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Research review paper

Fractional purification and bioconversion of hemicelluloses

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ABSTRACT

Hemicelluloses are types of plant cell wall polysaccharides, and the world's second most abundant renewable polymers after cellulose in lignocellulosic materials. They represent a type of hetero-polysaccharide with complex structure containing glucose, xylose, mannose, galactose, arabinose, rhamnose, glucuronic acid, and galacturonic acid in various amounts, depending on the source. Hemicelluloses are usually bonded to other cell-wall components such as cellulose, cell-wall proteins, lignin, and phenolic compounds by covalent and hydrogen bonds, and by ionic and hydrophobic interactions. This paper provides a review on hemicelluloses from lignocellulosic materials, especially in regard to their isolation and purification methods, and bioconversion. Current isolation and purification strategies are summarized, including: alkali peroxide extraction, organic solvent extraction, steam explosion, ultrasound-assisted extraction, microwave-assisted extraction, column chromatography, and membrane separation. In addition, the bioconversion of hemicelluloses including pretreatment, enzymatic hydrolysis, and fermentation are discussed.

Contents

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1. Introduction

Lignocellulosic biomass is a low-cost and uniquely sustainable resource that offers the production of numerous industrial and non-food consumer products such as fuels, chemicals and polymeric materials, which can reduce greenhouse gas emissions, be used as additives to enhance existing energy fuels, make use of solid waste, and improve air quality. The biomass has a complex composite structure consisting of three main polymeric components including cellulose, hemicelluloses and lignin. Cellulose is the main cell wall constituent, containing highly uniform β-(1→4)-linked polyglucan. Hemicelluloses are the second most abundant polysaccharide in plants, comprising a wide variety of monosaccharides including: xylose, arabinose, glucose, galactose, mannose, fucose, glucuronic acid, and galacturonic acid, depending upon the source (Ebringerova and Heinze, 2000). They are branched polymers with low molecular weights with a degree of polymerization of 80–200 (Cai and Paszner, 1988). Lignin is a network polymer made up of oxidative coupling of three major C_6-C_3 (phenylpropanoid) units with various carbon-to-carbon and ether linkages.

Hemicelluloses were originally considered to be intermediates in the biosynthesis of cellulose. It is now known that hemicelluloses belong to a group of heterogeneous polysaccharides, which are formed by biosynthetic routes different from that of cellulose (Sjöström, 1981). In 1891, Schulze (1891) introduced the term hemicelluloses, which referred to plant tissue which could be extracted by aqueous alkali. However, similar materials could be extracted using water. As such, hemicelluloses are generally defined as being polysaccharides that can be extracted by water or aqueous alkali from plant tissue (Gabrielii et al., 2000; Glasser et al., 2000a; Whistler, 1993). Recently, Scheller and Ulvskov (2010) reviewed the structure and biosynthesis of hemicelluloses, and stated that hemicelluloses are a group of wall polysaccharides that are characterized by being neither cellulose nor pectin and by having β-(1→4)-linked backbones of glucose, mannose, or xylose. Hemicelluloses are non-cellulosic and short-branched chain hetero-polysaccharides consisting of various different sugar units, which are arranged in different proportions and with different substituents (Glasser et al., 2000b). The average degree of polymerization (DP) of hemicelluloses is in the range of 80–200. They are usually associated with various other cell-wall components such as cellulose, cell-wall proteins, lignin, and other phenolic compounds by covalent and hydrogen bonding, and by ionic and hydrophobic interactions (Sun et al., 2000a).

Hemicelluloses have a very wide variety of applications (Fig. 1). They can be easily hydrolyzed into pentose (xylose and arabinose) and hexose (glucose, galactose, and mannose), and can be transformed into fuel ethanol and other value-added chemicals, such as 5-hydroxymethylfurfural (HMF), furfural, levulinic acid, and xylitol (Canilha et al., 2003). In addition, hemicelluloses can be converted into various biopolymers by modification, used as viscosity modifiers in food packaging film, as wet strength additives in papermaking, and as tablet binders (Watson, 1959). Moreover, hemicelluloses have also been investigated for their possible medical uses due to their ulcer protective (Cipriani et al., 2006), antitussive (Kardosova et al., 2002), immunostimulatory (Kulicke et al., 1997), and antitumor properties (Kitamura et al., 1994). For example, xylooligosaccharides have been shown to have economic utilization in the pharmaceutical industry for applications such as treating viral and cancer processes in the human body (Damonte et al., 1996; Hisado et al., 1985; Stone et al., 1998; Watson et al., 1999). The current review summarizes present knowledge on the structure, isolation and purification, and bioconversion of hemicelluloses.

2. Structure of hemicelluloses

Hemicelluloses are low-molecular-weight polysaccharides, associated in plant cell walls with cellulose and lignin. Large variations in hemicellulose content and chemical structure can occur between different biomass (i.e. maize stems (28.0%), barley straw (34.9%), wheat straw (38.8%), and rye straw (36.9%) (Fang et al., 2000), and between different components of an individual organism (stem, branches, roots, and bark)).

2.1. Hardwood hemicelluloses

2.1.1. Glucuronoxylan (GX)

Hemicelluloses in various hardwood species differ from each other both quantitatively and qualitatively. The main hemicelluloses of hardwood are *O*-acetyl-4-*O*-methylglucurono-β-D-xylan. Depending on the hardwood species, GX content varies with the limit of 15–30% of dry materials (Alen, 2000). GX consists of a backbone of (1→4)-linked β-D-xylopyranosyl (Xyl*p*) residues. Most of xylose residues contain an acetyl group at C-2 or C-3 (about seven acetyl residues per ten xylose units). GX is substituted with glucurononosyl and 4-*O*-methylglucuronosyl residues by α-(1→2) linkages (Table 1). The xylosidic bonds between the xylose units are easily hydrolyzed by acids, but the linkages between the uronic acid groups and xylose are very resistant. Acetyl groups are easily cleaved by alkali, and the acetate formed during kraft pulping of wood mainly originates from these groups (Sjöström, 1981). Some acidic xylans with the glucuronic acid (GlcA) side chain both in the 4-*O*-methylated and non-methylated forms are also isolated from monocotyl plants (Lindberg et al., 1990).

Hardwood xylans are generally composed of 4-*O*-methylglucuronoxylan, but several details regarding their structures are still uncertain. A question which remains unanswered is whether or not

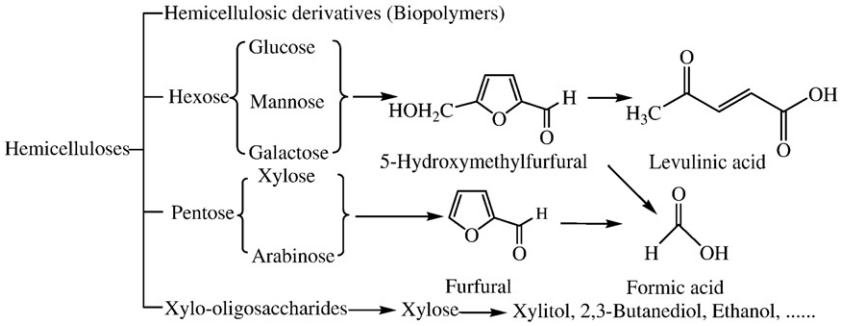

Fig. 1. The potential products from hemicelluloses.

galacturonic acid (GalA) and rhamnose (Rha) are structural components of xylan. Recently, some interesting features of xylan have been found in the hardwood xylans. The structure of -*O*-β-D-Xyl*p*-(1→4)-*O*-β-D-Xyl*p*-(1→3)-*O*-α-L-Rha*p*-(1→2)-*O*-α-D-GalA*p*-(1→4)-D-Xyl*p* have been revealed in the reducing end of hardwood xylans from birch (*Betula platyphylla*) and *Eucalyptus globules* (Ericsson et al., 1977; Evtuguin et al., 2003; Johansson and Samuelson, 1977; Shimizu et al., 1976).

2.1.2. Glucomannan (GM)

In addition to xylan, hardwoods contain 2–5% of a glucomannan, which is composed of β-glucopyranose and β-mannopyranose units linked by (1→4)-bonds (Table 1). However, the mannose/glucose monomer ratio may vary depending on the original source of GM. The ratio of glucose to mannose varies between 1:2 and 1:1. Galactose is not present in hardwood mannan. The mannosic bonds between the mannose units are more rapidly hydrolyzed by acid than the corresponding glycosidic bonds, and GM is easily depolymerized under acidic conditions (Sjöström, 1981).

The most commonly used type of GM is konjac glucomannan (KGM), which is extracted from tubers of *Amorphophallus* plants (Gao and Nishinari, 2007). It consists of D-mannose and D-glucose in a molar ratio of 1.6:1, with a β-1,4-linkage (Kato and Matsuda, 1969; Maeda et al., 1980). There may be certain short side branches at the C-3 position of the mannoses and acetyl groups randomly present at the C-6 position of a sugar unit. The acetyl groups frequently range from 1 per 9 sugar units to 1 per 20 sugar units (Koroskenyi and Mccarthy, 2001; Smith and Srivastava, 1959). Because of its renewability, good biocompatibility and biodegradability, KGM is a promising material for use in degradable plastics.

2.1.3. Xyloglucan(XG)

Besides xylan and glucomannan, xyloglucans are also present in the primary cell walls of some higher plants (mainly in hardwoods, and less in softwoods) (de Vries and Visser, 2001; Ebringerova et al., 2005). They can also appear in small amounts (2–5%) in grasses (Scheller and Ulvskov, 2010). Xyloglucans have a cellulosic β-(1→4)-glucopyranan backbone with α-D-xylose units attached at *O*-6. In the primary cell walls, xyloglucans are the principal interlocking polysaccharides, interacting with the cellulose microfibril by hydrogen bond (Carpita and Gibeaut, 1993).

Table 1
The main types of polysaccharides present in hemicelluloses (Gírio et al., 2010; Scheller and Ulvskov, 2010; Sjöström, 1981).

Polysaccharide	Biological origin	Amount[a]	Units			DP[b]
			Backbone	Side chains	linkages	
Arabinogalactan	Softwoods	5–35	β-D-Gal*p*	β-D-Galp α-L-Araf β-L-Arap	β-(1→6) α-(1→3) β-(1→3)	100–600
Xyloglucan	Hardwoods, softwoods, and grasses	2–25	β-D-Glc*p* β-D-Xyl*p*	β-D-Xylp β-D-Galp α-L-Araf α-L-Fucp Acetyl	β-(1→4) α-(1→3) β-(1→2) α-(1→2) α-(1→2)	
Galactoglucomannan	Softwoods	10–25	β-D-Man*p* β-D-Glc*p*	β-D-Galp Acetyl	α-(1→6)	40–100
Glucomannan	Hardwoods	2–5	β-D-Man*p* β-D-Glc*p*			40–70
Glucuronoxylan	Hardwoods	15–30	β-D-Xyl*p*	4-*O*-Me-α-D-GlcpA Acetyl	α-(1→2)	100–200
Arabinoglucuronoxylan	Grasses and softwoods	5–10	β-D-Xyl*p*	4-*O*-Me-α-D-GlcpA α-L-Araf	α-(1→2) α-(1→3)	50–185
Glucuronoarabinoxylan	Grasses	15–30	β-D-Xyl*p*	α-L-Araf 4-*O*-Me-α-D-GlcpA Acetyl	α-(1→2) α-(1→3)	
Homoxylan	Algae		β-D-Xyl*p*[c]			
β-(1→3, 1→4)-glucan	Grasses	2–15	β-D-Glc*p*		β-(1→3) β-(1→4)	

[a] %, Dry biomass.
[b] Degree of polymerization.
[c] May also present β-(1→3) linkages on the backbone.

2.2. Softwood hemicelluloses

2.2.1. Galactoglucomannans (GGM)

The major hemicelluloses in softwoods are acetylated galactoglucomannans (AcGGM), which can constitute up to 10% of dry wood. They are composed of a backbone of (1→4)-linked β-D-Glc*p* and β-D-Man*p* residues with α-(1→6)-D-Gal*p*. An important structural feature is the hydroxyl groups at the C-2 and C-3 positions in the main chain units being partially substituted by *O*-acetyl groups, with, on average, one group per 3–4 hexose units containing this substitution (Table 1). AcGGM isolated from spruce by microwave oven treatment or isolated from spruce wood holocellulose were recently shown to be partially substituted at the C-2 and C-3 positions on the β-D-Man*p* units and not on the β-D-Glc*p* units (Capek et al., 2002; Lundqvist et al., 2002). There are two main types of acetylgalactoglucomannans in softwoods, one being galactose-poor (5–8% of dry wood) and the other galactose-rich (10–15% of dry wood). The ratio of galactose:glucose:mannose is approximately 0.1:1:3 and 1:1:3, for the two woods, respectively. AcGGM have an approximate DP between 100 and 150, which is equivalent to a molecular weight (*Mw*) around 16,000–24,000 Da (Lundqvist et al., 2002; Timell, 1982). Willfor et al. (2003b) reported the AcGGM from native Norway spruce wood and thermomechanical pulp. About 65% of the mannose units in the GM backbone were acetylated at either the C-2 or C-3 positions in a ratio of 2.2:1.0. Mainly the mannose units, but also some glucose units, were partly substituted at C-6 by galactopyranose units. The number of galactose side groups at C-6 was considerably lower for AcGGM from thermomechanical pulp than from wood. GGMs are easily depolymerized by acids, especially the bonds between galactose and the main chain. The acetyl groups are much more easily cleaved by alkali and acid (Sjöström, 1981).

2.2.2. Arabinoglucuronoxylan (AGX)

Besides GGM, softwood contains an arabinoglucuronoxylan (5–10%). AGXs are the major component of non-woody materials. AGXs has single 4-*O*-methyl-D-glucuronic acid (MeGlcA) and α-L-arabinofuranosyl (α-L-Ara*f*) units attached at position 2 and 3, respectively, to the β-(1→4)-D-xylopranose backbone, which might also be slightly acetylated (Table 1). Generally, AGX has the backbone more heavily substituted by MeGlcA than that of the hardwood MGX, with 5–6 xylose residues per uronic acid group in the former and 10 on average in the latter (Ebringerova et al., 2005). In addition, because of their furanosidic structure, the arabinose side chains are easily hydrolyzed by acids. Both arabinose and uronic acid substituents stabilize the xylan chain against alkali-catalyzed degradation (Sjöström, 1981).

2.2.3. Arabinogalactan (AG)

The heartwood of larches contains exceptionally large amounts of water-soluble AG, which is only a minor constituent in other softwood species (Sjöström, 1981). AGs are highly branched polysaccharides with molecular weights ranging from 10,000–120,000 Da. All Larch AG isolated from the *Larix* sp. are of the 3,6-β-D-galactan type and consist of galactose and arabinose in a 6 to 1 ratio. Larch AG has a galactan backbone that features β-(1→3) linkages and galactose β-(1→6) and arabinose β-(1→6 and 1→3) sugar side chains (Fitzpatrick et al., 2004) (Table 1). The highly branched structure is responsible for the low viscosity and high solubility in water of this polysaccharide (Sjöström, 1981). Nature provides a uniquely high concentration of AG in the *Larix* genus of trees, which is used as the commercial source of Larch AG. The Larch AG is a highly water-soluble prebiotic fiber that is extracted with water from the lumen of cells found in the Western and Eastern Larch trees known as *Larix occidentalis* and *Larix laricina*, respectively. The concentration and quality of Larch AG is not affected by seasonal variability. This natural bioactive compound is extracted from the trees using a patented, eco-friendly process, which makes use of hot water and hence is solvent-free (Fitzpatrick et al., 2004). Larch AG has good solubility in cold and hot water in 30 s, extremely low viscosity of concentrated aqueous solutions, the ability to bind fat, retain liquid, and dispersing properties. It also possesses a highly biological activity, such as enhancing the immunity, protecting liver, digestive aid, prebiotic, hypolipidemic, and gastroprotective (Fitzpatrick et al., 2004; Medvedeva and Aleksandrova, 2003; Medvedeva et al., 2003). Therefore, these properties may allow Larch AG to be used in a variety of food, beverage, nutraceutical, and medicine applications.

2.3. Gramineae hemicelluloses

2.3.1. Arabinoxylan (AX)

Arabinoxylans are the main hemicelluloses of Gramineae. AXs have been generally present in a variety of tissue of the main cereals of commerce: wheat, rye, barley, oat, rice, corn, and sorghum, as well as other plants: pangola grass, bamboo shoot, and ray grass (Fincher and Stone, 1986; Hartley and Jones, 1976; Ishii, 1991). AXs are generally present in the starchy endosperm (flour) and outer layers (bran) of cereal grain (Bengtsson et al., 1992; Gruppen et al., 1992). They are similar to hardwood xylan, but the amount of L-arabinose is higher. In AX, the linear β-(1→4)-D-Xyl*p* backbone is substituted by α-L-Ara*f* units in the positions 2-*O* and/or 3-*O* (Table 1). In addition, the AXs are also substituted by α-D-glucopyranosyl uronic unit or its 4-*O*-methyl derivative in the position 2-*O*, such as wheat straw, bagasse and bamboo. *O*-acetyl substituents may also occur (Brillouet et al., 1982; Shibuya and Iwasaki, 1985; Sun et al., 1996, 2004b; Wen et al., 2011; Wende and Fry, 1997). Bacon et al. (1975) mentioned that cell walls of Gramineae plants account for 1–2% of the acetyl groups. According to the amount of glucuronic acid and arabinose, the types of AX are classified as arabinoglucuronoxylan (AGX) and glucuronoarabinoxylan (GAX), respectively (Ebringerova et al., 2005). AGX are the dominant hemicelluloses in the cell walls of grasses and cereals, such as sisal, corncobs and straw (Ebringerova and Heinze, 2000; Ebringerova et al., 2003). Compared to AGX, the GAX have an arabinoxylan backbone, which contains about ten times fewer uronic acid side chains than arabinose, and also contains xylan which is double-substituted by uronic acid and arabinose units. Ferulic acid and *p*-coumaric acid can occur esterified to the C-5 of arabinosyl units of GAXs (Kato and Nevins, 1985; Mueller-Harvey et al., 1986). Dimerization of esterified phenolic compounds may also lead to inter- and intra-molecular cross-links of xylan (Grabber et al., 1995). The physical and/or covalent interaction with other cell wall constituents restricts xylan extractability.

2.3.2. β-(1→3, 1→4)-glucans

β-(1→3, 1→4)-glucans consist of a linear chain of β-D-glucopyranosyl units linked by (1→3) and (1→4) bonds (Izydorczyk and Dexter, 2008) (Table 1). Previous studies suggested that Poaceae (grasses and cereals) was the only family with undelignified cell walls containing (1→3, 1→4)-β-D-glucans (Smith and Harris, 1999). However, recent studies show that β-(1→3, 1→4)-glucans are also present in *Equisetum*, liveworts and Charopytes (Popper and Fry, 2003; Sorensen et al., 2008). The mixed-linkage glucans are dominated by cellotriosyl and cellotetrasyl units linked by β-(1→3) linkages, but longer β-(1→4)-linked segments also occur (Stone and Clarke, 1992). Cellulose is also β-D-glucan, which is linked by (1→4)-glycosidic bonds, and thus cellulose has high stiffness (crystalline) and insoluble in most solvents. However, the (1→3) linkages exists in β-(1→3, 1→4)-glucans, which makes glucans flexible and soluble (Anderson and Bridges, 1993). Extensive research has been done about the structure, properties and functionality of β-(1→3, 1→4)-glucans (Cui et al., 2000; Lazaridou et al., 2004; Storsley et al., 2003). There were no significant differences in the structures of β-glucans obtaining from different botanical origin, whereas the difference may occur in the ratios of (1→4) to (1→3)

linkages, molecular weights, the molar ratio of cellotriosyl/cellotetraosyl residues, and the presence and amount of long cellulose-like fragments (Izydorczyk and Dexter, 2008; Lazaridou et al., 2007). Johansson et al. (2000) found that the β-glucan from oat bran with different molar masses has different solubility, and the β-glucan having lower molar masses (1.1 kD) is more readily soluble than that with higher molar masses (1.6 kD). They also investigated the structure of β-glucan by two-dimensional HSQC NMR spectroscopy, and found that the β-(1→3)-linked Glc*p* could also be separated.

3. Isolation of hemicelluloses

In conventional kraft pulping processes, most of the hemicelluloses from wood are degraded into oligomers or mono sugars, and dissolved in black liquor along with dissolved lignin and the pulping chemicals (inorganic substances). The black liquor is usually combusted for steam and electricity generation. However, since hemicelluloses have a considerably lower heating value than lignin, their combustion represents an uneconomical use of the feedstock resource. In addition, during the production of ethanol, the removal of lignin and hemicelluloses is desired to improve the accessibility of cellulosic material to hydrolytic enzymes: promoting high ethanol yields and an economically feasible production process. Removal also avoids the formation of degradation products that are inhibitory to the yeast used in the subsequent fermentation (Sun, 2008). Therefore, in an integrated lignocellulose biorefinery, pre-extraction and isolation of hemicelluloses and lignin followed by the production of value-added products such as ethanol, sugar-based polyesters, and other chemicals and biopolymers, offers a tremendous valued-added opportunity (Huang et al., 2008). The use of all three polymeric biomass components of cellulose, hemicelluloses and lignin has been discussed for several decades, with efforts being intensified in connection with emerging biorefinery strategies (Ahring et al., 2005). However, so far, it has been a great challenge to design production processes allowing the utilization of all components of biomass materials, primarily due to the hydrogen and covalent linkages between hemicelluloses and cellulose and lignin, respectively.

Interest in the isolation of hemicelluloses from biomass has greatly increased in recent years. The methods investigated include: alkaline extraction, alkaline peroxide extraction, liquid hot water extraction, steam explosion-based extraction, and so on. However, an appropriate method has yet to be reported for selective liberation of all hemicelluloses from other components of the cell wall without degradation during the isolation process. Therefore, much attention has been paid to developing effective isolation and purification methods to obtain hemicelluloses with both high purity and yield. In this section, the methods currently available in literature for isolating hemicelluloses from lignocellulosic materials will be reviewed.

3.1. Alkali treatment

Hemicelluloses are the most complex components in the cell wall of woods and annual plants. They form covalent bonds (mainly α-benzyl ether linkages) with lignin and ester linkage with acetyl units and hydroxycinamic acids, which restrict the liberation of the hemicellulose matrix. The extensive hydrogen bonding between the individual polysaccharide cell wall components may impede the isolation of hemicellulose components (Ebringerova and Heinze, 2000). Alkaline extraction has been shown to be very effective for the removal of hemicelluloses (Gabrielii et al., 2000; N'Diaye et al., 1996; Sun et al., 2005a). Alkali treatment of lignocellulosic materials such as cereal straw and bagasse disrupts the cell wall by dissolving hemicelluloses, lignin, and silica, hydrolyzing uronic and acetic esters, and swelling cellulose, decreasing its crystallinity (Jackson, 1977). In addition, the alkali treatment also cleaves the α-ether linkages between lignin and hemicelluloses, as well as the ester bonds between lignin and/or hemicelluloses and hydroxycinnamic acids, such as *p*-coumaric and ferulic acids (Spencer and Akin, 1980). Alkaline conditions at elevated temperatures can also be used to pre-treat biomass for bioethanol production, demonstrated in ammonia recycled percolation (ARP) and wet oxidation (Klinke et al., 2002; Mosier et al., 2005). However, alkali extractions have the disadvantage of deacetylating hemicelluloses.

For the isolation of hemicelluloses from woods and annual plants, various multi-step and two-step extraction procedures have been proposed. Hemicelluloses can be removed from the original or the delignified tissue by extraction with aqueous alkali or, less frequently, with water. For quantitative isolation of hemicelluloses from straw, the typical procedure is as follows: firstly, the material must be pre-extracted, preferably with ethanol-toluene (1/2, v/v), aiming to remove all lipophilic and hydrophilic nonstructural components. Secondly, the material is delignified with acidic sodium chlorite, after which the resulting holocellulose is extracted with alkali. The hemicelluloses extracted by alkali are, in part, precipitated in neutralization or mild acidification, and in part, by the subsequent addition of an excess of acetone or ethanol (O'Dwyer, 1926). However, the hazardous and expensive $NaClO_2$-delignification step is substituted by a procedure of aqueous alcohol treatment, and the hemicelluloses obtained have a relatively higher content of lignin and degradation products (Ebringerova and Heinze, 2000). Several aqueous alkali processing approaches have been established. One approach is to isolate the hemicelluloses from the holocellulose by aqueous alkali. The hemicelluloses isolated by this approach have a light brown color and contain a relatively smaller amount of associated lignin (1–2%). This method was developed for characterization purposes, but it can also be used as a preparative method. The isolation carried out by a gradient extraction using different concentrated alkali solutions yields a crude fractionation of the hemicelluloses (Selvendran and Oneill, 1987). Then the soluble hemicellulosic fractions may be precipitated with acidified ethanol. Another alkali approach is to isolate the hemicelluloses from the dewaxed materials directly. Hemicelluloses and lignin could be obtained by adding ethanol and adjusting the pH of the solution obtained after alkali extraction. This procedure is an eco-friendly extraction process, and has thus been recently used in the isolation of hemicelluloses from wheat straw, sugar beet pulp, barley straw, maize stems, rye straw, and rice straw (Ruzene et al., 2008; Sun and Hughes, 1999; Sun and Sun, 2002; Sun et al., 1996; Xiao et al., 2001). It was found that the treatment of dewaxed maize stems, rye straw, and rice straw with 1.0 M NaOH at 30 °C for 18 h resulted in a dissolution of 78.0, 68.8, and 82.1% of the original lignin, and 72.1, 72.6, and 84.6% of the original hemicelluloses, respectively (Xiao et al., 2001). However, because of the lignin hemicellulosic complex in the cell walls of straw, the content of phenolics in the hemicellulosic fractions extracted directly from dewaxed straw was 5–10 times higher than those in the hemicellulosic fractions extracted from straw holocelluloses. This indicates that the hemicelluloses obtained by this approach had higher amount of lignin than the hemicelluloses obtained by the alkali extraction from holocellulose.

Because of the relatively lower amount of lignin, the hemicelluloses from annual plants are extracted more easily than that from wood (Ebringerova and Hromadkova, 1999). Alkali type, concentration, time, and temperature all had a noticeable effect on the yield of hemicelluloses. Generally, aqueous solutions of sodium, potassium, lithium, barium, calcium, and ammonium hydroxide were appropriate for isolating hemicelluloses from lignocellulosic plants, where sodium and potassium hydroxide are typically preferred because of the high yield of hemicelluloses obtained (Lawther et al., 1996). The effects of aqueous sodium and potassium hydroxide and extraction duration on the yield of hemicelluloses were investigated by Xu, Sun and Curling (Curling et al., 2007; Sun et al., 1998; Xu et al., 2007). At equal concentrations, sodium hydroxide was slightly more effective than potassium hydroxide for the removal of hemicelluloses,

although potassium hydroxide resulted in higher purity. Gruppen et al. (1991) reported that barium hydroxide solvent is preferred for its selectivity towards arabinoxylans, where treatment with saturated $Ba(OH)_2$ containing $NaBH_4$ can solubilize pure arabinoxylan, representing approximately 80% of all arabinoxylan present in wheat endosperm cell wall material which cannot be extracted using water. Calcium hydroxide can also be applied for extracting hemicelluloses, but it has lower solubility and selectivity for hemicelluloses. Under the condition of alkali-treatment, the cleavage of *O*-acetyl groups cannot be avoided because the pH of the solution can be as high as 10, conditions at which all acetyl groups are split off. Moreover, the addition of boric acid or borate also has the ability to minimize the degradation of the reducing end groups and markedly facilitate the dissolution ability of alkaline solvents toward certain classes of polysaccharides, especially for glucomannans and galactoglucomannans, mainly because of the formation of borate complexes with hydroxyl groups of mannose units at C-2 and C-3 positions (Fig. 2a) (Fengel and Wegener, 1989; Lawther et al., 1996). The optimum extraction concentration of boric acid is either 2 or 5%, depending on which of the following criteria is considered most important: consumption of boric acid or total hemicellulose yield.

Recently, it has been reported that alkaline peroxide is an effective agent for both delignification and solubilization of hemicelluloses from lignocellulosic materials (Pan et al., 1998; Sun and Sun, 2002). It is, generally, accepted that the hydroperoxide anion (HOO^-) formed in alkaline media, is the principal active group in hydrogen peroxide (H_2O_2) bleaching systems. In contrast, H_2O_2 is unstable in alkaline conditions and readily decomposes into hydroxyl radicals ($HO^{\cdot}$) and superoxide anion radicals (O_2^{-}). This is particularly true in the presence of certain transition metals such as manganese, iron, and copper. These radicals are thought to cause the oxidation of lignin structures which lead to the introduction of hydrophilic (carboxyl) groups, cleavage of some inter-unit bonds and, eventually, the dissolution of lignin and hemicelluloses (Pan et al., 1998). The hemicelluloses isolated by aqueous alkali from wheat straw were, in general, brown due to higher content of lignin, thus impeding their industrial utilization. Sun et al. (2000c, 2000d, 2001) found that more than 80% of the original hemicelluloses and lignin were solubilized during the treatment of cereal straws (wheat, rice, and rye) and maize stems with 2% H_2O_2 at 48 °C for 16 h at pH 12.0–12.5. Under these conditions, carbohydrates were less damaged and delignification was more efficient. Furthermore, the hemicelluloses were whiter in color and contained very smaller amounts of associated lignin (3–5%) compared to those obtained from the traditional alkali extraction process. The dilute alkaline solutions of hydrogen peroxide removed 80% of the lignin present in materials such as wheat straw, yielding a cellulose-rich insoluble residue that can be enzymatically converted to glucose for the production of chemicals and ethanol.

3.2. Organic solvent treatment

Organic solvent treatment is another potential method in the isolation of hemicelluloses. In recent years, the hazardous and expensive $NaClO_2$ delignification step has been substituted by the procedure of aqueous organosolv treatment, which yields xylan-rich polysaccharide fractions contaminated to various extents with lignin and degradation products of the cell wall components (Shatalov and Pereira, 2002; Sun et al., 2000b). Dimethyl sulfoxide (DMSO) is the most common neutral (non-destructive) solvent that has been applied to extract hemicelluloses from holocellulose. Hagglund et al. (1956) first used DMSO as a solvent for the isolation of hemicelluloses from plant materials. The hemicelluloses may also be extracted by water, which has been found to be the best solvent for dissolving the original AcGGM (AL_Manasrah, 2008). A Combination of DMSO with water was used to extract GGM from spruce holocellulose (Hu et al., 2008; Willfor et al., 2008). Ebringerova and Heinze (2000) found that DMSO and DMSO/water mixtures were efficient solvents for low-branched heteroxylans. The hemicelluloses can be extracted without cleaving the acetyl ester compounds and the glycosidic linkages, which aids in the study of the hemicellulose structure. This solvent might be useful in pretreatment, where it would allow access to various components of the wood. However, the high cost and potential hazards of handling large volumes of DMSO limit its utilization (Kenealy et al., 2007).

In order to completely utilize the lignocellulosic materials, the hemicellulosic polymers and their degradation products, the sulfur-free and chlorine-free phenolic lignin polymer and cellulose can be obtained by the treatment with organic solvents (Dapía et al., 2002; Vázquez and Lage, 1992). The organosolv treatment has some advantages: easy recovery of solvent by distillation, low environmental impact, and low energy consumption (Aziz and Sarkanen, 1989; Pan et al., 2006; Vila et al., 2003). The low boiling point organic solvents (e.g. ethanol and methanol) in combination with water are usually used for dissolving hemicelluloses and lignin. Pisarnitskii et al. (2006) analyzed the hemicelluloses in the oak wood ethanol extracts by aqueous–alcoholic media (40–90%), and found that the hemicelluloses with different compositions were extracted from wood at the different concentrations of ethanol. In addition, a great variety of solvents with acidic and alkaline catalyst additions have been tested for wood pretreatment processes (Evtuguin et al., 1999; Neto et al., 1994). For example, Geng et al. (2003) reported that 18.7 and 17.8% hemicelluloses from dewaxed *Caligonum monogoliacum* and *Tamarix* spp could be obtained using ethanol-H_2O (60/40, v/v) under acid catalyst (0.2 N HCl). Xu et al. (2006b) compared the influence of various organosolv treatments, such as acetic acid-H_2O (65/35, v/v), acetic acid-H_2O (80/20, v/v), acetic acid-H_2O (90/10, v/v), formic acid-acetic acid-H_2O (20/60/20, v/v/v), formic acid-acetic acid-H_2O (30/60/10, v/v/v), methanol-H_2O (60/40, v/v), and ethanol-H_2O (60/40, v/v) using 0.1% HCl as a catalyst at 85 °C for 4 h, on the characterization of hemicelluloses from wheat straw. The optimum condition for isolation of hemicelluloses was found to use a mixture of formic and acetic acids and H_2O (30/60/10, v/v/v), which yielded 76.5% of the original hemicelluloses from the feedstock material. In addition, Jin et al. (2009) investigated isolation methods of hemicelluloses from barley straw and maize stems, which included the use of 90% neutral dioxane, 80% dioxane containing 0.05 M HCl, DMSO, and 8% aqueous KOH solvents. It was found that the structures of the hemicellulosic fractions released during the treatment with the neutral solvents of 90% dioxane and DMSO remained intact, while the extractions with 80% acidic dioxane and 8% KOH under the conditions used resulted in a partial depolymerization of the dissolved polysaccharides by cleavage of the glycosidic bonds and saponification of the ester groups in the polymers. The 90% neutral dioxane-soluble hemicellulosic fractions consisted mainly of the more branched arabinoxylans

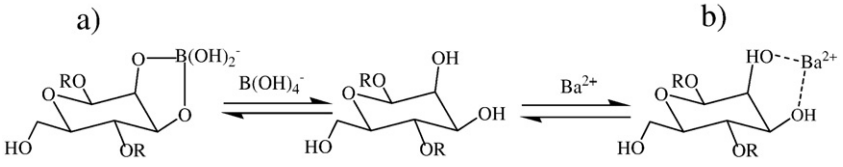

Fig. 2. Reaction of the *cis*-2,3-diol structure of a 1,4-linked β-D-mannopyranosyl unit with borate (a) and barium (b) ions (Sjöström, 1981).

and mixed-linkage glucans such as β-glucans, whereas the hemicellulosic fractions solubilized during the sequential treatments with 80% acidic dioxane, DMSO, and 8% KOH where primarily comprised of arabino-(4-*O*-methyl-D-glucurono) xylans.

3.3. Mechanical–chemical treatments

3.3.1. Ultrasonication

Another potential method for isolation of hemicelluloses combines chemical treatment with mechanical treatment. The application of ultrasonic irradiation during the isolation of plant materials has been found to significantly improve the extraction of polysaccharides, particularly for extracting low molecular weight substances (Mason et al., 1996; Panchev et al., 1994; Salisova et al., 1997). Recently, Ebringerova and Hromadkova (2010) summarized the importance and potential of ultrasound to enhance extraction of various polysaccharide components present in different plants and plant materials. The mechanisms of ultrasonic irradiation extraction are ascribed to the propagation of ultrasound pressure waves, and resulting in the phenomenon of cavitation. When the pressure waves pass through the surface of lignocellulosic materials, the cavitation bubble will implode, and cause microfractures (Mason, 1992; Vinatoru, 2001, Vinatoru et al., 1997). The ultrasound makes the cell wall of biomass swell and hydration results in increasing the diffusion and mass transfer (Ebringerova and Hromadkova, 2010; Toma et al., 2001; Vinatoru, 2001). Positive effects of ultrasound on the extractability of hemicelluloses from corn bran, buckwheat hulls wheat straw, sugarcane bagasse, poplar wood, and *Salvia officinalis L* have been reported (Ebringerova and Hromadkova, 2002; Hromadkova and Ebringerova, 2003; Hromadkova et al., 1999; Sun and Tomkinson, 2002, 2003; Sun et al., 2002, 2004a; Yuan et al., 2010). Higher yields of hemicelluloses can be achieved at lower temperatures and shorter extraction times (Ebringerova et al., 1995). The application of ultrasound has been shown to be very effective during the alkaline extraction. Sun and Tomkinson (2002) investigated the effect of extraction procedures (0.5 M KOH treatment) with and without the application of ultrasonic irradiation, and found that the ultrasonic irradiation extraction had a positive effect on the extraction process, and preserved the structure of hemicellulose and lignin. However, due to the disadvantage of ultrasonic irradiation caused by radical reaction, the glycosidic linkages among the sugars were cleaved, which resulted in the degaradation of polysaccharides (Lorimer and Mason, 1995; Portenlänger and Heusinger, 1994). Thus the ultrasound power used and operating parameters involved should be established for hemicellulose extraction, and should be chosen to preserve structural and molecular properties (Vodenicarova et al., 2006; Zhou and Ma, 2006).

3.3.2. Twin-screw extruder

The main application of twin-screw extruders is found in agro-industry for starch conversion by Harper (1989). He allowed the combination of two unit operations: extrusion and cooking, to occur simultaneously, and, therefore, the physical and chemical transformations of this biopolymer occurred in a single step. N'Diaye et al. (1996) extracted hemicellulose from poplar with a 5% NaOH solution as the extracting solvent, using an extruder-type twin-screw reactor equipped with a filtration system. This extruder or extrusion reactor, called a thermo-mechanico-chemical fractionation system, allows the integration of extrusion, cooking, liquid–solid extraction, and liquid/solid separation (filtration) in a single step, and operates in a continuous mode. Based on the content of pentosans, the twin-screw reactor allows extraction of up to 90% of the initial hemicellulose, the liquid/solid ratio being six times less than for a batch reactor and the solid reaction time being much lower. The sodium hydroxide concentration was found to have a strong influence on the consistency of the reaction mixture, defining two effective zones of operation for extraction of hemicellulose. Extraction yield was shown to depend markedly on temperature, while reduction in screw rotation speed and solid flow rate affected the mean residence time of the liquid phase, which enhanced the extraction yield of hemicellulose (N'Diaye and Rigal, 2000). In addition, Marechal et al. (2004) developed a process for direct atomization of the extract by considering twin-screw extrusion and ultrafiltration. Twin-screw extrusion was found to be very efficient for alkali impregnation of wheat bran, but xylan extraction was achieved only with large liquid/solid ratios. As xylan extraction from wheat bran alone is not feasible by twin-screw extrusion at low liquid/solid ratios, the coextrusions of wheat bran and wheat straw were investigated (Marechal et al., 2004; Zeitoun et al., 2010). Compared with a stirred reactor extraction, twin-screw extrusion gave a lower extraction yield, about 24% of hemicellulose in the wheat bran, however, it did provide shorter residence time for the vegetable matter and lower chemical and water consumption.

3.4. Hydrothermal treatments

3.4.1. Steam explosion

High-pressure steaming, with rapid decompression (explosion), has been claimed as one of the most successful pretreatment methods for fractionating lignocellulosic materials into their three major components and enhancing the susceptibility of cellulose to enzymatic attack (Excoffier et al., 1991; McMillan, 1994). In this method, biomass is treated with high pressure saturated steam, and then the pressure is suddenly reduced, causing the materials to undergo explosive decomposition. This decomposition breaks down the lignocellulosic structure so that the lignin is readily depolymerized and thus the hemicelluloses are easily hydrolyzed (Cara et al., 2006). Steaming of lignocellulosics in batch digesters followed by flash decompression (explosion) was developed by Mason (1928) for the production of hardboard in the process known as Masonite. Since then, steam explosion has become a well known method for separating lignocellulosic materials or disrupting different biomasses into their main components: cellulose, lignin, and hemicelluloses (Nguyen, 1989). The use of the Mason process for the effective separation of hemicelluloses and then the separation of lignin by successive extractions with water and alcohol solvents to obtain acceptable-quality cellulose has been described in a patent by DeLong (1981). The most important operational conditions in steam explosion pretreatment are: time, temperature, and chip size (Duff and Murray, 1996). Optimal conditions are defined as those in which the best substrate for hydrolysis is produced with the least material loss due to side reactions such as dehydration, and in which adequate carbohydrate linkages are disrupted by releasing the majority of the hemicelluloses into solution while leaving the cellulose fraction intact (Wu et al., 1999). The method is effective for a wide variety of plant biomass including wood and agricultural residues such as cereal straw (Dekker et al., 1987; Emmel et al., 2003; Ramos et al., 1992; Sun et al., 2005b; Tanaka and Matsuno, 1990). Sun et al. (2005b) utilized a two-stage process based on a steam explosion pretreatment followed by alkaline peroxide post-treatment. The two-stage treatments degraded 77.0–87.6% of the total original hemicelluloses and 92.3–99.4% of the total original lignin. Recently, Wang et al. (2009b, 2009c, 2010) studied the synergistic effect of steam explosion and alkaline aqueous/ethanol solution post-treatment on the fractionation of main components from *Lespedeza crytobytrya* stalks, and the influence of steaming explosion time and pressure on the physio-chemical properties of cellulose, hemicelluloses, and lignin were fully discussed. An eco-friendly process of wheat straw fractionation by steam explosion coupled with ethanol extraction was studied (Chen and Liu, 2007). The wheat straw was steam exploded for 4.5 min with moisture of 34.0% and a pressure of 1.5 MPa without acid or alkali. It was found that the total recovery rate of hemicelluloses was 80%, lignin yield was 75% by acid precipitation, and 85% of the ethanol solvent was recovered.

The cellulose recovery rate was 94%. Compared with alternative methods, the steam explosion is more environmentally friendly and it requires lower capital investment (Cara et al., 2006). However, it is difficult to control the degradation of hemicelluloses, lignin, and cellulose in this process (Josefsson et al., 2002).

3.4.2. Microwave irradiation

Microwave-assisted extraction is based on the direct application of electromagnetic radiation to a material (e.g. organic solvent, plant tissue) that has the ability to absorb electromagnetic energy (microwaves) and transform them into heat. Compared with traditional methods, microwave-assisted extraction has many advantages, such as shorter time, less solvent requirement, higher extraction rates, lower cost and better products obtained without changing the molecular structures involved (Ganzler et al., 1986). Microwave-assisted water extraction has been reported to be an efficient method for hemicellulose extraction, requiring a short treatment time (Buranov and Mazza, 2010; Jacobs et al., 2003; Palm and Zacchi, 2003; Roos et al., 2009). Palm and Zacchi (2003) investigated the extraction of hemicellulosic oligosaccharides from spruce using microwave treatment. The highest yield of oligosaccharides, measured as mannan, was 70% and was obtained with treatment in the microwave oven at 200 °C for 5 min. The amount of oligosaccharides extracted was 12.5 g per 100 g of dry wood. The water-soluble hemicelluloses obtained from flax shives using a hydrothermal microwave treatment were characterized with respect to molar mass, molar mass distribution, degree of polymerization (DP), and degree of substitution with acetyl moieties (DS). The major portion of the water-soluble flax hemicelluloses consisted of an *O*-acetyl-4-*O*-methylglucuronoxylan exhibiting a DP value of 28, and a DS value of 0.7. The combination of microwave irradiation for screening followed by steam pretreatment has previously been applied for the extraction of polysaccharides from, for example, barley husks (Palm and Zacchi, 2003; Roos et al., 2009). This treatment of husks was performed to extract arabinoxylan with high yield and high weight averaged M_W. Microwave irradiation was shown to be a good method for predicting the effects of heat treatment on a larger scale using steam pretreatment. A M_W of about 40,000 Da was achieved without the addition of chemicals, by both microwave irradiation and steam pretreatment, with a yield of about 9%. The yield was significantly increased by slightly increasing the severity factor, however, the M_W decreased below 20,000 Da at severity factors above 3.7. Heat treatment is often combined with the addition of chemicals, such as alkali, or acid, which can be crucial for to the final product due to different factors, such as solubility of components, and chemical reactions which could occur (Lai, 2001). Microwave-assisted fractionation of NaOH-impregnated spruce into hemicelluloses has also been carried out (Lundqvist et al., 2002). The highest mannan yield (78% based on the amount in the raw material) was obtained from water impregnated spruce chips heat-fractionated at 190 °C for 5 min (M_W of 3800). The highest M_W (14,000) was obtained from impregnation with 2% NaOH (190 °C, 5 min), but the yield of mannan was very low (3%). Impregnation with 0.025% NaOH and heat-fractionation at 190 °C for 5 min resulted in extraction of GGM with M_W of 9500 and a mannan yield of 31%. When the spruce chips were impregnated with <0.05% NaOH, an *O*-acetylgalactoglucomannan was extracted, whereas when higher NaOH charges were used in the impregnation, the extracted GGM lacked acetyl groups. Furthermore, the microwave-assisted alkali pretreatment of wheat straw and barley husks for removing hemicelluloses and lignin for ethanol production was also investigated (Palmarola-Adrados et al., 2005; Zhu et al., 2006). The difficulty with microwave-assisted extraction is how to achieve a good yield without extensive degradation of the hemicelluloses and contamination with dissolved lignin and cellulose. Compared with alternative methods, microwave-assisted extraction could be a novel eco-friendly way of isolation of hemicelluloses.

4. Fractionation and purification of hemicelluloses

Hemicelluloses are non-cellulosic and short-branched chain hetero-polysaccharides consisting of various different sugar units, which are arranged in different proportions and with different substituents (Glasser et al., 2000a, 2000b). In plant cell walls, there are large amounts of hemicelluloses with a wide variation in content and chemical structure. Hemicelluloses generally consist of several populations of polysaccharide molecules which vary in structural characteristics, and several fractionation techniques have been employed in order to obtain more homogeneous fractions and thus explore structure–property relationships for the hemicellulosic polymers.

4.1. Fractional isolation of hemicelluloses

In general, one-step dilute alkali treatments extract only part of the hemicelluloses from both holocellulose and lignified materials. Successive treatments with alkali of initially low, and then higher concentration avoid unnecessary exposure of hemicellulosic material to alkali that are more concentrated than that required for the extraction (Buchala et al., 1971; Xu et al., 2006a). In this case, the hemicellulosic materials from plant cell walls are frequently fractionated to give polysaccharides having different structural features. More importantly, studies of such fractionated materials have led to much structural information in hemicellulosic molecules recovered by the most commonly used procedures (Wilkie, 1979). Our research group intensively investigated the fractionation and characterization of hemicelluloses obtained by sequential treatments of organic solvent, organosolv-acid, organosolv-alkali, alkali, and alkaline peroxide from various grasses, such as wheat straw, sugarcane bagasse, barley straw and maize stems (Jin et al., 2009; Sun and Sun, 2002; Sun et al., 2004a, 2004b, 2005a). The aim was to develop techniques for fractionation of lignocellulosic materials into their primary polymer components, cellulose, hemicelluloses, and lignin. Sun et al. (2004b) reported the dewaxed bagasse was sequentially extracted with: distilled water, 0.5 M NaOH, 0.5, 1.0, 1.5, 2.0 and 3.0% H_2O_2 at pH 11.5, and 2.0 M NaOH at 55 °C for 2 h. The successive treatments released over 90% of the original hemicelluloses and 89.0% of the original lignin from the cell walls. This indicated that sequential treatments were very effective on the fractionation of hemicelluloses from bagasse, and the extraction strength, such as alkali and H_2O_2 concentration, had a great influence on the chemical and structural features of the hemicellulose, (i.e. in the content of associated lignin and molecular weight). In addition, Xu et al. (2008) proposed a novel three-step procedure for separation of hemicelluloses and lignin with a high yield and purity, where wood is mildly milled and successively extracted to produce three hemicellulosic and lignin fractions representing the total hemicelluloses and lignin present. The sequential treatments of the mild ball-milled *Periploca sepium* with 80% aqueous dioxane containing 0.05 M HCl at 85 °C for 4 h, DMSO at 85 °C for 4 h, and 8% NaOH at 50 °C for 3 h resulted in a total release of over 85% of the original hemicelluloses and 86% of the original lignin. In particular, approximately 36% of the original hemicelluloses and 50% of the original lignin were separated during the first mild acidolytic hydrolysis process after low intensity milling.

4.2. Fractional purification of hemicelluloses

Fractional isolation of hemicelluloses would be achieved by successive extractions with neutral or alkaline solvent. However, the isolated hemicelluloses are still mixtures of different polysaccharides. In order to determine the detailed structure of the polymers, the isolated hemicelluloses should be further purified to obtain homogeneous hemicellulosic fractions. Traditionally, hemicelluloses can be isolated from materials by extraction with alkaline solutions, and their

recovery by neutralization and precipitation with ethanol has led to a widely used conventional fractionation procedure originally established as early as 1926 by O'Dwyer. In this convention, "hemicelluloses A" represents the water-insoluble fraction which is precipitated on neutralization of an alkaline extract to pH 4.5–5.0 with acetic acid; while "hemicelluloses B" is isolated by precipitation with 3 volumes of ethanol, and the remaining portion, usually discarded, is designated "hemicelluloses C". Hemicelluloses A are generally believed to homogeneous, while hemicelluloses B are a mixture of several different polymers, both linear and branched (Gaillard, 1965). Hemicelluloses C are more branched and low molecular weight polymers (Verwimp et al., 2007).The separation and purification of hemicellulosic polymers in a pure state is tedious procedure, requiring method such as graded ammonium sulfate/ethanol precipitation, anion-exchange chromatography or DEAE-borate chromatography (Cyran and Saulnier, 2007; Peng et al., 2009; Rao and Muralikrishna, 2006). A variety of multistep fractionation and purification procedures have been proposed to prepare hemicelluloses of desired purity from plant cell walls.

4.2.1. Ethanol precipitation

Precipitation of the polysaccharide fractions by addition of miscible organic solvents to aqueous solutions is one of the main methods of recovery and purification of hemicelluloses. Ethanol is the most commonly used solvent, but methanol, acetone, and other organic solvents are also applied for hemicellulose fractionation. Under these conditions, part of the hemicellulosic material remains in solution, and is commonly not recovered. In a study by Reid and Wilkie (1969), an oat-leaf holocellulose was treated with aqueous acid, and the precipitated hemicelluloses were recovered after the addition of an excess amount of acetone. The solvents were removed from the remaining solution, and after dissolving the potassium acetate in ethanol, the hemicellulosic material recovered accounted for 13.2% of the hemicelluloses extracted. Repeated precipitations in ethanol can increase the purity, but this is not always the case since co-precipitation of other dissolved polysaccharides can be a problem (Willfor et al., 2008). The ethanol fractional precipitation is influenced by time and temperature. A solution subjected to ethanol treatment may initially yield no visible precipitate, but this precipitate may form if the solution is allowed to stand, or if cations are added. Possibly, invisible colloids flocculate on standing, but it is much more probable that precipitation is due to time-dependent, conformational changes leading to molecular aggregation (Blake and Richards, 1971; Blake et al., 1970; Wolf et al., 1952). Hemicelluloses from grasses disaggregate in solution on heating, and reaggregate with time on cooling (Blake and Richards, 1971; Dea et al., 1973).

To further understand structural information on fractionated hemicelluloses, the hemicellulosic fractions with different structural features could be subfractionated by means of a gradual ethanol precipitation. More recently, Peng et al. (2009, 2010b) used graded ethanol precipitation for fractionation of water and alkali-soluble hemicelluloses from the sugarcane bagasse (SCB). The hemicelluloses obtained by sequential extraction with H_2O, 1%, 3%, 5% and 8% NaOH aqueous solutions from the dewaxed SCB were fractionated by gradual precipitation at the ethanol concentrations of 15, 30, and 60% (v/v). It was also found that with an increasing ethanol concentration from 15% to 60%, the Arabinose/Xylose (Ara/Xyl) ratios, molecular weights and thermal stabilities of the hemicellulosic fractions increased, and the content of associated lignin decreased. There was no significant difference in the structural features of the precipitated hemicellulosic fractions, but the difference may have occurred in the distribution of branches along the xylan backbone. In addition, Bian et al. (2010) also reported that the alkali-extractable hemicelluloses from holocellulose of the hardwood (*Caragana korshinskii*) were successively fractionated by graded precipitation at final ethanol concentrations of 10%, 20%, 30%, 45%, 60%, and 80%, respectively. The results also revealed that the molecular weights and the distribution of branches along the xylan backbone were different among the hemicellulosic fractions obtained in various ethanol concentrations. The less-branched hemicelluloses with larger molecules were precipitated in lower ethanol percentages, while with increasing ethanol concentrations, more branched hemicelluloses with low molecular weights were obtained. Moreover, an increase in Ara/Xyl ratio for fractions with an increase of ethanol concentration in the extraction of arabinoxylans from wheat and barley flour was reported by Vietor et al. (1992) and Gruppen et al. (1992). However, Hoffmann et al. (1991) did not observe such relationship, finding instead that the highly branched AX fractions with a high Ara/Xyl ratio had higher *Mw* than their less branched counterparts. Peng et al. (2010c) and Bian et al. (2010) recently reported that the linear hemicelluloses of hardwood (*Populus gansuensis* and *C. korshinskii*) had a higher molecular weight than the branched hemicelluloses. These inconsistencies probably resulted from structural differences among hemicelluloses, differences in botanical origin, and differences in the techniques and solvent conditions employed during the measurement of the *Mw*, such as solvent quality and chain aggregation events (Izydorczyk and Biliaderis, 1995).

4.2.2. Ammonium sulfate precipitation

Compared to proteins, selective precipitation by $(NH_4)_2SO_4$ has received very little attention in the area of isolation and fractionation of polysaccharides from aqueous solution. Izydorczyk and Biliaderis (1992) reported that a water-soluble arabinoxylan from wheat flour was fractionated by a graded ammonium sulfate fractionation technique into four fractions obtained at 60%, 70%, 80%, 95% saturation with $(NH_4)_2SO_4$. With increasing concentration of $(NH_4)_2SO_4$ from 60% to 95%, the ratio of Ara/Xyl and the relative amount of doubly substituted xylose residues of arabinoxylan fractions increased, meanwhile, ferulic acid content and the molecular size decreased. Among the four fractions, the arabinoxylan fraction obtained by 60% saturation with $(NH_4)_2SO_4$ was the most effective in stabilizing protein foams from disruption heating, probably because of its higher molecular weight. The exact mechanism by which the salt precipitated neutral polysaccharides, remained obscure, and the results of the study indicated that fractional precipitation with this agent is affected not only by the molecular size but also by the fine structure of the polymer (Izydorczyk and Biliaderis, 1992). Izydorczyk et al. (1998) and Rao and Muralikrishna (2006) also investigated hemicelluloses by using stepwise addition of ammonium sulfate from red spring flour and Raji, respectively, and obtained results in good agreement with the results of Izydorczyk and Biliaderis (1994). In addition, Izydorczyk et al. (1998) also found that stepwise precipitation with $(NH_4)_2SO_4$ was useful in separating β-glucans from arabinoxylans as well as in fractionation of these polysaccharides into several subpopulations. Fractions obtained up to 45% saturation with $(NH_4)_2SO_4$ were essentially free of arabinoxylans, while fractions precipitated at higher saturation levels (>45%) contained progressively more arabinoxylans and less β-glucans.

4.2.3. Iodine-complex precipitation

Another fractionation technique, iodine-complex precipitation, was studied by Gaillard (1961). He found that a solution of the polysaccharide in aqueous calcium chloride could be partially precipitated by adding iodine-potassium iodide (3 g iodine and 4 g potassium iodide in 100 ml water). The precipitated polysaccharide was linear, and the remaining polysaccharide in the filtrate was branched. Many linear polysaccharides have been shown to give a blue coloration with iodine when they are dissolved in concentrated aqueous calcium chloride solution, although they will not do so when dissolved in salt-free aqueous solutions (Gaillard, 1961). Gaillard and Bailey (1996) investigated several plant hemicelluloses, and found that a primary requirement for the formation of a blue product is the presence of a sequence of at least three (1→4)-linked D-glucose,

D-xylose, or D-mannose residues in the polysaccharide. Morak and Thompson (1965) reported that highly branched polysaccharides do not react with iodine under these conditions, but a limited degree of branching could be formed in the colored product. It has also been shown that other multivalent ions may be substituted for calcium, and that bromine may be used instead of iodine in some instances. Recently, Peng et al. (2010c, 2012) reported that the alkali-soluble hemicelluloses from the delignified Peashrub (*C. korshinskii*) and Poplar (*Populus gansuensis*) were fractionated by the iodine-complex precipitation technique in the presence of aqueous potassium chloride instead of calcium chloride. The hemicellulosic fractions precipitated by iodine-potassium iodide solution were more linear as they contained more xylose (81.7–87.6%) and lower uronic acids/xylose ratios (0.10–0.14) than the hemicellulosic sub-fractions remaining in the solution, which were more branched and contained a higher content of uronic acids (15.7–19.7%). Therefore, it appeared that the reaction of hemicelluloses with iodine-potassium iodide solution could discriminate between linear and branched polysaccharides, which may be a useful method of obtaining low-branched xylans for further production of xylose, an intermediate for the production of xylitol, and a variety of xylo-oligosaccharides.

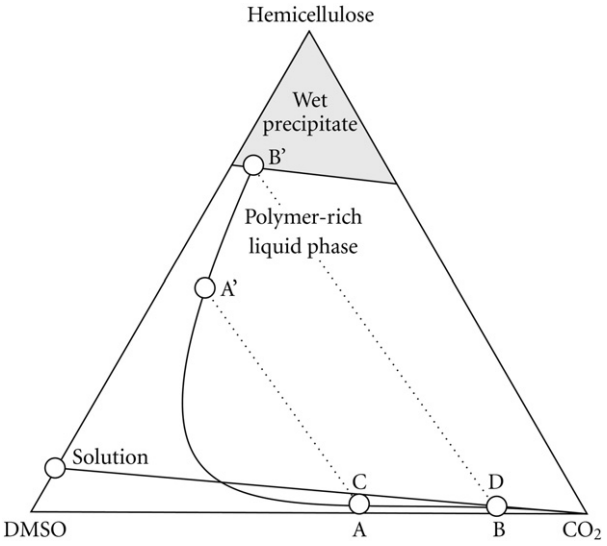

Fig. 3. Diagram of the ternary system of DMSO/CO_2/hemicellulose (Haimer et al., 2008).

4.2.4. Supercritical anti-solvent precipitation

Supercritical carbon dioxide has especial physico-chemical properties at the critical temperature (31.8 °C) and pressure (73.8 bar), and it is non-toxic, non-flammable, inert, cheap and easily removed from the product (McHugh and Krukonis, 1994). Owing to its low polarizability per unit volume and low cohesive energy density, supercritical carbon dioxide has attracted much interest as an alternative solvent for precipitation of biodegradable polymers, such as hemicelluloses and proteins (de Diego et al., 2005, 2006; Haimer et al., 2008, 2010). Recently, precipitation of hemicelluloses from DMSO/water mixtures using carbon dioxide as an anti-solvent was investigated by Haimer et al. (2008, 2010). The typical phase diagram of the ternary system composed of polymer, DMSO, and CO_2 above the critical conditions was proposed by several authors and given in Fig. 3 (de Diego et al., 2006; Dixon and Johnston, 1993; Haimer et al., 2008). Anti-solvent CO_2 was added into the hemicellulose solvent system, and transferred through the binodial, which causes supersaturation of hemicelluloses. The type of hemicellulose, water content of DMSO, and the precipitation pressure and temperature can influence the precipitation rate, the paticle size and shape of hemicelluloses. Haimer et al. (2010) obtained the precipitated xylans and mannans, their particle size within a wide range from less than 0.1 to more than 5 um. The obtained xylans and mannans having nano- and micro-structure can be used in various applications, e.g. active compounds encapsulation, slow release agents, or chromatographic separation materials. Therefore, this technology will be applied in many industries in the future.

4.2.5. Column chromatography

The hemicellulosic preparations can also be further fractionated and purified by using chromatography, including size-exclusion chromatography and anion-exchange chromatography. Generally, the principle of anion-exchange chromatography is based on the ion exchange mechanism. Compared with the precipitation techniques, anion-exchange chromatography is more likely to obtain pure hemicellulosic fraction. However, Lineback et al. (1977) suggested that the principle is probably due to adoption of arabinoxylans to the cellulose material rather than to an ion exchange mechanism. Diethyl aminoethyl (DEAE) cellulose chromatography is usually applied to fractionate the hemicelluloses from the plant materials. The hemicellulosic fractions are generally obtained by eluting with a stepwise gradient of increasing borate, NaOH, or NaCl concentration. Dupont and Selvendran (1987) and Gruppen et al. (1992) used DEAE anion-exchange chromatography for fractionation of alkali-soluble arabinoxylans from beeswing bran and endosperm of wheat. They found that the bound arabinoxylan in the DEAE column had an acidic character, and the DEAE-bound arabinoxylans had higher overall Ara/Xyl ratios than the corresponding unbound fractions. Thus, acidic polysaccharides can be removed using anionic exchange chromatography. Peng et al. (2010a) investigated that the water- and alkali-soluble hemicelluloses from dewaxed sugarcane bagasse were fractionated on DEAE-cellulose-52 chromatography and obtained six hemicellulosic sub-fractions. It was found that with increasing concentration of NaCl aqueous solution, the hemicellulosic fractions containing higher arabinose to xylose ratios, molecular weights, thermal stabilities and contents of associated lignin were eluted. These results are consistent with the other research results using rye grain and wheat flour (Gruppen et al., 1992; Mondal et al., 2001). Sjöström (1993) and Thornton et al. (1994) fractionated the polysaccharides, mainly *O*-acetylgalactoglucomannans, from Norway spruce, by using different hydrodynamic, cationic, and anion columns. The strength of chromatographic methods is more in removing non-polysaccharide substances, such as lignin and lipophilic extractives, than in separating different polysaccharides in pure fractions (Willfor et al., 2008).

Size-exclusion chromatography can also be used for fractionation according to molar mass or more correctly according to hydrodynamic volume (Willfor et al., 2008). Generally, the various purified polysaccharides can be obtained by using size-exclusion chromatography and anion-exchange chromatography. Wang et al. (2008) found a novel heteropolysaccharide RBPS2a with anti-complementary activity from defatted rice bran, obtained by Q-Sepharose big bead anion-exchange chromatography and Sepharose CL-6B gel chromatography. RBPS2a was found to be a novel hetero-polysaccharide, with a molecular weight of 90,000 Da and the main chain contained β-(1→3)-linked D-galacopyranosyl. In addition, Palm and Zacchi (2004) investigated the preparation of hemicellulosic oligomers from spruce wood using steam treatment, and separation of the liquid fraction following steam treatment by size-exclusion chromatography. Size-exclusion chromatography was employed to exclude monomer sugars and other low-molecular-weight compounds. It also can be used to reduce the lignin content, but not to remove it completely. Moreover, size-exclusion chromatography can be applied to determine the molecular weight of polysaccharides.

4.2.6. Membrane

Membrane fractionation technologies such as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) have drawn great attention in the green biorefinery for

separation and purification of lignocellulosic products. Most extraction procedures of hemicelluloses, other than water extraction, are often operated under more severe pretreatment conditions, producing hemicelluloses with smaller molecular weight, such as protein, lignin, and inorganic salts (Huang et al., 2008). It is very difficult to purify the hemicelluloses, and this involves many steps, such as filtration, neutralization, ethanol precipitation, and centrifugation (Sun et al., 2000d; Swennen et al., 2005). Hence membrane technologies are often used for the separation and purification of hemicelluloses from lignocellulosic materials (Gabrielii et al., 2000; Krawczyk et al., 2008; Vegas et al., 2008; Willfor et al., 2003a; Zeitoun et al., 2010). Zeitoun et al. (2010) designed a new process for the fractionation and purification of wheat bran hemicelluloses by using efficient extraction and purification methods such as twin-screw extrusion, membrane and chromatographic separation techniques. UF was investigated as a means to reduce alcohol consumption. Trials were made with hollow fiber polyethersulfone membranes with a molecular weight cut-off of 30 kDa. UF mainly concentrated the extract and removed small molecules such as monosaccharides and minerals. The combination of the anion-exchange chromatography and UF allowed for the removal of colored compounds (Zeitoun et al., 2010). Recently, Swennen et al. (2005) used graded ethanol precipitation and UF for fractionating an arabinoxylooligosaccharides (AXOS) containing stock solution into fractions of different sizes and structures. Their fractionation by graded ethanol precipitation or UF with different molecular mass cut-off yielded fractions, which differed both in degree of polymerization (DP) and degree of substitution (DS). Although the UF fractions were more heterogeneous than the ones obtained by ethanol precipitation, AXOS fractions with similar DP and DS could be obtained with both methods. The process had been optimized and the right membrane had been chosen, it is feasible that an economically viable separation process could be developed. In addition, UF processes have been applied to separation and purification of arabinoxylan, glucuronoxylan, and acetyl-galactoglucomannans (Gabrielii et al., 2000; Krawczyk et al., 2008; Willfor et al., 2003b). NF, usually defined as having a membrane cut-off from 150 to 1000 g mol^{-1}, could provide a relatively cost-competitive separation step, offering a commercial alternative to chromatographic methods for purification of hemicelluloses and oligosaccharides (Matsubara et al., 1996; Mok et al., 1995). Vegas et al. (2008) studied the ability of low molecular weight cut-off (MWCO) for fractionation and purification of xylooligosaccharides (XO) from monosaccharides and other low molar mass materials, such as salt. An XO concentration with purity over 91% was obtained, with an overall purity of 71%. Schlesinger et al. (2006) investigated the performance of five polymeric NF and tight UF membranes during the separation of hemicelluloses from process liquors containing 200 g/l sodium hydroxide. The experimental data showed that hemicelluloses were quantitatively retained at molar masses above 1000 g/mol. MWCOs of the membranes at 49 °C were between 380 and 1090 g/mol. The NTR-7470 and MPF-34 membranes showed the highest overall hemicellulose retention and lowest MWCO among the studied membranes. The N30F and GE membranes were also able to separate hemicelluloses and sodium hydroxide efficiently. Two of the membranes exhibiting excellent overall hemicellulose retention (around 90%) were found to be the most suitable for efficient removal of hemicelluloses from the process.

4.2.7. Other techniques

Other precipitation agents are have also been investigated, such as barium hydroxide for glucomannans, which can form complexes with 2,3-vicinal hydroxide on mannose units (Fig. 2b), Fehling's solution, other copper salts and basic lead acetate for glucomannans, and cetyltrimethylammonium hydroxide or bromide for acid polysaccharides, such as glucuronoxylans. In addition, the borate complexes react with cetyltrimethylammonium hydroxide, and form selective salt, which can be used for the fractionation of neutral arabinogalactans (Browning, 1967; Sjöström, 1993; Timell, 1965; Vuorinen and Alen, 1999). Specific enzymes can also be used for purification of hemicelluloses (Sjöström, 1993).

5. Bioconversion of hemicelluloses

Acid-catalyzed processes employing chemicals such as sulfuric acid and other mineral acids (hydrochloric, nitric, and trifluoracetic acids) are mainly confronted with challenges in regards to the environment and climate. The main drawback of those processes is that hemicellulosic polysaccharides may be degraded to organic acids and furan derivatives which potentially act as microbial inhibitors during the subsequent fermentation step. As an alternative to the conventional chemical processes, enzymatic bioconversion, which has the advantages of being environmentally friendly, mild, specific and low in byproduct-formation, has been favored in recent years. For example, three key steps, pretreatment, enzymatic hydrolysis and fermentation, have attracted considerable research interest (Ge et al., 2011; Kazi et al., 2010; Maas et al., 2008; Yang et al., 2009). The development and upgrading of sustainable bioconversion technologies has been successfully applied in food, value-added chemicals, bioenergy, and pulp and paper industries (Mussatto et al., 2010; Valls et al., 2010; Zhu et al., 2010). The following section focus on the enzymatic strategies for conversion of hemicelluloses.

5.1. Enzyme family

There are many literature reports about the production and applications of various hemicellulases from fungi, bacteria, yeast, marine algae, protozoans, snails, crustaceans, insects, and seeds (Table 2), in which the enzymes are mostly xylanases. Generally, xylanases refer to a large group of enzymes responsible for the hydrolysis of xylan. The main enzymes involved are endo-1,4-β-xylanase (EC 3.2.1.8) and β-xylosidase (EC 3.2.1.37). Endo-xylanases randomly internally cleave the main chain of xylan, producing a mixture of xylo-oligosaccharides, while β-xylosidase liberates xylose by removing terminal monosaccharides from the non-reducing end of the short oligosaccharides. Additionally, the debranching activities of several accessory enzymes have been proposed in the breakdown of plant cell wall polysaccharides. α-L-arabinofuranosidase (EC 3.2.1.55) hydrolyzes the L-arabinose residues substituted at positions 2 and/or 3 of the backbone xylan, α-D-glucuronidase (EC 3.2.1.139) is required to cleave the α-1,2 bonds between the glucuronic acid residues and β-D-xylopyranosyl, and acetylxylan esterase (EC 3.1.1.72) removes the *O*-acetyl of acetyl xylan, while ferulic acid and coumaroyl esterases (EC 3.1.1.73) hydrolyse the respective phenolic acids linked to the position 5 of arabinofuranoside residues. A schematic view on the degradation of arabinoxylan is shown in Fig. 4 as an example of a hemicellulolytic system (Aro et al., 2005; Chávez et al., 2006). For complete breakdown of glactoglucomannans into mono- and oligosaccharides, the β-mannosidases (EC 3.2.1.25) and endo-1,4-β-mannanases (EC 3.2.1.78) are involved in cleaving the polymer backbone, respectively. Similar to xylanases, mannanases also include several accessory enzymes that remove side chains attaching at various points on mannans, such as α-galactosidases (EC 3.2.1.22), β-glucosidases (EC 3.2.1.21), and acetyl mannan esterases. Furthermore, the minor form of xyloglucanase has been characterized and analyzed to find its role in effecting the enzymatic hydrolysis. In fact, the biodegradation of hemicelluloses is a complex process that requires the coordinated action of several hemicellulases (Latha and Muralikrishna, 2009; Sørensen et al., 2007a).

In order to improve efficiency, production, and properties of enzymes, some enzyme engineering strategies have been carried out. It is well known that the production of enzymes by microbial cells is governed by genetic and biochemical controls. Therefore, rapid

Table 2
Hemicellulolytic enzymes.

Enzyme	Linkage hydrolysed	Origin	Purification	MW (SDS-PAGE, kDa)	Optimal condition			Reference
					pH	Temp (°C)	Buffer solution	
Endo-1,4-β-Xylanase (3.2.1.8)	Internal β-1,4	*Aspergillus niger* B03	Ultrafiltration, anion exchange and chromatography	33	6.0	60	Citrate	Dobrev et al. (2009)
		Acrophialophora nainiana	Gel filtration and ion exchange chromatographies	22.1	7.0	55	50 mM Acetate	Salles et al. (2000)
		Bacteroides xylanisolvens XB1A	Metal affinity resin and batch/gravity-flow column	40	6.0	37	50 mM phosphate	Mirande et al. (2010)
		Sulfolobus solfataricus	Ultrafiltration and AKTA Fast Protein Liquid Chromatography system	57	7.0	90	50 mM Tris–HCl	Cannio et al. (2004)
		Streptomyces sp. strain AMT-3	Filtration and overnight dialysis	170–700	6.0	55–65	50 mM sodium citrate	Nascimento et al. (2002)
		Glaciecola mesophila KMM 241	Precipitation with 60% saturation of ammonium sulfate and His-Tag Ni-affinity column	43	7.0	30	50 mM citrate	Guo et al, (2009)
		Bacillus sp. YJ6	Sephacryl S-100 HR chromatography	190	5.0	50	20 mM citrate (pH 3.0–6.0), sodium phosphate (pH 6.0–8.0), Tris–HCl (pH 8.0–9.0), sodiumcarbonate (pH 8.0–11.0)	Yin et al. (2010)
		Streptomyces mayensis	Ammonium sulfate precipitation, ion exchange and gel filtration chromatography	21.2	7.0	65	50 mM MOPS	Yan et al. (2009)
		Paenibacillus barcinonensis	Precipitation with 20% ammonium sulfate and cation exchange chromatography	31	6.5	60	50 mM phosphate	Valenzuela et al. (2010)
		Aspergillus versicolor	DEAE-Sephadex and HPLC GF-510 gel filtration	32	6.0–7.0	55	McIlvaine (pH 4.0–8.0), Tris–HCl (pH 8.0–9.0), glycine–NaOH (9.0–9.5)	Carmona et al. (2005)
		Humicola grisea var. *thermoidea*	Ultrafiltration, ion-exchange, DEAE-Sepharose and Phenyl-Sepharose resins	29	4.5–6.5	55–60	100 mM sodium acetate	Lucena-Neto and Ferreira-Filho (2004)
		Penicillium capsulatum	Ultrafiltration and DEAE–cellulose anion-exchange column	22	3.8	48	100 mM citrate–phosphate	Ryan et al., 2003
β-Xylosidase (3.2.1.37)	Terminal β-1,4	Cell wall of maize	Precipitated with ammonium sulfate and CM-sephadex cation-exchange chromatography	68.5	4.5	37	50 mM sodium acetate	Han and Chen (2010)
				60/66	4.2	60	50 mM sodium acetate	

α-L-Arabinofuranosidase (EC 3.2.1.55)	Terminal α-1,2, α-1,3	Germinated barley	Ultrafiltration, SP-Sepharose column, Superdex 200 size-exclusion column, Superdex 200 column and Mono P chromatofocusing column					Ferré et al. (2000)
		Cytophaga xylanolytica	Anion-exchange hydrophobic interaction column chromatography	160–210	5.8	45	2 mM Sodium citrate (pH 3–6), MES (pH 5.5–6.5), MOPS (pH 6.5–8.0.)	Renner and Breznak (1998)
α-D-Glucuronidase (EC 3.2.1.139)	Terminal α-1,2	*Bispora* sp. MEY-1	Ultrafiltration, HiTrap Q Sepharose XL FPLC column	49.8	3.0	60	0.1 M citric acid-Na_2HPO_4	Luo et al. (2010)
Acetylxylan esterase (EC 3.1.1.72)	Ester bond	*Aspergillus ficuum*	Ni^{2+}–NTA agarose column chromatography	29.5	7.0	37	0.1 M potassium phosphate	Chung et al. (2002)
		Streptomyces sp. PC22	Ammonium sulfate precipitation, Macro-Prep DEAE, t-butyl hydrophobic interaction and hydroxyapatite chromatography	34	6.5–7.0	50	50% Methanol, 0.5 M sodium phosphate	Chungool et al. (2008)
Ferulic acid and coumaric acid esterases (EC 3.1.1.73)	Ester bond	*Eleusine coracana*, Indaf-15	Ammonium sulfate precipitation, DEAE-cellulose, Sephacryl S-200 and phenyl-Sepharose column chromatography	19.7	7.5	45	75 mM sodium acetate (pH 4.0–6.0), sodium phosphate (pH 6.0–8.0), Tris–HCl (pH 7.0–9.0)	Latha et al. (2007)
Endo-1,4-β-Mannanases (EC 3.2.1.78)	Internal β-1,4	*Escherichia coli*	DEAE-Sepharose column, ultrafiltration, phenyl-Sepharose CL-4B column and DEAE-Sepharose column	38	6.0	60	50 mM sodium citrate	Yoon et al. (2008)
		Bacillus subtilis WY34	Gel filtration and ion exchange chromatography Q-Sepharose fast flow column	39.6	6.0	65	50 mM citrate (pH 3.0–6.5), phosphate (pH 6.7–8.2), CHES (pH 8.7–11.0)	Jiang et al. (2006)
β-Mannanases (EC 3.2.1.25)	Terminal β-1,4	*Helix pomatia* and *A. oryzae*	DEAE-Sepharose chromatography column and CMSepharose	72	3.5–4.5	30	50 mM sodium acetate	Scigelova et al. (1999)
		Thermobifida fusca TM51	Fast-performance liquid chromatography by anion-exchange chromatography on a Mono-Q column and gel chromatography on an FPLC Superose-12 column	94	7.17	53	0.1 M sodium phosphate	Béki et al. (2003)
β-Glucosidase (EC 3.2.1.21)	Terminal β-1,4	*Pichia guilliermondii* K123-1	Ammonium sulfate precipitation, ion exchange column chromatography, gel filtration and liquid chromatogram	45	4.5	45	100 mM (pH 3.0–7.0), sodium phosphate (pH 6.5–8.0), Tris–HCl (pH 7.5–9.0)	So et al. (2010)
α-Galactosidase (EC 3.2.1.22)	Terminal α-1,6	*Aspergillus niger*	Sephadex DEAE A-50, S-Sepharose fast flow, Source Q, Superose 12 and MonoS HR	54	4.0	60–65	Mcllvaine	Manzanares et al. (1998)
		Streptomyces sp. S27	Ni^{2+}-NTA column	82	7.4	35	0.1 mM cIlvaine (pH 2.0–8.0), 0.1 M glycine–NaOH (pH 9.0–11.0)	Cao et al. (2010)
β-Galactosidase (EC 3.2.1.23)	Terminal β-1,3, β-1,4, β-1,6	*Guehomyces pullulans* 17-1	Merckenschlager cell homogenizer with CO_2		4.0	50	100 mM Citrate	Song et al. (2010)
endo-β-1,4-Glucanase	Terminal β-1,4 in Xyl substituted Glc	*Thermobifida fusca*		94.7–96.2 (MALDI-TOF MS)	6.0–9.4	60	30 mM solutions of citric acid, NaH_2PO_4, boric acid and barbita	Irwin et al. (2003)

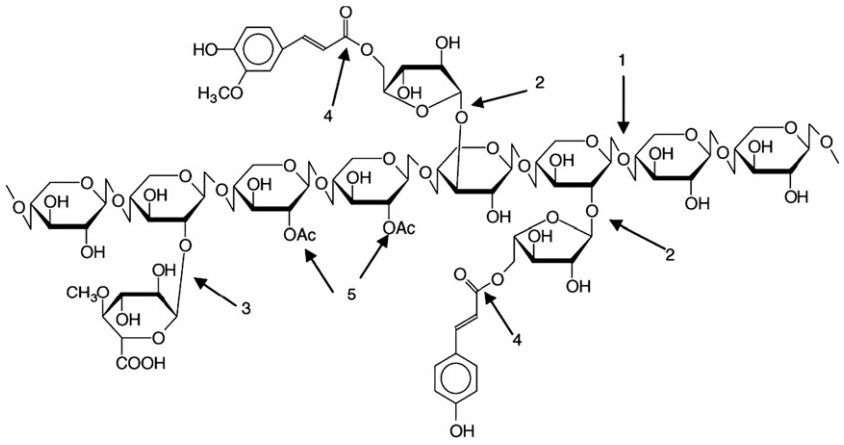

Fig. 4. The structure of xylan and site of action of the enzymes of the xylanase complex. 1: endoxylanases; 2: α-L-arabinofuranosidases; 3: glucuronidases; 4: feruloyl and coumaroyl esterases; 5: acetyl xylan esterases (Chávez et al., 2006).

developments in molecular biology techniques such as genetic manipulation and co-cultivation approaches have made it possible to increase the enzyme levels in cultured bacteria. These strategies may be roughly classified into three different classes as follows: random mutagenesis, site-directed mutagenesis, and recombinant DNA. In random mutagenesis, strain improvement has been successfully performed to produce more efficient hemicellulases by using various physical (e.g. UV and microwave irradiation) and chemical (e.g. *N*-methyl-*N*′-nitrosoguanidine (NTG), ethyl methane sulfonate (EMS), sodium azide, and colchicine) methods (Gírio et al., 2010). The UV and NTG mutagenesis of *Aspergillus ochraceus* enhanced the active and thermostable of xylanase and ß-xylosidase (Biswas et al., 1990). *Thermomyces lanuginosus* subjected to UV/NTG mutagenesis showed a 1.5 fold higher xylanase production than the parent strain on oat spelts xylan (Kumar et al., 2009). In site-directed mutagenesis, depending on the structure-function correlation of the xylanases, a protein can be redesigned via the substituted individual amino acids, which makes it possible to have enzymes with higher specificity towards a certain substrate, secretion, and condition (pH and temperature). For instance, the thermostability of *Geobacillus stearothermophilus* was improved by directed evolution and site-directed mutagenesis (Zhang et al., 2010). Finally, using recombinant DNA strategies, hemicellulase genes with desirable molecular properties in suitable hosts (homologous or heterologous) may be cloned to obtain desirable tailor-made enzymes (Gírio et al., 2010). For example the recombinant xylanase B of *Thermotoga maritime* was shown to produce exclusively xylobiose from xylans by Jiang et al. (2004).

5.2. Enzymatic hydrolysis of hemicelluloses

As mentioned before, hemicelluloses can be hydrolyed into mono- and oligosaccharides by enzymes. Compared to monosaccharides, the functional oligosaccharides present important physicochemical and physiological properties beneficial to the health of consumers, and they have been extensively used as pharmacological supplements, and food ingredients, reducing serum lipid levels in hyperlipidemics (Xu et al., 2009b). In recent years, among the functional oligosaccharides, xylo-oligosaccharides (XOS) have received special attention due to their favorable features including stability in acidic media, resistance to heat, lower available energy, and significant biological effects at low daily intakes (Christakopoulos et al., 2003; Manisseri and Gudipati, 2010; Moure et al., 2006; Vázquez et al., 2000). This part mainly focuses on the current research surrounding the manipulation of XOS by enzymes.

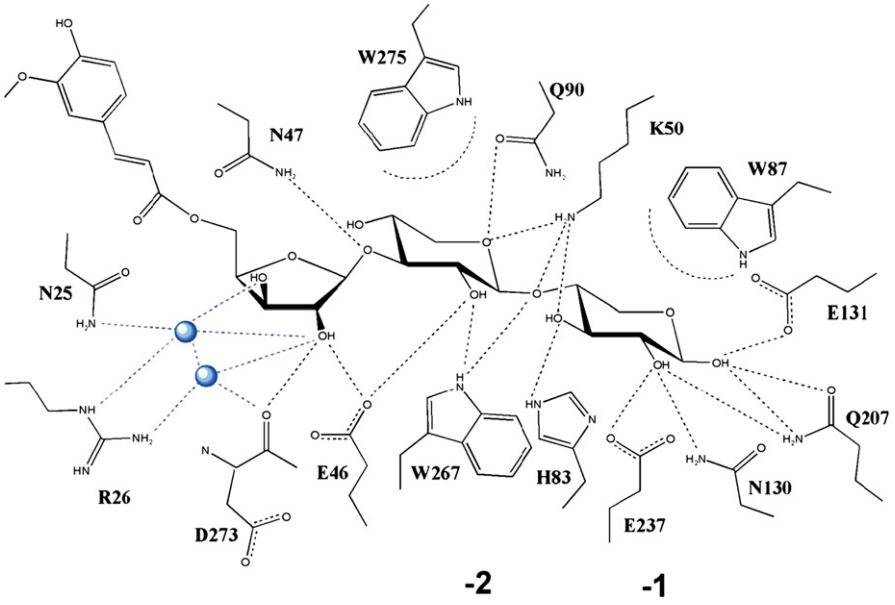

Fig. 5. Schematic of the interactions of FAX2 with TaXyn10. The interations were determined using PyMol (Vardakou et al., 2005).

There have been two approaches divulged for producing XOS from suitable feedstocks by enzymatic hydrolysis. One is direct enzymatic hydrolysis of xylan-rich material, and another is isolation and purification of xylan through chemical fractionation, followed by enzymatic hydrolysis. The production of XOS with direct enzymatic treatment is the only suitable method for susceptible materials (e.g. citrus peels) and is limited by obstacles such as crystalline structure of lignocelluloses and inhibitors, resulting in low yield (Alonso et al., 2003). In contrast to direct enzymatic hydrolysis, the latter method is more desirable because it avoids the above drawbacks. The detailed preparation of hemicelluloses has been described beforehand, while the classical enzymatic hydrolysis of hemicelluloses is given as follows: the hemicellulosic sample (2–4%, w/v) in 50 mM sodium acetate (citrate and Na-phosphate, etc.) buffer is incubated with endo-(1,4)-β-D-xylanase (commercial or cultivated hemicellulases) at its optimal condition (enzymatic activity, pH, and temperature) for 24 or 48 h. After inactivation of the enzyme (100 °C, 10 min) the produced xylo-oligosaccharides are further purified and identified. Methods of step-wise, sequential and combined enzymatic hydrolysis were examined to obtain XOS (Pastell et al., 2008; Sørensen et al., 2007c). Intriguingly, Vardakou et al. (2005) portrayed that the arabinofuranose linked α1,3 to Xyl2 in FAX_2 made several direct and indirect interactions with *Ta*Xyn10 (Fig. 5). Moreover, inactivated enzymes as probes of the structure of arabinoxylans were observed by atomic force microscopy (AFM). The xyn11A enzyme was shown to bind randomly along arabinoxylan molecules (Fig. 6), and the xylanase binding was also monitored following *Aspergillus niger* arabinofuranosidase pretreatment of the samples (Fig. 7), which demonstrated that removal of the arabinose side chain significantly altered the binding pattern of the inactivated enzyme, while the xyn10A enzyme was found to show deviations from random binding to the arabinoxylan chains (Fig. 8) (Adams et al., 2004).

Large ranges of XOS yield have been achieved from different substrates, enzyme and methods during enzymatic hydrolysis of (glucurono)arabinoxylans from wheat bran with several combinations of xylanolytic enzymes (endoxylanase I (EXI), glucuronoxylanase (GX), arabinoxylan arabinofuranohydrolase (AXH) and arabinofuranosidase B (AfB)) yielding relatively detailed data between various hemicellulosic fractions and enzymes (Table 3) (Schooneveld-Bergmans et al., 1999). The degradability of different fractions by EXI was shown to range from 2 to 26%, the lowest of which was observed for BE.70 and the highest found for BE.10. GX gave generally less degradation than EXI and AXH (2–5%), ranging from 0 to 5%. In comparison with the single enzyme, the synergistic effect of combined enzymes on degradation of hemicelluloses was much better. Removal of arabinose by AXH likely created new sites for hydrolysis by EXI. From the proportions of arabinose released, it was shown that this substance was always released when AXH was also present. On the other hand, substitution of the backbone is known to restrict the activity of endoxylanases in general. Brillouet and Joseleau (1987) also observed a lack of degradation of their highly-substituted wheat bran arabinoxylan by an endoxylanase from *Polyporus tulipiferae*.

After the production of XOS, the most important step is the refining step, namely purification, in order to separate the high molecular weight polysaccharides and low molecular weight monosaccharides, or obtain food grade XOS. Activated carbon adsorption, membrane separation, and chromatographic methods (Biogel P-2 column, HPLC, HPAEC, and HPSEC) can be used for these purposes. Treatment with activated carbon has been shown to be an effective, simple, and low-cost process for removing impurities from carbohydrate products and decolorization. In a study by Montané et al. (2006), the appropriate amount of activated carbon (from 1.5 to 50.0 mg/ml) was added in XOS solution (20 g/L), and then rotated at 30 °C for 24 h. The activated carbon was then removed by centrifugation, and the solution containing XOS was obtained by further filtering the supernatant liquid. It was found that adsorption for lignin-related products

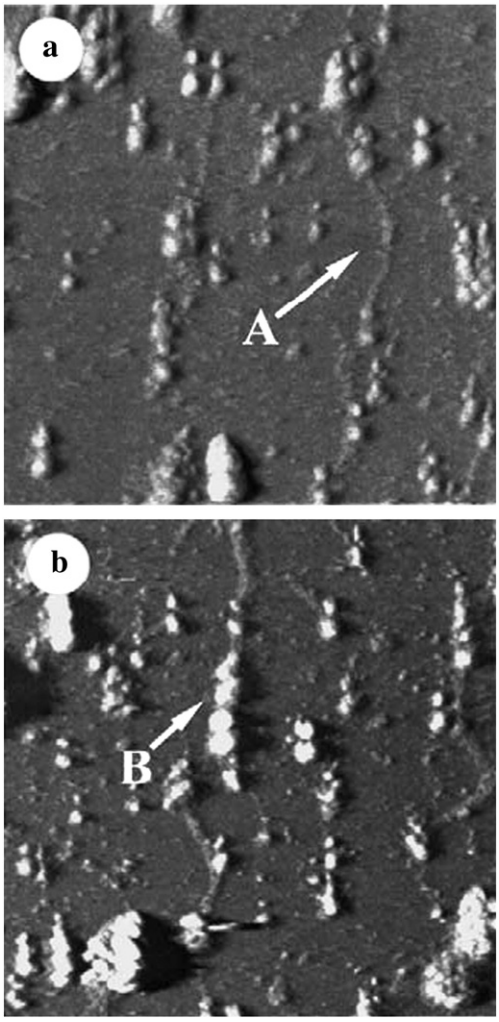

Fig. 6. AFM images showing the binding of mutant Xyn11A xylanases (E386A) to arabinoxylan chains. Image size 600 × 600 nm. A indicates a region of the polysaccharide chain devoid of bound enzymes, B shows a cluster of bound enzymes (Adams et al., 2004).

was higher than for xylo-oligosaccharides, and the selectivity toward lignin adsorption was better when the carbon was highly microporous and had small mesopore diameters (Montané et al., 2006). Currently, the technique of activated carbon adsorption has been used on an industrial scale (Sato et al., 2010). Membrane separation may be a promising method for purification of XOS. The size-dependent selection of the membrane process mechanism results in various concentrations of molecules with different molecular weights. Ultrafiltration and nanofiltration are two well known membrane separation processes for preparation of several XOS (Nabarlatz et al., 2007; Vegas et al., 2006; Yang et al., 2003). Finally, chromatography, based on the mechanism of solid–liquid extractions, ion exchange or size-dependent selection can be considered. Eleven fractions were obtained from the enzymatically hydrolyzed arabinoxylan on a Biogel P-2 column by Höije et al. (2006). HPLC and HPAEC (high performance anion-exchange chromatography) are the most commonly used methods in separation and purification of XOS, while HPSEC (high-performance size exclusion chromatography) which relies on dividing the elution profiles into fixed time intervals and utilizing the linear refractive index response (area under the curve) of defined standard compounds may be used as well. The molecular weight distribution of XOS was evaluated in detail by Rasmussen and Meyer (2010) with HPSEC.

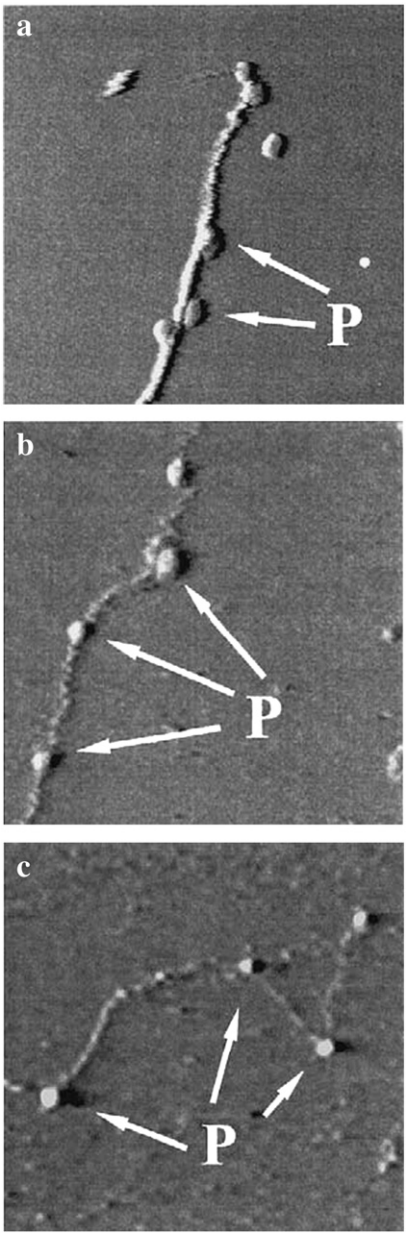

Fig. 7. AFM images showing the binding of mutant Xyn10A xylanases (E246A) to arabinoxylan chains. Scan size 600×600 nm. The enzymes (P) are shown attached to the polysaccharide chains. In (a) there are two enzymes that have become detached from the chain (Adams et al., 2004).

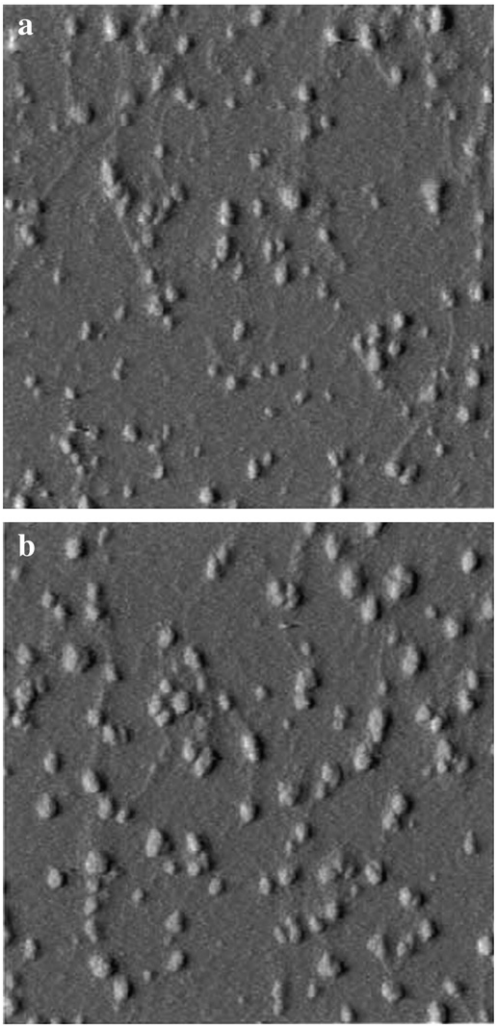

Fig. 8. AFM images showing the binding of mutant Xyn11A xylanases (E386A) to arabinoxylan chains that have been treated with an arabinofuranosidase for 3 h. Scan size 600×600 nm (Adams et al., 2004).

The efforts targeted for identification of XOS not only help us understand the structure–function relationships, but also map the structural characterization of their parent hemicelluloses. For example, the evidence of the presence of 2-*O*-β-D-xylopyranosyl-α-L-arabinofuranose side chains in barley husk arabinoxylan was provided by analysis of XOS structure after enzymatic hydrolysis (Höije et al., 2006). As for simple XOS (DP 1–6), quantitative and qualitative identification of XOS were simultaneously performed by HPLC or HPAEC basing on the retention time comparison with those of standard compounds (Figs. 9 and 10). For the relatively complex XOS, NMR technology undoubtedly provides a powerful tool for the structural characterization of XOS. Hoffmann et al. (1992) investigated the ^{1}H NMR of enzymically generated wheat-endosperm arabinoxylan oligosaccharides, and exhibited a large number of XOS and their corresponding ^{1}H chemical shift data in detail. The development of ^{13}C and 2D (HSQC, TOCSY, COSY, and HMBC) NMR technologies were also involved in characterization of various XOS structures (Ishii et al., 2010). In addition, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), electrospray ionization mass spectrometry (ESI-MS), quadrupole ion trap (QIT) and Fourier transform ion cyclotron resonance (FT-ICR) as well as Raman spectroscopy play important roles in identification of branched XOS such as AXOS and GXOS (Barron et al., 2006; Goncalves et al., 2009; Kabel et al., 2001; Pasanen et al., 2007). In general, these techniques can be used either alone or in combination (Gullón et al., 2010; Reis et al., 2003). Table 4 summarizes the current knowledge about the enzymatic hydrolysis of hemicelluloses for XOS production.

5.3. Pretreatment

The conventional bioprocesses for lignocellulose-to-value-added-products conversion require three events: pretreatment, enzymatic hydrolysis, and microbial fermentation, in which pretreatment plays a key role in deciding the subsequent efficiency of enzymes. This is due to the fact that pretreatment is intended to break the lignin seal, decrease crystallinity of cellulose, expose cellulose and hemicelluloses for available area, and allow the enzymes to effectively penetrate into the fibers. Obviously, it should be noted that the pretreatment step should also minimize the loss of carbohydrate and formation of inhibitory by-products. At present, the pretreatment methods can be divided into physical, chemical, and biological methods,

Table 3
Degradation and release of arabinose from the barium hydroxide extract of wheat bran WUS and some fractions derived therefrom by anion-exchange chromatography (BE.DU) and graded ethanol precipitation (BE.10, BE70 and BE.80S) after 24 h incubation with various enzymes (Schooneveld-Bergmans et al., 1999).

	% Degradation[a]					% Arabinose released[b]				
	BE	BE.DU	BE.10	BE.70	BE.80S	BE	BE.DU	BE.10	BE.70	BE.80S
EXI	13.8	6.6	25.6	1.6	4.9	0	0	0	0	0
GX	1.1	4.9	0.2	0.1	2.1	0	0	0	0	0
AXH	3.2	4.8	3.9	1.9	5.2	8.4	16.0	24.9	5.0	9.3
AfB	0.9	1.7	0.4	0.4	5.5	2.9	7.6	5.2	2.1	17.1
EXI + AXH	15.1	14.2	31.5	4.8	7.9	6.4	10.9	23.4	4.1	8.8
EXI + AfB	13.4	7.4	28.4	3.1	11.1	5.0	6.1	17.4	2.3	15.4
EXI + GX	12.8	10.4	26.2	2.4	6.7	0	0	0	0	0
EXI + GX + AXH	18.6	18.4	29.2	5.1	9.7	7.3	13.6	22.3	4.1	7.2
EXI + GX + AfB	17.4	11.9	29.7	3.6	1.7	5.2	6.0	18.3	2.4	15.4

EXI = endoxylanase I; GX = glucuronoxylanase; AXH = arabinoxylan arabinofuranohydrolase; AfB = arabinofuranosidase B.
[a] Expressed as percentage of reducing sugars produced from total sugars present.
[b] Expressed as percentage of arabinose released from total arabinose present.

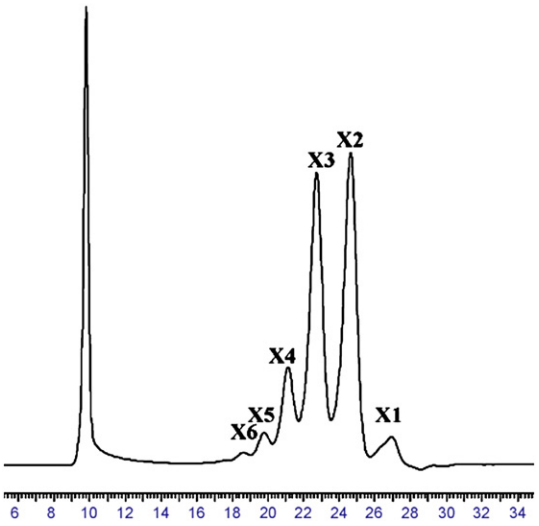

Fig. 9. HPLC chromatogram of XOs from sunflower xylan (Akpinar et al., 2009).

as well as combinations of these three. Physical pretreatment includes steaming, grinding, milling, and irradiation, with the most commonly used approach being chemical pretreatment, including alkali, acid, ammonia, organosolv, steam explosion or autohydrolysis and hydrothermal treatments as well as treatment with ionic liquids. Biological pretreatment is referred to the use of fungi for delignification, and offers some conceptually important advantages such as low chemical and energy use. Additionally, combinations of physical–chemical, physica–biological, and chemica–biological methods are also involved in pretreatment. For example, the effects of combinations of ammonia and xylanase pretreatments on enzymatic xylan and cellulose recovery from wheat straw were evaluated by Rémond et al. (2010). Moreover, the effectiveness of pretreatment has been studied in many cases, and the results exhibited the desired features, for instance, compared to untreated substrate, an approximate 6-fold increase in the enzyme activity was displayed for a lime pretreated sugarcane substrate (Beukes and Pletschke, 2010). The pretreatment methods have been well reviewed and compared by many authors recently (Binod et al., 2010; Carvalheiro et al., 2008; Gírio et al., 2010; Olofsson et al., 2008a; Taherzadeh and Karimi, 2007). As a general rule, the type of feedstock and economic viability must be taken into account in the choice of pretreatment method.

5.4. Biofuels and chemicals from hemicellulosic fermentation

Processes based on microbial fermentation are currently regarded as alternatives having the most potential in converting hemicelluloses into biofuels and chemicals such as ethanol, butanol, hydrogen, and succinic acid due to the advantages of low cost and environmental friendliness. The recent studies on biofuels and chemicals from hemicellulosic fermentation are shown in Table 5. This process is a simple approach that uses fermentable sugars (xylose, arabinose, galactose, mannose, and glucose) as carbon sources and directly fermenting them to produce hydrogen by microorganisms. The maximum fermentative hydrogen production by *Clostridium amygdalinum* strain C9 was found to be 2.2–2.5 and 1.78 mol H_2/mol xylose and arabinose, respectively (Jayasinghearachchi et al., 2010), with the presence of 10 g/L NaCl. Similar hydrogen production was also obtained by Li et al. (2010b), the maximum H_2 yields using the *Δldh* mutant were 2.71, 1.45 and 2.28 mol H_2/mol sugar under glucose, xylose, and glucose/xylose mixture tests, respectively. Even though hydrogen, ethanol, and lactic acid were observed by fermentation of hemicelluloses from municipal waste and commercial xylans (Murty and Chandra, 1997), the hemicellulose polysaccharides still lack of the ability to be directly fermented. Qureshi et al. (2006) provided evidence that the corn fiber arabinoxylan was not fermented until either xylose or glucose plus xylanase enzyme were added to support initial growth and fermentation. In that system, 9.60 g/L acetone, butanol, and ethanol (ABE) were produced by *Clostridium acetobutylicum*, indicating that hydrolysis of hemicelluloses to these monomer sugars was an important prerequisite for further fermentation.

As one of the most successful methods for biofuels and chemicals production from lignocelluloses, simultaneous saccharification and fermentation (SSF) is a combination of the enzymatic hydrolysis of materials with high xylan contents and fermentation in one step.

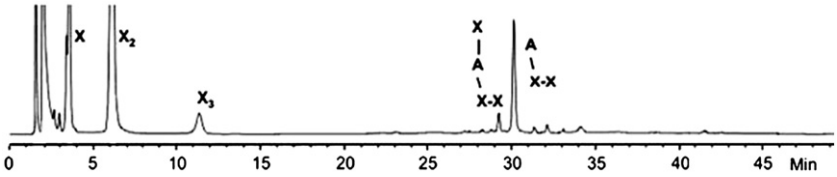

Fig. 10. HPAEC-PAD chromatograms of the OSAX (Pastell et al., 2009).

Table 4
Enzymatic hydrolysis of hemicelluloses for XOS production.

Substrate	Origin	Enzyme	pH	Temp (°C)	Time (h)	Buffer	Yield (%)	Analysis	Main results	Reference
Arabinoxylans (5 g/L)	Rye	Endo-1,4-β-D-Xylanase (10,000 nkat xylanase/g AX)	5.0	40	48	20 mM NaAc	12	HPAEC-PAD, MALDI-TOF-MS and NMR	α-L-Ara*f*-(1→3)-β-D-Xyl*p*-(1→4)-D-Xyl*p*	Pastell et al. (2008)
Xylan (2%)	Cotton stalks	Commercial xylanase (1.1 units/ml)	5.4	40	24	50 mM Citrate	53	Membrane separations and HPLC	XOS in the DP range of 2–7 (X6≈X5>X2>X3) with minor quantities of xylose	Akpinar et al. (2007)
Xylan (1%, w/v)	Birchwood and wheat bran	ReBlxA (122.9 U/mg)	6.0	40	24	McIlvaine	43	HPLC	Xylotriose (X3)	Liu and Liu (2008)
Xylan (2%)	Tobacco stalk, cotton stalk, sunflower stalk and wheat straw	Xylanase from *A. niger* (4 U/ml)	5.5	40	8–24	50 mM Citrate	19.3–28.6	HPLC	*X2*>X3>X4>X5>X6, >X6	Akpinar et al. (2009)
		Xylanase from *T. longibrachiatum* (4 U/ml)	4.6	50			18.4–27.9			
(Glucurono)arabinoxylans (1%)	Barley husks	Endo-β-Xylanase (5 U/ml)	6.0	50	24	0.05 M Na-phosphate		Biogel P-2 column, NMR, and MALDI-TOF-MS	β-D-Xyl*p*-(1→4)-[α-L-Ara*f*-(1→3)]-β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-β-D-Xyl*p*	Höije et al. (2006)
Arabinoxylan (1.0%, w/w)	Wheat	Combination of Ultraflo L (5%, w/w), β-xylosidase (0.25 g/kg), esterase (0.1 g/kg) and acetyl xylan esterase (0.1 g/kg).	5.0	50	48	0.2 M phosphate	45	HPAEC, HPLC and MALDI-TOF MS	Ferulic acid (1.6 mg), acetic acid (24 mg), arabinose (51 mg), xylose (167 mg), and solubilized oligosaccharides (244 mg) per gram substrate dry matter	Sørensen et al. (2007b)
Water-unextractable arabinoxylans (1 wt.%,)	Birchwood and wheat bran	*B. subtilis* xylanase (0.12 μmol/g)	6.0	40	24	0.1 M sodium acetate		HPSEC	Molecules of high (DP>25), medium (DP 9–25), and low (DP<9) molecular weights	Rasmussen and Meyer (2010)
Water extractable polysaccharides (50 mg/ml)	Wheat bran and bengal gram husk	Driselase (0.28 U/mg)	4.8	50	2	0.1 M acetate		Biogel P-2 column, HPLC, ESI-MS and ^{1}H NMR	→4)[α-L-Ara*f*-(1→2)][α-L-Ara*f*-(1→3)]-ß-D-Xyl*p*-(1→	Madhukumar and Muralikrishna (2010)
Xylan (4 mg/ml)	*Eucalyptus* wood, brewery's spent grain	End-(1,4)-β-D-xylanase I (0.2 μg/ml)	5.0	30	24	50 mM sodium acetate		HPAEC HPSEC MALDI-TOF MS Nanospray mass spectrometry	X_nAc_m, $X_n(GlcA_{me})_1Ac_m$, $X_n(GlcA_{me})2Ac_m$, and $X_n(GlcA_{me})_1$ or $_2Ac_mH$	Kabel et al. (2002)
Arabinoxylan (5 g/L)	Oat spelts	Termamyl®120 L (0.7 U/mg)	5.0	40	48	10 mM NaOAc		HPAEC-PAD, ESI-MS and NMR	β-D-Xyl*p*-(1→2)-a-LAra*f*-(1→3)-β-D-Xyl*p*-(1→4)-D-Xyl	Pastell et al. (2009)
Xyloglucan (0.1%, w/v)	*Benincasa hispida*	Endo-(1→4)-β-D-Xylanase (10 units)	5.0	37	24	100 mM NaOAc		HPAEC, MALDI-TOF-MS and GC-MS	XXXG type, and containing XXXG, XXFG, XXLG and XFLG as major oligomeric building sub-units	Mazumder et al. (2005)
Xylan (0.1%, w/v)		Endo-(1→4)-β-D-Xylanase (22.5 units)							Exhibiting a classical structure with a backbone of β-(1→4)-linked xylopyranosyl residues substituted with three 4-*O*-methyl glucuronic acid per 97 xylopyranosyl unit	
Insoluble dietary fiber (40 g/L)	Wheat bran	Sunzymes (0.4%, w/w)	5.0	50	16	50 mM Acetate	31.2	HPLC	Degree of polymerization of 2–7 and the ratio of arabinose to xylose of 0.27, and XOS was strongly resistant to lower acidic conditions	Wang et al. (2009a)
4-*O*-Methyl-D-glucuronoxylan	Beechwood	Family 10 EXs, (0.2 U/ml)	5.5	30	15	0.05 M acetate		TLC	$MeGlcA^3Xyl_3$, $MeGlcA^3Xyl_5$	Kolenová et al. (2006)
4-*O*-Methyl-D-glucuronoxylan (2%, w/v)	Birchwood	Endoxylanase (0.1 U/ml)		50	20	Deionized water	15	HPLC, HPAEC-PAD and ^{13}C NMR	Aldotetrauronic acid	Katapodis et al. (2002)

Table 5
Biofuels and chemicals from hemicellulosic fermentation.

Raw material	Type of pretreatment	Amount of solids	Strain	pH	Temp (°C)	Time (h)	Cultivation on hydrolyzate	Product	Final conc. (g/L)	Yield (%)	Mode of operation	Reference
Salix chips	Steam of SO_2	9%, w/w WIS	*Saccharomyces cerevisiae*	5.0	37	78	Yes	Ethanol	32	76	SSF Batch	Sassner et al. (2006)
Oat spelt xylan	No	2.5%	*Debaromyces hansenii*	10	40	36	Yes	Ethanol	9.1	46	SSF	Menon et al., (2010)
Wheat bran hemicellulose						48			9.5		Batch	
Barley hull	Ammonia	3% w/v Glucan	Recombinant *E. coli* (KO11)	7.0 (Begining), 5.5 (End)	38	72	No	Ethanol	24.1	89.4	SSCF Batch	Kim et al. (2008)
Xylan	Xylanase	13.1 g/L xylose	*Clostridium butyricum* CGS5	7.5	37	0–20	Yes	Hydrogen	62.5 (ml/h/L)	70	SSF two-stage process	Lo et al. (2010)
Rice straw	NaOH	9.2 g/L xylose							26.8 (ml/h/L)	76	batch-dark fermentation	
Wheat straw	Steam	9% WIS	*Saccharomyces cerevisiae*, TMB3400	5.0	34	96	Yes	Ethanol	38	80	SSF Fed-batch	Olofsson et al. (2008b)
Corn stover	Lactic acid and/or acetic acid	2% w/v Glucan	De Danske SpritfabrikkerA/S	4.8	32	0–170	No	Ethanol	31.5	88.7	SSF batch	Xu et al. (2009a)
Wheat straw	Lime	6.3% Dry matter	*B. coagulans* DSM 2314	6.0	50	55	Yes	Lactic acid	40.7	43	SSF Fed-batch	Maas et al. (2008)
Sweet sorghum bagasse	Ammonia fiber Expansion	1% Glucan	*Saccharomyces cerevisiae* 424A	6.0	30	Overnight	No	Ethanol	42.3	96.9	SSF	Li et al. (2010a)
Switchgrass	Ammonia fiber expansion	4% of Cellulases	*Saccharomyces cerevisiae* 424A	5.5	35	144	Yes	Ethanol	22.0	72.7	Two-step SSCF Fed-batch	Jin et al. (2010)
Convert corn	Ammonia	3% w/v Glucan	Recombinant *Escherichia coli* KO11	7.0 (First phase), 5.0 (Secondphase)	37	12	Yes	Ethanol	22.3	84	TPSSF	Li et al. (2010c)
Corn stover	Diluted alkaline	10.5% WIS	*Actinobacillus succinogenes*	6.5–7.0	38	48	No	Succinic acid	47.4	72	SSF Batch	Zheng et al. (2010)
Xylan	No		*Clostridium amygdalinum* strain C9	7.5	37	24	Yes	Hydrogen		40 mmol H_2/g xylan	Dark fermentation batch	Jayasinghearachchi, et al. (2010)
Switchgrass	Hydrothermolysis	41 g/L glucan	*Kluyveromyces marxianus* IMB4	4.8	45	0–168	Yes	Ethanol	16.8	72	SSF batch	Suryawati et al. (2009)
Wheat straw	Lime		*E. coli*	6.0	35	72	Yes	Ethanol	20.6	26	SSF Batch	Saha and Cotta (2007)

SSF from xylan and hemicelluloses-rich wheat straw hydrolysate to generate H_2 were conducted by Lo et al. (2010) and Kongjan and Angelidaki (2010), respectively. In the case of pretreated lignocelluloses, although the pretreatment solubilizes partial hemicelluloses mainly in the oligomeric form with minor furan-aldehydes, it is possible to still retain some amounts of hemicellulosic sugars in the solids, reaching up to 80% xylan for corn stover (Li et al., 2010c). From this point of view, the SSF from pretreated lignocelluloses could be considered to be the formation with combinations of hemicelluloses and cellulosic fractions. Among many studies, the production of ethanol has attracted most attention. The highest ethanol yield (96.9%) was obtained when fermentation was done using ammonia fiber expansion (AFEX) treated sweet sorghum bagasse (Li et al., 2010a). Additionally, succinic acid and lactic acid as important platform chemicals were also produced from diluted alkaline-treated corn stover and lime-treated wheat straw by SSF, respectively (Mass et al., 2008; Zheng et al., 2010). Recently, the two-phase simultaneous saccharification and fermentation (TPSSF) technology was developed to convert ammonia-treated corn stover to ethanol, leading to 84% of the theoretical ethanol yield based on the total carbohydrates (glucan + xylan) in the untreated corn stover (Li et al., 2010c). Further work on SSF should focus on improving the process by increasing the substrate loading, decreasing enzyme and yeast concentration, and varying temperature and pH.

The simultaneous saccharification and co-fermentation (SSCF) process is considered to be an improvement to SSF, and it refers to the fermentation of both hexoses and pentoses to ethanol, in which the hydrolyzed hemicelluloses during pretreatment and the solid celluloses are not separated and the hemicellulose sugars are allowed to be converted to ethanol together with SSF of the cellulose (Taherzadeh and Karimi, 2007). The study of SSCF from the ammonia-treated barley hull resulted in 24.1 g/L ethanol concentration, which corresponded to 89.4% of the maximum theoretical yield based on glucan and xylan (Kim et al., 2008). Furthermore, two-step SSCF was developed to convert AFEX-treated switchgrass to ethanol, and the results showed that two-step SSFC had higher xylose consumption and ethanol yield compared to traditional SSCF (Jin et al., 2010).

Currently, the *Escherichia coli*, *zymomonas mobillis*, *Saccharomyces cerevisiae* and *Pichia stipitis* are the most commonly used microorganisms in fermentation processes. In order to enhance yield and extend the type of production, physical immobility and biological engineering are proposed. For example, enhancement of biogas production was carried out by addition of hemicellulolytic bacteria immobilized on activated zeolite (Weiß et al., 2010). More importantly, Steen et al. (2010) demonstrated the engineering of *E. coli* to produce structurally tailored fatty esters (biodiesel), fatty alcohols, and waxes directly from hemicelluloses (Fig. 11).

6. Conclusion

In an integrated lignocellulose biorefinery, pre-exaction and isolation of hemicelluloses and lignin followed by the production of value-added products such as ethanol, sugar-based polyesters, other chemicals and biopolymers offer a potential opportunity. Isolation and purification of hemicelluloses using efficient methods would be beneficial to increasing their utilization. Among the isolation and purification methods, alkali treatment has been proven to be an efficient method for extracting the most available hemicelluloses from the cell wall of biomass, but the alkali extractions have the disadvantage of deacetylating the hemicelluloses. Alkali peroxide treatment is an effective agent for both delignification and solubilization of hemicelluloses from lignocellulosic materials. Dimethyl sulfoxide is the most common neutral solvent for extracting hemicelluloses, and the obtained hemicelluloses maintained their structural integrity. Steam explosion, microwave, and ultrasound-assisted extraction represent methods for the acceleration of hemicellulose extraction and reduction of the consumption of chemicals. Membrane technologies such as microfiltration, ultrafiltration, nanofiltration and reverse osmosis have drawn great attention in the green biorefinery for separation and purification of lignocellulosic products, which provide relatively cost-competitive separation steps, and offer a commercial alternative to chromatographic methods for purification of hemicelluloses. The combination of twin-screw extrusion, ultrafiltration, and anion-exchange chromatography efficiently produced hemicellulosic polymers with high purity rates. Bioconversion of hemicelluloses into

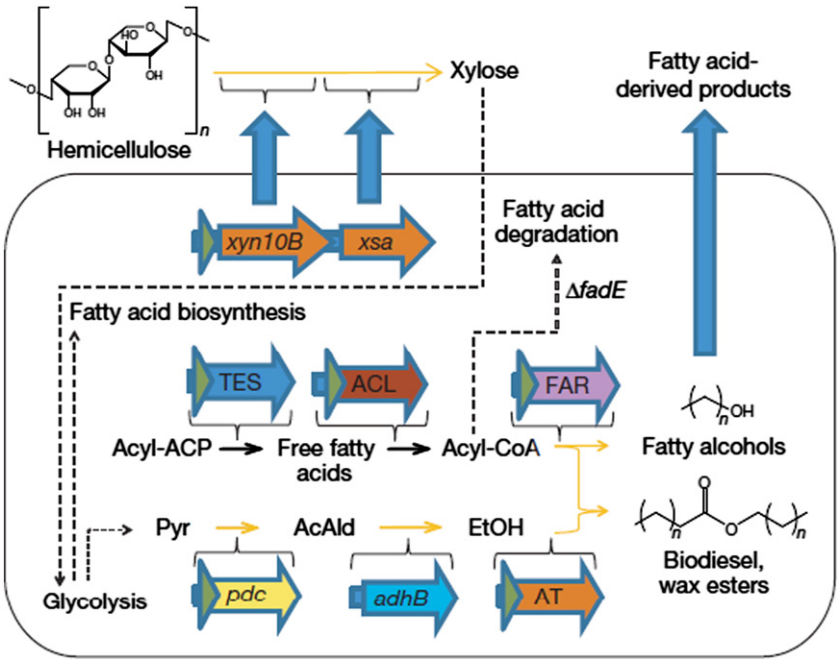

Fig. 11. Engineered pathways for production of fatty acid-derived molecules from hemicelluloses or glucose and depiction of the synthetic operations used in this study (Steen et al., 2010).

high value-added products involves three key steps: pretreatment, enzymatic hydrolysis and fermentation. By developing pretreatment technologies and engineered hemicellulase, sustainable bioconversion technologies have been successfully applied in food, value-added chemicals, bioenergy, and pulp and paper industries.

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