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REVIEW PAPER

Badal C. Saha

Hemicellulose bioconversion

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Abstract Various agricultural residues, such as corn fiber, corn stover, wheat straw, rice straw, and sugarcane bagasse, contain about 20–40% hemicellulose, the second most abundant polysaccharide in nature. The conversion of hemicellulose to fuels and chemicals is problematic. In this paper, various pretreatment options as well as enzymatic saccharification of lignocellulosic biomass to fermentable sugars is reviewed. Our research dealing with the pretreatment and enzymatic saccharification of corn fiber and development of novel and improved enzymes such as endo-xylanase, β-xylosidase, and α-L-arabinofuranosidase for hemicellulose bioconversion is described. The barriers, progress, and prospects of developing an environmentally benign bioprocess for large-scale conversion of hemicellulose to fuel ethanol, xylitol, 2,3-butanediol, and other value-added fermentation products are highlighted.

Keywords Hemicellulose · Arabinoxylan · Bioconversion · Hemicellulase · Xylanolytic enzymes

Introduction

Hemicelluloses, the second most common polysaccharides in nature, represent about 20–35% of lignocellulosic biomass. Xylans are the most abundant hemicelluloses. In recent years, bioconversion of hemicellulose has received much attention because of its practical applications in various agro-industrial processes, such as efficient conversion of hemicellulosic biomass to fuels and chemicals, delignification of paper pulp, digestibility enhancement of animal feedstock, clarification of juices, and improvement in the consistency of beer [134, 139, 144]. Enzymes that degrade, or help to degrade, hemicellulose are of great interest to the paper and pulp industry due to their bleach-boosting properties (biobleaching of pulp), which reduces environmentally unfriendly chlorine consumption [91, 135]. Cellulase-free xylanase can facilitate lignin removal from paper pulp without any harmful effect. The utilization of hemicellulosic sugars is essential for efficient conversion of lignocellulosic materials to fuel ethanol and other value-added fermentation products. Xylan-degrading enzymes hold great promise in saccharifying various pretreated agricultural and forestry residues to fermentable sugars. Other potential applications of hemicellulases include biopulping of wood, coffee processing, fruit and vegetable maceration, and preparation of high fiber baked goods [19]. In addition, xylan-degrading enzymes play a great role in elucidating the structures of complex xylans. In this article, a brief review on the bioconversion of hemicellulose—particularly arabinoxylans present in various agricultural residues—to fuel ethanol, xylitol and 2,3-butanediol, is presented.

B. C. Saha (✉)
Fermentation Biotechnology Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, U. S. Department of Agriculture, 1815 North University Street, Peoria, IL 61604, USA
E-mail: sahabc@ncaur.usda.gov
Tel.: +1-309-6816276
Fax: +1-309-6816427

Structure of hemicellulose

Hemicelluloses are heterogeneous polymers of pentoses (xylose, arabinose), hexoses (mannose, glucose, galactose), and sugar acids. Unlike cellulose, hemicelluloses are not chemically homogeneous. Hardwood hemicelluloses contain mostly xylans, whereas softwood hemicelluloses contain mostly glucomannans [84]. Xylans of many plant materials are heteropolysaccharides with homopolymeric backbone chains of 1,4-linked β-D-xylopyranose units. Besides xylose, xylans may contain arabinose, glucuronic acid or its 4-*O*-methyl ether, and acetic, ferulic, and *p*-coumaric acids. The frequency and composition of branches are dependent on the source of xylan [1]. The backbone consists of *O*-acetyl, α-L-

arabinofuranosyl, α-1,2-linked glucuronic or 4-*O*-methylglucuronic acid substituents. However, unsubstituted linear xylans have also been isolated from guar seed husk, esparto grass, and tobacco stalks [35]. Xylans can thus be categorized as linear homoxylan, arabinoxylan, glucuronoxylan, and glucuronoarabinoxylan.

Xylans from different sources, such as grasses, cereals, softwood, and hardwood, differ in composition. Birch wood (Roth) xylan contains 89.3% xylose, 1% arabinose, 1.4% glucose, and 8.3% anhydrouronic acid [68]. Rice bran neutral xylan contains 46% xylose, 44.9% arabinose, 6.1% galactose, 1.9% glucose, and 1.1% anhydrouronic acid [126]. Wheat arabinoxylan contains 65.8% xylose, 33.5% arabinose, 0.1% mannose, 0.1% galactose, and 0.3% glucose [51]. Corn fiber xylan is one of the complex heteroxylans containing β-(1,4)-linked xylose residues [117]. It contains 48–54% xylose, 33–35% arabinose, 5–11% galactose, and 3–6% glucuronic acid [31]. About 80% of the xylan backbone is highly substituted with monomeric side-chains of arabinose or glucuronic acid linked to *O*-2 and/or *O*-3 of xylose residues, and also by oligomeric side chains containing arabinose, xylose, and sometimes galactose residues (Fig. 1) [122]. A model for the corn fiber cell wall is shown in Fig. 2 [121]. The heteroxylans, which are highly cross-linked by diferulic bridges, constitute a network in which the cellulose microfibrils may be imbedded. Structural wall proteins might be cross-linked together by isodityrosine bridges and with feruloylated heteroxylans, thus forming an insoluble network [60]. In softwood heteroxylans, arabinofuranosyl residues are esterified with *p*-coumaric acids and ferulic acids [88]. In hardwood xylans, 60–70% of the xylose residues are acetylated [131]. The degree of polymerization of hardwood xylans (150–200) is higher than that of softwoods (70–130).

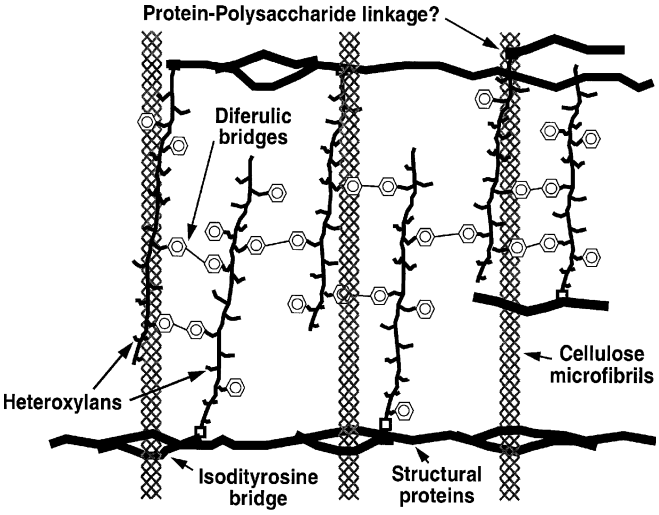

Fig. 2 Model for corn fiber cell walls. Reprinted from [121], with permission from John Wiley & Sons on behalf of SCI

Pretreatment of hemicellulose

Lignocellulosic biomass includes various agricultural residues (straws, hulls, stems, stalks), deciduous and coniferous woods, municipal solid wastes, waste from the pulp and paper industry, and herbaceous energy crops. The compositions of these materials vary. The major component is cellulose (35–50%), followed by hemicellulose (20–35%) and lignin (10–25%). Table 1 gives the composition of some lignocellulosics. Proteins, oils, and ash make up the remaining fraction of lignocellulosic biomass [140]. The structure of these materials is very complex, and native biomass is generally resistant to an enzymatic hydrolysis. In the current model of the structure of lignocellulose, cellulose fibers are embedded in a lignin-polysaccharide matrix.

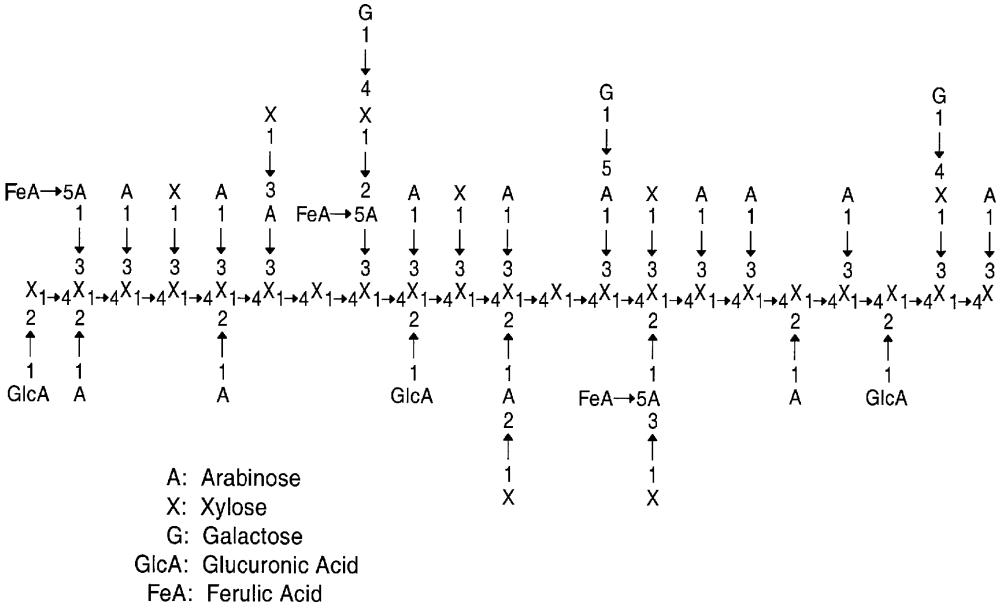

Fig. 1 Schematic structure of corn fiber heteroxylan. Reprinted from [122], with permission from Elsevier, Amsterdam

Table 1 Composition of some agricultural lignocellulosic biomass

	Composition (%, dry basis)		
	Cellulose	Hemicellulose	Lignin
Corn fiber[a]	15	35	8
Corn cob	45	35	15
Corn stover	40	25	17
Rice straw	35	25	12
Wheat straw	30	50	20
Sugarcane bagasse	40	24	25
Switchgrass	45	30	12
Coastal bermuda grass	25	35	6

[a] Contains20% starch

Xylan may play a significant role in the structural integrity of cell walls by both covalent and non-covalent associations [130].

The pretreatment of lignocellulosic biomass is crucial before enzymatic hydrolysis. Various pre-treatment options are available now to fractionate, solubilize, hydrolyze and separate cellulose, hemicellulose, and lignin components [8, 21, 137, 141]. These include concentrated acid [45], dilute acid [117], alkaline [71], SO_2 [17], hydrogen peroxide [48], steam explosion (autohydrolysis) [40], ammonia fiber explosion (AFEX) [22], wet-oxidation [123], lime [64], liquid hot water [73], CO_2 explosion [21], and organic solvent treatments [15]. In each option, the biomass is reduced in size and its physical structure is opened. Two categories of dilute acid pretreatments are used: high temperature (>160°C) continuous-flow for low solids loading (5–10%, w/w) and low temperature (<160°C) batch process for high solids loading (10–40%, w/w) [128]. Dilute acid pretreatment at high temperature usually hydrolyzes hemicellulose to its sugars (xylose, arabinose and other sugars), which are water soluble [8]. The residue contains cellulose and often much of the lignin. The lignin can be extracted with solvents such as ethanol, butanol, or formic acid. Alternatively, hydrolysis of cellulose with lignin present produces water-soluble sugars and insoluble residues, which are lignin plus unreacted materials. The use of SO_2 as a catalyst during steam pretreatment results in the enzymatic accessibility of cellulose and enhanced recovery of the hemicellulose-derived sugars [7]. Steam pretreatment at 200–210°C with the addition of 1% SO_2 (w/w) was superior to other forms of pretreatment of willow [36]. A glucose yield of 95%, based on the glycan available in the raw material, was achieved. By steam explosion, optimal solubilization, and degradation of hemicellulose can generally be achieved by either high temperature and short residence time (270°C, 1 min) or lower temperature and longer residence time (190°C, 10 min) [33]. Morjanoff and Gray [87] reported that enzymatic saccharification of 100 g sugarcane bagasse after steam explosion with 1% H_2SO_4 at 220°C for 30 s at a water:solid ratio of 2:1 yielded 65.1 g sugar.

Super critical carbon dioxide explosion was effective for pretreatment of cellulosic materials before enzymatic hydrolysis [65, 147]. Zheng et al. [148] compared CO_2 explosion with steam and ammonia explosion for pretreatment of sugarcane bagasse and found that CO_2 explosion was more cost-effective than ammonia explosion and did not cause the formation of inhibitory compounds, which could occur in steam explosion. Cao et al. [9] reported a pretreatment method that involves steeping of the lignocellulosic biomass (using corn cob as a model feedstock) in dilute NH_4OH at ambient temperature to remove lignin, acetate, and extractives. This is followed by a dilute acid treatment that readily hydrolyzes the hemicellulose fraction to simple sugars, primarily xylose. The residual cellulose fraction of biomass can then be enzymatically hydrolyzed to glucose. Kurakake et al. [72] pretreated sugarcane bagasse, corn husk, and switchgrass with ammonia water to enhance enzymatic hydrolysis. Garrote et al. [43] treated *Eucalyptus* wood substrates with water under selected operational conditions (autohydrolysis reaction) to obtain a liquid phase containing hemicellulose decomposition products (mainly acetylated xylooligosaccharides, xylose, and acetic acid). In a further acid-catalyzed step (posthydrolysis reaction), xylooligosaccharides were converted to xylose. The wet oxidation method can be used for fractionation of lignocellulosics into solubilized hemicellulose fraction and a solid cellulose fraction susceptible to enzymatic saccharification. Bjerre et al. [3] found that a combination of alkali and wet oxidation did not generate furfural and 5-hydroxymethyl furfural (HMF). Klinke et al. [67] characterized the degradation products from alkaline wet oxidation (water, sodium carbonate, oxygen, high temperature, and pressure) of wheat straw. Apart from CO_2 and water, carboxylic acids were the main degradation products from hemicellulose and lignin. Aromatic aldehyde formation was minimized by temperature control and the addition of alkali. Draude et al. [32] reported that oxygen delignification of kraft pulp removed up to 67% of the lignin from softwood pulp and improved the rate of, and yield from, enzymatic hydrolysis by up to 111% and 174%, respectively.

Phenolic compounds from lignin degradation, furan derivatives (furfural and HMF) from sugar degradation, and aliphatic acids (acetic acid, formic acid and levulinic acid) are considered to be fermentation inhibitors generated from pretreated lignocellulosic biomass [94]. Various methods for detoxification of the hydrolyzates have been developed [93]. These include treatment with ion-exchange resins, charcoal or the ligninolytic enzyme laccase, pre-fermentation with the filamentous fungus *Trichoderma reesei*, removal of non-volatile compounds, extraction with ether or ethyl acetate, and treatment with alkali (lime) or sulfite. Persson et al. [97] employed countercurrent flow supercritical fluid extraction to detoxify a dilute acid hydrolyzate of spruce prior to ethanol fermentation with baker's yeast. A summary of various pretreatment options is given in Table 2. Each pretreatment method offers distinct advantages and disadvantages.

Table 2 Methods for pretreatment of lignocellulosic biomass (taken from [112])

Method	Example
Thermo-mechanical	Grinding, milling, shearing, extruder
Autohydrolysis	Steam pressure, steam explosion, supercritical carbon dioxide explosion
Acid treatment	Dilute acid (H_2SO_4, HCl), concentrated acid (H_2SO_4, HCl)
Alkali treatment	Sodium hydroxide, ammonia, alkaline hydrogen peroxide
Organic solvents treatment	Methanol, ethanol, butanol, phenol

Enzymatic saccharification of hemicellulose

Total biodegradation of xylan requires endo-β-1,4-xylanase, β-xylosidase, and several accessory enzymes, such as α-L-arabinofuranosidase, α-glucuronidase, acetylxylan esterase, ferulic acid esterase, and *p*-coumaric acid esterase, which are necessary for hydrolyzing various substituted xylans. Table 3 lists the enzymes involved in the degradation of xylan and their modes of action. The endo-xylanase attacks the main chains of xylans, and β-xylosidase hydrolyzes xylooligosaccharides to xylose. The α-arabinofuranosidase and α-glucuronidase remove the arabinose and 4-*O*-methyl glucuronic acid substituents, respectively, from the xylan backbone. The esterases hydrolyze the ester linkages between xylose units of the xylan and acetic acid (acetylxylan esterase) or between arabinose side chain residues and phenolic acids, such as ferulic acid (ferulic acid esterase) and *p*-coumaric acid (*p*-coumaric acid esterase).

Many microorganisms, such as *Penicillium capsulatum* and *Talaromyces emersonii*, possess complete xylan-degrading enzyme systems [41]. Bachmann and McCarthy [2] reported significant synergistic interaction among endo-xylanase, β-xylosidase, α-arabinofuranosidase, and acetylxylan esterase of the thermophilic actinomycete *Thermomonospora fusca*. Synergistic action between depolymerizing and side-group cleaving enzymes has been verified using acetylated xylan as a substrate [99]. Many xylanases do not cleave glycosidic bonds between xylose units that are substituted. The side chains must be cleaved before the xylan backbone can be completely hydrolyzed [76]. On the other hand, several accessory enzymes only remove side chains from xylooligosaccharides. These enzymes require a partial hydrolysis of xylan before the side chains can be cleaved [100]. Although the structure of xylan is more complex than cellulose and requires several different enzymes with different specificities for complete hydrolysis, the polysaccharide does not form tightly packed crystalline structures like cellulose and is, thus, more accessible to enzymatic hydrolysis [44].

Corn fiber, a byproduct of corn wet milling facilities, contains about 20% starch in addition to 15% cellulose and 35% hemicellulose [120]. Saha and Bothast [117] evaluated several pretreatments (hot water, alkali, and dilute acid) and enzymatic saccharification procedures for conversion of corn fiber starch, cellulose, and hemicellulose to fermentable sugars. Hot water pretreatment (121°C, 1 h) facilitated the enzymatic saccharification of starch and cellulose but not hemicellulose. Hydrolysis of corn fiber pretreated with alkali (10:1, w/w, 121°C, 3 h) with hemicellulase enzymes gave similar results. The results indicate that there are no suitable commercial hemicellulase preparations that can hydrolyze corn fiber hemicellulose to monomeric sugars efficiently. Hemicellulose and starch components were then converted to simple sugars by dilute acid pretreatment, and the residual cellulose component was converted to glucose using commercially available enzymes. The procedure involves pretreatment of corn fiber (15% solid, w/v) with dilute acid (0.5% H_2SO_4, v/v) at 121°C for 1 h, neutralization to pH 5.0, then saccharification of the pretreated corn fiber material with commercial cellulase and β-glucosidase preparations. The yield of monomeric sugars from corn fiber was typically 85–100% of the theoretical yield. This procedure did not generate inhibitors such as furfural and HMF, which are generally considered inhibitors for fermentative microorganisms. Thus, dilute acid pretreatment at a relatively low temperature to minimize the formation of inhibitory compounds, followed by enzymatic saccharification of the cellulosic portion, is an excellent workable process for generating fermentable sugars from corn fiber.

Leathers and Gupta [75] described a partial saccharification of corn fiber using a crude enzyme prepa-

Table 3 Enzymes involved in the hydrolysis of complex heteroarabinoxylans (taken from [116])

Enzyme	Mode of action
Endo-xylanase	Hydrolyzes mainly interior β-1,4-xylose linkages of the xylan backbone
Exo-xylanase	Hydrolyzes β-1,4-xylose linkages releasing xylobiose
β-Xylosidase	Releases xylose from xylobiose and short chain xylooligosaccharides
α-Arabinofuranosidase	Hydrolyzes terminal nonreducing α-arabinofuranose from arabinoxylans
α-Glucuronidase	Releases glucuronic acid from glucuronoxylans
Acetylxylan esterase	Hydrolyzes acetylester bonds in acetyl xylans
Ferulic acid esterase	Hydrolyzes feruloylester bonds in xylans
ρ-Coumaric acid esterase	Hydrolyzes ρ-coumaryl ester bonds in xylans

ration from *Aureobasidium* sp. Christov et al. [14] showed that crude enzyme preparation from *Aureobasidium pullulans* was only partially effective in the removal of xylan from dissolving pulp. Saha [108, 110] isolated three fungal cultures (*Fusarium proliferatum* NRRL 26517, *Fusarium verticillioides* NRRL Y-26518, and *Mucor circinelloides* NRRL Y-26519) that had the capability to utilize corn fiber xylan as growth substrate after screening 132 soil samples surrounding decaying corn and wood. The crude enzyme preparations from these newly isolated cultures were able to degrade corn fiber xylan well but the purified endo-xylanases from two fungal cultures could not degrade corn fiber xylan. The purified β-xylosidase released xylose from xylobiose and other short-chain xylooligosaccharides [109]. A summary of the characteristics of purified endo-xylanase and β-xylosidase from *Fusarium verticillioides* NRRL 26518 is presented in Table 4. For effective hydrolysis of xylan substrates, a proper mix of endo-xylanase with several accessory enzymes is essential. β-Xylosidase was competitively inhibited by xylose with a K_i value of 6 mM [109]. It is thus essential that a xylose tolerant β-xylosidase is also developed in order to make enzymatic saccharification of any xylan substrate a commercial success.

Saha and Bothast [114] found that *A. pullulans* grown on oat spelt xylan produced a highly thermostable novel extracellular α-L-arabinofuranosidase that had the ability to rapidly hydrolyze arabinan and debranched arabinan, and released arabinose from various arabinoxylans. The purified enzyme had a half-life of 8 h at 75°C and displayed optimal activity at 75°C and pH 4.0–4.5. The enzyme production was induced most by arabinose [115]. This indicates that arabinose-rich hemicellulose hydrolyzates can be used for production of the enzyme.

Production of fuel ethanol from hemicellulosic hydrolyzates

The utilization of hemicellulosic sugars is essential for efficient and cost-effective conversion of lignocellulosis material to fuel ethanol. In 2002, over 2 billion gallons of ethanol was produced, mainly by fermenting corn starch. The demand for fuel ethanol is expected to rise very sharply as a safer alternative to methyl tertiary butyl ether (MTBE), the most common additive to gasoline used to provide cleaner combustion. MTBE has been found to contaminate groundwater.

Various waste and underutilized lignocellulosic agricultural residues can serve as low-cost feedstocks for production of fuel ethanol. Any hemicellulose containing lignocellulose generates a mixture of sugars upon pretreatment alone or in combination with enzymatic hydrolysis. The sugar mixture may contain any combination of xylose, arabinose, glucose, galactose, mannose, fucose and rhamnose depending on the source. Although traditional *Saccharomyces cerevisiae* and *Zymomonas mobilis* ferment glucose to ethanol rapidly and efficiently, they cannot ferment other sugars such as xylose and arabinose to ethanol. The yeasts *Pachysolen tannophilus*, *Pichia stipitis*, and *Candida shehate* have the capability to ferment xylose to ethanol [4, 124, 136]. Commercial exploitation of these yeasts for ethanol production from xylose is restricted mainly by their low ethanol tolerance, slow rates of fermentation, difficulty in controlling the rate of oxygen supply at the optimal level, plus sensitivity to inhibitors generated during pretreatment and hydrolysis of lignocellulosic substrates [34, 54]. However, xylose can be converted to xylulose using the enzyme xylose isomerase and traditional yeasts can ferment xylulose to ethanol [46, 53], although the process is not cost-effective. Arabinose, another pentose sugar, is often present in hemicellulosic hydrolyzate depending on the source. However, only a few yeast strains can, barely, ferment arabinose to ethanol [24, 111]. Thus, no naturally occurring yeast can ferment all these sugars to ethanol.

Some bacteria, such as *Escherichia coli*, *Klebsiella*, *Erwinia*, *Lactobacillus*, *Bacillus*, and *Clostridia*, can utilize mixed sugars but produce no, or only a limited quantity of, ethanol. These bacteria generally produce mixed acids (acetate, lactate, propionate, succinate, etc.) and solvents (acetone, butanol, 2,3-butanediol, etc.). Several microorganisms have been genetically

Table 4 Biochemical characteristics of purified xylanase and β-xylosidase from *Fusarium verticillioides* NRRL 26518 capable of utilizing corn fiber xylan (taken from [108,110])

Property	Xylanase	β-Xylosidase
Specific activity (U/mg protein)	492	57
Molecular weight	24,000	94,500
Isoelectric point (pI)	8.6	7.8
Optimum temperature	50°C	65°C
Optimum pH	5.5	4.5
Substrate	Xylan	Xylobiose and xylooligosaccharides
Product	Xylobiose and xylooligosaccharides	Xylose
Mode of action	Endo-acting	Exo-acting
K_m	9.5 mg/ml (oat spelt xylan, pH 5.0, 50°C	0.85 mM (pNP-β-D-xyloside, pH 4.5, 50°C)
K_i (at pH 4.5, 50°C)		6.0 mM xylose
Metal ion requirement	None	None

engineered to overproduce ethanol from mixed sugar substrates by using two different approaches: (1) divert carbon flow from native fermentation products to ethanol in efficient mixed sugar utilizers such as *Escherichia*, *Erwinia*, and *Klebsiella*, and (2) introduce the pentose-utilizing capability into efficient ethanol producers such as *Saccharomyces* and *Zymomobilis* [55, 58, 61, 145]. Various recombinant strains, e.g., *E. coli* K011, *E. coli* SL40, *E. coli* FBR3, *Zymomonas* CP4 (pZB5), and *Saccharomyces* 1400 (pLNH32), have been evaluated for fermentation of mixed sugar substrates and corn fiber hydrolyzates [5, 25, 26, 27, 28, 29, 62, 86]. These strains fermented corn fiber hydrolyzates to ethanol in the range of 21–34 g/l with yields ranging from 0.41–0.50 g ethanol per gram of sugar consumed [6]. Recently, Nichols et al. [89] constructed ethanologenic *E. coli* strains with a glucose phospho-transferase (*ptsG*) mutation. The *ptsG* mutants fermented mixed sugars—glucose, xylose, and arabinose—simultaneously rather than sequentially to ethanol with yields of 87–94%, i.e., similar to wild type. Martinez et al. [82] reported that increasing gene expression through the replacement of promoters and the use of a higher gene dosage (plasmids) substantially eliminated the apparent requirement for large amounts of complex nutrients of ethanologenic recombinant *E. coli* strain. Ethanol-tolerant mutants of recombinant *E. coli* have been developed that can produce up to 6% ethanol [142]. Recombinant *Z. mobilis*, into which four genes from *E. coli* [*xylA* (xylose isomerase), *xylB* (xylulokinase), *tal* (transaldolase) and *tktA* (transketolase)] were inserted, grew on xylose as the sole carbon source and produced ethanol at 86% of the theoretical yield [145]. Deng and Ho [23] demonstrated that phosphorylation is a vital step for metabolism of xylose through the pentose phosphate pathway (PPP). The gene *XKS1* (encoding xylulokinase) from *S. cerevisiae* and the heterologous genes from *XYL1* and *XYL2* (from *P. stipitis*) were inserted into a hybrid host, obtained by classical breeding of *Saccharomyces uvarum* and *Saccharomyces diastaticus*, which resulted in *Saccharomyces* strain pLNH32, capable of growing on xylose alone. Eliasson et al. [37] reported that chromosomal integration of a single copy of the *XYL1-XYL2-XYLS1* cassette in *S. cerevisiae* resulted in strain TMB3001. This strain attained specific uptake rates (g g^{-1} h^{-1}) of 0.47 and 0.21 for glucose and xylose, respectively, in continuous culture using a minimal medium. Recently, Sedlak and Ho [125] expressed genes [*arab* (L-ribulokinase), *araA* (L-arabinose isomerase) and *araD* (L-ribulose-5-phosphate)] from the *araBAD* operon encoding the arabinose-metabolizing genes from *E. coli* in *S. cerevisiae* but the transformed strain was not able to produce any detectable amount of ethanol from arabinose. Zhang et al. [146] constructed one strain of *Z. mobilis* (PZB301) with seven plasmid-borne genes encoding xylose- and arabinose-metabolizing genes and PPP genes. This recombinant strain was capable of fermenting both xylose and arabinose in a mixture of sugars with 82–84% theoretical yield in 80–100 h at 30°C. Richard et al. [102] reported that overexpression all five enzymes (aldose reductase, L-arabinitol 4-dehydrogenase, L-xylulose reductase, xylitol dehydrogenase and xylulokinase) of the L-arabinose catabolic pathway in *S. cerevisiae* led to growth of *S. cerevisiae* on L-arabinose.

With much global research effort being directed towards developing a stable, ethanol-tolerant, robust recombinant ethanologenic organism capable of tolerating common fermentation inhibitors generated during pretreatment, competitive and economical production of fuel ethanol from hemicellulosic biomass holds strong promise.

Production of xylitol from hemicellulosic hydrolyzate

Xylitol, a five-carbon sugar alcohol, has attracted much attention because of its potential use as a natural food sweetener, a dental caries reducer, and a sugar substitute for diabetics [113]. It is currently produced by chemical reduction in alkaline conditions of the xylose derived mainly from wood hydrolyzate [81]. The recovery of xylitol from the xylan fraction is about 50–60% or 8–15% of the raw material employed [138]. The value depends on the xylan content of the raw material. Drawbacks of the chemical process are the requirements of high pressure (up to 50 atm) and temperature (80–140°C), use of an expensive catalyst (Raney-Nickel), and use of extensive separation and purification steps to remove the by-products, which are derived mainly from the hemicellulose hydrolyzate [85]. The bulk of xylitol produced is consumed in various food products such as chewing gum, candy, soft drinks, and ice cream. It gives a pleasant cool and fresh sensation due to its high negative heat of solution.

Production of xylitol by fermentation is becoming more attractive because of the problems associated with its chemical production. Many yeasts and mycelial fungi possess NADPH-dependent xylose reductase (EC 1.1.1.21), which catalyzes the reduction of xylose to xylitol as a first step in xylose metabolism [12]. Xylitol can be subsequently oxidized to xylulose by the action of xylitol dehydrogenase, which preferentially uses NAD as an acceptor [59]. In xylose-fermenting yeasts, the initial reactions of xylose metabolism appear to be rate-limiting [92]. This results in accumulation of xylitol in the culture medium, the degree varying with the culture conditions, and the yeast strain used [133]. A surplus of NADH during transient oxygen limitation inhibits the activity of xylitol dehydrogenase resulting in xylitol accumulation [50]. Some natural xylose-fermenting yeasts known to produce xylitol are: *Candida boidini*, *Candida guillermondii*, *Candida tropicalis*, *Candida parapsilosis*, and *Debaryomyces hansenii* [113, 127].

Saha and Bothast [118] evaluated the ability of *Candida peltata* NRRL Y-6888 to ferment xylose to xylitol under different fermentation conditions such as pH, temperature, aeration, substrate concentration, and in the presence of glucose, arabinose, ethanol, methanol,

and organic acids. A maximum xylitol yield of 0.56 g/g xylose was obtained when the yeast was cultivated at pH 6.0, 28°C, and 200 rpm on 50 g/l xylose. The yeast produced ethanol (0.41 g/g in 40 h) from glucose (50 g/l) and arabitol (0.55 g/g in 87 h) from arabinose (50 g/l). It preferentially utilized glucose > xylose > arabinose from mixed substrates. Glucose (10 g/l), ethanol (7.5 g/l), and acetate (5 g/l) inhibited xylitol production by 61, 84, and 68%, respectively. Arabinose (10 g/l) had no inhibitory effect on xylitol production by the yeast. Saha and Bothast [111] also studied xylitol production from xylose and corn fiber acid hydrolyzate by two other yeasts (*Candida entomaea* NRRL Y-7785 and *Pichia guilliermondii* NRRL Y-2075). During the screening of 49 yeast strains capable of growing on arabinose, Saha and Bothast [111] observed that these two strains were superior secretors of arabitol as a major extracellular product of arabinose. Both strains produced about 0.70 g arabitol per gram arabinose.

Chen and Gong [11] studied the fermentation of sugarcane bagasse hemicellulose hydrolyzate to xylitol by a hydrolyzate-acclimatized yeast strain *Candida* sp. B-22. With this strain, a final xylitol concentration of 94.74 g/l was obtained from 105.35 g/l xylose in hemicellulose hydrolyzate after 96 h of incubation. *C. guilliermondii* FTI 20037 was able to ferment a sugar cane bagasse hydrolyzate producing 18.4 g/l xylitol from 29.5 g/l of xylose, at a production rate of 0.38 g l^{-1} h^{-1} [98]. This lower value, compared to that (0.66 g l^{-1} h^{-1}) of the synthetic medium, may be attributed to the various toxic substances that interfere with microbial metabolism (e.g., acetic acid). Dominguez et al. [30] studied different treatments (neutralization, activated charcoal and neutralization, cation-exchange resins and neutralization) of sugar cane bagasse hemicellulose hydrolyzate to overcome the inhibitory effect on xylitol production by *Candida* sp. 11-2. The highest xylitol productivity (0.205 g l^{-1} h^{-1}), corresponding to 10.54 g/l, was obtained from hydrolyzates treated with activated charcoal (initial xylose, 42.96 g/l). To obtain higher xylitol productivity, treated hydrolyzates were concentrated by vacuum evaporation in rotavapor to provide higher initial xylose concentration. The rate of xylitol production increased with increasing initial xylose concentration from 30 to 50 g/l, reaching a maximum of 28.9 g/l after 48 h fermentation. The decrease in xylitol production was dramatic with further increases in the initial xylose concentration. Parajo et al. [96] later reported a xylitol production of 39–41 g/l from concentrated *Eucalyptus globulus* wood acid hydrolyzate containing 58–78 g xylose/l by *D. hansenii* NRRL Y-7426 using an initial cell concentration of 50–80 g/l.

Roberto et al. [103, 104] tested hydrolyzed hemicellulosic fractions of sugar cane bagasse and rice straw for xylitol production in batch fermentation by *C. guilliermondii* under semi-aerobic condition and compared these with synthetic medium containing xylose. For all media tested, simultaneous utilization of hemicellulosic sugars (glucose and xylose) was observed, and the highest substrate uptake rate was attained in sugar cane bagasse medium. Increased xylitol concentration (40 g/l) was achieved in synthetic and rice straw media, although the highest xylitol production rate was obtained in sugar cane bagasse hydrolyzate. They concluded that both hydrolyzates can be converted into xylitol with satisfactory yields and productivities. Roberto et al. [105, 106] evaluated xylitol production by *C. guilliermondii* in a rice straw hemicellulose hydrolyzate under different conditions of initial pH, nitrogen sources, and inoculum level. The xylitol yields were 0.68 g/g for the medium containing ammonium sulfate at pH 5.3 and 0.66 g/g with urea at pH 4.5. Under appropriate inoculum conditions, rice straw hemicellulose hydrolyzate was converted into xylitol by the yeast with efficiency values as high as 77% of the theoretical maximum. Mayerhoff et al. [83] evaluated 30 different yeast strains belonging to four different genera (*Candida*, *Debaryomyces*, *Hansenula* and *Pichia*) for xylitol production from rice straw hemicellulose hydrolyzate. The best performer was *Candida mogii* NRRL Y-17032, which yielded 0.65 g xylitol/g at 0.40 g l^{-1} h^{-1} over 75 h. Preziosi-Belloy et al. [101] investigated the production of xylitol from aspenwood hemicellulose hydrolyzate by *C. guilliermondii*. The hydrolyzate had to be supplemented with yeast extract, and the maximum xylitol yield (0.8 g/g) and productivity (0.6 g l^{-1} h^{-1}) were reached by controlling oxygen input. Leathers and Dien [74] developed a two-stage sequential fermentation scheme for production of xylitol and arabitol from a mixture of sugars. Following glucose consumption, cells were removed from mixed sugar cultures and replaced with cells from cultures grown on xylose alone. In the second fermentation stage, xylose and arabinose were successfully fermented to xylitol and arabitol. Dilute acid hydrolyzates of corn fiber were suitable for the two-stage fermentation process, but only after treatment with a mixed-bed deionization resin. Cruz et al. [20] studied xylitol production from barley bran hydrolyzates by continuous fermentation with *Debaryomyces hansenii*. The optimum xylitol productivity (2.53 g l^{-1} h^{-1}) was reached at a dilution of rate of 0.284/h with cell recycle after membrane separation. Xylitol was produced in a two-substrate (xylose, glucose) batch fermentation with cell recycling of *C. tropicalis* [13]. The optimized cell recycle fermentation resulted in xylitol yield of 0.823 g/g xylose with a productivity of 4.94 g l^{-1} h^{-1} and a final xylitol concentration of 189 g/l.

Carvalho et al. [10] obtained maximum xylitol concentration of 20.6 g/l with a volumetric productivity of 0.43 g l^{-1} h^{-1} and yield of 0.47 g/g after 48 h fermentation during batch xylitol production from concentrated sugarcane bagasse hydrolyzate and *C. guilliermondii* cells, immobilized in calcium-alginate beads. The production of xylitol from various hemicellulosic hydrolyzates is presented in Table 5. A number of recombinant *S. cerevisiae* strains have been created by expressing the xylose reductase gene (*XYL1*) from *P. stipitis* and *C. shehate* and production of xylitol from xylose by

Table 5 Xylitol production from detoxified hemicellulosic hydrolyzates by fermentation

Substrate	Yeast	Fermentation time (h)	Xylose (g/l)	Xylitol (g/l)	Xylitol (g/g)
Sugarcane bagasse [11]	*Candida* sp.B-22	96	105.4	96.8	0.89
Sugarcane bagasse [30]	*Candida* sp.11-2	48	42.96	10.54	
Sugarcane bagasse [98]	*Candida guilliermondii* FTI 20037		29.50	18.40	
Rice straw [106]	*C. guilliermondii* FTI 20037	72	64	37.6	0.62
Corn cob [66]	*Candida parapsilosis*	59	50	36	0.72
Wood [95]	*Debaryomyces hansenii* NRRL Y-7426	78	78	41	0.73
Hardwood [18]	*Pachysolen tannophilus*	96	89	39.5	

these recombinant strains in batch and fed batch fermentations have been investigated [16, 49, 56, 57, 77]. These strains converted xylose to xylitol with over 95% yield.

Gurgel et al. [52] studied xylitol recovery from fermented sugarcane bagasse hydrolyzate. The best clarifying treatment was found by adding 20 g activated carbon to 100 ml fermented broth at 80°C for 1 h at pH 6.0. The clarified medium was treated with ion-exchange resins after which xylitol crystallization was attempted. The ion exchange resins were not efficient but the crystallization technique showed good performance, although the crystals were involved in a viscous and colored solution. Recently, Faveri et al. [39] reported xylitol recovery by crystallization from synthetic solutions and fermented hemicellulose hydrolyzates. The method involves evaporation of dilute solution up to super saturation, cooling of the super saturated solution, separation of crystals by centrifugation and final filtration. Using two sets of tests on xylitol-xylose synthetic solutions and one set on fermented hardwood hemicellulose hydrolyzate, the best results in terms of either crystallization yield (0.56) or purity degree (1.00) were obtained with quite concentrated solutions of 730 g/l at relatively high temperature (−5°C). They concluded that xylitol separation by crystallization from fermented hemicellulose hydrolyzate is feasible.

Nidetzky et al. [90] studied continuous enzymatic production of xylitol with simultaneous coenzyme regeneration in a charged membrane reactor. An NADH-dependent xylose reductase from *Candida tenuis* catalyzed the reduction of xylose, which was coupled to enzymatic oxidation of glucose by glucose dehydrogenase from *Bacillus cereus* to make achievable an up to 10,000-fold regeneration of NADH per cycle of discontinuous conversion. Under suitable conditions, 300 g/l substrate could be converted in yields above 96% in one single batch reaction.

The demand for xylitol in the food and pharmaceutical industries as an alternative sweetener has created a strong market for the development of low cost xylitol production process. Various xylose-rich hemicellulosic materials can serve as abundant and cheap feedstocks for production of xylitol by fermentation. The cellulosic fraction can be converted to glucose, which is then fermented to fuel ethanol by conventional *S. cerevisiae*. Much research needs to be done to select a suitable microorganism that can convert xylose into xylitol efficiently in the presence of other hemicellulosic sugars such as glucose, and to understand the regulation and optimization of xylitol production by fermentation. It is possible to introduce the pathway for conversion of arabinose to xylitol into xylitol-producing yeast. In that case, xylitol can be produced from both xylose and arabinose. There is strong interest in creating a recombinant yeast that can produce xylitol from glucose.

Production of 2,3-butanediol from hemicellulosic hydrolyzate

2,3-Butanediol, otherwise 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. 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 [132]. Dehydration of 2,3-BD yields the industrial solvent methyl ethyl ketone, which is much more suited as a fuel because of its much lower boiling point. Further dehydration yields 1,3-butanediene, which is the starting material for synthetic rubber and is also an important monomer in the polymer industry [79]. During World War II, it was needed for conversion to 1,3-butanediene. Methyl ethyl ketone can be hydrogenated to yield high octane isomers suitable for high quality aviation fuels. Diacetyl, formed by catalytic dehydrogenation of the diol, is a highly valued food additive [80]. A wide variety of chemicals can also be easily prepared from 2,3-BD [47, 143]. There is interest in industrial scale production of 2,3-BD from various agricultural residues as well as from logging, pulp and paper, and food industry wastes [80].

2,3-BD can occur in two enantiomeric forms: D-(−) and L-(+) as well as an optically inactive meso-form. *Bacillus polymyxa* produces D-(-)2,3-BD whereas *Klebsiella pneumoniae* (*Aerobacter aerogenes*) produce meso-form and also some of the L-(+) form. *Bacillus subtilis*, *Seratia marcescens* and *Aerobacter hydrophia* produce mixtures of different forms [69]. Saha and Bothast [119] isolated *Enterobacter cloacae* NRRL B-23289 from local decaying wood/corn soil samples while screening for microorganisms for conversion of arabinose to fuel ethanol. The major product of fermentation

Table 6 Fermentation of various sugars by *Enterobacter cloacae* NRRL B-23289[a] (taken from [119])

Substrate (50 g/l)	Time (h)	Butanediol (g/l)	Acetoin (g/l)	Butanediol (g/g substrate)
Glucose	63	18.6	3.1	0.37
Xylose	63	18.9	3.7	0.38
Arabinose	39	21.7	0.6	0.43
Mixture A[b]	48	19.6	3.5	0.39
Mixture B[c]	48	19.5	4.1	0.39
Mannose	72	18.6	0	0.37
Galactose	63	18.9	3.7	0.38
Fructose	39	21.7	0.6	0.43
Sucrose	48	17.7	2.4	0.35

[a]At pH 6.0, 30°C and 200 rpm
[b]Glucose:xylase:arabinose (1:1:1)
[c]Glucose:xylase:arabinose (1:2:1)

by the bacterium was meso-2,3-BD (Table 6). In a typical fermentation, a 2,3-BD yield of 0.43 g/g arabinose was obtained at an initial arabinose concentration of 50 g/l. The bacterium utilized sugars from acid plus enzyme saccharified corn fiber and produced 2,3-BD (0.35 g/g available sugars). It also produced 2,3-BD from dilute acid pretreated corn fiber acid by simultaneous saccharification and fermentation (0.34 g/g theoretical sugars). The 2,3-BD yield (0.35–0.43 g/g sugar) by the newly isolated *E. cloacae* NRRL B-23289 compares favorably with other 2,3-BD-producing organisms reported in the literature (0.30–0.45 g/g sugar) [79].

Butanediol is produced during oxygen-limited growth, by a fermentative pathway known as the mixed acid-butanediol pathway [70]. 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. The theoretical maximum yield of 2,3-BD from monosaccharides is 0.5 g/g [63]. The efficient biological conversion of all available sugars in agricultural biomass residues to fuels and chemicals is crucial to the efficiency of any process intended to compete economically with petrochemical products [143].

The high boiling point of 2,3-BD, its high affinity for water, and the dissolved and solid substances of the fermentation broth make it difficult for 2,3-BD to be purified and recovered from fermentation slurry [129]. Various methods, such as solvent extraction, liquid-liquid extraction and salting out, have been used to recover butanediol. Another feasible method to recover butanediol is countercurrent stream stripping [42].

Production of other value-added products from hemicellulose hydrolyzates

Lactic acid is used in the food, pharmaceutical, and cosmetic industries. It is a component of biodegradable plastic polylactate, the market for which is expected to grow significantly. Dien et al. [28] constructed recombinant *E. coli* carrying the lactate dehydrogenase gene from *Streptococcus bovis* on a low copy number plasmid for production of L-lactate. The recombinant strains (FBR 9 and FBR 11) produced 56–63 g L-lactic acid from 100 g/l xylose at pH 6.7 and 35°C. The catabolic repression mutants (*ptsG*) of the recombinant *E. coli* strains have the ability to simultaneously ferment glucose and xylose [29]. The *ptsG*$^-$ strain FBR19 fermented 100 g sugar (glucose and xylose, 1:1) to 77 g lactic acid/l. This strain has great potential to be used for conversion of lignocellulosic substrates to lactic acid.

Ferulic acid is the major cinnamic acid found in a variety of plant cell walls. Corn fiber contains about 3% ferulic acid. Wheat bran is another source of ferulic acid (0.5–1%). Faulds et al. [38] developed a laboratory scale procedure to produce free ferulic acid (5.7 g) from wheat bran (1 kg) by using a *Trichoderma* xylanase preparation and *Aspergillus niger* ferulic acid esterase. Using filamentous fungi, a two-stage process for vanillin formation was developed in which a strain of *A. niger* was first used to convert ferulic acid to vanillic acid, which was then reduced to vanillin by a laccase-deficient strain of *Pycnoporus cinnabarinus* [78].

Concluding remarks

Conversion of hemicellulose into value-added useful products by enzymatic and/or fermentation routes holds strong promise for the use of a variety of unutilized and underutilized agricultural residues for practical purposes. Currently, the conversion of hemicellulosic substrates to fermentable sugars is problematic. Some of the emerging pretreatment methods, such as alkaline peroxide and AFEX, generate solubilized and partially degraded hemicellulosic biomass that needs to be treated further with enzymes or other means in order to produce fermentable sugars. With the development of a suitable pretreatment method, minimizing the formation of inhibitory compounds for fermentative organisms, and with the proper mix of hemicellulases (enzyme cocktail) tailored for each biomass conversion, this vast renewable resource can be utilized for production of fuels and chemicals by fermentation. Much research needs to be done to develop efficient and cost-effective pretreatment methods, enzymes for cellulose and hemicellulose conversion at an industrial scale, robust efficient microorganisms to ferment hemicellulosic sugars simultaneously in a cost-competitive way, and methods for cost-effective recovery of fermentation products.

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