze the hemicellulose sugars and the cellulosic sugars in a countercurrent process using a batch reactor, and results in poor yields of glucose and xylose using a mineral acid. Further, the process scheme is complicated and the economic potential on a large-scale to produce inexpensive sugars for fermentation is low. U.S. Pat. No. 5,188,673 [84] employs concentrated acid hydrolysis which has benefits of high conversions of biomass,
but suffers from low product yields due to degradation and the requirement of acid recovery and recycles. Sulfuric acid concentrations used are 30-70 weight percent at temperatures less than 100°C. Although 90% hydrolysis of the cellulose and hemicellulose is achieved by this process, the concentrated acids are toxic, corrosive and hazardous and require reactors that are resistant to corrosion. In addition, the concentrated acid must be recovered after the hydrolysis to make the process economically feasible [85]. A multi-function process for hydrolysis and fractionation of lignocellulosic biomass to separate hemicellulosic sugars using mineral acid like sulfuric acid, phosphoric acid or nitric acid has been described [86]. A process for treatment of hemicellulose and cellulose in two different configurations is described [87]. Hemicellulose is treated with dilute acid in a conventional process. The cellulose is separated out from the "prehydrolyzate" and then subjected to pyrolysis at high temperatures. Further, the process step between the hemicellulose and cellulose reactions require a drying step with a subsequent pyrolysis high temperature step at 400-600°C for conversion of the cellulose to fermentable products.

3.2 Steam explosion

Autohydrolysis and steam explosion processes selectively hydrolyze hemicellulose from lignocellulosic biomass. The process is economic and ecofriendly as it doesn’t involves chemical catalyst. A relatively high hemicellulose recovery in the range of 55–84%, together with low levels of inhibitory by-products, has been obtained through autohydrolysis [88-91]. Cellulose and lignin are not significantly affected, yielding a cellulose- and lignin-rich solid phase together with a liquid fraction with a relative low concentration of potential fermentation inhibitors. The main drawback of this process is that the solubilised hemicellulose appears mainly in oligomeric form [16, 22, 90, 92, 93,]. In principle steam explosion is one of the attractive pretreatment methods that can cause disintegration of the material, thereby creating a large surface area on which cellulase enzyme complex can act upon. Simultaneously hemicellulose is separated during the steam explosion process thereby improving the accessibility to the enzymes and enhancement of the over all lignocellulose degradation [94]. Most steam treatments yield high hemicellulose solubility (producing mainly oligosaccharides) along
with slight lignin solubilisation. Studies without added catalyst report sugars recoveries between 45% and 69% [95-98]. Cara et al., [99] have reported a 30% increase in maximum pentose yield for olive tree prunings when steam explosion was followed by acid hydrolysis.

3.3 Enzymatic hydrolysis

The most promising method for hydrolysis of polysaccharides to monomer sugars is by use of enzymes, i.e., cellulases and hemicellulases. Moreover, hemicellulases facilitate cellulose hydrolysis by exposing the cellulose fibres, thus making them more accessible [100]. The enzymatic hydrolysis of the lignocellulosic biomass is preceded by a pretreatment process in which the lignin component is separated from the cellulose and hemicellulose to make it amenable to the enzymatic hydrolysis. The lignin interferes with the hydrolysis by blocking the access of the cellulases to the cellulose and by irreversibly the binding hydrolytic enzymes. Therefore, the removal of the lignin can dramatically increase the hydrolysis rate [101]. Recently the enzymatic hydrolysis of lignocellulosic biomass has been optimized using enzymes from different sources and mixing in an appropriate proportion using statistical approach of factorial design. A twofold reduction in the total protein required to reach glucan to glucose and xylan to xylose hydrolysis targets (99% and 88% conversion, respectively), thereby validating this approach towards enzyme improvement and process cost reduction for lignocellulose hydrolysis [102].

3.3.1 Hemicellulose degrading enzymes

3.3.1.1 Xylanases and Mannanases

Hemicellulases are multi-domain proteins and generally contain structurally discrete catalytic and non-catalytic modules. The most important non-catalytic modules consist of carbohydrate binding domains (CBD) which facilitate the targeting of the enzyme to the polysaccharide, interdomain linkers, and dockerin modules that mediate the binding of the catalytic domain via cohesion-dockerin interactions, either to the microbial cell surface or to enzymatic complexes such as the cellulosome [100]. Based on the amino acid or nucleic acid sequence of their catalytic modules hemicellulases are
either glycoside hydrolases (GHs) which hydrolyse glycosidic bonds, or carbohydrate esterases (CEs), which hydrolyse ester linkages of acetate or ferulic acid side groups and according to their primary sequence homology they have been grouped into various families. Most studies on hemicellulases have focused until now on enzymes that hydrolyse xylan [7]. Enzymes that hydrolyse mannan have been largely neglected, even though it is an abundant hemicellulose, therefore the application of mannanases for catalysing the hydrolysis of β-1,4 mannans is as important as the application of xylanases.

Due to the complex structure of hemicelluloses, several different enzymes are needed for their enzymatic degradation or modification. The two main glycosyl hydrolases depolymerising the hemicellulose backbone are endo-1, 4- β-D-xylanase and endo-1, 4-β-D-mannanase [5]. Endo-1,4-β-xylanase cleaves the glycosidic bonds in the xylan backbone, bringing about a reduction in the degree of polymerization of the substrate. Xylan is not attacked randomly, but the bonds selected for hydrolysis depend on the nature of the substrate molecule, i.e., on the chain length, the degree of branching, and the presence of substituents [103]. Initially, the main hydrolysis products are β-D-xylopyranosyl oligomers, but at a later stage, small molecules such as mono-, di- and trisaccharides of β-D-xylopyranosyl may be produced [104]. These enzymes are produced by fungi, bacteria, yeast, marine algae, protozoans, snails, crustaceans, insect, seeds, etc. [105]. Filamentous fungi are particularly interesting producers of xylanases since they excrete the enzymes into the medium and their enzyme levels are much higher than those of yeasts and bacteria [7]. *Aspergillus niger*, *Humicola insolens*, *Termomonospora fusca*, *Trichoderma reesei*, *T. longibrachiatum*, *T. koningii* have been used as industrial sources of commercial xylanases. Nevertheless, commercial xylanases can also be obtained from bacteria, e.g., from *Bacillus* sp. Xylanases have many commercial uses, such as in paper manufacturing, animal feed, bread-making, juice and wine industries, or xylitol production [104, 106]. Several investigations so far have indicated that xylanases are usually inducible enzymes [107], and different carbon sources have been analysed to find their role in effecting the enzymatic levels. Xylanase biosynthesis is induced by xylan, xylose, xylobiose or several β-D-xylopyranosyl
residues added to the culture medium during growth [7, 104, 108]. However, constitutive production of xylanase has also been reported [109]. Catabolite repression by glucose is a common phenomenon observed in xylanase biosynthesis [7].

The main sugar moiety of galactoglucomannans (GGM) is D-mannose, but for its complete breakdown into simple sugars, the synergistic action of endo-1,4-β-mannanases (EC 3.2.1.78) and exoacting β-mannosidases (EC 3.2.1.25) is required to cleave the polymer backbone. Additional enzymes, such as β-glucosidases (EC 3.2.1.21), α-galactosidases (EC 3.2.1.22) and acetyl mannan esterases are required to remove side chain sugars that are attached at various points on mannans [110, 112]. The property of mannanolysis is widespread in the microbial world. A vast variety of bacteria, actinomycetes, yeasts and fungi are known to be mannan degraders [113, 114]. Mannanases of microbial origin have been reported to be both induced as well as constitutive enzymes and are usually being secreted extracellularly [110]. Although a number of mannanase-producing bacterial sources are available, only a few are commercially exploited as wild or recombinant strains, of these, the important ones are: *Bacillus* sp., *Streptomyces* sp., *Caldibacillus cellulovorans*, *Caldicellulosiruptor* Rt8B, *Caldocellum saccharolyticum* [115-117].

### 3.3.1.2 Accessory enzymes for hemicellulose hydrolysis

Since xylan is a complex component of the hemicelluloses in wood, its complete hydrolysis requires the action of a complete enzyme system, which is usually composed of β-xylanase, β-xylosidase, and enzymes such as α-L-arabinofuranosidase, α-gluconidase, acetylxylan esterase, and hydroxycinnamic acid esterases that cleave side chain residues from the xylan backbone. Figure 1 describes the mechanism of xylanolytic enzymes involved in the degradation of hemicellulose. All these enzymes act cooperatively to convert xylan to its constituents [5]. Xylanases attack randomly the backbone of xylan to produce both substituted and non-substituted shorter chain oligomers, xylobiose and xylose. Xylosidases are essential for the complete breakdown of xylan as they hydrolyse xylooligosaccharides to xylose [118]. The enzymes arabinosidase, α-gluconidase and acetylxylan esterase act in synergy with the xylanases
and xylosidases by releasing the substituents on the xylan backbone to achieve a total hydrolysis of xylan to monosaccharides [4]. Table 2 lists the microorganisms producing different hemicellulases [119].

3.3.1.3 Xyloglucanases

Many endoglucanases have been reported to hydrolyze xyloglucan as a substrate analog [120], however few endo-β-1,4-glucanases have high activity toward xyloglucan, with little or no activity towards cellulose or cellulose derivatives [121, 122]. They have been assigned a new EC number (EC 3.2.1.151) and designated as xyloglucanase, xyloglucan hydrolase (XGH), or xyloglucan-specific endo-β-1,4-glucanases (XEGs) belonging to families 5, 12, 44, and 74, according to a recent classification of glycoside hydrolases (GHs) available at http://afmb.cnrs-mrs.fr/CAZY/ [123–126]. They represent a new class of polysaccharide degrading enzymes which can attack the backbone at substituted glucose residues. Among these enzyme families, xyloglucanases placed in GH family 74 are known to have high specific activity towards xyloglucan, with inversion of the anomeric configuration, and both endo-type and exo-type hydrolases have been found in several microorganisms [127–134]. The exo-type enzymes recognize the reducing end of xyloglucan oligosaccharide (oligoxyloglucan reducing- end-specific cellobiohydrolase, EC 3.2.1.150, from Geotrichum sp. M128 [129] and oligoxyloglucan reducing- end-specific xyloglucanobiolydrolase from Aspergillus nidulans [134]), whereas the endo-type enzymes hydrolyze xyloglucan polymer randomly. In addition, XEG74 from Paenibacillus sp. KM21 and Cel74A from Trichoderma reesei have been reported to have endo-processive or dual-mode endo-like and exo-like activities [127, 132]. The complete hydrolysis of galactoxyloglucan requires an accessory enzyme, β-galactosidase which cleaves the galactose residue attached to the branched xylose moiety in the β-D-glucopyranose backbone [135]. The xyloglucan and the enzymes responsible for its modification and degradation are finding increasing prominence, reflecting the drive for diverse biotechnological applications in fruit juice clarification, textile processing, cellulose surface modification, pharmaceutical delivery and production of food thickening agents [136].
3.3.2 Role of thermostable hemicellulases in hydrolysis

Thermostable enzymes are gaining wide industrial and biotechnical interest due to the fact that they are more stable and thus generally better suited for harsh process conditions. Thermostability is expressed as a measure of the half-life of enzyme activity at elevated temperatures [137]. Thermostable enzymes are produced both by thermophilic and mesophilic organisms. Although thermophilic microorganisms are a potential source for thermostable enzymes, the majority of industrial thermostable enzymes originate from mesophilic organisms. Thermophilic bacteria have, however, received considerable attention as sources of highly active and thermostable enzymes. Thermostable enzymes in the hydrolysis of lignocellulosic materials have several potential advantages: higher specific activity (decreasing the amount of enzyme needed), higher stability (allowing elongated hydrolysis times) and increased flexibility for the process configurations [138]. The two first characteristics would expectedly improve the overall performance of the enzymatic hydrolysis even at the range of conventional enzymes active at around 50°C. Thus, carrying out the hydrolysis at higher temperature would ultimately lead to improved performance, i.e. decreased enzyme dosage and reduced hydrolysis time and, thus, potentially decreased hydrolysis costs. Thermostable enzymes would expectedly also allow hydrolysis at higher consistency due to lower viscosity at elevated temperatures and allow more flexibility in the process configurations. In addition to improved performance in the hydrolysis of lignocellulosic substrates, thermophilic enzymes allow the design of more flexible process configurations [138]. Menon et al, [139] have analyzed the comparative data on hydrolysis of hemicellulosic substrates, oat spelt xylan and wheat bran hemicellulose at different temperatures with thermostable xylanase from alkalothermophilic Thermomonospora sp. At higher temperature, rapid hydrolysis of hemicellulosic substrates were achieved, favouring a reduction in process time and enzyme dosage.
4 Production of biochemicals from hemicellulosic hydrolysates

4.1 Fermentation of hemicellulosic hydrolysates to fuel ethanol

D-xylose, the predominant sugar of hemicellulose can be produced from xylan/hemicellulose by the action of acid/enzymes. D-xylose is not readily utilized as D-glucose for the production of ethanol by microorganisms such as *Saccharomyces cerevisiae*. Bacteria, yeast and mould differ in their mode of conversion of D-xylose to xylulose, the initial step in xylose fermentation. Few yeasts uses the enzymes xylose reductase to reduce xylose to xylitol which is subsequently oxidized to D-xylulose by xylitol dehydrogenase. Yeasts such as *Pichia*, *Klyveromyces*, *Pachysolen* are reported to ferment xylose to ethanol under micro-aerophilic condition. The general requirements of an organism to be used in ethanol production is that it should give a high ethanol yield, a high productivity and be able to withstand high ethanol concentrations in order to keep distillation costs low. In addition to these general requirements, inhibitor tolerance, temperature tolerance and the ability to utilize multiple sugars are also essential. Figure 2 describes the catabolism of hemicellulose derived monosaccharide.

Essentially, there are three different types of processes namely: Separate Hydrolysis and Fermentation (SHF), Direct Microbial Conversion (DMC) and Simultaneous Saccharification and Fermentation (SSF). SSF has been shown to be the most promising approach to biochemically convert cellulose to ethanol in an effective way [140].

4.1.1 Separate hydrolysis and fermentation (SHF)

SHF is a conventional two step process where the hemicellulose is hydrolysed using the enzymes to form the reducing sugars in the first step and the sugars, thus formed, are fermented to the ethanol in the second step using *Pichia* or *Zymomonas*. The advantage of this process is that each step can be carried out at its optimum conditions. Table 3 summarizes the ethanol production from hemicellulosic hydrolysates. A recent study by Wilkins et al, [149] reveals an ethanol production by *Kluyveromyces* sp from D-xylose at 40°C and 45°C with a yield of 0.15g/g and 0.08g/g respectively under anaerobic
condition. *H. polymorpha* could ferment both glucose and xylose up to 45°C [150]. *Debaryomyces sp* used both pentoses and hexoses to similar extents in sugar mixtures and a preference for one carbohydrate did not inhibit the consumption of other. This could be important, since the usual substrates of hemicelluloses, which often consists of sugar mixtures [151].

4.1.2 Direct microbial conversion (DMC)

This process involved three major steps, namely enzyme production, hydrolysis of the lignocellulosic biomass and the fermentation of the sugars all occurring in one step [152]. The relative lower tolerance of the ethanol is the main disadvantage of this process. A lower tolerance limit of about 3.5% has been reported as compared to 10% of ethanologenic yeasts. Acetic acid and lactic acid are also formed as the by-products in this process in which a significant amount of carbon get utilized [153]. *Neurospora crassa* is known to produce ethanol directly from the cellulose/hemicellulose, since it produces both the cellulase and the xylanase and also has the capacity to ferment the sugars to ethanol anaerobically [154].

4.1.3 Simultaneous saccharification and fermentation (SSF)

The saccharification of the lignocellulosic biomass by the enzymes and the subsequent fermentation of the sugars to ethanol by the yeast such as *Saccharomyces* or *Zymomonas* takes place in the same vessel in this process [155]. The compatibility of both saccharification and fermentation process with respect to various conditions such as pH, temperature, substrate concentration etc. is one of the most important factors governing the success of the SSF process. The main advantage of using SSF for the ethanol bioconversion is enhanced rate of hydrolysis of lignocellulosic biomass (cellulose and hemicellulose) due to removal of end product inhibition. Several inhibitory compounds are formed during hydrolysis of the raw material, the hydrolytic process has to be optimized so that inhibitor formation can be minimized. When low concentrations of inhibitory compounds are present in the hydrolyzate, detoxification is easier and fermentation is cheaper. The choice of a detoxification method has to be based on the degree of microbial inhibition caused by the compounds. As each detoxification method
is specific to certain types of compounds, better results can be obtained by combining two or more different methods. Another factor of great importance in the fermentative processes is the cultivation conditions, which, if inadequate, can stimulate the inhibitory action of the toxic compounds. Thermotolerant yeast is an added advantage for SSF and thermotolerant yeast strains, e.g. *Fabospora fragilis, Saccharomyces uvarum, Candida brassicae, C. lusitaniae*, and *Kluyveromyces marxianus*, have been evaluated for future use in SSF, to allow fermentation at temperatures closer to the optimal temperature for the enzymes. However, in all these cases saccharification of pure cellulose (e.g. Sigmaticell-50) or washed fibers, in defined fermentation medium, were applied. SSF of cellulose with mixed cultures of different thermotolerant yeast strains have also been carried out [156]. However, there is scarcity of literature from SSF experiments in which hemicelluloses have been used together with thermotolerant strains. Menon et al., [139] have reported for the first time the SSF experiments using hemicellulosic substrates and thermotolerant *Debaryomyces hansenii*. Maximum ethanol concentrations of 9.1 g/L and 9.5 g/L were obtained in SSF with oat spelt xylan and wheat bran hemicellulose respectively. These concentrations were attained in 36h for OSX and 48h for WBH from the onset of SSF. The increased ethanol yield in SSF systems is evidently due to removal of xylose formed during hydrolysis which causes end product inhibition.

### 4.2 Production of xylitol from hemicellulosic hydrolysates

The conversion of xylose to value-added product xylitol will have significant role in the economic viability of cellulose bioconversion process. The pretreatment (acid or enzyme) of hemicellulose predominantly liberates xylose and arabinose and hydrogenation products are xylitol and arabinitol. Xylitol is a naturally occurring five-carbon sugar alcohol [157], has important applications in pharmaceuticals and food industries due to high sweetening properties, non- and anticariogenicity property and microbial growth inhibition capacity [158, 159]. Xylitol is used as sugar substitute for diabetics, as it does not require insulin for its metabolism [160]. Recently it has been demonstrated that xylitol can prevent acute otitis media (AOM) [161] in children. The value of xylitol market is currently $340 million with applications in mouthwashes,
toothpastes and chewing gums as well as in foods for special dietary uses. Global xylitol consumption was 43,000 t in 2005, the U.S. and Western Europe accounting for 30 and 37% of the total xylitol consumption [162]. Currently the major product of xylan which is of considerable importance is xylitol derived mainly from agricultural and wood residues respectively.

Xylitol is produced from xylan-rich biomass by both chemical and biological methods [163]. The chemical process adapted so far is not ecofriendly and hydrogenation of xylose demands huge production costs in terms of temperature and pressure input as well as the formation of by-products that require expensive separation and purification steps. In addition, xylose should be purified before it is hydrogenised, which further increases the capital investment and costs for xylitol production [164]. Certain microbial species have potential to utilize xylose as carbon source by converting it to xylitol. Yeasts naturally produce xylitol as an intermediate during D-xylose metabolism. Xylose reductase (XR) is typically an NADPH or NADH-dependent enzyme which oxidizes xylose to xylitol [165], while enzyme xylitol dehydrogenase (XDH) requires NAD+ [166] for reduction of xylitol to xylulose. The cofactors imbalance results in the secretion of xylitol as a xylose fermentation by-product [167]. Various bioconversion methods have been exploited for the production of xylitol from hemicellulose using microorganisms or their enzymes [168]. Xylitol production from hemicellulosic hydrolysates using various microorganisms is illustrated in Table 4. Xylitol production by fermentation is usually operated under mild conditions and could achieve higher yield and will lead to an ecofriendly process. Hence the biological process for xylitol production is receiving wider attention.

4.3 Production of other value added products from hemicellulosic hydrolysates

4.3.1 Butanediol

2,3-Butanediol, also known as 2,3-butylene glycol (2,3-BD) is a valuable chemical feedstock because of its application as a solvent, liquid fuel, and as a precursor
of many synthetic polymers and resins [3]. Butanediol is produced during oxygen-limited
growth, by a fermentative pathway known as the mixed acid-butanediol pathway [175].
The 2,3-BD pathway and the relative proportions of acetoin and butanediol serve to
maintain the intracellular NAD/NADH balance under changing culture conditions. All of
the sugars commonly found in hemicellulose and cellulose hydrolysates can be converted
to butanediol, including glucose, xylose, arabinose, mannose, galactose, and cellobiose.
The theoretical maximum yield of butanediol from sugar is 0.50 kg per kg. With a
heating value of 27,200 J/g, 2,3-BD compares favorably with ethanol (29,100 J/g) and
methanol (22,100 J/g) for use as a liquid fuel and fuel additive [176].

Hexose and pentose can be converted to 2,3-BD by several microorganisms
including Aeromonas [177], Bacillus [178], Paenibacillus [179], Serratia, Aerobacter
[180], Enterobacter [11] and also Klebsiella [181–186]. Among these, especially
Klebsiella is often used for 2,3-BDO production because of its broad substrate spectrum
and cultural adaptability. At first, 2,3-BD was produced worldwide from glucose or
xylose by fermentation. Starch [177,187], molasses [188], water hyacinth [189] and
Jerusalem artichoke tubers [190] were also tested for their suitability as substrates for 2,3-
BD fermentation. Some studies have been conducted utilizing the hemicellulose portion
of forest residues like wood for 2,3-BD production [191]. Cheng et al, [186] have
reported production of 2, 3-butanediol from corn cob hydrolysate by fed batch
fermentation using Klebsiella oxytoca. A maximal 2,3-butanediol concentration of 35.7
g/l was obtained after 60 h of fed-batch fermentation, giving a yield of 0.5 g/g reducing
sugar and a productivity of 0.59 g/h l.

4.3.2 Ferulic acid and Vanillin

Ferulic acid is the major phenolic acid of cereal grains such as wheat, triticale,
and rye [192, 193, 194]. It can exist as an extractable form, as free, esterified, and
glycosylated phenolic constituents [192] as well as an insoluble-bound occurring in the
outer layers of wheat grains [195]. Alkaline hydrolysis is reported to release ferulic acid
from the insoluble form [195, 196]. Also, enzyme preparations are used for the same
There are numerous reports on antiradical, oxidase inhibitory, antiinflammatory, antimicrobial, anticancer activities of ferulic acid and its derivatives in the literature [198-200]. Feruloyl esterases (FAEs) act synergistically with xylanases to hydrolyze ester-linked ferulic (FA) and diferulic (diFA) acid from cell wall material and therefore play a major role in the degradation of plant biomass [201]. FAEs are used as a tool for the release of Ferulic acid from agroindustrial byproducts such as wheat bran [202-216], maize bran [217-219], maize fiber [210], brewer’s (or barley) spent grain [202,220-227], sugar beet pulp [216,217,228-231], coastal bermudagrass [232], oat hulls [233-235], jojobameal [236], wheat straw [217], coffee pulp [217] and apple marc [217]. Vanillin can be obtained by fermentation using suitable microorganisms in the stationary growth phase [237-240]. The microbial transformation of ferulic acid is recognized as one of the most attracting alternatives to produce natural vanillin. Bacteria belonging to different genera are able to metabolize ferulic acid as the sole carbon source, producing vanillin, vanillic acid and protocatechuic acid as catabolic intermediates [241,242].

Vanillin is industrially used as fragrance in food preparations, intermediate in the productions of herbicides, antifoaming agents or drugs [243], ingredient of household products such as air fresheners and floor polishes, and, because of its antimicrobial and antioxidant properties [244,245], also as food preservative [246]. The much higher price of the natural vanillin compared to the synthetic vanillin has been leading to growing interest of the flavor industry in producing it from natural sources by bioconversion [243,247–250]. *Pseudomonas fluorescens* was shown to produce vanillic acid from ferulic acid [251,252], with formation of vanillin as an intermediate [253]. Promising vanillin concentrations were obtained from ferulic acid by *Amycolatopsis* sp. [250,254] and *Streptomyces setonii* [241,255,256]. However, the process development is difficult because of the well known slow growth of actinomycetes and high viscosity of broths fermented by them; therefore, the construction of new recombinants strains of quickly growing bacteria able to overproduce vanillin is attractive. The biotechnological process to produce vanillin from various agro by-products had been investigated using different microorganisms as biocatalysts. *Aspergillus niger* I-1472 and *Pycnoporus cinnabarinus* MUCL39533 were used in a two-step bioconversion using sugar-beet pulp [257,258],
maize bran [259], rice bran oil [260], and wheat bran [261]. Wheat bran [262] and corn cob [263] is recently reported as a good substrate for biovanillin production by *Escherichia coli* JM 109/pBB1.

### 4.3.3 Lactic acid

Lactic acid is widely used in food, pharmaceutical and textile industries. It is also used as a source of lactic acid polymers which are being used as biodegradable plastics [264,265]. The physical properties and stability of polylactides can be controlled by adjusting the proportions of the L(+) and D(−)-lactides [266]. Optically pure lactic acid is currently produced by the fermentation of glucose derived from corn starch using various lactic acid bacteria [267,268]. However, the fastidious lactic acid bacteria have complex nutritional requirements [269] and the use of corn is not favoured as the feedstock competes directly with the food and feed uses. The use of lignocellulose biomass will significantly increase the competitiveness of lactic acid-based polymers compared to conventional petroleum based plastics.

*Lactobacillus* spp. are used extensively in industry for starch-based lactic acid production, the majority of which lack the ability to ferment pentose sugars such as xylose and arabinose [266]. Although, *Lactobacillus pentosus*, *L. brevis* and *Lactococcus lactis* ferment pentoses to lactic acid, pentoses are metabolized using the phosphoketolase pathway which is inefficient for lactic acid production [270,271]. In the phosphoketolase pathway, xylulose 5-phosphate is cleaved to glyceraldehyde 3-phosphate and acetyl-phosphate. With this pathway, the maximum theoretical yield of lactic acid is limited to one per pentose (0.6 g lactic acid per g xylose) due to the loss of two carbons to acetic acid. Sugarcane bagasse [272], steam exploded wood [273], soybean stalk [274], corncob molasses [275], trimming vine shoots [276] and wheat straw [270] hydrolysate are used for lactic acid production. John et al, [277] have reported the production of lactic acid from agro residues using *Lactobacillus delbrueckii* in solid state and simultaneous saccharification and fermentation [278].
### 4.3.4 Furfural

Furfural is commercially synthesized from lignocellulosic biomass in a process which involves acid hydrolysis of the pentosans or xylans, present in the hemicelluloses of some agriculture residues and hardwoods into pentoses or xylose and successive cyclodehydration of the latter to form furfural. The second step of dehydration is comparatively slower than that of mineral acid hydrolysis. There is no synthetic route available for the production of furfural. The potential furfural yield for typical feedstock is expressed in terms of kg of furfural per metric ton of dry biomass. It is reported to be 220 for corncobs, 170 for bagasse, 160 for cornstalks, 160 for sunflower hulls, and approximately 150–170 for hardwoods. Furfural, derived from renewable biomass, is used for the production of a wide spectrum of important non-petroleum derived chemicals. World market for furfural currently is estimated to be about 200,000 to 210,000 tpa of which about 60–62% is used for the production of furfuryl alcohol. Furfural is well known for its thermosetting properties, physical strength, and corrosion resistance. It is mainly used for the production of resin which accounts for 70% of the market. Currently, commercial production of furfural suffers from technological challenges and maintenance problems [279]. The use of conventional acid catalysts (mineral acids such as H2SO4 or HCl) for the production of furfural poses serious environmental problems and subsequently results in few undesirable side reactions causing the formation of huge amounts of toxic waste [280]. Hence, there is a need to develop ecofriendly catalytic processes to eliminate environmental corrosion as well as waste-disposal problems. *Coniochaeta ligniaria* NRRL30616 metabolizes furfural and 5-hydroxymethylfurfural (HMF) as well as aromatic acids, aliphatic acids and aldehydes. NRRL30616 grew in corn stover dilute-acid hydrolysate and converted furfural to both furfuryl alcohol and furoic acid [281].

### 4.3.5 Butanol

Butanol is a 4-carbon alcohol (butyl alcohol), an advanced biofuel, offers a number of advantages and can help accelerate biofuel adoption in countries around the world. It can be produced through processing of domestically grown crops, such as corn
and sugar beets, and other biomass, such as fast-growing grasses and agricultural waste products. Biobutanol's primary use is as an industrial solvent in products such as lacquers and enamels and is also compatible with ethanol blending and can improve the blending of ethanol with gasoline. Using fermentation to replace chemical processes in the production of butanol depends largely on the availability of inexpensive and abundant raw materials and efficient conversion of these materials to solvents. Solventogenic Acetone Butanol Ethanol (ABE)-producing *Clostridia* have an added advantage over many other cultures as they can utilize both hexose and pentose sugars [282], which are released from wood and agricultural residues upon hydrolysis, to produce ABE. Parekh et al. [283] produced ABE from hydrolysates of pine, aspen, and corn stover using *Clostridium acetobutylicum* P262. Similarly Marchal et al. [284] used wheat straw hydrolysate and *C. acetobutylicum*, while Soni et al. [285] used bagasse and rice straw hydrolysates and *C. saccharoperbutylacetonicum* to convert these agricultural wastes into ABE. Qureshi et al., [286] have studied the production of butanol from corn fibre hydrolysate using *Clostridium beijerinckii* BA101. Sun and Liu, [287] have reported the production of butanol from sugar maple hemicellulose hydrolysate using *Clostridia acetobutylicum* ATCC824.

### 4.3.6 Biohydrogen

Hydrogen is considered to be an ideal energy alternative for the future. Compared with the conventional hydrogen generation process (thermochemical and electrochemical), bio-hydrogen production processes are more environment-friendly and less-energy intensive. Hydrogen could be produced from renewable materials, such as waste water, organic wastes, corn straws and waste water sludge, etc. Biohydrogen has the potential to considerably reduce costs and environmental impact as it can be produced with sunlight and minimal nutrients or organic waste effluents. Hydrogen (H2) producing microorganisms can be rapidly grown in bioreactors with relatively small energy and environmental footprints, making biohydrogen a renewable and low impact technology. Biological hydrogen, biohydrogen, production may provide a renewable, more sustainable alternative but has yet to reach a scale large enough for consideration in replacing a significant portion of the hydrogen supply [288]. Various hemicellulosic
hydrolysates from wheat straw [289,290], Corn stover [291], sugarcane bagasse [292], Sweet sorghum [293], Corn straw [294] have been evaluated for biohydrogen production.

4.3.7 Chitosan

Chitosan, as an important kind of biomacromolecules, has protective and supportive functions in cell walls of zygomycetes [295]. Chitosan synthesis is a complex process, which involves changes of many intermediate metabolites and energy charges in cells. Chitosan itself has found numerous applications in food, cosmetics and pharmaceutical industries because of their unique properties such as nontoxicity, biodegradability, biocompatibility, film-forming and chelating properties along with their antimicrobial activity [296-298]. Tai et al, [299] have studied the potential of hemicellulose hydrolysate of corn straw for the production of chitosan by *Rhizopus oryzae.*

4.3.8 Xylo-oligosaccharides

Xylo-oligosaccharides are xylose-based oligomers that may have variable proportions of substitute groups like acetyl, uronic, and phenolic acids, depending on the lignocellulosic from which they are extracted and the process of production. Substituted xylo-oligosaccharides have some specific characteristics that are driving research efforts to develop applications in fields related to the food and pharmaceutical industries. They may be used as soluble dietary fiber because of its low calorific value and acceptable organoleptic properties. Furthermore, they are non-carcinogenic and act as prebiotics promoting the growth of beneficial *Bifidobacteria* in the colon [300,301], and are considered a possible ingredient in functional foods [302,303]. Some studies point to the beneficial effect of xylo-oligosaccharides may have on reducing the risk of colon cancer [304-306]. Recently, xylo-oligosaccharides extracted by autohydrolysis of bamboo have been found to posses a cytotoxic effect on human leukemia cells [307]. Also xylo-oligosaccharides can be used as a source of xylose for the production of xylitol, a well-known low-calorie sweetener [308].
5 Recombinant approaches for ethanol and xylitol production

Recombinant DNA methods are being currently used for overexpression of cellulases and hemicellulases for lignocellulose hydrolysis and production of bioproducts. *Saccharomyces cerevisiae* and *Z. mobilis* have been explored for utilizing lignocellulosic biomass by genetic manipulations. Several metabolic and evolutionary engineering techniques have been applied to: increase substrate range, like in *S. cerevisiae* and *Z. mobilis*; maximize ethanol production like in *E. coli*; supply other important traits to improve lignocellulose conversion to ethanol and xylitol. In general, approaches involve genetic engineering towards those specific traits, often followed by strain optimization through adaptation. Since the molecular basis for ethanol and inhibitor tolerance is not fully understood, random mutagenesis and evolutionary engineering have also been applied to improve those traits. Moreover, as a result of technological developments, systems biology approaches have been recently applied to characterise the functional genomics of microorganisms and to evaluate the impact of metabolic and evolutionary engineering strategies. This advanced characterisation (genomics, transcriptomics, proteomics, metabolomics) is already contributing to better understand physiological responses and to identify crucial targets for metabolic engineering [309-313]. Table 5 illustrates the production of xylitol and ethanol using recombinant strains.

6 Future prospects

The implementation of lignocellulosic material for the development of alternative energy is an important aspect for the lucrative gratification of biofuel vision. For an economically viable bioconversion process it is necessary to utilize both the cellulosic and hemicellulosic fractions of biomass. The research focus on cellulosic ethanol is tremendously enhanced all over the world. On the other hand, corn and other grains can be readily hydrolyzed to glucose with high yield by low-cost enzymes. However this causes the debate on food versus fuel. Therefore a hemicellulose based process, wherein hemicellulose can be readily hydrolyzed to value added products is foreseen to be significant from this aspect. The fermentation of C₆ sugars by *Saccharomyces* is a well
established technology whereas the utilization of C₅ sugars is a challenging area. Extensive research on xylose fermenting yeasts such as \textit{Pachysolen tannophilus}, \textit{Candida shehatae} and \textit{Pichia stipitis}, has been conducted. However, low ethanol tolerance, conversion rates and catabolite repression in xylose conversion due to glucose need to be addressed. Another potential ethanologen is recombinant \textit{Zymomonas mobilis} in view of its ability to ferment both xylose and glucose. E. coli strain developed by Ingram’s group deserves special recognition, as it not only ferments all five sugars (glucose, xylose, arabinose, mannose and galactose) present in the synthetic sugar mixtures to ethanol but also performs competently in real hydrolyzates like that of \textit{Pinus Saccharomyces} LNH-ST is another promising recombinant capable of fermenting dilute acid treated corn fiber hydrolyzates.

Pervasive research needs to be performed to develop an efficient cost-effective and eco-friendly pretreatment methods, enzymes or cocktails of various enzymes for complete conversion of hemicellulose to monomers, efficient and robust microorganisms for fermentation of hemicellulosic hydrolysates to value added products in a cost-competitive way. Although bioethanol production has been greatly improved by new technologies there are still challenges that need further investigations. These challenges include maintaining a stable performance of the genetically engineered yeasts in commercial scale fermentation operations and integrating the optimal components into economic ethanol production systems.

Metabolic engineering and other classical techniques such as random mutagenesis address the further enhancement of microorganism capabilities by adding or modifying traits such as tolerance to ethanol and inhibitors, efficient hydrolysis of cellulose/hemicellulose, thermotolerance, reduced need for nutrient supplementation and improvement of sugars transport. The improvement achieved in the fermentation step with the help of metabolic engineering is just one of the aspects of an integrated process. Keeping a realistic perspective one can conclude that several pieces still remain to be properly assembled and optimized before an efficient industrial configuration is acquired.
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Table 1 Composition of representative hemicellulosic feedstocks

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Xylose</th>
<th>Arabinose</th>
<th>Galactose</th>
<th>Mannose</th>
<th>Rhamanose</th>
<th>Uronic acid</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Agricultural and agro-industrial biomass</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn cob</td>
<td>28-30</td>
<td>3-5</td>
<td>1-1.2</td>
<td>-</td>
<td>1</td>
<td>3</td>
<td>15,16,17,18</td>
</tr>
<tr>
<td>Corn stover</td>
<td>15-25</td>
<td>2-3.6</td>
<td>0.8-2.2</td>
<td>0.3-0.4</td>
<td>-</td>
<td>-</td>
<td>15,19,20</td>
</tr>
<tr>
<td>Corn stalks</td>
<td>26</td>
<td>4.1</td>
<td>&lt;2.5</td>
<td>&lt;3.0</td>
<td>-</td>
<td>-</td>
<td>21</td>
</tr>
<tr>
<td>Corn fibre</td>
<td>21.6</td>
<td>11.4</td>
<td>4.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>22</td>
</tr>
<tr>
<td>Rice husk</td>
<td>17.7</td>
<td>1.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>23</td>
</tr>
<tr>
<td>Rice straw</td>
<td>15-23</td>
<td>3-5</td>
<td>0.4</td>
<td>1.8</td>
<td>-</td>
<td>-</td>
<td>22,20</td>
</tr>
<tr>
<td>Sugarcane bagasse</td>
<td>21-26</td>
<td>2.5-7</td>
<td>1.6</td>
<td>0.5-0.6</td>
<td>-</td>
<td>-</td>
<td>24-26</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>19-21</td>
<td>2.4-4</td>
<td>2-2.5</td>
<td>0-0.8</td>
<td>-</td>
<td>-</td>
<td>27,20</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>16</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>16</td>
</tr>
<tr>
<td>Barley straw</td>
<td>15</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>28</td>
</tr>
<tr>
<td><strong>Hardwoods</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birch</td>
<td>19-25</td>
<td>0.3-0.5</td>
<td>0.7-1.3</td>
<td>1.8-3.2</td>
<td>0.6</td>
<td>4-7</td>
<td>29,30</td>
</tr>
<tr>
<td>Maple</td>
<td>18-19</td>
<td>0.8-1</td>
<td>1.0</td>
<td>1.3-3.3</td>
<td>-</td>
<td>4.9</td>
<td>15,30</td>
</tr>
<tr>
<td>Eucalypt</td>
<td>14-19</td>
<td>0.6-1</td>
<td>1-1.9</td>
<td>1-2</td>
<td>0.3-1</td>
<td>2</td>
<td>16, 31-33</td>
</tr>
<tr>
<td>Oak</td>
<td>22</td>
<td>1</td>
<td>1.9</td>
<td>2.3</td>
<td>-</td>
<td>3</td>
<td>34</td>
</tr>
<tr>
<td>Aspen</td>
<td>18-28</td>
<td>0.7-4</td>
<td>0.6-2</td>
<td>0.9-2.5</td>
<td>0.5</td>
<td>5-6</td>
<td>27,29,30,35</td>
</tr>
<tr>
<td>Poplar</td>
<td>18-21</td>
<td>0.9-1.4</td>
<td>1</td>
<td>3.5</td>
<td>-</td>
<td>2.3-3.7</td>
<td>30, 36</td>
</tr>
<tr>
<td><strong>Softwoods</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spruce</td>
<td>5-10</td>
<td>1.2</td>
<td>1.9-4.3</td>
<td>9.4-15</td>
<td>0.3</td>
<td>1.5-6</td>
<td>29,30,37-39</td>
</tr>
<tr>
<td>Douglas fir</td>
<td>6</td>
<td>3</td>
<td>3.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>35</td>
</tr>
<tr>
<td>Pine</td>
<td>5-10</td>
<td>2-4</td>
<td>2-4</td>
<td>6-13</td>
<td>-</td>
<td>3-6</td>
<td>30</td>
</tr>
</tbody>
</table>
Table 2 List of microorganisms with high specific activity (μmol.min⁻¹.mg⁻¹) for hemicellulases (119)

<table>
<thead>
<tr>
<th>ENZYME</th>
<th>Organism</th>
<th>Substrate</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feruloyl esterase</td>
<td><em>Clostridium stercorarium</em></td>
<td>Ethyl ferulate</td>
<td>88</td>
</tr>
<tr>
<td>β-1,4-xylosidase</td>
<td><em>Thermoanaerobacter ethanolicus</em></td>
<td>o-nitrophenyl-β-D-xylopyranoside</td>
<td>1073</td>
</tr>
<tr>
<td>Exo-β-1,4-mannosidase</td>
<td><em>Pyrococcus furiosus</em></td>
<td>p-nitrophenyl-β-D-galactoside</td>
<td>31.1</td>
</tr>
<tr>
<td>Endo-β-1,4-mannanase</td>
<td><em>Bacillus subtilis</em></td>
<td>Galactoglucomannan/glucomannans/mannan</td>
<td>514</td>
</tr>
<tr>
<td>α-Larabinofuranosidase</td>
<td><em>Clostridium stercorarium</em></td>
<td>alkyl-α-arabinofuranoside/ aryl-α-arabinofuranoside/Larabinogalactan/L-arabinoxylan/methylumbelliferyl-α-Larabinofuranoside</td>
<td>883</td>
</tr>
<tr>
<td>α-Glucuronidase</td>
<td><em>Thermoanaerobacterium saccharolyticum</em></td>
<td>4-O-methyl-glucuronosyl-xylotriose</td>
<td>9.6</td>
</tr>
<tr>
<td>α-Galactosidase</td>
<td><em>Escherichia coli</em></td>
<td>Raffinose</td>
<td>27350</td>
</tr>
<tr>
<td>Endo-galactanase</td>
<td><em>Bacillus subtilis</em></td>
<td>Arabinogalactan</td>
<td>1790</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td><em>Bacillus polymyx</em></td>
<td>4-nitrophenyl-β-D-glucopyranoside</td>
<td>2417</td>
</tr>
<tr>
<td>Acetyl xylan esterase</td>
<td><em>Fibrobacter succinogenes</em></td>
<td>Acetylxylan/ α-naphthyl acetate</td>
<td>2933</td>
</tr>
<tr>
<td>Feruloyl esterase</td>
<td><em>Aspergillus niger</em></td>
<td>Methyl sinapinate</td>
<td>156</td>
</tr>
<tr>
<td>Endo-1,4-β-xylanase</td>
<td><em>Trichoderma longibrachiatum</em></td>
<td>1,4-β-D-xylan</td>
<td>6630</td>
</tr>
<tr>
<td>β-1,4-xylosidase</td>
<td><em>Aspergillus nidulans</em></td>
<td>p-nitrophenyl-β-D-xylopyranoside</td>
<td>107.1</td>
</tr>
<tr>
<td>Exo-β-1,4-mannosidase</td>
<td><em>Aspergillus niger</em></td>
<td>β-D-Man-(1-4)-β-D-GlcNAc-(1-4)-β-DGlcNAc-Asn-Lys</td>
<td>188</td>
</tr>
<tr>
<td>Endo-β-1,4-mannanase</td>
<td><em>Sclerotium rolfsii</em></td>
<td>Galactoglucomannan/mannans galactomannans/glucomannans/</td>
<td>380</td>
</tr>
<tr>
<td>Endo-α-1,5-arabinanase</td>
<td><em>Aspergillus niger</em></td>
<td>1,5-α-L-arabinan</td>
<td>90.2</td>
</tr>
<tr>
<td>α-L-arabinofuranosidase</td>
<td><em>Aspergillus niger</em></td>
<td>1,5-α-L-</td>
<td>396.6</td>
</tr>
<tr>
<td>Enzyme Type</td>
<td>Organism</td>
<td>Substrates</td>
<td>Unit(s)</td>
</tr>
<tr>
<td>---------------------</td>
<td>-------------------------------</td>
<td>------------------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>α-Glucuronidase</td>
<td>Phanerochaete chrysosporium</td>
<td>arabinofuranohexaose/ 1,5-α-L-arabinotriose/ 1,5-L-arabinan/ α-L-arabinofuranotriose</td>
<td>4.5</td>
</tr>
<tr>
<td>α-Galactosidase</td>
<td>Mortierella vinacea</td>
<td>melibiose</td>
<td>2000</td>
</tr>
<tr>
<td>Endo-galactanase</td>
<td>Aspergillus niger</td>
<td>4-O-methyl-glucuronosyl-xylobiose</td>
<td>6593</td>
</tr>
<tr>
<td>β-glucosidase</td>
<td>Humicola insolvens</td>
<td>(2-hydroxymethylphenyl)-β-D-glucopyranoside</td>
<td>266.9</td>
</tr>
<tr>
<td>Acetyl xylan esterase</td>
<td>Schizophyllum commune</td>
<td>4-methylumbelliferyl acetate/ 4-nitrophenyl acetate</td>
<td>227</td>
</tr>
</tbody>
</table>
Table 3 Ethanol production from hemicellulosic hydrolysates

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Pretreatment</th>
<th>Detoxification</th>
<th>Microorganisms</th>
<th>Ethanol (g/L)</th>
<th>Ethanol yield (g/g)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bagasse</td>
<td>Steam pretreatment</td>
<td>-</td>
<td><em>Pichia Stipitis</em> CBS 6054</td>
<td>19.5</td>
<td>0.22</td>
<td>141</td>
</tr>
<tr>
<td>Sunflower seed hull</td>
<td>Acid hydrolysis</td>
<td>Over-liming combined with Na$_2$SO$_3$</td>
<td><em>Pichia Stipitis</em> NRRLY-7124</td>
<td>11</td>
<td>0.32</td>
<td>142</td>
</tr>
<tr>
<td>Bagasse</td>
<td>Acid Hydrolysis</td>
<td>Over-liming or electrodialysis</td>
<td><em>Pachysolen tannophilus</em> DW 06</td>
<td>21</td>
<td>0.35</td>
<td>143</td>
</tr>
<tr>
<td>Prosopsis Julifora</td>
<td>Acid hydrolysis</td>
<td>Over-liming</td>
<td><em>Pichia stipitis</em> NCIM 3498</td>
<td>7.13</td>
<td>0.39</td>
<td>144</td>
</tr>
<tr>
<td>Water hyacinth</td>
<td>Acid hydrolysis</td>
<td>-</td>
<td><em>Pichia stipitis</em></td>
<td>-</td>
<td>0.425</td>
<td>145</td>
</tr>
<tr>
<td>Olive Tree</td>
<td>Acid hydrolysis</td>
<td>Over-liming and Activated charcoal</td>
<td><em>Pichia stipitis</em></td>
<td>-</td>
<td>0.42</td>
<td>146</td>
</tr>
<tr>
<td>Yellow poplar wood meal</td>
<td>Alkaline pretreatment</td>
<td>Over-liming</td>
<td><em>Pichia stipitis</em></td>
<td>28.7</td>
<td>0.48</td>
<td>147</td>
</tr>
<tr>
<td>Oat spelt xylan</td>
<td>Enzymatic hydrolysis</td>
<td>-</td>
<td><em>Debaromyces hansenii</em></td>
<td>7.55</td>
<td>0.42</td>
<td>139</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>Enzymatic hydrolysis</td>
<td>-</td>
<td><em>Debaromyces hansenii</em></td>
<td>6.42</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>Tamarind kernel powder</td>
<td>Acid hydrolysis</td>
<td>Over-liming and Activated charcoal</td>
<td><em>Debaromyces hansenii</em></td>
<td>16</td>
<td>0.35</td>
<td>148</td>
</tr>
<tr>
<td>Tamarind kernel powder</td>
<td>Enzymatic hydrolysis</td>
<td>-</td>
<td><em>Debaromyces hansenii</em></td>
<td>5.18</td>
<td>0.39</td>
<td>148</td>
</tr>
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</table>
Table 4 Xylitol production from detoxified hemicellulosic hydrolysates

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Pretreatment</th>
<th>Detoxification</th>
<th>Microorganisms</th>
<th>Xylitol (g/L)</th>
<th>Xylitol yield (g/g)</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Sugarcane bagasse</td>
<td>Acid hydrolysis</td>
<td>Activated charcoal</td>
<td>Candida guilliermondii</td>
<td>35.5</td>
<td>0.72</td>
<td>164</td>
</tr>
<tr>
<td>Eucalyptus</td>
<td>Acid hydrolysis</td>
<td>Activated charcoal Ion exchange resins</td>
<td>Candida guilliermondii</td>
<td>32.7</td>
<td>0.57</td>
<td>169</td>
</tr>
<tr>
<td>Corn fibre</td>
<td>Acid hydrolysis</td>
<td>Over-liming Activated charcoal Ion exchange resins</td>
<td>Candida tropicalis</td>
<td>129</td>
<td>0.43</td>
<td>170</td>
</tr>
<tr>
<td>Brewery spent-grain</td>
<td>Acid hydrolysis</td>
<td>Over-liming Ion exchange resins</td>
<td>Debaromyces hansenii</td>
<td>18</td>
<td>0.38</td>
<td>171</td>
</tr>
<tr>
<td>Rice straw</td>
<td>Acid hydrolysis</td>
<td>Over-liming Activated charcoal</td>
<td>Candida subtropicalis WF79</td>
<td>-</td>
<td>0.73</td>
<td>172</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>Acid hydrolysis</td>
<td>Activated charcoal</td>
<td>Candida guilliermondii FTI 20037</td>
<td>24.2</td>
<td>0.48</td>
<td>173</td>
</tr>
<tr>
<td>Corn cob</td>
<td>Acid hydrolysis</td>
<td>Over-liming, solvent extraction</td>
<td>Candida tropicalis W103</td>
<td>68.4</td>
<td>0.7</td>
<td>174</td>
</tr>
<tr>
<td>Sugars</td>
<td>Genetic manipulations</td>
<td>Microorganism</td>
<td>Yield and/or productivity</td>
<td>Reference</td>
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<td>--------</td>
<td>-----------------------</td>
<td>---------------</td>
<td>----------------------------</td>
<td>-----------</td>
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</tr>
<tr>
<td><strong>Xylitol</strong></td>
<td></td>
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</tr>
<tr>
<td>Xylose + glucose</td>
<td>Expressed P.stipitis XR</td>
<td><em>L.lactis</em></td>
<td>2.7 g/l/h</td>
<td>314</td>
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<td>Xylose + glycerol</td>
<td>Disrupted XYL2</td>
<td><em>C.tropicalis</em></td>
<td>3.2 g/l/h</td>
<td>315</td>
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<tr>
<td>Xylose + glucose</td>
<td>Expressed XylE and K.lactis XR, deleted xylA,yhbC-deficient</td>
<td><em>E.coli</em></td>
<td>0.81 g/l/h</td>
<td>316</td>
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<tr>
<td>Xylose + glucose</td>
<td>Overexpressed G6PDH, expressed Xyl1, attenuated PGI activity</td>
<td><em>S.cerevisiae</em></td>
<td>0.34 g/g</td>
<td>317</td>
<td></td>
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<tr>
<td><strong>Ethanol</strong></td>
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<tr>
<td>Xylose</td>
<td>Disrupted <em>CRC 1</em></td>
<td><em>P.stipitis</em></td>
<td>0.46 g/g</td>
<td>318</td>
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<td></td>
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<tr>
<td>Xylose + glucose</td>
<td>Genomic DNA integration</td>
<td><em>Z.mobilis</em></td>
<td>0.4 g/g</td>
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<tr>
<td>Xylose</td>
<td>Overexpressed native P.stipitis XDH, xylulokinase, deleted GRE3</td>
<td><em>S.cerevisiae</em></td>
<td>0.39 g/g</td>
<td>320</td>
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</tr>
<tr>
<td>Xylose</td>
<td>Evolutionary engineering, increased PDH activity</td>
<td><em>E.coli</em></td>
<td>0.46 g/g</td>
<td>321</td>
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XylE- xylose permease; XR- xylose reductase; G6PDH- glucose-6-phosphate dehydrogenase; PGI- phosphoglucone isomerase; PDH- pyruvate dehydrogenase; XDH- xylitol dehydrogenase; CRC 1- cytochrome c
Mechanism of action of the hemicellulases

Figure 1: Xylanolytic enzymes involved in the degradation of hard wood and soft wood xylan. Ac, acetyl group; Ara, α-arabinofuranose; MeGlcA, α-4-Omethylglucuronic acid; Xyl xylose.
Figure 2 Microbial conversion of xylose to ethanol and xylitol

Hemicellulose \[\rightarrow\] D - XYLOSE

Xylose isomerase (XI)

Xylose reductase (XR)

Xylitol dehydrogenase

NAD(P)

D-Xylose

NAD(P)

NAD

Xylitol dehydrogenase

D-Ribose - 5-

D-Xylose - 5- Phosphate

Glyceraldehyde-3-Phosphate

D-Fructose-6-Phosphate

Transketolase (TKL)

Transaldolase (TAL)

NAD

NADH

ADP

ATP

ADP

ATP

Pyruvate

Pyruvate formate lyase

Pyruvate dehydrogenase

Acetaldehyde

NADH

NAD

Alcohol dehydrogenase

ETHANOL

Acetyl

Aldehyde dehydrogenase

D-XYLOSE

XYLITOL

D-Xylose kinase (XK)

ATP

ADP

NADH

NAD

D-XYLOSE

XYLITOL

D-Xylose

NAD(P)

NAD(P)

NAD

Xylitol dehydrogenase

D-Ribose - 5-

D-Xylose - 5- Phosphate

Glyceraldehyde-3-Phosphate

D-Fructose-6-Phosphate

Transketolase (TKL)

Transaldolase (TAL)

NAD

NADH

ADP

ATP

ADP

ATP

Pyruvate

Pyruvate formate lyase

Pyruvate dehydrogenase

Acetaldehyde

NADH

NAD

Alcohol dehydrogenase

ETHANOL

Acetyl

Aldehyde dehydrogenase

D-XYLOSE

XYLITOL

D-Xylose

NAD(P)

NAD(P)

NAD

Xylitol dehydrogenase

D-Ribose - 5-

D-Xylose - 5- Phosphate

Glyceraldehyde-3-Phosphate

D-Fructose-6-Phosphate

Transketolase (TKL)

Transaldolase (TAL)

NAD

NADH

ADP

ATP

ADP

ATP

Pyruvate

Pyruvate formate lyase

Pyruvate dehydrogenase

Acetaldehyde

NADH

NAD

Alcohol dehydrogenase

ETHANOL

Acetyl

Aldehyde dehydrogenase

D-XYLOSE

XYLITOL

D-Xylose

NAD(P)

NAD(P)

NAD

Xylitol dehydrogenase

D-Ribose - 5-

D-Xylose - 5- Phosphate

Glyceraldehyde-3-Phosphate

D-Fructose-6-Phosphate

Transketolase (TKL)

Transaldolase (TAL)

NAD

NADH

ADP

ATP

ADP

ATP

Pyruvate

Pyruvate formate lyase

Pyruvate dehydrogenase

Acetaldehyde

NADH

NAD

Alcohol dehydrogenase

ETHANOL

Acetyl

Aldehyde dehydrogenase