Hemicelluloses for Fuel Ethanol: A Review

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Review

Hemicelluloses for fuel ethanol: A review

F.M. Gírio *, C. Fonseca, F. Carvalheiro, L.C. Duarte, S. Marques, R. Bogel-Łukasik

Laboratório Nacional de Energia e Geologia, I.P., Unidade de Bioenergia, Estrada do Paço do Lumiar 22, 1649-038, Lisboa, Portugal

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ABSTRACT

Hemicelluloses currently represent the largest polysaccharide fraction wasted in most cellulosic ethanol pilot and demonstration plants around the world. The reasons are based on the hemicelluloses heterogeneous polymeric nature and their low fermentability by the most common industrial microbial strains. This paper will review, in a "from field to fuel" approach the various hemicelluloses structures present in lignocellulose, the range of pre-treatment and hydrolysis options including the enzymatic ones, and the role of different microbial strains on process integration aiming to reach a meaningful consolidated bioprocessing. The recent trends, technical barriers and perspectives of future development are highlighted.

1. Introduction

Efficient conversion of lignocellulosic materials (LCMs) into fuel ethanol has become a world priority for producing environmentally friendly renewable energy at reasonable price for the transportation sector. Fuel ethanol can be utilized as oxygenate of gasoline elevating its oxygen content, allowing a better oxidation of hydrocarbons and reducing the amounts of greenhouse gas emissions into the atmosphere (Hill et al., 2006).

LCMs are composed of carbohydrate polymers (cellulose and hemicellulose), lignin and a remaining smaller part comprising extractives and minerals in an intricated structure, that is recalcitrant to deconstruction. The cellulose and hemicellulose typically comprise up to two thirds of the LCM and are the substrates for second generation ethanol production. The microbial conversion of the hemicellulose fraction, either in the monomeric form or in the oligomeric form, is essential for increasing fuel ethanol yields from LCM. Unlike cellulose, hemicelluloses are not chemically homogeneous (see below) and different hydrolytic technologies and various biological and non-biological pre-treatment options are available both for fractionation or solubilisation of hemicellulose from LCM. Depending on the process and conditions used during pre-treatment, hemicellulose sugars may be degraded to weak acids and furan derivatives which potentially act as microbial inhibitors during the fermentation step to ethanol. Also lignin derived products can be formed and further interfere along the process.

Therefore, recent trends favour the integration of, at least, three key steps (Fig. 1) – pre-treatment, hydrolysis and fermentation – to both improve bioethanol yields and productivities and to lower capital and operating costs (Lynd et al., 2002, 2008; Margeot et al., 2009; van Zyl et al., 2007). Microorganisms, with unique genotype features, obtained either through recombinant DNA technology (Dien et al., 2003; Hahn-Hägerdal et al., 2007b; Jeffries, 2008; Sonderegger et al., 2004b) and/or through evolutionary engineering techniques (Kuyper et al., 2005; Sauer, 2001; Sonderegger et al., 2004b; Sonderegger and Sauer, 2003; Wisselink et al., 2009), represent the best option to overcome the barriers to the commercial exploitation of lignocellulosic bioethanol.

In this paper, a review on biomass pre-treatment and on the biotechnological aspects for hemicelluloses conversion to fuel ethanol are presented.

2. Hemicelluloses structure

Hemicelluloses are a heterogeneous class of polymers representing, in general, 15–35% of plant biomass and which may contain pentoses (β-D-xylose, α-L-arabinose), hexoses (β-D-mannose, β-D-glucose, α-D-galactose) and/or uronic acids (α-D-glucuronic, α-D-4-*O*-methylgalacturonic and α-D-galacturonic acids). Other sugars such as α-L-rhamnose and α-L-fucose may also be present in small amounts and the hydroxyl groups of sugars can be partially substituted with acetyl groups. Homopolymers of xylose, so-called homoxylans only occur in seaweeds (red and green algae).

The most relevant hemicelluloses are xylans and glucomannans, with xylans being the most abundant. Xylans are the main

* Corresponding author. Tel.: +351 210924721; fax: +351 217163636.
E-mail address: francisco.girio@lneg.pt (F.M. Gírio).

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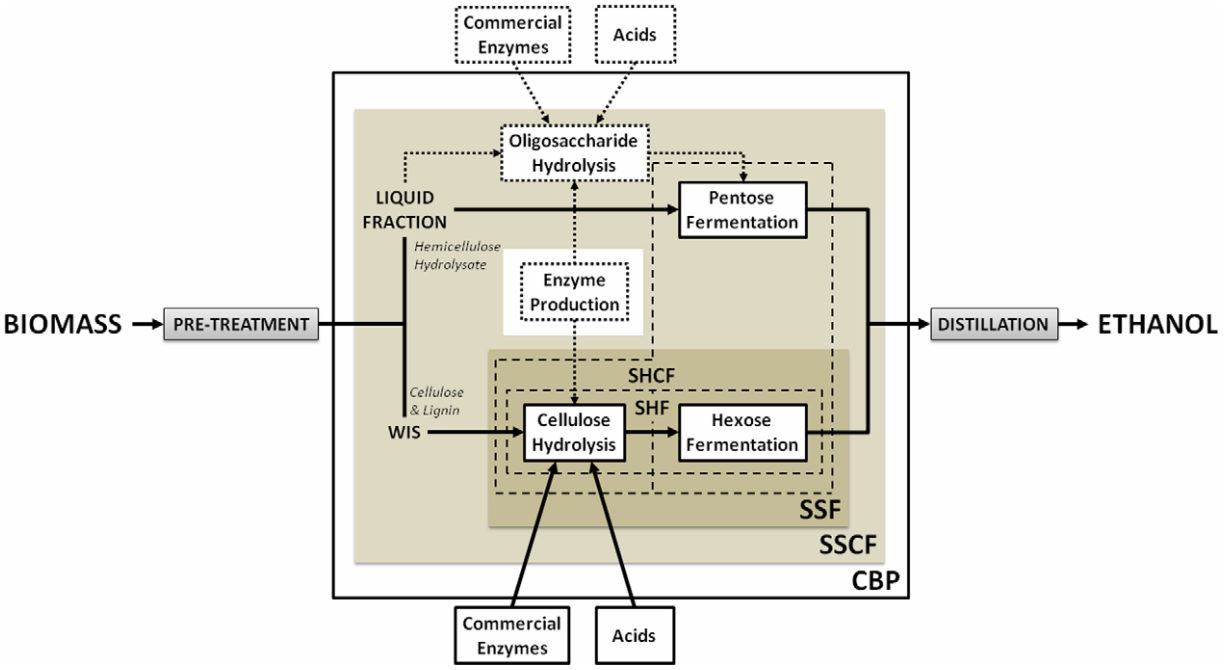


Fig. 1. Lignocellulosic ethanol bioprocesses. In most cases, pre-treatment produces water-insoluble solids (WIS), containing cellulose and lignin, and a liquid fraction composed of hemicellulose. The hemicellulose is more or less intact, depending on the pre-treatement: when hydrolysed to monosaccharides, it proceeds to fermentation; when not completely hydrolysed, i.e., composed of oligosaccharides, it requires further hydrolysis before fermentation. Cellulose is hydrolysed by cellulases (currently, commercial enzymes are used) and converted to glucose, which is fermented. When hydrolysis of cellulose and fermentation occurs separately, the process is designated separate hydrolysis and fermentation (SHF). When the pentose fraction is fermented together with the hexose fraction after a separate hydrolysis it is designated separate hydrolysis and co-fermentation (SHCF). When hydrolysis of cellulose is performed in simultaneous with fermentation it is named simultaneous saccharification and fermentation (SSF). When SSF includes the co-fermentation of glucose and xylose, i.e., with the whole slurry (WIS and liquid fraction) it is called simultaneous saccharification and co-fermentation (SSCF). Finally, when enzymes are produced also during the process, in a way enzyme production, hydrolysis and fermentation of all sugars are performed in one step, it is denominated consolidated bioprocessing (CBP).

hemicellulose components of secondary cell walls constituting about 20–30% of the biomass of hardwoods and herbaceous plants. In some tissues of grasses and cereals xylans can account up to 50% (Ebringerová et al., 2005). Xylans are usually available in huge amounts as by-products of forest, agriculture, agro-industries, wood and pulp and paper industries. Mannan-type hemicelluloses like glucomannans and galactoglucomannans are the major hemicellulosic components of the secondary wall of softwoods whereas in hardwoods they occur in minor mounts.

Depending on their biological origin, different hemicelluloses structures can be found (Table 1). Upon hydrolysis, the hemicelluloses are breakdown into their monomers (Table 2).

2.1. Glucuronoxylans (GX)

Glucuronoxylans (*O*-acetyl-4-*O*-methylglucuronoxylan) are the main hemicellulose of hardwoods, which can also contain small amounts of glucomannans (GM). In hardwoods, GX represent 15–30% of their dry mass (Alén, 2000) and consist of a linear backbone of β-D-xylopyranosyl units (xyl*p*) linked by β-(1,4) glycosidic bonds. Some xylose units are acetylated at C2 and C3 and one in ten molecules has an uronic acid group (4-*O*-methylglucuronic acid) attached by α-(1,2) linkages. The percentage of acetyl groups ranges between 8% and 17% of total xylan, corresponding, in average, to 3.5–7 acetyl groups per 10 xylose units (Alén, 2000). The 4-*O*-methylglucuronic side groups are more resistant to acids than the Xyl*p* and acetyl groups. Besides these main structural units, GX may also contain small amounts of L-rhamnose and galacturonic acid. The later increases the polymer resistance to alkaline agents. The average degree of polymerization (DP) of GX is in the range of 100–200 (Pereira et al., 2003).

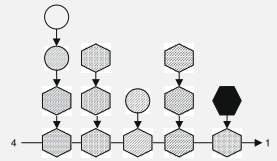
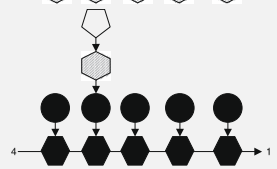
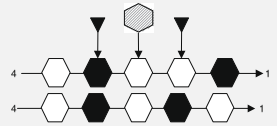
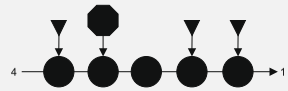
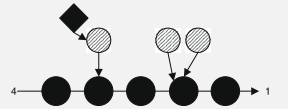
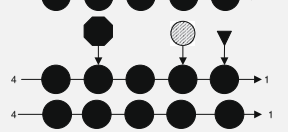
2.2. Galactoglucomannans (GGMs)

Galactoglucomannans (*O*-acetyl-galactoglucomannans) are the main hemicelluloses of softwoods, accounting up to 20–25% of their dry mass (Pereira et al., 2003). GGM consist of a linear backbone of β-D-glucopyranosyl and β-D-mannopyranosyl units, linked by β-(1,4) glycosidic bonds, partially acetylated at C2 or C3 and substituted by α-D-galactopyranosyl units attached to glucose and mannose by α-(1,6) bonds. Acetyl groups content of GGM is around 6%, corresponding, on average, to 1 acetyl group per 3–4 hexoses units (Alén, 2000). Some GGM are water soluble, presenting in that case a higher galactose content than the insolubles (Timell, 1967). Glucomannans (GM) occur in minor amounts in the secondary wall of hardwoods (<5% of the dry wood mass) (Alén, 2000). As GGM, they have linear backbone of β-D-glucopyranosyl (Glc*p*) and β-D-mannopyranosyl (Man*p*) units but the ratio Glc*p*:Man*p* is lower. In GGM and GM the extent of galactosylation governs their association tendency to the cellulose microfibrils and hence, their extractability from the cell wall matrix (Ebringerová et al., 2005). The average DP of AGX ranges between 40 and 100 (Pereira et al., 2003).

2.3. Arabinoglucuronoxylans (AGXs)

Arabinoglucuronoxylans (arabino-4-*O*-metylglucuronoxylans) are the major components of non-woody materials (e.g., agricultural crops) and a minor component for softwoods (5–10% of dry mass). They also consist of a linear β-(1,4)-D-xylopyranose backbone containing 4-*O*-metil-α-D-glucopiranosyl uronic acid and α-L-arabinofuranosyl linked by α-(1,2) and α-(1,3) glycosidic bonds (Timell, 1965; Woodward, 1984). The typical ratio arabinose:

Table 1
Main types of polysaccharides present in hemicelluloses (information based mainly on (Alén, 2000; Carpita and Gibeaut, 1993; de Vries and Visser, 2001; Ebringerová et al., 2005; Pereira et al., 2003)).

Polysaccharide type	Biological origen	Abbreviation	Amount[a]	Units			DP[b]	Schematic representation
				Backbone	Side chains	Linkage		
Arabinogalactan	Softwoods	AG	1–3;35[*]	β-D-Gal*p*	β-D-Gal*p* α-L-Ara*f* β-L-Ara*p*	β-(1 → 6) α-(1 → 3) β-(1 → 3)	100–600	
Xyloglucan	Hardwoods, grasses	XG	2–25	β-D-Glc*p* β-D-Xyl*p*	β-D-Xyl*p* β-D-Gal*p* α-L-Ara*f* α-L-Fuc*p* Acetyl	β-(1 → 4) α-(1 → 3) β- (1 → 2) α-(1 → 2) α-(1 → 2)		
Galactoglucomannan	Softwoods	GGM	10–25	β-D-Man*p* β-D-Glc*p*	β-D-Gal*p* Acetyl	α-(1 → 6)	40–100	
Glucomannan	Softwoods and hardwoods	GM	2–5	β-D-Man*p* β-D-Glc*p*			40–70	
Glucuronoxylan	Hardwoods	GX	15–30	β-D-Xyl*p*	4-O-Me-α-D-Glc*p*A Acetyl	α-(1 → 2)	100–200	
Arabinoglucuronoxylan	Grasses and cereals, softwoods	AGX	5–10	β-D-Xyl*p*	4-O-Me-α-D-Glc*p*Aβ-L-Ara*f*	α-(1 → 2) α-(1 → 3)	50–185	
Arabinoxylans	Cereals	AX	0.15–30	β-D-Xyl*p*	α-L-Ara*f*Feruloy	α-(1 → 2) α-(1 → 3)		
Glucuronoarabinoxylans	Grasses and cereals	GAX	15–30	β-D-Xyl*p*	α-L-Ara*f* 4-O-Me-α-D-Glc*p*A Acetyl	α-(1 → 2) α-(1 → 3)		
Homoxylans	Algae	X		β-D-Xyl*p*[c]				

[a] %, dry biomass.
[b] Degree of polymerization.
[c] May also present β-(1 → 3) linkages on the backbone; [*] (up to) in the heartwood of larches ⬡, β-D-Gal*p*; ⬢, β-D-Glc*p*; ⬡, β-D-Man*p*; ●, β-D-Xyl*p*; ◍, β-L-Ara*f*; ○, β-L-Ara*p*; ⬠, α-L-Fuc*p*; ⬢, 4-O-Me-α-D-Glc*p*A; ▼, Acetyl; ♦, Feruloyl.

Table 2
Hemicelluloses composition of various lignocellulosic materials.[a,b,c]

Raw material	Xyl	Ara	Man	Gal	Rha	UA	AcG	References[d]
Softwoods								
Douglas fir	6.0	3.0	–	3.7	–	–	–	(1)
Pine	5.3–10.6	2.0–4.2	5.6–13.3	1.9–3.8	–	2.5–6.0	1.2–1.9	(2, 3)
Spruce	5.3–10.2	1.0–1.2	9.4–15.0	1.9–4.3	0.3	1.8–5.8	1.2–2.4	(2, 4–7)
Hardwoods								
Aspen	18–27.3	0.7–4.0	0.9–2.4	0.6–1.5	0.5	4.8–5.9	4.3	(1, 2, 4, 8)
Birch	18.5–24.9	0.3–0.5	1.8–3.2	0.7–1.3	0.6	3.6–6.3	3.7–3.9	(2, 4)
Black locust	16.7–18.4	0.4–0.5	1.1–2.2	0.8	–	4.7	2.7–3.8	(2, 9)
Eucalypt	14–19.1	0.6–1	1–2.0	1–1.9	0.3–1	2	3–3.6	(10–13)
Maple	18.1–19.4	0.8–1	1.3–3.3	1.0	–	4.9	3.6–3.9	(2, 9)
Oak	21.7	1.0	2.3	1.9	–	3	3.5	(14)
Poplar	17.7–21.2	0.9–1.4	3.3–3.5	1.1	–	2.3–3.7	0.5–3.9	(2, 15)
Sweet gum	19.9	0.5	0.4	0.3	–	2.6	2.3	(16)
Sycamore	18.5	0.7	1.0	–	–	–	3.6	(9)
Willow	11.7–17.0	2.1	1.8–3.3	1.6–2.3	–	–	–	(4, 17)
Agricultural and agro-industrial materials								
Almond shells	34.3	2.5	1.9	0.6	–	–	–	(18)
Barley straw	15	4.0	–	–	–	–	–	(19)
Brewery's spent grain	15	8	0	1	0	2	0.8	(12)
Cardoon	26.0	2.5	3.7	1.4	0.9	–	–	(20)
Corn cobs	28–35.3	3.2–5.0	–	1–1.2	1	3	1.9–3.8	(9, 13, 21, 22)
Corn fibre	21.6	11.4	–	4.4	–	–	–	(23)
Corn stalks	25.7	4.1	<3.0	<2.5	–	–	–	(24)
Corn stover	14.8–25.2	2–3.6	0.3–0.4	0.8–2.2	–	–	1.7–1.9	(9, 25, 26)
Olive stones	2.0–3.7	1.1–1.2	0.2–0.3	0.5–0.7	0.3–0.5	1.2–2.2	–	(27)
Rice husks	17.7	1.9	–	–	–	–	1.62	(28)
Rice straw	14.8–23	2.7–4.5	1.8	0.4	–	–	–	(23, 26)
Sugar cane bagasse	20.5–25.6	2.3–6.3	0.5–0.6	1.6	–	–	–	(29–31)
Wheat bran	16	9	0	1	0	2	0.4	(13)
Wheat straw	19.2–21.0	2.4–3.8	0–0.8	1.7–2.4	–	–	–	(8, 26)

[a] Non-glycosidic units.
[b] Expressed as g/100 g of dry material.
[c] The percentages of oses were, in some cases, calculated from the corresponding "polymers". Xyl, xylose; Ara, arabinose; Man, mannose; Gal, galactose; Rha, rhamnose; UA, uronic acids; AcG, acetyl groups.
[d] 1. Schell et al. (1999), 2. Fengel and Wegener (1983), 3. Ballesteros et al. (2000), 4. Taherzadeh et al. (1997), 5. Tengborg et al. (1998), 6. Söderström et al. (2002), 7. Söderström et al. (2004), 8. Grohmann et al. (1985), 9. Torget et al. (1991), 10. Pereira (1988), 11. Miranda and Pereira (2002), 12. Garrote et al. (1999b), 13. Kabel et al. (2002), 14. Conner (1984), 15. Torget and Hsu (1994), 16. Torget et al. (1990), 17. Sassner et al. (2004), 18. Montané et al. (1994), 19. Magee and Kosaric (1985), 20. Gominho et al. (2001), 21. Ropars et al. (1992), 22. Garrote et al. (2001b), 23. Allen et al. (2001b), 24. Rubio et al. (1998), 25. Torget et al. (2000), 26. Lee (1997), 27. Fernández-Bolaños et al. (2004), 28. Vila et al. (2002), 29. Dekker and Wallis, (1983), 30. Neureiter et al. (2002), 31. Aguilar et al. (2002).

glucuronic acid:xylose is 1:2:8 (Alén, 2000). Conversely to hardwoods xylan, AGX might be less acetylated, but may contain low amounts of galacturonic acid and rhamnose. The average DP of AGX ranges between 50 and 185 (Pereira et al., 2003).

2.4. Xyloglucans (XGs)

Xyloglucans are quantitatively predominant hemicellulosic polysaccharide type in the primary cell walls of hardwoods (mainly in dicotyledonae and less in monocotyledonae) (de Vries and Visser, 2001) and it can also appear in small amounts in grasses. Xyloglucans consist of β-1,4-linked D-glucose backbone with 75% of these residues substituted at *O*-6 with D-xylose. L-arabinose and D-galactose residues can be attached to the xylose residues forming di-, or triglycosyl side chains. Also L-fucose has been detected attached to galactose residues. In addition, xyloglucans can contain *O*-linked acetyl groups (Maruyama et al., 1996; Sims et al., 1996). Xyloglucans interact with cellulose microfibrils by the formation of hydrogen bonds, thus contributing to the structural integrity of the cellulose network (Carpita and Gibeaut, 1993; de Vries and Visser, 2001).

2.5. Arabinoxylans (AXs)

Arabinoxylans (AXs) represent the major hemicellulose structures of the cereal grain cell walls. AXs are similar to harwoods xylan but the amount of L-arabinose is higher. In AX, the linear β-(1,4)-D-xylopyranose backbone is substituted by α-L-arabinofuranosyl units in the positions 2-*O* and/or 3-*O* and by α-D-glucopyranosyl uronic unit or its 4-*O*-methyl derivative in the position 2-*O* (Brillouet et al., 1982; Shibuya and Iwasaki, 1985). *O*-acetyl substituents may also occur (Ishii, 1991; Wende and Fry, 1997). Arabinofuranosyl residues of AX may also be esterified with hydroxycinnamic acids residues, e.g., ferulic and *p*-coumaric acids (Wende and Fry, 1997). Dimerization of esterified phenolic compounds may also lead to inter- and intra-molecular cross-links of xylan. The physical and/or covalent interactions with other cell-wall constituents, restricts the extractability of xylan. In lignified tissues for example, xylan is ester linked through its uronic acid side chains to lignin (Ebringerová et al., 2005).

2.6. Complex heteroxylans (CHXs)

Complex heteroxylans are present in cereals, seeds, gum exudates and mucilages and they are structurally more complex (Stephen, 1983). In this case the β-(1,4)-D-xylopyranose backbone is decorated with single uronic acid and arabinosyl residues and also various mono- and oligoglycosyl side chains.

3. Selective fractionation of hemicelluloses

The first step of lignocellulose ethanol bioprocesses is pre-treatment (Fig. 1), and different pre-treatment methods have been developed (Table 3). For fuel ethanol production, hemicelluloses are commonly removed during the initial stage of biomass

Table 3
Comparison of advantages and disadvantages of different pre-treatment options for lignocellulosic materials.

Desirable features	Concentrated acid	Dilute acid	Steam explosion	Autohydrolysis	Organosolv	Solid Superacids	Alkaline	Ionic liquids	Supercritical fluids
High hemicellulose solubilisation	++	++	++	++	+	+	+	++	+
High hemicellulosic monosaccharides production	++	++	0	–	–/0	+	–/0	0/+	–
Low hemicellulosic oligosaccharides production	+	+	0	–	–/0	+	–/0	0/+	–
High cellulose recovery	++	++	++	++	–	++	+	+	++
High cellulose digestibility	++	++	+	+	–	+	++	+	0/+
High lignin quality	–	–	0/+	+	+	–/0	–	+	+
High chemicals recycling	–	–	0	n.r.	–	+	–/0/+	+	n.r./+
Low inhibitors formation	–	–	0	0	+	0	+	–	0
Low corrosion problems	–	–	0	0	–	0	–	–/0	–/0
Low need for chemicals	–	–	0	++	–	0/+	–/0	+	++
Low neutralisation requirements	–	–	0	n.r.	+	–/0	–/0	0	n.r.
Low investment costs	+	+	–	+	0	0	0/+	+	–
Low operational costs	–	0	++	+	–	0	– / 0	–	–
Low energy use	0	–	0	0	0	+	+	++	+

+, Advantage; –, disadvantage; 0, neutral; n.r., not relevant.

processing aiming to reduce structural constraints for further enzymatic cellulose hydrolysis.

The main process options for the selective fractionation of hemicelluloses from biomass include the use of acids, water (liquid or steam), organic solvents and alkaline agents. The later two are not selective towards hemicellulose as they also remove lignin, which in turn can hinder the valorisation process, e.g., fermentation or bioconversion, as the lignin-derived compounds are usually microbial growth inhibitors. Therefore, acid/water/steam pre-treatments are the most commonly applied technologies yielding a selectively solubilisation of hemicelluloses and producing hemicellulose-rich liquids totally or partially hydrolysed to oligomeric and monomeric sugars and cellulose-enriched solids for further bioprocessing. Depending on the operational conditions degradation products are also formed, both from sugars (furan and its derivates and weak acids) and, to a less extent, from lignin (phenolics) (Olsson and Hahn-Hägerdal, 1996). These compounds may also inhibit the later fermentation processes, leading to lower ethanol yields and productivities and, therefore, might be required to carry out a prior detoxification treatment.

The following section reviews the fundamental modes of action of most relevant biomass fractionation methods.

3.1. Acid hydrolysis

Acid catalysed processes can be divided in two general approaches, based on concentrate-acid/low temperature and dilute-acid/high temperature hydrolysis. Sulphuric acid is the common acid employed although other mineral acids such as hydrochloric, nitric and trifluoracetic acids have also been assayed. In dilute-acid processes phosphoric acid and weak organic acids have also been reported.

3.1.1. Concentrated acid

Concentrate-acid processes enable the hydrolysis of both hemicelluloses and cellulose. The solubilisation of polysaccharides is reached using different acid concentrations, like 72% H_2SO_4, 41% HCl or 100% TFA (Fengel and Wegener, 1983). HCl and TFA have the advantage to be easier recovered. Concentrate-acid based processes have the advantage to allow operating at low/medium temperatures leading to the reduction in the operational costs. The formation of degradation products is low, although its formation rate can be severely affected by slight changes in temperature (Camacho et al., 1996). One of the key steps of these pre-treatments is the acid recovery, which is mandatory for their economical viability (Goldstein, 1983). Moreover, the equipment corrosion is an additional disadvantage. Nevertheless, there seems to be a renewable interest in these processes (Zhang et al., 2007) owing to the moderate operation temperatures and because no enzymes are required.

3.1.2. Dilute acid

Dilute-acid processes have been viewed primarily as a means of pre-treatment for the hydrolysis of hemicelluloses rendering the cellulose fraction more amenable for a further enzymatic treatment. Both cellulose and hemicellulose components can also be hydrolysed using dilute-acid catalysed processes but in this case a two step-hydrolysis is required. The difference between these two steps is mainly the operational temperature, which is high in the second step (generally around 230–240 °C) (Lee et al., 1999b; Wyman, 1999). Nevertheless, dilute-acid hydrolysis was not a preferable pre-treatment for cellulose hydrolysis since the high temperatures required for cellulose hydrolysis also lead to formation of very high amount of degradation products. However, the use of counter-current shrinking-bed reactors gives very high cellulose hydrolysis yields without significant formation of degradation products (Lee et al., 1999a; Torget et al., 1997). Typical sulphuric acid concentrations for hemicellulose hydrolysis are in the range 0.5–1.5% and temperatures above 121–160 °C. From hemicelluloses, dilute-acid processes yield sugar recoveries from 70% up to >95% (Allen et al., 2001a; Carvalheiro et al., 2004a; Marzialetti et al., 2008; Monavari et al., 2009). However, for both dilute- and concentrate-acid hydrolysis approaches, the acid has to be removed/neutralised before fermentation, yielding large amounts of waste.

Compared to the concentrate-acid hydrolysis, one of the advantages of dilute-acid hydrolysis is the relatively low acid consumption, limited problem associated with equipment corrosion and less energy demanding for acid recovery. Under controlled conditions, the levels of the degradation compounds generated can also be low.

As an alternative to the conventional dilute-acid processes, the addition of CO_2 to aqueous solutions, taking advantage of the carbonic acid formation, has been described (van Walsum and Shi, 2004) but the results obtained were not enough interesting.

3.2. Hydrothermal treatments

Hydrothermal processes mainly include liquid hot water (autohydrolysis) and steam-explosion treatments. The aim of both

processes is to selectively hydrolyse hemicelluloses from LCM. No chemical catalyst is added in these processes making them economically and environmentally attractive. The operational conditions used vary widely, and mainly depend on the pre-treatment type: autohydrolysis or steam explosion. Typically autohydrolysis is carried out at slightly lower temperatures, but for longer periods as compared to steam explosion (see below).

3.2.1. Autohydrolysis

The autohydrolysis process uses compressed hot water (pressure above saturation point). The operation temperatures usually range between 150 °C and 230 °C (Garrote et al., 1999a) and reaction time may vary from seconds up to hours, depending on the temperature (Fig. 2). Solids concentration, usually referred as the liquid-to-solid ratio (LSR), may range between 2 and 100 (w/w) (Garrote et al., 1999a; van Walsum et al., 1996), although the most common values are around 10. Higher LSR are usually associated to continuous reactors. Autohydrolysis has a similar mechanism to dilute-acid hydrolysis. Both are hydronium ions catalysed processes. The catalysts in autohydrolysis are hydronium ions generated in situ by water autoionization and acetic acid resulting from acetyl substituents of hemicelluloses, the later having a much higher contribution to the hydrolysis (Carrasco, 1989; Heitz et al., 1986). It has also been suggested that uronic acids may also contribute to the formation of hydronium ions (Conner, 1984) but their role in the hydrolysis is still not completely understood.

A relatively high hemicellulose recovery in the range of 55–84%, together with low levels of inhibitory by-products, has been obtained through autohydrolysis (Aoyama et al., 1995; Carvalheiro et al., 2009; Conner and Lorenz, 1986; Garrote and Parajó, 2002). Cellulose and lignin are not significantly affected, yielding a cellulose- and lignin-rich solid phase together with a liquid fraction with a relative low concentration of potential fermentation inhibitors. Owing to the mild pH the corrosion problems are reduced, and the steps of acid recycling and precipitates removal are no longer necessary. This reduces both the capital and operational costs with beneficial consequences on the environment compared to other hydrolytic technologies.

The main drawback of this process when using the liquid stream for fuel ethanol fermentation is that the solubilised hemicellulose appears mainly in oligomeric form (Allen et al., 2001b; Carvalheiro et al., 2004b; Conner and Lorenz, 1986; Garrote et al., 1999b; Kabel et al., 2002).

3.2.2. Steam explosion

Steam explosion has been described as a thermomechanochemical process where the breakdown of structural components is aided by heat in the form of steam (thermo), shear forces due to the expansion of moisture (mechano), and hydrolysis of glycosidic bonds (chemical) once it is (self)-catalysed (e.g., by the biomass derived acetic acid, and possibly by added catalysts) (Chornet and Overend, 1988). Experimentally, the material is heated (preferably to temperatures below 240 °C) using high-pressure steam up to few minutes. The steam condenses under the high pressure thereby "wetting" the material, that it is then "exploded" when the pressure within the reactor is rapidly released. The forces resulting from decompression lead to a desegregation of lignocellulosic matrix, breaking down inter- and intra-molecular linkages (Carrasco, 1989).

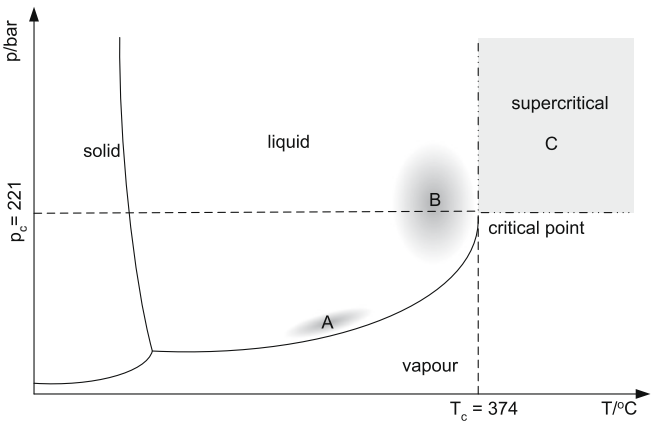


Fig. 2. Typical ranges for different water-based pre-treatments as a function of temperature and pressure. (A) Autohydrolysis, LHW; (B) subcritical conditions; (C) supercritical conditions. Lines represent the phase diagram for water.

The effectiveness of steam explosion pre-treatment has been studied for many LCM. Moreover, some steam explosion processes were even implemented at pilot and industrial scale mostly used as pre-treatment methods for kraft pulps bleaching in paper and pulp industry. The most known is STAKE continuous steam explosion digester (Kokta, 1991).

Most steam treatments yield high hemicellulose solubility (producing mainly oligosaccharides) along with slight lignin solubilisation. Studies without added catalyst report sugars recoveries between 45% and 69% (Ballesteros et al., 2002a; Heitz et al., 1991; Martín et al., 2008; Ruiz et al., 2008). The impregnation of biomass with acid catalysts, namely H_2SO_4 or SO_2, is also common. For example, when 1% H_2SO_4 is added, maximum pentose yield can increase about 30% for olive tree prunings (Cara et al., 2008). A similar trend was observed for wheat straw for which 0.9% H_2SO_4 enabled a maximum overall sugar yield of 85% (Ballesteros et al., 2005). The employment of catalysts are particularly important for softwoods (typically less acetylated), resulting in lower treatment temperatures and shorter reaction times, thereby improving hemicellulose recovery and reducing the formation of sugar degradation products (Boussaid et al., 2001; Bura et al., 2003; Galbe and Zacchi, 2007; Shevchenko et al., 2000).

3.3. Alkaline treatments

The alkaline pre-treatments can be divided into two major groups, depending on the catalyst used: pre-treatments that use alkaline/alkaline-earth metals based agents (typicaly sodium, potassium, or calcium) and those that use ammonia. Conversely to acid or hydrothermal processes, alkaline pre-treatments are very effective for lignin solubilisation exhibiting only minor cellulose and slighthly higher hemicellulose solubilisation and are not considered in this review with two exceptions: wet oxidation and the ammonia based treatments.

Wet oxidation was first described as a LCM pre-treatment involving oxygen and water at elevated temperatures and pressure, promoting the oxidation of lignin and decomposing it to CO_2, H_2O and carboxylic acids (Bjerre et al., 1996; Klinke et al., 2002). Combining alkaline agents (in particular Na_2CO_3) it is possible to get higher rates of hemicellulose solubilisation, up to 82% xylan for sugarcane bagasse (Bjerre et al., 1996). The hemicellulosic sugars remain mainly in the oligomeric form, and although there is a low formation of furan-aldehydes, a significant formation of carboxylic acids still exists (Bjerre et al., 1996; Klinke et al., 2002; Martín et al., 2008).

Ammonia recycling percolation (ARP) is carried out using aqueous ammonia in a flow-through mode at high temperatures (typically around 170 °C) (Kim and Lee, 2005). This treatment also solubilises hemicelluloses, reaching values between 40% and 60%, mainly in the oligomeric form. Cellulose fraction is almost not degraded (solubilisation <10%), but in the following cellulose hydrolysis step yields are close to the theoretical (Kim et al., 2000, 2008, 2003; Kim and Lee, 2005; Yoon et al., 1995). Again, selectivity is low, as delignification is high and can reach 60–85% (Kim et al.,

2003, 2008; Kim and Lee, 2005). ARP has already been successfully applied to hardwoods (Kim et al., 2008; Yoon et al., 1995) and corn stover (Kim et al., 2003, 2006; Kim and Lee, 2005; Zhu et al., 2006) with treated biomass presenting a cellulose digestibility above 93%, and with a slight less efficiency to wastepaper and softwood pulp mill sludges (Kim et al., 2000). The aqueous ammonia reaction mechanism is similar to lime- and NaOH-catalysed pre-treatments, namely in the swelling of biomass and in the cleavage of ether and ester bonding in lignin carbohydrate complex. The main economical constrains of ARP are the cost of ammonia recovery, the considerable safety issues regarding the practical use of ammonia and the high lignin solubilisation that will require the use of suitable detoxification processes. On the other hand, the economy is positively influenced by the high sugar recovery achieved, without the presence of sugar degradation products (Mosier et al., 2005). Alternatively the ammonia fibre explosion/expansion (AFEX) process has also been reported (Huber et al., 2006). Recently, AFEX (Lau and Dale, 2009) has been successfully used for simultaneous solubilisation of hemicellulose and cellulose from corn stover with yields over 85%, with a minimized formation of sugar degradation compounds. Both polysaccharides are recovered in monomeric and oligomeric form, even after enzymatic hydrolysis with xylo-oligosaccharides (XOS) accounting for more than 35% of the soluble hemicellulose (Lau and Dale, 2009). The ammonia was recycled at 99%, with residual ammonia positively contributing to the subsequent fermentation step that can be carried out with no further supplementation.

3.4. Organosolv

The organosolv process has been recently reviewed (Zhao et al., 2009). In this type of treatment the direct action of water dissolved organic solvents (such as ethanol, methanol, or acetone), usually in combination with an acid, acts together to solubilise the lignin and hydrolyse the hemicellulose fraction (Kin, 1990; Pan et al., 2005; Vázquez et al., 1992; Zhao et al., 2009). The process temperatures may vary from room temperature up to 205 °C, depending mainly on the organic solvent use. Overall economy is strongly dependend on the solvent recycling. Among the most effective processes are those based on water:ethanol blends catalysed by sulphuric acid (Pan et al., 2005, 2006). The operational temperature usually ranges between 180 °C and 200 °C. Ethanol has the advantage that it can be easely recycled by distillation. Under these conditions cellulose digestibility is high, but although hemicellulose sugars both from softwoods and hardwoods can be recovered from the water-soluble stream, about half are in oligomeric form. As a drawback, there is a significant amount of furfural produced, which implies a lower recovery of pentoses. Peracid based processes are more specific for lignin removal, but are more expansive and technologically demanding for safety reasons (Kham et al., 2005; Zhao et al., 2009).

3.5. Solid (super)acids

Superacids may be defined as acids stronger than 100% sulphuric acid (also known as Brønsted superacids), or as acids that are stronger than anhydrous aluminum trichloride (also known as Lewis superacids). Solid superacids are composed of solid media that are treated with either Brønsted or Lewis acids. Conversely to the petrochemical refinery industry, in which the solid acids are already greatly applied, there is a considerable need for scientific and technical knowledge considering the potential and use of these catalysts in a biorefinery framework. Although the use of solid superacids for hydrolysing oligosaccharides and polysaccharides has already been proposed in the 1980s (Hahn-Hägerdal et al., 1984; Kim and Lee, 1986), only recently there has been a considerable renew interest in their use, and the main focus has been on the study of the hydrolysis of pure cellulose.

Solid acids are environmental benign catalysts with respect to corrosiveness, safety and waste (Okuhara, 2002), that are better proton donors than pure sulphuric acid for hydrolysis of polysaccharides and exhibit higher selectivity towards the hydrolytic reaction. Other advantages of these catalysts are the use of lower temperatures and pressures, together with a decrease in water usage, which improves process water economy. Furthermore, particulate catalysts can be readily separated from the sugar solution for re-use without loss of activity and with lower energy consumption. The main process variable to be controlled is the water availability (Yamaguchi et al., 2009), as it influences the effective acid concentration and biomass/catalyst contact.

Examples of solid acids studied for LCM pre-treatment include niobic acid ($Nb_2O_5{\cdot}nH_2O$), H-mordenite (zeolite), Nafion NR50 (perfluorosulfonated ionomer), Amberlyst-15 (polystyrene-based cation-exchangeable resin with SO_3H), sulfonated activated-carbon, and amorphous carbon bearing SO_3H, COOH and OH (Chung et al., 1992; Onda et al., 2009; Suganuma et al., 2008), as well as other materials such as bentonite, kaolin and acid-treated alumina (Blair et al., 2009). The later materials and amorphous carbon bearing SO_3H, COOH and OH have been reported to effectively treat the solid biomass at relatively mild temperatures ($\leqslant$100 °C).

In the case of the amorphous carbon bearing SO_3H, COOH and OH, this better catalytic performance is attributed to the ability of this material to adsorb β-1,4 glucan, which does not adsorb to other solid acids (Kitano et al., 2009). The process seems to be not very selective. As to the polysaccharides, both cellulose and hemicellulose are solubilised (to mono and oligosaccharides) with close to 100% recoveries. Lignin may mainly remains insoluble (Suganuma et al., 2008; Yamaguchi et al., 2009), or also be solubilised (Blair et al., 2009), in what is probably a function of the catalyst nature. Recent studies are focussed on the use of solid acids together with ionic liquids (ILs), exploring possible synergies (Rinaldi et al., 2008; Zhang and Zhao, 2009).

3.6. Ionic liquids

ILs can be alternative to the conventional media in fractionation processes. ILs are organic salts with melting points usually below 100 °C. Their high thermal stability (Domanska and Bogel-Lukasik, 2005; Ngo et al., 2000) and negligible vapour pressure (Earle et al., 2006; Paulechka et al., 2003) allow sometimes to classify them as "green solvents". A claimed advantage of using ILs is the possibility of a complete dissolution of wood in its native form, which has opened new possibilities to fractionate, derivatise and process LCM.

Some ILs, e.g., 1-butyl-3-methylimidazolium chloride [bmim][Cl] and 1-allyl-3-methylimidazolium chloride [amim][Cl], are particularly useful to dissolve cellulose (Dadi et al., 2007). In 2002, Rogers and co-workers (Swatloski et al., 2002) demonstrated that some imidazolium ILs dissolve up to 25 wt.% of cellulose, forming the highly viscous solutions. They also suggested that dissolution occurs via breaking the extensive hydrogen bonding network of the polysaccharide by the anion of the IL. Other crucial factor reported by them is water content, which was found to considerably decrease the solubility of carbohydrate by promoting the re-aggregation of the polymer's chains through the competitive hydrogen bonding. The influence of water is particularly important in case of the further modification because the aggregation decreases accessibility, and thus the reactivity of the polymer. On the other hand, the same feature allows for the easy regeneration of the already dissolved carbohydrates forming the solution, by the simple addition of water, alcohol or acetone (Swatloski et al., 2002).

The major efforts in the dissolution of lignocellulose-based materials were focused on the employment of chloride ILs, as the chloride anion act as a strong proton acceptor in the interaction between IL and the hydroxyl groups of the carbohydrate. Nevertheless, the high melting point and viscosity of most of the chloride salts causes the processing of carbohydrates expensive and inefficient. This is the reason why the newly designed ILs exhibiting a low melting temperature, a low viscosity and a sufficient polarity, are preferably being investigated. ILs built up from carboxylate anions show relatively low viscosities and stronger (than chloride ions) hydrogen bonding basicity (Fukaya et al., 2006). Additionally, they dissolve more efficiently a considerably higher amount of cellulose at lower temperatures than chloride ILs. Nevertheless, the low thermal stability, due to the decarboxilation, is considered to be an important limitation in the employment of this class of ILs. A different example showed that 1-ethyl-3-methylimidazolium dimethylphosphate ([emim][$(MeO)_2PO_2$]) revealed a moderate ability to destroy the crystalline structure of the carbohydrate as well. It was also showed that ILs containing dialkylimidazolium cation and dicyanamide anion, are also good solvents for mono- and more bulky carbohydrates (Liu et al., 2005).

ILs have been shown as very effective in cellulose solubilisation (Ohno and Fukaya, 2009), although the solubility study of hemicellulose and lignin in ILs was reported rarely (Zavrel et al., 2009) and it is required to be investigated in detail. Nevertheless, presented examples show that there are two possible approaches for wood fractionation. The first leads to the complete dissolution followed by selective precipitation of the different components (Edye and Doherty, 2008; Fort et al., 2007; Ren et al., 2007; Sun et al., 2009). The second assumes the selective dissolution of one or more of the components as it is reported on the examples of selective dissolution of lignin and (hemi)cellulose (Lee et al., 2009; Sievers et al., 2009).

One of the main drawbacks of using ILs is the limited amount of data about their toxicity and biodegradability. Although, this field noticed a slightly progressive increase (Ranke et al., 2007), the study is still in its infancy.

3.7. Supercritical fluids

Supercritical fluid (SCF) is a compound above its critical temperature, T_c, and critical pressure, p_c, but below the pressure required to condense it into a solid (Jessop and Leitner, 1999). As the temperature increases the liquid becomes less dense due to thermal expansion and as the pressure increases the gas becomes denser. Once the densities become equal, the phase distinction between liquid and gas disappears and the critical point has been reached (Fig. 2). The most popular supercritical fluids are carbon dioxide (T_c = 31.0 °C, p_c = 73.8 bar), water (T_c = 374.0 °C, p_c = 221.0 bar) and propane (T_c = 96.7 °C, p_c = 42.5 bar) (Jessop and Leitner, 1999). Above the critical point, there is only one gas phase. Its properties takes values that are somewhat in between the values usually taken by liquids and gases, leading many authors to claim important advantages for the use of supercritical fluids in chemical processes. Additionally, supercritical fluids show tuneable properties such as partition coefficients and solubility. Small changes in temperature or pressure close to critical point can result in up to 100-fold changes in solubility, which simplifies separation.

Up to now there are only a few literature reports about the pre-treatment of LCM by SCF, mostly by supercritical water, e.g., (Miyafuji et al., 2005), or by supercritical CO_2, (Kim and Hong, 2001). Water under sub or supercritical conditions behaves very differently from water under normal pressure and temperature. Supercritical water or liquid hot water (LHW) treatments can be expected to be superior in economic efficiency, compared with ordinary acid hydrolysis and enzymatic saccharification because the feasibility of breaking chemical bonds with water are facilitated by the fact that water develops acidic characteristics at high temperatures (Schacht et al., 2008). The ionic product for water, K_w, increases with the temperature up to a maximum of 6.34×10^{-12} at 250 °C, resulting in a pH of 5.5 for water at a temperature of 220 °C. Thus hemicellulose could be completely separated from the lignocellulose and enzymatic digestibility of cellulose can be significantly increased by treating the lignocellulosic material under such conditions (Kim and Lee, 2006; Sasaki et al., 2003). Furthermore it was found that the amount of dissolved hemicellulose rises with increasing temperature and time of the treatment. However, the hydrolysed hemicellulose monomers can further react to furfural or HMF and other toxic by-products.

The use of pure supercritical CO_2 did not cause any significant change in microscopic morphology of wood (Ritter and Campbell, 1991) but it can significantly increase cellulose hydrolysis (Kim and Hong, 2001). Addition of e.g., organic acids to CO_2 allows enhancing the yield of pre-treatment. What is more, the mineral acids can be avoided and corrosiveness of acid catalysed process is significantly reduced. Furthermore, the employment of CO_2 usually reduces the temperature of the process that diminishes the xylose degradation and increases the yield of the reaction (Zheng et al., 1998). Additionally, the employment of CO_2 is also relevant in separation of the products (Persson et al., 2002a,b; Schacht et al., 2008).

Summarising, the employment of high dense (either sub or supercritical) fluids is beneficial but there is still a wide range of improvements to be achieved before these fluids will be implemented in larger scale for hemicellulose pre-treatment.

4. Looking for the ideal "pre-treatment for hemicelluloses

Each pre-treatment technology has advantages and disadvantages, and the "ideal" pre-treatment possibly will not exist. The most appropriated treatment(s) depends on the various factors, namely on the type of material, i.e., its recalcitrance. Therefore, the challenge of any lignocelluloses pre-treatment strategy is the adequate fractionation of hemicelluloses, cellulose and lignin, together with a minor degradation, in order to get maximal fermentation yields and rates.

As a general rule, optimal pre-treatment operational conditions (e.g., acid concentration, temperature, pressure, severity factor) for hemicellulose recovery from LCM hardly lead to optimal process integration for fuel ethanol fermentation from cellulose. Indeed, maximal hemicellulose recoveries are usually obtained for lower severity factors (e.g., for autohydrolysis, values of log R_0 between 3.5 and 4.0), which prevent the degradation of the hemicellulosic sugars (Carvalheiro et al., 2004b, 2009; Garrote et al., 1999a; Moura et al., 2007; Petersen et al., 2009; Vegas et al., 2008). Conversely, effective cellulose enzymatic hydrolysis is only attained after biomass pre-treatment using higher severity conditions (Boussaid et al., 1999; Söderström et al., 2002; Xu et al., 2009; Yourchisin and van Walsum, 2004). Autohydrolysis pre-tratment has been suggested to be the best trade-off for a standard pre-treatment technology for lignocellulose-based biorefineries, even though it may require a further hydrolysis step in order to convert oligosaccharides to monosaccharides (Carvalheiro et al., 2008).

4.1. Posthydrolysis of hemicellulosic oligosaccharides

Most effective hemicelluloses pre-treatments render the soluble hemicellulose, either totally or in a very significant amount, in oligomeric form (e.g., for autohydrolysis, values up to 60–80%) and since the most efficient ethanol producing microrganisms

are not able to assimilate hemicellulosic oligosaccharides, their hydrolysis is almost a compulsory requirement for upgrading the hemicellulosic sugar stream.

The posthydrolysis options for the XOS hydrolysis can be reduced to acid (Boussaid et al., 2001; Duarte et al., 2004; Garrote et al., 2001a; Shevchenko et al., 2000), or enzymatic catalysed processes (Belkacemi et al., 2002; Duarte et al., 2004; Puls et al., 1985; Vázquez et al., 2001).

The main factors affecting monosaccharide recovery in dilute-acid posthydrolysis are catalyst concentration, reaction time, and temperature. This process has been applied to hydrolysates obtained from softwoods (Boussaid et al., 2001; Shevchenko et al., 2000), hardwoods (Garrote et al., 2001a) and herbaceous materials (Allen et al., 1996; Duarte et al., 2004, 2009; Garrote et al., 2001c; Saska and Ozer, 1995). The main catalyst reported is sulphuric acid (Duarte et al., 2004, 2009; Garrote et al., 2001a,c; Saska and Ozer, 1995; Shevchenko et al., 2000). Under fully optimized posthydrolysis conditions, sugar recovery can be close, or even surpass, 100% (Duarte et al., 2004, 2009; Garrote et al., 2001a,c; Saska and Ozer, 1995; Shevchenko et al., 2000), as compared to the standard dilute-acid hydrolysis (121 °C, 4% H_2SO_4 and 60 min) which is generally used for the quantitative acid hydrolysis of oligosaccharides.

5. Hemicellulases: microbial producers, enzyme applications and gene expression

Without a profitable use of the hemicellulose fraction, bioethanol is too expensive to compete in commercial markets (Wyman, 1999). Therefore to foster the commercial production of lignocellulosic ethanol, bioconversion of the hemicelluloses into fermentable sugars is essential. The most promising method for hydrolysis of polysaccharides to monomer sugars is by use of enzymes, i.e., cellulases and hemicellulases. Moreover, hemicellulases facilitate cellulose hydrolysis by exposing the cellulose fibres, thus making them more accessible (Shallom and Shoham, 2003). Most studies on hemicellulases have focused until now on enzymes that hydrolyse xylan. Enzymes that hydrolyse mannan have been largely neglected, even though it is an abundant hemicellulose, therefore the application of mannanases for catalysing the hydrolysis of β-1,4 mannans is as important as the application of xylanases.

5.1. Xylanolysis and mannanolysis

5.1.1. Xylanases

Xylanases are a group of enzymes responsible for the hydrolysis of xylan. The main enzymes involved, which cleave the glycosidic bonds in the xylan backbone, are endo-1,4-β-xylanase (1,4-β-D-xylan xylanohydrolase; EC 3.2.1.8) and β-xylosidase (1,4-β-D-xylan xylohydrolase; EC 3.2.1.37). The activity of several accessory enzymes (cleaving the various side chains) is also essential (Gray et al., 2006; Polizeli et al., 2005; Ryabova et al., 2009). α-D-glucuronidase (EC 3.2.1.139) hydrolyses the α-1,2 bonds between the glucuronic acid residues and β-D-xylopyranosyl backbone units found in glucuronoxylan. α-L-arabinofuranosidase (EC 3.2.1.55) removes L-arabinose residues substituted at positions 2 and 3 of the β-D-xylopyranosil. Acetylxylan esterase (EC 3.1.1.72) removes the *O*-acetyl groups from positions 2 and/or 3 on the β-D-xylopyranosyl residues of acetyl xylan (Chavez et al., 2006; Polizeli et al., 2005). Ferulic acid and *p*-coumaric acid esterases (EC 3.1.1.73) hydrolyse ester bonds on xylan, liberating the respective phenolic acids linked to the arabinofuranoside residues (Chavez et al., 2006; Christov and Prior, 1993; Topakas et al., 2007; Williamson et al., 1998).

Endo-1,4-β-xylanase cleaves the glycosidic bonds in the xylan backbone, bringing about a reduction in the degree of polymerization of the substrate. Xylan is not attacked randomly, but the bonds selected for hydrolysis depend on the nature of the substrate molecule, i.e., on the chain length, the degree of branching, and the presence of substituents (Li et al., 2000). Initially, the main hydrolysis products are β-D-xylopyranosyl oligomers, but at a later stage, small molecules such as mono-, di- and trisaccharides of β-D-xylopyranosyl may be produced (Polizeli et al., 2005).

β-Xylosidase hydrolyses small XOS and xylobiose, releasing β-D-xylopyranosyl residues from the non-reducing terminus. Purified β-xylosidases usually do not hydrolyse xylan; their best substrate is xylobiose and their affinity for XOS is inversely proportional to its degree of polymerization (Polizeli et al., 2005).

These enzymes are produced by fungi, bacteria, yeast, marine algae, protozoans, snails, crustaceans, insect, seeds, etc. (Sunna and Antranikian, 1997). Filamentous fungi are particularly interesting producers of xylanases since they excrete the enzymes into the medium and their enzyme levels are much higher than those of yeasts and bacteria (Kulkarni et al., 1999b). *Aspergillus niger*, *Humicola insolens*, *Termomonospora fusca*, *Trichoderma reesei*, *T. longibrachiatum*, *T. koningii* have been used as industrial sources of commercial xylanases. Nevertheless, commercial xylanases can also be obtained from bacteria, e.g., from *Bacillus* sp. Xylanases have many commercial uses, such as in paper manufacturing, animal feed, bread-making, juice and wine industries, or xylitol production (Collins et al., 2005; Polizeli et al., 2005).

Several investigations so far have indicated that xylanases are usually inducible enzymes (Beg et al., 2001), and different carbon sources have been analysed to find their role in effecting the enzymatic levels. Xylanase biosynthesis was induced by xylan, xylose, xylobiose or several β-D-xylopyranosyl residues added to the culture medium during growth (Kulkarni et al., 1999b; Kumar et al., 2008; Polizeli et al., 2005). However, constitutive production of xylanase has also been reported (Khasin et al., 1993). Catabolite repression by glucose is a common phenomenon observed in xylanase biosynthesis (Kulkarni et al., 1999b).

5.1.2. Mannanases

The main sugar moiety of galactoglucomannans (GGM) is D-mannose, but for its complete breakdown into simple sugars, the synergistic action of endo-1,4-β-mannanases (EC 3.2.1.78) and exoacting β-mannosidases (EC 3.2.1.25) is required to cleave the polymer backbone. Additional enzymes, such as β-glucosidases (EC 3.2.1.21), α-galactosidases (EC 3.2.1.22) and acetyl mannan esterases are required to remove side chain sugars that are attached at various points on mannans (Dhawan and Kaur, 2007; Wyman, 2003).

The property of mannanolysis is widespread in the microbial world. A vast variety of bacteria, actinomycetes, yeasts and fungi are known to be mannan degraders (Puchart et al., 2004; Talbot and Sygusch, 1990). Mannanases of microbial origin have been reported to be both induced as well as constitutive enzymes and are usually being secreted extracellularly (Dhawan and Kaur, 2007). Although a number of mannanase-producing bacterial sources are available, only a few are commercially exploited as wild or recombinant strains, of these, the important ones are: *Bacillus* sp., *Streptomyces* sp., *Caldibacillus cellulovorans*, *Caldicellulosiruptor* Rt8B, *Caldocellum saccharolyticum* (Hatada et al., 2005; Morris et al., 1995; Sunna et al., 2000; Zhang et al., 2006).

5.2. Strategies for overproducing hemicellulases

In order to be cost competitive with grain-derived ethanol, the enzymes used for fuel ethanol from LCM, including hemicellulases, must become more efficient, produced at high yields and far less expensive (Gray et al., 2006; Dhawan and Kaur, 2007). Cost reduction has been achieved by a combination of enzyme engineering

and fermentation process development (Gray et al., 2006; Wong et al., 1988).

The production of enzymes by microbial cells is governed by genetic and biochemical controls including induction, catabolite repression, or end-product inhibition. The major hemicellulolytic microbial strains, isolated so far, including fungi or bacteria, produce limiting levels of one or more hydrolases. Attempts have been made to increase the enzyme levels either by genetic manipulation (Kulkarni et al., 1999b) or by co-cultivation approaches (Kumar et al., 2008). In the later, co-cultivation of *Thermoanaerobacter mathranii* strain A3M1 with the xylanase-producing *Dictyoglomus* sp. B4 in wheat straw hydrolysates was investigated to improve the ethanol production (Ahring et al., 1999; Gunter et al., 2008). Classical mutation and selection procedures have been applied to develop mutants with higher hemicellulolytic activities. Mutagenic treatments of wild strains have been carried out by altering the factors regulating the enzyme synthesis, e.g., by introducing end-product inhibition resistance. Engineering of hemicellulolytic microorganisms for hemicellulase production can also be performed through alteration of the metabolic flux by blocking undesirable pathways, typically via homologous recombination-mediated "gene knockout" and/or overexpression of genes associated with desirable pathways (Kumar et al., 2008).

Random mutagenesis has been used for strain improvement. Various physical (e.g., with ultraviolet (UV) or microwave irradiation) and chemical (e.g., applying *N*-methyl-*N'*-nitrosoguanidine (NTG), sodium azide, ethyl methane sulfonate (EMS) or colchicine) methods have been used to develop bacterial and fungal strains producing higher amounts of hemicellulases (Kanotra and Mathur, 1995; Kumar and Singh, 2001; Ray et al., 2000). For instance, *Thermomyces lanuginosus* was subjected to UV and NTG mutagenesis and the obtained mutant showed a 1.5-fold increase in xylanase production on oat spelts xylan (Kumar et al., 2009). A mutant was also obtained by consecutive mutagenesis by UV irradiation and NTG treatment using the alkali-tolerant cellulase-free xylanase producer *Pseudomonas* sp. WLI-11 (Xu et al., 2005). The UV/NTG mutagenesis of *T. lanuginosus* BS1 also resulted in xylanase-hyperproducing mutants (Chadha et al., 1999). Mutagenesis of *Fusarium oxysporum* DSM 841 enhanced the activity of xylanase and β-xylosidase enzymes by more than threefold following UV or NTG treatments (Singh et al., 1995). A high yield xylanase producing strain, *Aspergillus usamii* L336-23, was screened out from its parent strain after microwave irradiation, with a 35.7%-increase on xylanase productivity (Li et al., 2003). A strategy of mixed mutagenesis was followed using UV irradiation and ethidium bromide for development of mutant from wild type *Penicillium oxalicum* SA-8ITCC 6024, which had resulted into 1.87-fold increases in the activity of the enzyme (Dwivedi et al., 2009).

Site-directed mutagenesis has also been applied. The identification of active site residues by chemical modification, X-ray crystallographic data and site-directed mutagenesis has provided basic information regarding the structure–function correlation of the xylanases. The residues essential for substrate binding or catalysis are identified (Kulkarni et al., 1999b). Rapid developments in molecular biology techniques have made it possible for the individual amino acids to be substituted by site-specific mutagenesis, providing the technology required to redesign the protein. Protein engineering is applied towards substrate specificity, secretion, pH optima and thermal stability (Kulkarni et al., 1999b). Variants displaying increased catalytic efficiency, with different substrate and inhibitor specificities, have been obtained by site-directed mutagenesis to improve xylanases produced by *Penicillium griseofulvum* (Tison et al., 2009). Mutagenesis has been applied to change amino acids with impact in the signal peptide controlling the secretion of xylanase by *Streptomyces lividans* (Li et al., 2006). Deoxyglucose-resistant mutants of and *Cellulomonas biazotea* secreting high levels of xylanases have also been obtained (Rajoka, 2005).

Using recombinant DNA techniques the efforts have been targeted for cloning hemicellulase genes with desirable molecular properties in suitable hosts (homologous or heterologous), resulting in the overproduction of tailor-made enzymes. The fact that endo-1,4-β-xylanases are the most abundant components of xylan-degrading enzyme systems (Wong et al., 1988), together with the ease with which these enzymes can be detected during the screening of gene libraries, may explain why most of the genes isolated to date encode endoxylanases. Genes encoding other relevant enzymes have also been cloned, e.g., genes from *Aureobasidium pullulans* encoding α-glucuronidase (De Wet et al., 2006) and α-arabinofuranosidase (De Wet et al., 2008) have been expressed in *Saccharomyces cerevisiae*.

A large number of xylanase genes have been isolated from different microbial genera and expressed in *Escherichia coli* (Coughlan and Hazlewood, 1993; Kulkarni et al., 1999b; Polizeli et al., 2005). The expression in *E. coli* is generally found to be lower than the parent organism. In bacteria, xylanases are not only produced at lower activity levels than in fungi, but are also restricted to the intracellular or periplasmic fractions. Furthermore, enzymes expressed in bacteria are not subjected to post-translation modifications, such as glycosylation (Kulkarni et al., 1999b; Polizeli et al., 2005). Glycosylation, a common phenomenon among many eukaryotic xylanases (Wong et al., 1988), has been implicated in the stabilization of glycanases against extreme environments (Kulkarni et al., 1999b). The expression of extracellular xylanases in *E. coli* recombinants that express genes from alkalophilic *Aeromonas* and *Bacillus* species and alkalophilic and thermophilic *Bacillus* species as also been reported (Horikoshi, 1996; Kulkarni et al., 1999a). The heterologous expression of the gene *xyn* A, encoding an endoxylanase from *Bacillus*, in the yeast *S. cerevisiae*, has also been described (Nuyens et al., 2001). Besides the expression of bacterial hemicellulases, either in bacteria and yeast, fungal xylanases have also been heterologously expressed either in bacteria, yeast or filamentous fungi, as recently reviewed elsewere (Ahmed et al., 2009). For example, endoxylanase genes from *T. reesei* and *Aspergillus kawachii* have been cloned and expressed in *S. cerevisiae* (Dalboge, 1997). The cloning and expression of xylanases has been mainly carried out in yeast and bacteria such as *Saccharomyces*, *Pichia*, *Kluyveromyces*, *Lactobacillus*, *E. coli* and *Bacillus subtilis* (Cho et al., 1995; Hahn-Hägerdal et al., 2005; Kulkarni et al., 1999b).

It is well known that higher expression levels are usually obtained in the homologous host system. Nevertheless, studies on the homologous expression of cloned xylanase genes are scarce (Kulkarni et al., 1999b). The level of expression of the xylanases from *Bacillus* sp. is relatively higher in a homologous host system than that in *E. coli*. In the case of *B. pumilus* IPO, the extracellular secretion of the xylanase was achieved using the homologous host *B. subtilis* (Panbangred et al., 1985).

The development of recombinant mannanases altering the enzyme properties to suit its commercial applications has been recently reviewed (Dhawan and Kaur, 2007). Mannanases from several bacteria, such as *Bacillus stearothermophilus* (Ethier et al., 1998), *C. cellulovorans* (Sunna et al., 2000) and *Caldicellulosiruptor saccharolyticus* (Morris et al., 1995), have been successfully cloned and expressed into a non-mannanase producing strain of *E. coli* (Dhawan and Kaur, 2007). Amongst host fungi it is noteworthy the 13-fold increase level of production of mannanase from *Aspergillus aculeatus* through expression in *A. niger* in comparison with the level originally obtained in *S. cerevisiae* (van Zyl et al., 2009).

6. Fuel ethanol from hemicellulosic hydrolysates

The production of fuel ethanol from hemicellulosic hydrolysates is foressen to be essential for the economical success of lignocellulosic ethanol. Moreover, this should be achieved by co-fermentation with the cellulosic fraction. Microorganisms are envisaged to contribute to the four events required for the lignocellulose-to-ethanol conversion process (production of saccharolytic enzymes, hydrolysis of the polysaccharides present in pre-treated biomass, fermentation of hexose sugars, and fermentation of pentose sugars) in one reactor, in the so-called consolidated bioprocessing (CBP) (Cardona and Sanchez, 2007; Lynd et al., 2005). CBP is gaining increasing recognition as a potential breakthrough for low-cost LCM processing. Although no natural microorganism exhibits all the features desired for CBP, a number of microorganisms, both bacteria and fungi, possess some of the desirable properties for the production of lignocellulosic ethanol.

6.1. Microorganisms in fuel ethanol production from hemicellulose

The microorganisms considered in fuel ethanol production from hemicellulose are reviewed according to:

- Natural ability to convert LCM into ethanol – ability to metabolize lignocellulose-derived sugars (polymers, oligomers and/or monomers); efficiency in ethanol production (ethanol yield and specific productivity); tolerance to lignocellulose-derived inhibitors.
- Metabolism of hemicellulose and derived oligo- and monosaccharides – microbial hydrolysis; transport of hemicellulose-derived oligo- and monosaccharides; metabolic pathways for sugar utilization and ethanol production.
- Metabolic and evolutionary engineering strategies for strain improvement – introduction of pathways for pentose utilization; overexpression of existent pathways (Pentose Phosphate Pathway); expression of efficient ethanologenic pathways, deletion of pathways conducing to by-product formation; expression of hemicellulolytic enzymes; expression of detoxifying enzymes; adaptation and evolution.
- Process integration and the role of the microorganisms – advantages, disadvantages and challenges of the utilization of different microorganisms in the processes of ethanol production from hemicellulose – simultaneous saccharification and co-fermentation (SSCF) and CBP as emerging processes.

6.2. Microbial natural ability to convert lignocellulosic materials into ethanol

Several microorganisms, including bacteria, yeasts and filamentous fungi, have been reported as able to ferment lignocellulosic hydrolysates generating ethanol. Among them *E. coli*, *Zymomonas mobilis*, *S. cerevisiae* and *Pichia stipitis* are the most relevant in the context of lignocellulosic ethanol bioprocesses. These microorganisms have different natural characteristics that can be regarded as either advantages or disadvantages in processes of ethanol production from LCM (Table 4).

E. coli presents several advantages as a biocatalyst for ethanol production, such as the ability to ferment a wide range of sugars that includes D-xylose and L-arabinose, simple genetic manipulation and prior industrial use (e.g., for the production of recombinant proteins). However, several drawbacks can be enunciated: the narrow and neutral pH range (6–8) makes bacterial fermentation susceptible to contaminations; low tolerance to lignocellulose-derived inhibitors; low ethanol tolerance; and mixed-product formation (ethanol, acetic acid, lactic acid and others), reducing ethanol yield (Dien et al., 2003).

Table 4
Characteristics of most relevant microorganisms considered for ethanol production from hemicelluloses.

Characteristics	Microorganism			
	E. coli	*Z. mobilis*	*S. cerevisiae*	*P. stipitis*
D-glucose fermentation	+	+	+	+
Other hexose utilization (D-galactose and D-mannose)	+	–	+	+
Pentose utilization (D-xylose and L-arabinose)	+	–	–	+
Direct hemicellulose utilization	–	–	–	w
Anaerobic fermentation	+	+	+	–
Mixed-product formation	+	w	w	w
High ethanol productivity (from glucose)	–	+	+	w
Ethanol tolerance	w	w	+	w
Tolerance to lignocellulose-derived inhibitors	w	w	+	w
Osmotolerance	–	–	+	w
Acidic pH range	–	–	+	w
GRAS microorganism	–	+	+	+

+, Positive; –, negative; w, weak.

The high ethanol yield and specific productivity observed for *Z. mobilis* are a consequence of anaerobic D-glucose utilization using the Entner–Doudoroff (ED) pathway. The ED pathway yields only 1 ATP per molecule of glucose in contrast to 2 ATP per molecule of glucose via the Embden–Meyerhoff–Parnas (EMP), the common glycolytic pathway which is present in both *E. coli* and *S. cerevisiae* (see below). As a consequence, *Z. mobilis* produces less cell mass. Therefore, this microorganism has higher specific ethanol productivity when compared to *S. cerevisiae*. However, *Z. mobilis* presents disadvantages similar to *E. coli* in what concerns neutral pH range and low tolerance to lignocellulose-derived inhibitors. The overall tolerance to ethanol allows fermentation to proceed up to concentration levels of 12–13.5% (v/v) but ethanol cause significant inhibition of fermentation from concentrations as low as 2% (v/v) (Osman and Ingram, 1985). Moreover, *Z. mobilis* has a narrow substrate range, lacking the ability to utilize all main sugars from lignocellulose except D-glucose.

The yeast *S. cerevisiae* is the most commonly used microorganism in traditional industrial fermentations, including current sucrose-, starch- and cellulose-based bioethanol production. *S. cerevisiae* is also generally recognized as safe (GRAS) and can ferment efficiently simple hexose sugars, such as D-glucose, D-mannose and D-galactose, and disaccharides like sucrose and maltose, reaching ethanol concentrations as high as 20% (v/v). Moreover, *S. cerevisiae* has a relatively good tolerance to lignocellulose-derived inhibitors and high osmotic pressure. Also, the fermentation rate is not significantly reduced at ethanol concentrations below 10% (v/v) (Casey and Ingledew, 1986). The major inconvenience to the use of *S. cerevisiae* for lignocellulosic fermentation is its lack of natural ability to utilize the pentose sugars D-xylose and L-arabinose.

Contrary to *S. cerevisiae*, the yeast *P. stipitis* is able to metabolize the main hemicellulose sugar monomers and efficiently ferments xylose to ethanol. Therefore it receives special attention when considering hemicellulose conversion to ethanol. *P. stipitis*, its anamorph *Candida shehatae*, and *Pachysolen tanophilus* efficiently ferment xylose but only under oxygen-limited conditions (Ligthelm et al., 1988). Also, these yeasts are less tolerant to pH, ethanol and hydrolysate inhibitors when compared to *S. cerevisiae* (Hahn-Hägerdal et al., 1994). *P. stipitis* is also described as able to produce hemicellulolytic enzymes (Jeffries et al., 2007; Ozcan et al., 1991).

There are other microorganisms harbouring specific characteristics of interest for the production of lignocellulosic ethanol. Several filamentous fungi are the most efficient in hemicellulose degradation, being the source of most commercial enzymes used in the production of lignocellulosic ethanol. Some can convert hemicellulose and derived oligosaccharides and monosaccharides to ethanol (Singh et al., 1992). However, filamentous fungi produce ethanol at low rates and have limited tolerance to this alcohol (Singh et al., 1992). In another perspective, some yeasts, like *Candida* (*Pichia*) *guilliermondii*, *D. hansenii*, are able of efficient glucose fermentation to ethanol and xylose fermentation to xylitol in non-detoxified hemicellulose hydrolysates (Barbosa et al., 1988; Carvalheiro et al., 2005; Mussatto and Roberto, 2004). The inability to produce ethanol, as major end-metabolite, from xylose is a major drawback in the use of these yeasts for hemicellulosic ethanol. Finally, most bacteria have a broad substrate range but ethanol is rarely the single product of their metabolism. Other enteric bacteria (*Klebsiella oxytoca*, *Erwinia chrysanthemi*) were considered for ethanol production, having similar advantages and disadvantages of *E. coli*. Some bacteria like *Klebsiella*, *Bacillus*, *Clostridium*, *Thermotoga*, are able to produce cellulases and hemicellulases. Extreme thermophilic bacteria, besides the capacity of producing hydrolytic enzymes, have optimal growing temperatures above 70 °C (Blumer-Schuette et al., 2008). These bacteria are also candidate as suitable cell factories under a complete CBP approach since the distillation process could be coupled to enzyme production, hydrolysis and fermentation. However, most bacteria are strongly inhibited by ethanol, hydrolysates and low pH.

6.3. Microbial metabolism of hemicellulose and derived monomers

6.3.1. Hydrolysis of hemicelluloses

As mentioned before, some microorganisms are able to degrade cellulose and hemicellulose to oligosaccharides and monosaccharides. However, that is not a characteristic of the most promising ethanol producers (Table 4). In natural environments, hydrolysis of hemicellulose is performed by a variety of enzymes that work synergistically and, in some cases, organized in complexes. Microorganisms can be divided in three groups according to their strategies to hydrolyse hemicellulose (Shallom and Shoham, 2003):

- Complete hydrolysis to monosaccharides and disaccharides by several and synergic extracellular hemicellulases. Several fungi, like *Fusarium*, *Thrichoderma* and *Aspergillus* species, adopt this strategy.
- Partial extracellular hydrolysis to oligosaccharides further hydrolysed by cell-associated or intracellular hemicellulases. Bacteria like *Bacilli* perform this type of hydrolysis, which has advantages regarding sugar competition against non-hemicellulolytic microorganisms.
- Hydrolysis by cellulosomes, extracellular cell-associated multienzyme complexes, harbouring cellulases and hemicellulases. This structure is considered a versatile extracellular organelle whose functions can be tailored by incorporating different dockerin-containing subunits, as described for the thermophilic anaerobic bacteria *Clostridium thermocellum* (Demain et al., 2005).

Among the microorganisms considered for ethanol production from hemicelluloses, *P. stipitis* is the only described as harbouring xylanases (Basaran et al., 2001; Jeffries et al., 2007; Ozcan et al., 1991). In addition, a mannosidase was identified in its genome (Jeffries et al., 2007).

6.3.2. Sugar transport

After hemicellulose hydrolysis, monosaccharides, disaccharides and oligosaccharides need to be transported across the cell membrane. Transport systems for a specified sugar depend on the microorganism and the surrounding conditions, including growth substrate, sugar concentration, oxygen availability, temperature and pH. It has often been found that more than one transporter for the same sugar may operate in simultaneous. Two classes of mediated transport systems are common for microbial sugar uptake: facilitated diffusion, which does not require metabolic energy but only a concentration gradient; and active transport, which requires energy: as membrane potential (e.g., proton-symporters); as ATP (ATP-binding cassette – ABC-transporters); or as phosphoenolpyruvate (PEP) (PEP-transferase systems – PTS-transporters). Hemicellulose-derived oligo- and disaccharides, especially XOS, have been reported to be taken up by some bacteria – Sodium-XOS symporters (Qian et al., 2003); PTS-XOS symporters (Ohara et al., 2006); and XOS ABC-transporters (Tsujibo et al., 2004). Interestingly, in the extremophilic bacteria *Thermotoga maritima*, archeal homologues were assigned to ABC-transporters responsible for the uptake of several hemicellulose-derived oligosaccharides, including manno-oligosaccharides (Nanavati et al., 2006). Arabino-oligosaccharides were suggested to be taken up by ABC-transporters in *B. subtilis* (Sá-Nogueira et al., 1997).

In the yeast-like fungi *Aureobasidium pullulans*, hemicelluloses-derived disaccharides, xylobiose, and mannobiose, were reported to be taken up by energy-dependent permeases, which are part of xylanolytic and mannanolytic systems (Lubomir and Peter, 1998). Also, a permease for XOS was reported in *Cryptococcus albidus* (Krátký and Biely, 1980).

As for oligosaccharides, bacteria generally employ active transporters for the uptake of hemicelluloses-derived monosaccarides. Transport of glucose, mannose and galactose is mainly performed by PTS-systems in bacteria. In *E. coli*, D-xylose is taken up by two specific transport systems, a high-affinity ABC-xylose transporter and a low affinity proton-symporter (Lam et al., 1980; Sumiya et al., 1995). However, the efficiency of xylose utilization in this organism is suboptimal due to these energetic requirements for xylose transport (Khankal et al., 2008). *E. coli* has the same set of specific transporters for L-arabinose, a high-affinity ABC-arabinose transporter and a low affinity arabinose-symporter (Kolodrubetz and Schleif, 1981; Novotny and Englesberg, 1966) with the same consequences.

Z. mobilis holds a high-capacity facilitated diffusion transport system for glucose (Dimarco and Romano, 1985; Parker et al., 1995; Weisser et al., 1995). D-mannose, D-xylose and D-galactose, by this order of preference, are also substrates of the glucose transporter (Weisser et al., 1996).

S. cerevisiae was extensively studied with respect to D-glucose transport (Reifenberger et al., 1997), where hexose transporters (Hxts) are all facilitators. In other yeasts, low-affinity/high-capacity passive (facilitated diffusion) transporters are present together with high-affinity/low-capacity active transport systems (sugar-proton symporters) (Gárdonyi et al., 2003b; Nobre et al., 1999; Spencer-Martins, 1994; Stambuk et al., 2003). In regard to the transport of hemicellulose monomers, *P. stipitis* (Does and Bisson, 1989; Kilian and van Uden, 1988; Weierstall et al., 1999) and *Candida shehatae* (Lucas and van Uden, 1986) have been the most extensively studied yeasts. In general, D-glucose, D-mannose and D-xylose share the same, unspecific, transporters and the active transport systems are repressed by both D-glucose and high substrate concentrations. *P. stipitis* appears to have at least two proton-symporters (Kilian and van Uden, 1988) and produces also facilitators accepting both D-glucose and D-xylose and encoded by the *SUT1–SUT3* genes (Weierstall et al., 1999).

SUT1 is strongly induced by glucose and independent of the oxygen supply, whereas *SUT2* and *SUT3* are almost identical, constitutive and only expressed under aerobic conditions. More recently, two genes encoding D-glucose/D-xylose transporters were identified in *Candida intermedia* (Leandro et al., 2006), a facilitated diffusion (*GXF1*) and a sugar-proton symporter (*GXS1*). Filamentous fungi, seems to harbour similar transport systems for hexoses and xylose (Scarborough, 1973; Torres et al., 1996; vanKuyk et al., 2004). Recently, a putative specific D-xylose permease was identified in *T. reesei* (*Hypocrea jecorina*) (Saloheimo et al., 2007).

High-affinity L-arabinose proton-symporters are common in several non-*Saccharomyces* yeasts, but a second and low-affinity transport system is apparently restricted to very few L-arabinose-utilizing yeasts (Fonseca et al., 2007a; Knoshaug et al., 2009).

Even though not metabolized by wild-type strains of *S. cerevisiae*, D-xylose and L-arabinose are taken up through non-specific hexose transporters. D-xylose uptake is mainly carried out by the high- or intermediate-affinity transporters Hxt4p, Hxt5p, Hxt7p and Gal2p (Hamacher et al., 2002; Sedlak and Ho, 2004), although the affinity for xylose is about 100-fold lower than for D-glucose (Kötter and Ciriacy, 1993; Kotyk, 1967; Lee et al., 2002). Both D-xylose and L-arabinose can hardly be taken up by the D-galactose transporter Gal2p (Cirillo, 1968).

6.3.3. Catabolism of hemicelluloses-derived monosaccharides

The hemicellulose-derived hexoses enter in the upper part of glycolysis (in the case of *E. coli* and *S. cerevisiae*) through transport-dependent or independent phosphorylation followed by: isomerisation in the case of D-mannose (through mannose-6P isomerase-PMI); the Leloir Pathway for D-galactose (Fig. 3).

The catabolism of hemicellulose-derived pentoses, L-arabinose and D-xylose, is closely related, sharing common intermediates (Fig. 3). Both D-xylose and L-arabinose are metabolized through the Pentose Phosphate Pathway (PPP). These pentoses can be converted into D-xylulose 5-phosphate, the PPP intermediate, through different pathways (Fig. 3): those, usually attributed to bacteria, starting with an isomerisation step followed by a phosphorylation reaction (and additional epimerization, in the case of L-arabinose catabolism); and pathways generally ascribed to fungi, which follow redox reactions and use NAD(P)(H) as cofactors.

Most bacteria utilize the xylose isomerase pathway in D-xylose catabolism (Fig. 3). Bacterial D-xylose isomerases have an optimal temperature in the range between 50 °C and 90 °C and an optimal pH of 7–9 (Chen, 1980). The presence of D-xylose isomerase was also reported in eukaryotes, namely in some yeasts (Tomoyeda and Horitsu, 1964; Vongsuvanlert and Tani, 1988) and filamentous fungi (Banerjee et al., 1994; Harhangi et al., 2003; Madhavan et al., 2009). Eukaryotic xylose isomerases display optimal activity at

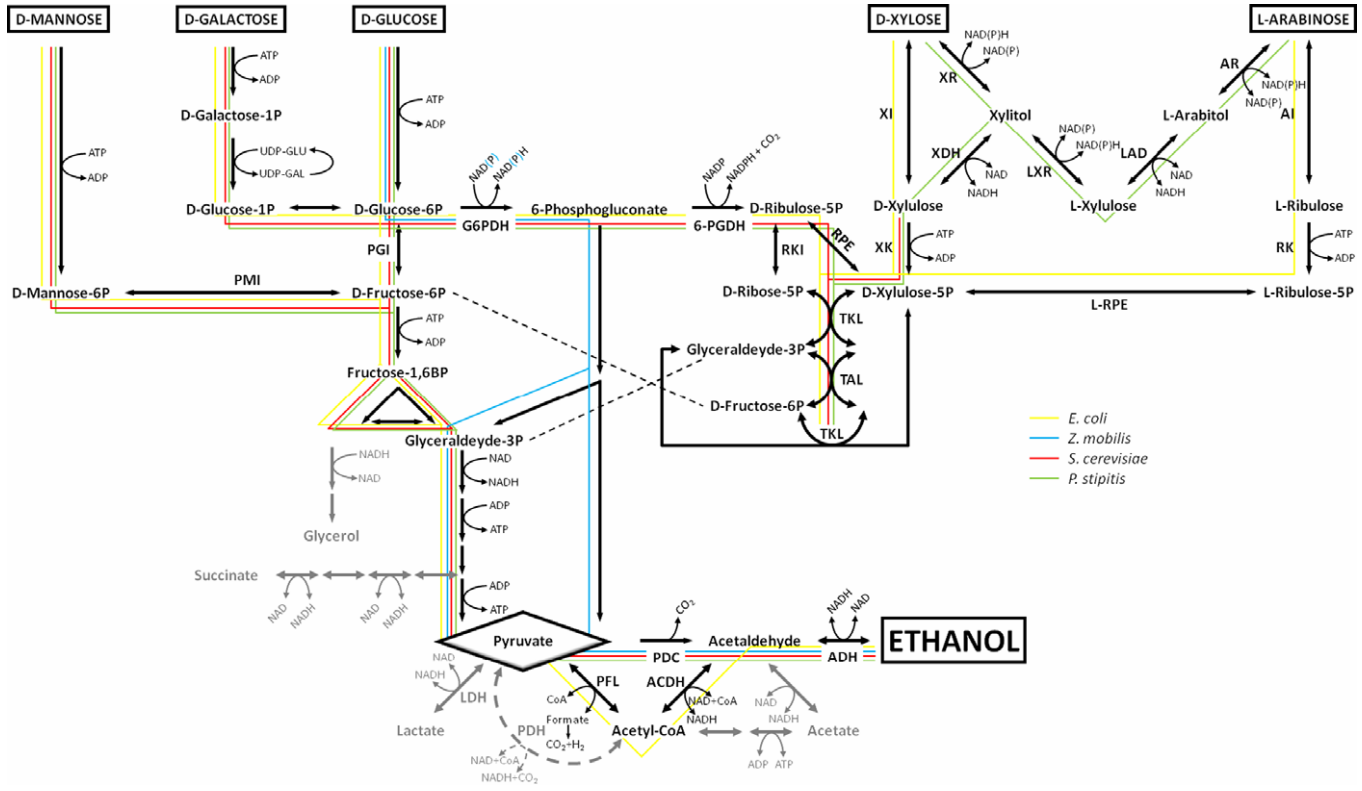


Fig. 3. Fermentative pathways of hemicellulose-derived monosaccharidesin *E. coli*, *Z. mobilis*, *S. cerevisiae* and *P. stipitis*. Natural traits are represented for each microorganism. *E. coli*, *S. cerevisiae* and *P. stipitis* use glycolysis (or EMP pathway) and pentose phosphate pathway (PPP) to convert monosaccharides into pyruvate (D-fructose-6P and glyceraldeyde-3P are common intermediates between glycolysis and PPP). *Z. mobilis* utilize the Entner–Doudoroff (ED) pathway to convert glucose into pyruvate. In *Z. mobilis*, *S. cerevisiae* and *P. stipitis*, pyruvate is converted to ethanol via PDC/ADH, while in *E. coli*, acetyl-CoA is an intermediate. In *E. coli*, *S. cerevisiae* and *P. stipitis*, D-galactose is metabolized through the Leloir pathway and, as D-mannose, is converted into a glycolysis intermediate. *E. coli* and *P. stipitis* utilize pentoses (D-xylose and L-arabinose) through different pathways: through isomerases in the bacteria and reductases/dehydrogenases in the yeast. *Abbreviations:* PGI, glucose-6P isomerase (EC 5.3.1.9); G6PDH, glucose-6P dehydrogenase (EC 1.1.1.49); 6-PGDH, 6-phosphogluconate dehydrogenase (EC 1.1.1.44); RPE, D-ribulose-5P 3-epimerase (EC 5.1.3.1); RKI, D-ribose-5P ketol-isomerase (EC 5.3.1.6); TKL, transketolase (EC 2.2.1.1); TAL, transaldolase (EC 2.2.1.2); XI, D-xylose isomerase (EC 5.3.1.5); XR, D-xylose reductase (EC 1.1.1.21); XDH, xylitol dehydrogenase (EC 1.1.1.9); XK, xylulokinase (EC 2.7.1.17); AI, L-arabinose isomerase (EC 5.3.1.4); RK, L-ribulokinase (EC 2.7.1.16); L-RPE, L-ribulose-5P 4-epimerase (EC 5.1.3.4); AR, L-arabinose reductase (EC 1.1.1.21); LAD, L-arabitol 4-dehydrogenase (EC 1.1.1.12); LXR, L-xylulose reductase (EC 1.1.1.10); PMI, mannose-6P isomerase (EC 5.3.1.8); LDH, lactate dehydrogenase (EC 1.1.1.27); PFL, pyruvate formate lyase (EC 2.3.1.54); PDH, pyruvate dehydrogenase (acetyl-transferring) (EC 1.2.4.1); ACDH, acetaldehyde dehydrogenase (acetylating) (EC 1.2.1.10); PDC, pyruvate decarboxylase (EC 4.1.1.1); ADH, alcohol dehydrogenase (EC 1.1.1.1)

30–40 °C and may act together with XR and XDH (Banerjee et al., 1994).

The first enzyme in the D-xylose catabolic pathway of yeasts and filamentous fungi is D-xylose reductase (XR), which converts D-xylose to xylitol (Fig. 3). Xylitol is then oxidized to D-xylulose by xylitol dehydrogenase (XDH) (Chiang and Knight, 1959; Rizzi et al., 1988) (Fig. 3). Both enzymes were identified and/or characterised in several yeasts (Gírio et al., 1994; Rizzi et al., 1988; Yablochkova et al., 2003). *S. cerevisiae* carries genes encoding an unspecific aldose reductase with XR activity (Kuhn et al., 1995) and an unspecific sugar alcohol dehydrogenase with XDH activity (Richard et al., 1999), but it is generally recognized as unable to metabolize xylose to ethanol. However, D-xylulose can be metabolized by *S. cerevisiae*. For industrial purposes, it was considered to add xylose (glucose) isomerase to the fermentation medium in order to have *S. cerevisiae* producing ethanol from D-xylose (Wang et al., 1980). Recently, it was found that native strains of *S. cerevisiae* can grow on xylose as a sole carbon source, albeit very slowly (Attfield and Bell, 2006).

After D-xylose conversion to D-xylulose through XR/XDH or XI, the metabolism proceeds via phosphorylation of D-xylulose, a reaction catalysed by xylulokinase (XK) (Fig. 3). XK was identified in several yeasts, including *S. cerevisiae* (Chang and Ho, 1988; Jin et al., 2002; Stevis et al., 1987). The resulting xylulose-5P is then metabolized through the PPP (Fig. 3).

Among the yeasts known to ferment D-xylose, *P. stipitis*, *C. shehatae* and *Pachysolen tannophilus* were pointed out as the most efficient D-xylose fermenters (du Preez and van der Walt, 1983; Maleszka and Schneider, 1982; Toivola et al., 1984). However, the specific ethanol productivity either from glucose or xylose of D-xylose-fermenting yeasts is 20% or less than the values obtained for *S. cerevisiae* on D-glucose (du Preez et al., 1989; Ligthelm et al., 1988).

The performance of pentose-fermenting yeasts depends on a large number of factors such as temperature, pH, aeration, nutritional factors and substrate concentrations (du Preez, 1994). The aeration level is critical for growth, ethanol production and xylitol accumulation. Low and well-controlled levels of aeration are required for maximal ethanol production in *P. stipitis* and *C. shehatae* (Delgenes et al., 1986; Skoog and Hahn-Hägerdal, 1990). Xylitol accumulation tends to increase with the sugar concentration, even in *P. stipitis*, which usually produces no detectable amounts of xylitol under oxygen-limited conditions (du Preez, 1994).

The influence of aeration on xylitol formation seems to be closely related to the characteristics of XR and XDH. These enzymes were extensively characterised with respect to enzymatic activity, specificity and cofactor requirement in yeasts grown under different experimental conditions. In some yeasts, like *D. hansenii* and *C. utilis*, XR is strictly NADPH-dependent and XDH is NAD^+-linked (Bruinenberg et al., 1984b; Gírio et al., 1994), while in others, like *P. stipitis*, *C. shehatae* and *P. tannophilus*, XR has dual co-factor specificity, i.e., it uses either NADPH or NADH, although NADPH is still the preferred cofactor (Bruinenberg et al., 1984a; Rizzi et al., 1988; Verduyn et al., 1985). The different cofactor requirement of XR and XDH (NADPH and NAD^+, respectively) leads to $NADP^+$ and NADH accumulation. In addition, the absence of transhydrogenase in yeast prevents cofactor interconversion (Bruinenberg et al., 1985). Whereas $NADP^+$ can be reduced through recycling fructose-6P (via glucose-6P) in the oxidative PPP, NADH is mainly oxidized in the respiratory chain. Under oxygen limitation, NAD^+ is not efficiently regenerated leading to xylitol accumulation. Yeasts harbouring strictly NADPH-dependent XR, like *D. hansenii* and *Candida* (*Pichia*) *guilliermondii*, produce xylitol as the major product of xylose fermentation under oxygen-limited conditions (Gírio et al., 1994; Silva et al., 1996). Yeasts producing a XR with dual co-factor specificity can oxidize NADH to NAD^+ in this step, thereby reducing xylitol formation and allowing D-xylose fermentation to proceed under oxygen-limited conditions (Bruinenberg et al., 1983). A direct relationship between the dual cofactor dependence of XR and the ability to ferment xylose with higher efficiency was established (Bruinenberg et al., 1984a). Moreover, alternative pathways may be recruited during xylose fermentation under oxygen-limited conditions, as occurs in *P. stipitis* with the induction of the NADH-specific glutamate dehydrogenase in the glutamate decarboxylase bypass (Jeffries, 2008).

The L-arabinose catabolic pathway and involved genes were identified in bacteria, namely in *E. coli* (Englesberg, 1961; Englesberg et al., 1969), *B. subtilis* (Sá-Nogueira and de Lencastre, 1989) and *Lactobacillus plantarum* (Burma and Horecker, 1958a; Burma and Horecker, 1958b; Heath et al., 1958). The enzymes L-arabinose isomerase (AI), L-ribulokinase (RK) and L-ribulose-5P 4-epimerase (L-RPE) (Fig. 3) were characterised and the respective genes, *araA*, *araB* and *araD*, were found to be organized in an operon. An alternative L-arabinose catabolic pathway, was identified and characterised in some bacteria and it is evolutionary related to the ED pathway (Novick and Tyler, 1982; Watanabe et al., 2006).

The first enzyme in the fungal L-arabinose pathway is L-arabinose reductase (AR), which converts L-arabinose into L-arabitol (Chiang and Knight, 1961) (Fig. 3). This enzyme is usually an unspecific aldose reductase, with substrates that include D-xylose and L-arabinose (Verduyn et al., 1985) and it is present in several yeasts that are unable to grow on L-arabinose (McMillan and Boynton, 1994). L-arabitol is then oxidized to L-xylulose by L-arabitol 4-dehydrogenase (LAD) and L-xylulose is converted into xylitol by another reductase, L-xylulose reductase (LXR) (Fig. 3). Xylitol follows the pathway indicated for D-xylose metabolism (Fig. 3). The genes encoding LAD and LXR, *lad1* and *lxr1*, respectively, were identified in the filamentous fungi *H. jecorina* (Richard et al., 2001; Richard et al., 2002). More recently, *alx1*, the gene encoding the LXR from *A. monospora*, was also identified (Verho et al., 2004).

The fungal L-arabinose catabolic pathway was first described for *Penicillium chrysogenum* (Chiang and Knight, 1961) and subsequently for *A. niger* (Witteveen et al., 1989) and *H. jecorina* (Richard et al., 2002). In yeast, only recently, the catabolism of L-arabinose in yeast has been characterised, in *C. arabinofermentans* and *P. guilliermondii* (Fonseca et al., 2007a,b). The cofactor dependence of enzymes in this pathway follows the pattern observed in the redox reactions of D-xylose catabolism. Reductases (AR and LXR) utilize NADPH as cofactor, while dehydrogenases (LAD and XDH) use NAD^+. Since under oxygen limitation NADH cannot be re-oxidized in the respiratory chain, a shortage in NAD^+ occurs, which leads to L-arabitol accumulation and excretion (Fonseca et al., 2007b). However, in *P. stipitis*, the aldose reductase can use either NADPH or NADH (Verduyn et al., 1985) and the LXR described in the yeast *Ambrosiozyma monospora* is NADH-dependent (Verho et al., 2004). *C. arabinofermentans* apparently also have a LXR NADH-dependent (Fonseca et al., 2007a). This apparently determines the even lower number of yeasts able to ferment L-arabinose compared to D-xylose. A extensive survey of 116 yeasts revealed that, in L-arabinose medium, low amounts of ethanol were produced in rich medium with high L-arabinose concentration by only four species (Dien et al., 1996): *Candida auringiensis*, *Candida succiphila*, *A. monospora* and *Candida* sp. (NRRL Y-2248), later designated *Candida arabinofermentans* (Kurtzman and Dien, 1998).

Efficient ethanol producers, like *S. cerevisiae* and *Z. mobilis*, utilize different routes to convert sugars to pyruvate, glycolysis (EMP Pathway) and ED Pathway, respectively (Fig. 3). However, these microorganisms convert pyruvate into ethanol through pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) (Fig. 3). *E. coli* produces ethanol through mix-acid metabolism involving the production of acetyl-CoA as intermediate of ethanol production (Fig 3).

6.4. Metabolic and evolutionary engineering for ethanol production

Several metabolic and evolutionary engineering techniques have been applied to: increase substrate range, like in *S. cerevisiae* and *Z. mobilis*; maximize ethanol production like in *E. coli*; supply other important traits to improve lignocellulose conversion to ethanol. In general, approaches involve genetic engineering towards those specific traits, often followed by strain optimization through adaptation. Since the molecular basis for ethanol and inhibitor tolerance is not fully understood, random mutagenesis and evolutionary engineering have also been applied to improve those traits. Moreover, as a result of technological developments, systems biology approaches have been recently applied to characterise the functional genomics of microorganisms and to evaluate the impact of metabolic and evolutionary engineering strategies. This advanced characterisation (genomics, transcriptomics, proteomics, metabolomics) is already contributing to better understand physiological responses and to identify crucial targets for metabolic engineering (Bengtsson et al., 2008; Grotkjaer et al., 2005; Jeffries and Van Vleet, 2009; Karhumaa et al., 2009; Van Vleet and Jeffries, 2009).

6.4.1. E. coli

In *E. coli* the obvious and successful strategy to increase ethanol production has been the expression of the ethanologenic pathway from *Z. mobilis*, with the genes encoding pyruvate decarboxylase (PDC) and ADH II (Fig. 3) organized in a single plasmid, the PET operon (Ingram et al., 1987; Ingram and Conway, 1988), latter integrated in the chromosome (Ohta et al., 1991a). Subsequent selection of mutants with high ADH activity and disrupted fumarate reductase (for succinate production) originated KO11 strain, which produce ethanol at a yield of 95% (Ohta et al. 1991). However, this strain is unable to grow in ethanol concentrations of 3.5% (Yomano et al., 1998). Evolutionary engineering strategies were then applied during a 3-month period, by alternating selection for ethanol tolerance in liquid media and selection for increased ethanol production in solid media (Yomano et al., 1998). The resulting strain, LY01, was able to grow in ethanol concentrations of 5%. Coincidentally, this strain became more resistant to aldehydes (including HMF and furfural), organic acids and alcohol compounds found in hemicelluloses hydrolysates (Zaldivar et al., 1999, 2000; Zaldivar and Ingram, 1999). However, LY01 strain performed poorly in mineral medium compared to rich medium (Yomano et al., 1998). To avoid dependence on nutritional supplementation, a new strain was produced from SZ110 (Yomano et al., 2008), which parental strain KO11 was engineered for lactate production in mineral medium (Zhou et al., 2005). SZ110 was re-engineered from a lactate producer strain for an ethanologenic strain, LY160, using PDC and ADHs from *Z. mobilis* (Yomano et al., 2008). This strain produces 4% ethanol from 9% xylose in mineral media which is comparable to the values obtained for KO11 in rich media (Yomano et al., 2008). Another drawback in KO11 strain is the phenotypic instability after repeated batch or continuous cultivations, with loss of ethanologenicity in the absence of antibiotics. Using the same heterologous *Z. mobilis* genes, another set of engineered ethanologenic strains was produced from *E. coli* FMJ39 (K12) to improve strain stability. FMJ39 strain is unable of anaerobic growth because its lactate dehydrogenase (LDH) and pyruvate formate lyase (PFL) encoding genes have been deleted (Fig. 3); consequently, pyruvate reduction and NAD^+ regeneration are impaired. Xylose-metabolizing mutants of the FMJ39 strain were transformed with the plasmid carrying *Z. mobilis* PDC/ADH encoding genes. The strain FBR3 resulted from the direct selection for anaerobic xylose growth, since ADH is able to restore the oxidation of NADH under anaerobiosis (Dien et al., 1999). However, stability was only maintained under anaerobiosis. FBR3 ferments arabinose, glucose and xylose alone or in mixture to ethanol at 90% of the theoretical yield. The fermentation performance of FBR3 and KO11 are comparable for maximum ethanol concentration and yield, but FBR3 denoted lower productivity (Dien et al., 1999). Non-recombinant ethanologenic *E. coli* have been developed from *E. coli* K12 and *E. coli* B (Kim et al., 2007; Zhou et al., 2008). An alternative pathway based on endogenous pyruvate dehydrogenase (PDH) activity became active under anaerobiosis either by mutagenesis, strain SE2378 (Kim et al., 2007), or by overexpression under a native anaerobic inducible promoter, strain SZ420 (Zhou et al., 2008) (Fig 3). Ethanol was produced from glucose and xylose fermentation with over than 80% of the theoretical yield. Moreover, an improved mutant (KC01, derived from SZ420) obtained through a three-month evolutionary engineering process, significantly increased PDH activity, productivity and ethanol tolerance, producing 2.3% ethanol from 5% xylose (Chen et al., 2010). Sugar consumption has also been improved in *E. coli* strains. The heterologous expression of PDC/ADH from *Z. mobilis* in *E. coli* strains not only improves ethanol production but also growth rate and glycolytic flux in 30–50% during anaerobic fermentation of xylose (Tao et al., 2001). Very recently, the deletion of the gene encoding methylglyoxal synthase improved co-metabolism of glucose and xylose in the strain LY168 (Yomano et al., 2009) and accelerated the metabolism of a mixture of hemicellulose-derived monosaccharides (mannose, glucose, arabinose, xylose and galactose) to ethanol. A XOS utilization operon from *K. oxytoca*, encoding a xylobiose/cation symporter and a xylosidase, was expressed in *E. coli* KO11, leading to successful consumption of XOS containing up to six xylosyl residues (Qian et al., 2003).

6.4.2. K. oxytoca

As for recombinant *E. coli* ethanologenic strains, *K. oxytoca* M5A1 was engineered with PDC/ADH from *Z. mobilis* for ethanol production from glucose and xylose (Ohta et al., 1991b). The maximal volumetric productivity from xylose was comparable to glucose and almost twice that previously obtained with *E. coli* KO11. Stabilization was achieved by chromosomal integration of the heterologous genes (Doran et al., 1994), allowing the strain to be used in hydrolysates and in simultaneous saccharification and fermentation (SSF) processes. This strain co-ferment glucose, arabinose and xylose to ethanol, by this order of preference (Bothast et al., 1994). Of notice is the fact that *K. oxytoca* is able to naturally metabolize XOS, as mentioned above (Qian et al., 2003).

6.4.3. Z. mobilis

Contrary to *E. coli*, *Z. mobilis* is an ethanologenic bacterium and lacks the ability to metabolize hemicelluloses-derived monosaccharides, except glucose (Fig. 3). Therefore, most of the engineering strategies applied to this bacterium intended to broaden the substrate range. In an early survey the strain CP4 has shown to be the best ethanol producer from glucose (Skotnicki et al., 1981). This strain was the first engineered towards xylose utilization by the expression, on a plasmid, of the *E. coli* genes encoding for xylose isomerase (XI), xylulokinase (XK), transaldolase (TAL) and transketolase (TKL) under the control of strong constitutive promoters (Zhang et al., 1995) (Fig. 3). Ethanol yield from xylose fermentation attained 86% of the theoretical. The same approach was used to engineer the strain ATCC 39676 towards arabinose fermentation (Deanda et al., 1996). The genes from the *E. coli* operon *araBAD*, encoding L-arabinose isomerase (AI), L-ribulokinase (RK), L-ribulose-5P 4-epimerase (L-RPE), together with TAL and TKL (Fig. 3) allowed L-arabinose fermentation at high yield (96%) but low rate. This was ascribed to very low affinity of the glucose facilitator to L-arabinose. The same ATCC 39676 strain was used to express the xylose pathway, followed by successful long-term (149 d) adaptation in continuous fermentation of hemicellulose hydrolysate

containing xylose, glucose and acetic acid (Lawford et al., 1999). Finally, co-fermentation of glucose, xylose and arabinose was obtained by genomic DNA integration (AX101 strain) of the xylose and arabinose pathways (Mohagheghi et al., 2002). Co-fermentation process yield was about 84%, with preferential order in sugar utilization: glucose first, then xylose, and arabinose last.

6.4.4. S. cerevisiae

S. cerevisiae cannot hydrolyse either cellulose or hemicellulose. Over the past two decades around ethanol production from LCM some attention was given to the heterologous expression of genes encoding cellulolytic and hemicellulolytic enzymes in *S. cerevisiae*, aiming at the fermentation of these polysaccharides by a single microorganism (CBP). Cellulolytic enzymes were extensively expressed in *S. cerevisiae* (Den Haan et al., 2007; Fujita et al., 2004; Hong et al., 2003; Takada et al., 1998; Van Rensburg et al., 1996; van Rooyen et al., 2005). Xylanases were expressed on the cell surface of *S. cerevisiae* strains, leading to fermented birchwood xylan to ethanol with a yield of 0.3 g/g (Fujita et al., 2004; Katahira et al., 2004). Several other hemicellulases were successfully expressed in *S. cerevisiae* including: mannan-degrading enzymes (Setati et al., 2001; Stalbrand et al., 1995); xylanases and side chain-splitting enzymes (La Grange et al., 2001; Pérez-González et al., 1996). The hydrolytic enzymes from *T. reesei* (*H. jecorina*), α-L-arabinofuranosidase and β-xylosidase were expressed together in *S. cerevisiae* (Margolles-Clark et al., 1996), releasing L-arabinose and D-xylose, respectively. Recently, the first industrial *S. cerevisiae* strain was engineered towards the fermentation of cellobiose (Saitoh et al., 2008), but the expression of hemicellulolytic enzymes was not reported so far in the same background. An important benchmark for efficient microbial hydrolysis of lignocellulose was achieved by the expression, in the cell surface of *S. cerevisiae*, of a minicellulosome (Lilly et al., 2009). However, the expression of so many enzymes in a single microorganism may represent a metabolic burden that negatively influences the fermentation capacity (Gorgens et al., 2001). Most of the effort in lignocellulosic ethanol production with *S. cerevisiae* has been devoted to the improvement of pentose fermentation. The expression of the *P. stipitis* genes *XYL1*, encoding a xylose reductase (XR), and *XYL2*, encoding a xylitol dehydrogenase (XDH), was the first successful approach for D-xylose utilization by *S. cerevisiae* (Kötter and Ciriacy, 1993; Tantirungkij et al., 1993) (Fig. 3). The first recombinant strains produced xylitol from D-xylose rather than ethanol. It was then suggested that the endogenous xylulokinase (XK), encoded by *XKS1*, could be limiting the performance of *S. cerevisiae* on D-xylose (Fig. 3). The additional overexpression of *XKS1* in *S. cerevisiae* (Eliasson et al., 2000b; Ho et al., 1998; Johansson et al., 2001; Toivari et al., 2001) led to lower xylitol accumulation and higher ethanol yield, but the strains still exhibited low specific growth rates in D-xylose medium. Improvement of D-xylose growth and fermentation by *S. cerevisiae* was subsequently achieved working both on the D-xylose catabolic enzymes (XR, XDH and XK) and on other enzymes of yeast sugar metabolism. Expression of different levels of *XYL1* and *XYL2* revealed an "ideal" XR:XDH ratio of 0.06, resulting in higher ethanol yields and lower xylitol accumulation (Walfridsson et al., 1997). Chromosomal integration of *XYL1*, *XYL2* and *XKS1* under a strong promoter resulted in stable expression of the enzymes XR, XDH and XK (Eliasson et al., 2000b). The overexpression of *XKS1* provided contradictory results, probably due to significant variations in ATP levels (Toivari et al., 2001; Träff et al., 2001). In strains already harbouring integrated *XYL1*, *XYL2* and *XKS1*, the genes encoding the enzymes involved in the non-oxidative PPP were overexpressed – transaldolase (TAL), transketolase (TKL), D-ribose-5P ketol-isomerase (RKI) and D-ribulose-5P 3-epimerase (RPE) (Johansson and Hahn-Hägerdal, 2002) (Fig. 3). Growth on D-xylulose was improved, but not on D-xylose, suggesting a limitation at the transport or XR/XDH levels. Simultaneous overexpression of non-oxidative PPP together with *XYL1* and *XYL2* in an episomal plasmid led to more efficient growth in D-xylose (Karhumaa et al., 2005). Moreover, it appears that the heterologous genes encoding the xylulokinase (*XYL3*) and the transaldolase (*PsTAL1*) from *P. stipitis* are valid alternative targets for overexpression in *S. cerevisiae* (Jin et al., 2005). In addition, protein engineering of *P. stipitis* XR and XDH towards the modification of co-factor specificity (Watanabe et al., 2005, 2007) revealed as a promising approach to overcome the redox imbalance in D-xylose catabolism. In fact, using recombinant xylose-fermenting *S. cerevisiae* strains, site-directed mutagenesis in XR or XDH, modified the co-factor specificity towards NADH or $NADP^+$, respectively, and led to higher xylose consumption rate, higher ethanol yield and lower xylitol yield compared to control strains (Bengtsson et al., 2009; Krahulec et al., 2009; Petschacher and Nidetzky, 2008). Due to its involvement in NADPH regeneration, modulation of the oxidative PPP has also been addressed while trying to improve the performance of recombinant xylose-fermenting *S. cerevisiae* strains (Fig. 3). Deletion of *GND1*, the gene encoding the enzyme 6-phosphogluconate dehydrogenase (6-PGDH), or *ZWF1*, which encodes the first enzyme of the oxidative part of the PPP, D-glucose-6P dehydrogenase (G6PDH), did not result in real progress in terms of D-xylose fermentation (Jeppsson et al., 2002, 2003). The impairment of trehalose synthesis (Hohmann et al., 1996) and the reduction of glucose-6P isomerase (PGI) activity (Boles et al., 1993) led to an increase of 20% and 15%, respectively, on ethanol yields from D-xylulose (Eliasson et al., 2000a). This was explained by a higher intracellular accumulation of fructose-6P and fructose-1,6-bisphosphate, which are required for the induction of ethanologenic enzymes. On the other hand, in D-xylulose-fermenting cells of *S. cerevisiae* the levels of fructose-1,6-bisphosphate were approximately 10 times lower than in glucose-fermenting cells (Senac and Hahn-Hägerdal, 1990). The previous examples reveal the need of a high flux all over the catabolic pathway for efficient D-xylose fermentation. The cofactor imbalance in the "fungal" pathway could, in principle, be overcome by the introduction of the "bacterial" pathway for D-xylose utilization. The xylose isomerase from *Thermus thermophilus* (Walfridsson et al., 1996) and, more recently, a xylose isomerase from the anaerobic filamentous fungus *Piromyces* sp. (Kuyper et al., 2003) were expressed in *S. cerevisiae*. Despite the fact that at 30 °C (the temperature used in the *S. cerevisiae* fermentation) the activity of the XI from the extremophilic bacteria decreases to less than 5%, the yeast was able to produce ethanol. However, xylitol was also detected due to the presence of an endogenous unspecific aldo–keto reductase (Kuhn et al., 1995). The deletion of *GRE3*, encoding this reductase, reduced xylitol formation twofold, but none of the recombinant strains grew on xylose (Träff et al., 2001). Evolutionary adaptation, by repeated cultivation on xylose medium, of the *S. cerevisiae* strain TMB 3045, expressing *xylA* from *T. thermophilus* and overexpressing four genes encoding enzymes of the non-oxidative PPP resulted in a strain, TMB 3050, capable of aerobic xylose growth and anaerobic ethanol production at 30 °C (Karhumaa et al., 2005). The engineered *S. cerevisiae* strain expressing the XI from the mesophilic and anaerobic fungus grew very slowly on xylose (Kuyper et al., 2003). However, growth on D-xylose was improved from 0.005 h^{-1} to 0.090 h^{-1} through evolutionary engineering and further selection pressure under anaerobic continuous culture. The resulting strain showed improved D-xylose uptake (Kuyper et al., 2005). Evolutionary engineering of *S. cerevisiae* TMB 3001, in continuous culture under microaerobic conditions, resulted in two mutant subpopulations with distinct and improved characteristics for xylose utilization (Sonderegger and Sauer, 2003). One of the resulting strains was able to grow in D-xylose under anaerobiosis. Breeding of those yeast strains in a mixture with industrial

recombinant strains able to ferment xylose resulted in a strain (*S. cerevisiae* BH42) with improved xylose consumption rate and higher ethanol yield (Fonseca et al., 2004; Sonderegger et al., 2004a). Comparison of several engineered strains of *S. cerevisiae* (Sonderegger et al., 2004a) showed that the mutagenized, selected and evolved polyploidy strain TMB 3400 (Wahlbom et al., 2003) produced ethanol from xylose in comparable amounts to other industrial recombinant strains. Moreover, this strain is able to ferment several non-detoxified hemicelluloses hydrolysates (Öhgren et al., 2006; Olofsson et al., 2008b; Rudolf et al., 2008) (see below). Also, F12 strain (Sonderegger et al., 2004a) and other industrial xylose-fermenting *S. cerevisiae* strains were tested in similar conditions being able to metabolize inhibitors (Olsson et al., 2006; Tomás-Pejó et al., 2008). Efforts have been directed to improve D-xylose uptake in recombinant xylose-fermenting strains of *S. cerevisiae*. Two glucose/xylose transporters from *C. intermedia*, a facilitator (Gxf1p) and a proton-symporter (Gxs1p), have been expressed in a recombinant hxtnull/*XYL1*/*XYL2*/*XKS1* strain of *S. cerevisiae* (Leandro et al., 2006), conferring week growth in xylose. Gxf1p, which have an affinity to xylose at least three times higher than Hxts, was expressed in an efficient xylose-utilizing *S. cerevisiae* strain, improving xylose metabolism only at low sugar concentrations (Runquist et al., 2009). In fact, transport appears to be limiting at low xylose concentration only in the most efficient xylose-fermenting *S. cerevisiae* strains (Gárdonyi et al., 2003a). Sut1p from *P.stipitis* (Katahira et al., 2008) and two sugar transporters from *Arabidopsis thaliana* (Hector et al., 2008) were also expressed in a xylose-fermenting *S. cerevisiae* improving xylose consumption and ethanol production, apparently by a significant increase in xylose uptake capacity (V_{max}), suggesting that those strains were limited at the transport level. A putative specific D-xylose transporter from *T. reesei* (*H. jecorina*), *Trxlt1*, was cloned in a xylose-utilizing *S. cerevisiae* (Saloheimo et al., 2007), but the real benefits of this transporter in a good xylose-fermenting strain need to be clarified. The identification of specific xylose transporters, preferably energy-independent, is of major interest when considering glucose and xylose co-fermentation. This issue will be further discussed in the context of the lignocellulosic ethanol bioprocesses. Noteworthy, a wild strain of *S. cerevisiae* was recently found to be able to grow on D-xylose as a sole carbon source, albeit very slowly. By applying natural selection and breeding over an extended period, the strain was able to grow at 0.12 h^{-1} in D-xylose, showing improved reductase (XR) and dehydrogenase (XDH) activities (Attfield and Bell, 2006). This work opens new insights in exploring non-recombinant *S. cerevisiae* for pentose fermentation.

Metabolic engineering of *S. cerevisiae* for L-arabinose utilization has been first attempted by expressing *E. coli araBAD* with poor results (Sedlak and Ho, 2001). By replacing the *E. coli* L-arabinose isomerase (encoded by *araA*) with the one produced by *B. subtilis*, as well as overexpressing the gene encoding the relatively unspecific endogenous L-arabinose transporter *GAL2*, ethanol production from L-arabinose by *S. cerevisiae* proved to be possible (Becker and Boles, 2003). The overexpression of *araA*, *araB* and *araD* from *L. plantarum* together with native non-oxidative PPP genes, improved significantly L-arabinose fermentation by *S. cerevisiae* (Wisselink et al., 2007). The entire fungal L-arabinose catabolic pathway has been expressed in a *S. cerevisiae* strain already carrying the genes for D-xylose metabolism (Richard et al., 2003). Since the aldose reductase (encoded by *XYL1*) catalyses the reduction of both D-xylose and L-arabinose, only LAD and LXR were required to complete the pathway for L-arabinose utilization (Fig. 3). The genes *lad1* and *lxr1* from *T. reesei* (*H. jecorina*) were successfully expressed in *S. cerevisiae*, allowing weak growth on L-arabinose and the detection of traces of ethanol (Richard et al., 2001, 2002, 2003). Subsequently, the fungal *lxr1* was replaced by *ALX1* from the yeast *A. monospora*, encoding a NADH-dependent LXR (Verho et al., 2004). Despite improvement in growth, no significant amount of ethanol was produced from L-arabinose.

Pentoses-fermenting yeast *S. cerevisiae* strains were recently produced. The first strategy combined the expression of both *XYL1*/*XYL2* for D-xylose utilization and the bacterial pathway (*E. coli araB*, *araD* and *B. subtilis araA*) for L-arabinose utilization in both laboratory and industrial strains (Karhumaa et al., 2006). The presence of the unspecific aldose reductase led to reduction of L-arabinose to arabitol and its consequent accumulation. Improvement of L-arabinose utilization was achieved with the overexpression of *araA*, pushing the flux to L-ribulose through L-arabinose isomerisation (Karhumaa et al., 2006). Bacterial L-arabinose isomerase pathway was combined either with XR/XDH (from *P. stipitis*) or XI (from *Piromyces* sp.) to compare the xylose catabolic pathways in a pentose-fermenting *S. cerevisiae* (Bettiga et al., 2008). Higher pentose uptake and ethanol production was obtained when expressing the XR/XDH pathway. More recently, *S. cerevisiae* was engineered for xylose and arabinose fermentation either by the expression of the fungal redox pathways (Bettiga et al., 2009) or the isomerase pathways (Wisselink et al., 2009). In the first approach, L-arabitol produced under anaerobic conditions is considerable less than in other reports where isomerase pathaway for L-arabinose utilization was combined with XR/XDH. In those cases XR converts L-arabinose in L-arabitol, which should accomplish the reverse reaction and proceed with isomerisation in order to be consumed (Bettiga et al., 2008; Karhumaa et al., 2006). The expression of LAD/LXR allows the conversion of L-arabitol into L-xylulose and then to xylitol, reducing L-arabitol accumulation (Bettiga et al., 2009). In the second approach, XI from *Piromyces* sp. was combined with *araA*, *araB* and *araD* from *L. plantarum*. This later laboratory strain was improved in specific xylose and arabinose consumption rates through evolutionary engineering, using several cycles of consecutive batch cultivations in three media with different compositions (glucose, xylose and L-arabinose; xylose and L-arabinose; and only L-arabinose) (Wisselink et al., 2009). Among the microorganisms evaluated in lignocellulosic hydrolysates, *S. cerevisiae* was considered the least sensitive to this inhibitory environment. In order to improve fermentation in those hydrolysates, several strategies have been applied to overcome the effect of inhibitors. These approaches include the improvement of natural tolerance, controlled inhibitor concentration during fermentation (Modig et al., 2008), the overexpression of homologous (Petersson et al., 2006) or heterologous (Larsson et al., 2001) genes encoding enzymes that confer resistance towards inhibitors, mutagenesis and evolutionary engineering (Liu et al., 2009) as reviewed elsewere (Almeida et al., 2007). Industrial *S. cerevisiae* are more robust than laboratory strains as demonstrated by superior performance in lignocellulosic hydrolysate. In fact, these strains reaching higher yield and productivity due to their higher tolerance to fermentation inhibiting compounds (Almeida et al., 2007; Hahn-Hägerdal et al., 2007a; Martín and Jönsson, 2003).

6.4.5. P. stipitis

Contrary to *S. cerevisiae*, *P.stipitis* is able to naturally utilize L-arabinose and/or D-xylose and efficiently ferments xylose to ethanol, being the gene donor of the xylose catabolic pathway successfully expressed in *S. cerevisae*. Also, it has been considered for fermentation of hemicellulose hydrolysates to ethanol (Jeffries et al., 2007; Jeffries, 2008; Jeffries and Van Vleet, 2009; Marques et al., 2008; Nigam, 2001b,c; van Zyl et al., 1988). Several auxotrophic mutants with higher fermentation capacities and improved xylose utilization have been developed in order to obtained suitable *P. stipitis* strains for further hemicellulose-to-ethanol metabolic engineering (reviewed in Jeffries (2008)). *P. stipitis* is however unable to grow unaerobically and is more sensitive to

ethanol and inhibitors than *S. cerevisiae*. The *S. cerevisiae* gene that confers the ability to grow under anaerobiosis (*URA1*, encoding the dihydroorotate dehydrogenase) was successfully expressed in *P. stipitis*, allowing anaerobic fermentation of glucose to ethanol (Shi and Jeffries, 1998). In addition, the disruption of the cytochrome *c* gene increased xylose fermentation and consequently ethanol yield (Shi et al., 1999). In an evolutionary engineering approach, *P. stipitis* was adapted in hemicellulose hydrolysate containing glucose, xylose and arabinose, improving tolerance to acetic acid and pH (Nigam, 2001a). In a CBP perspective, xylan conversion to ethanol was enhanced by the heterologous expression of fungal xylanases in *P. stipitis* (Den Haan and van Zyl, 2003). The recent progress in genomic and transcriptomic characterisation of *P. stipitis* (Jeffries and Van Vleet, 2009) opens new perspectives for metabolic engineering towards efficient hemicellulose fermentation.

6.5. Process integration and the role of the microorganisms

The process of hemicellulose conversion to ethanol requires adequate deconstruction of LCM, complete hemicelluloses hydrolysis, efficient fermentation of all hemicellulose-derived sugars, and low-cost ethanol recovery. Efficient fermentation in high sugar content, usually in the form of water-insoluble solids (WIS), is essential to maximize final ethanol concentration, with a consequent cost reduction in ethanol recovery. In fact, ethanol recovery by distillation becomes economically feasible when its concentration in broth exceeds 5% (v/v) (Zacchi and Axelsson, 1989). During LCM pre-treatment hemicellulose-derived sugars are obtained, to various extents, either in the liquid fraction as mono- or oligosaccharides, or in the solid material. Therefore, the requirement for hemicellulases in the following step, enzymatic hydrolysis, will vary. The C5 liquid fraction, containing most of microbial inhibitors (acetic acid, furfural, HMF and also some phenolics) is frequently separated from the WIS and not used in the fermentation step. This will represent a significant carbon lost if it contains hemicellulose-derived sugars (mainly xylose and XOS). Consequently, lignocellulosic ethanol economy is affected due to lower ethanol yield. The utilization of microorganisms able to ferment hemicellulose-derived sugars to ethanol and, simultaneously showing high inhibitor tolerance, are therefore required. Several of the best strains obtained from metabolic and evolutionary engineering towards the conversion of hemicelluloses-derived sugars to ethanol were tested in hemicelluloses hydrolysates. The sugar consumption, ethanol production and tolerance to inhibitors are the main topics discussed. *S. cerevisiae* appears to be the least sensitive to inhibitors (Almeida et al., 2007; Delgenes et al., 1996). In fact, most of the fermentation performed with recombinant *E. coli* (Barbosa et al., 1992; Farrell et al., 2006; Fiaux et al., 2003; Lawford and Rousseau, 1991; O'Brien et al., 2004; Saha et al., 2005) and *Z. mobilis* (McMillan et al., 1999; Mohagheghi et al., 2004) or *P. stipitis* (Ferrari et al., 1992; Nigam, 2001b,c; van Zyl et al., 1988), detoxification is required prior to fermentation or fermentation performance is significantly affected (Olsson and Hahn-Hägerdal, 1996). Industrial cellulosic ethanol production has been carried out by separate hydrolysis and fermentation (SHF). However, simultaneous saccharification and fermentation (SSF) processes have been proved to be more efficient compared to SHF (Marques et al., 2008; Olsson et al., 2006; Wingren et al., 2003). Several advantages of SSF can be enumerated (reviewed in Olofsson et al. (2008a)): the major advantage is the release of end-product inhibition of cellulases (and hemicellulases) by continuous sugar removal during simultaneous fermentation, reducing enzyme load and increasing productivity; the combination of two steps in the same bioreactor reduces investment costs; also sugar recovery between hydrolysis and fermentation is virtually 100%. Moreover, detoxification and co-consumption of xylose and glucose are favoured using an SSF process, as discussed below. Some disadvantages are inevitable: in SHF enzymatic hydrolysis is often performed at higher temperatures compared to fermentation, while in SSF a compromise has to be found; microbial re-use is not possible since it is difficult to separate from lignin. In order to have maximal conversion of sugars to ethanol and minimal investment costs, the whole slurry produced in the pre-treatment step, that is the liquid fraction together with the WIS, should be fermented in an SSF process. Since this involves the fermentation of cellulose- and hemicelluloses-derived sugars, this process is often called simultaneous saccharification and co-fermentation (SSCF). However, the fermentation of whole slurry implies high concentration of WIS (sugars) and inhibitors. In these conditions, the unique properties of *S. cerevisiae* with respect to osmotolerance, inhibitor tolerance and ethanol tolerance play a crucial role (Hahn-Hägerdal et al., 2007a). Nevertheless, the operation mode may be adjusted to optimize maximal ethanol production. Instead of the traditional batch, the SSF may proceed in fed-batch mode, which has several advantages: final dry matter conversion is increased due to gradual hydrolysis of added WIS (Ballesteros et al., 2002b; Rudolf et al., 2005); gradual microbial conversion of toxic compounds alleviates inhibition of enzymes and fermentation (Taherzadeh et al., 2000). For current *S. cerevisiae* strains, SSCF in fed-batch mode is of particular relevance for the co-fermentation of xylose, together with low glucose concentrations (Öhgren et al., 2006; Olofsson et al., 2008b). As mentioned above, *S. cerevisiae* does not harbour specific xylose transporters, actually, xylose is taken up by hexose transporters with affinities of two-order of magnitude lower. Therefore, xylose co-consumption in recombinant xylose-fermenting *S. cerevisiae* strains is only possible at low glucose concentrations. On the other hand, glucose has a positive effect on xylose fermentation (Meinander and Hahn-Hagerdal, 1997), since xylose is not effectively recognized as a fermentable sugar (Jin et al., 2004; Salusjärvi et al., 2008; Souto-Maior et al., 2009), while glucose plays an important role in maintaining high glycolytic flux (Gancedo, 1998) and repression of respiratory pathway (Salusjärvi et al., 2008). Also, based on kinetics and gene expression of sugar transporters (Hxts), it was demonstrated that xylose uptake benefits from the presence of (low) glucose, which induce the expression of common transporters during SSCF (Bertilsson et al., 2008). Fed-batch SSCF can therefore be modulated to maintained glucose at low concentration all over the SSCF process (Rudolf et al., 2005). Other microorganisms were tested in SSCF, like *P. stipitis* and *Z. mobilis*, but micro-aeration and pH adjustment (Marques et al., 2008; Rudolf et al., 2008) or prior detoxification (McMillan et al., 1999), respectively, were required to perform the fermentation process. The apparent success of SSF and SSCF processes led to the utilization of thermotolerant microorganisms. The yeast *Kluyveromyces* could be an interesting possibility in the context of SS(C)F (fed-batch) processes since it can efficiently ferment at temperatures as high as 42 °C, when compared to 30–35 °C of *S. cerevisiae*. However the inhibitory effects of the whole slurry and the hydrolytic enzymes in yeast cells represent significant drawbacks (Tomás-Pejó et al., 2009a,b). Also, the optimization of SSC and SSCF processes may conduct, in a short-term perspective, to the effective development of the consolidated bioprocessing (CBP). A case study of this process is the consortium between *Clostridium thermocellum*, as enzyme producer and glucose fermenter, and *C. thermosaccharolyticum*, as a pentose fermenter (Demain et al., 2005).

However, the cellulosic ethanol is currently produced with a "modified consortium" between the enzymes from *T. reesei* (or other fungi), and *S. cerevisiae*, as the glucose fermenter, in SHF processes. The heterologous expression of cellulases and hemicellulases in traditional ethanologenic microorganisms, like *S. cerevisiae*, has been successfully accomplished. Several cellulases and

hemicellulases (see above) have been expressed in the cell surface of *S. cerevisiae* allowing the conversion of cellulose and xylan to ethanol (Den Haan et al., 2007; Katahira et al., 2004). The development of *S. cerevisiae* strains towards a CBP is reviewed elsewere (van Zyl et al., 2007). The recent report on the successful heterologous expression of a minicellulosome in the cell surface of *S. cerevisiae* (Lilly et al., 2009), represents a breakthrough for the efficient hydrolysis of lignocellulose by recombinant *S. cerevisiae* and consequently to the perspective of a CBP configuration. The construction of minicellulosomes optimized for each substrate will be a challange. However, several variants of process configuration will be considered, depending on the LCM, pre-treatment and strain characteristics. Expressing, in the ethanologenic microorganism some hydrolytic enzymes, in a mixed process configuration somehow in between SSF and CBP, may reduce enzyme loading for saccharification.

7. Conclusions

The removal of hemicellulose during the initial stage of LCM processing, is still the dominating strategy for fuel ethanol from biomass. Among the developed pre-treatment technologies, the most promissing are based on hot water or steam leading to the formation of two parallel streams: the hemicellulose-rich liquids (totally or partially hydrolysed to oligomeric and monomeric sugars) and a solid cellulose-rich stream (usually converted to ethanol using SSF process). Current research intends to optimize ethanol production through the convertion of the whole slurry using SSCF approach, or the separated upgrade of the hemicellulosic stream aiming to improve the overall process economy. Process integration through the so-called consolidated bioprocessing (CBP) aiming at the combination of four biological events is still far from reached in pratical terms, although significant advances have been reached for a recombinant *S. cerevisiae*.

Future developments are foressen on both LCM innovative pretreatment (e.g., the use of ILs) and recombinant DNA biotechnological advances. There is also a search for novel (natural) microorganisms, taking in mind the large biodiversity. Better physiological characteristics in relation to temperature, pH of the medium, wider range of substrate fermentation, toxicity resistance and adaptability to low-cost substrates are still required.

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References

Aguilar, R., Ramírez, J.A., Garrote, G., Vázquez, M., 2002. Kinetic study of the acid hydrolysis of sugar cane bagasse. J. Food Eng. 55 (4), 309–318.
Ahmed, S., Riaz, S., Jamil, A., 2009. Molecular cloning of fungal xylanases: an overview. Appl. Microbiol. Biotechnol. 84 (1), 19–35.
Ahring, B.K., Licht, D., Schmidt, A.S., Sommer, P., Thomsen, A.B., 1999. Production of ethanol from wet oxidised wheat straw by *Thermoanaerobacter mathranii*. Bioresour. Technol. 68 (1), 3–9.
Alén, R., 2000. Structure and chemical composition of wood. In: Stenius, P. (Ed.), Forest Products Chemistry. Fapet Oy, Helsinki, pp. 12–57.
Allen, S.G., Kam, L.C., Zemann, A.J., Antal, M.J., 1996. Fractionation of sugar cane with hot, compressed, liquid water. Ind. Eng. Chem. Res. 35 (8), 2709–2715.
Allen, S.G., Schulman, D., Lichwa, J., Antal, M.J., Jennings, E., Elander, R., 2001a. A comparison of aqueous and dilute-acid single-temperature pretreatment of yellow poplar sawdust. Ind. Eng. Chem. Res. 40 (10), 2352–2361.
Allen, S.G., Schulman, D., Lichwa, J., Antal, M.J., Laser, M., Lynd, L.R., 2001b. A comparison between hot liquid water and steam fractionation of corn fiber. Ind. Eng. Chem. Res. 40 (13), 2934–2941.
Almeida, J.R.M., Modig, T., Petersson, A., Hahn-Hägerdal, B., Lidén, G., Gorwa-Grauslund, M.F., 2007. Increased tolerance and conversion of inhibitors in lignocellulosic hydrolysates by *Saccharomyces cerevisiae*. J. Chem. Technol. Biotechnol. 82 (4), 340–349.
Aoyama, M., Seki, K., Saito, N., 1995. Solubilization of bamboo grass xylan by steaming treatment. Holzforschung 49 (3), 193–196.
Attfield, P.V., Bell, P.J., 2006. Use of population genetics to derive nonrecombinant *Saccharomyces cerevisiae* strains that grow using xylose as a sole carbon source. FEMS Yeast Res. 6 (6), 862–868.
Ballesteros, I., Negro, M.J., Oliva, J.M., Cabañas, A., Manzanares, P., Ballesteros, M. (2005). Ethanol production from steam-explosion pretreated wheat straw. 27th Symposium on Fuels and Chemicals, Poster 3B-34, Denver, Colorado, USA.
Ballesteros, I., Oliva, J.M., Navarro, A.A., González, A., Carrasco, J., Ballesteros, M., 2000. Effect of chip size on steam explosion pretreatment of softwood. Appl. Biochem. Biotechnol. 84–6, 97–110.
Ballesteros, I., Oliva, J.M., Negro, M.J., Manzanares, P., Ballesteros, M., 2002a. Enzymic hydrolysis of steam exploded herbaceous agricultural waste (*Brassica carinata*) at different particule sizes. Process Biochem. 38 (2), 187–192.
Ballesteros, I., Oliva, J.M., Negro, M.J., Manzanares, P., Ballesteros, M., 2002b. Ethanol production from olive oil extraction residue pretreated with hot water. Appl. Biochem. Biotechnol. 98–100, 717–732.
Banerjee, S., Archana, A., Satyanarayana, T., 1994. Xylose metabolism in a thermophilic mould *Malbranchea pulchella* var. *sulfurea* TMD-8. Curr. Microbiol. 29 (6), 349–352.
Barbosa, M.D.S., Beck, M.J., Fein, J.E., Potts, D., Ingram, L.O., 1992. Efficient fermentation of *Pinus* sp. acid hydrolysates by an ethanologenic strain of *Escherichia coli*. Appl. Environ. Microbiol. 58 (4), 1382–1384.
Barbosa, M.F.S., Medeiros, M.B., Mancilha, I.M., Schneider, H., Lee, H., 1988. Screening of yeasts for production of xylitol from D-xylose and some factors which affect xylitol yield in *Candida guilliermondii*. J. Ind. Microbiol. 3 (4), 241–251.
Basaran, P., Hang, Y.D., Basaran, N., Worobo, R.W., 2001. Cloning and heterologous expression of xylanase from *Pichia stipitis* in *Escherichia coli*. J Appl Microbiol 90 (2), 248–255.
Becker, J., Boles, E., 2003. A modified *Saccharomyces cerevisiae* strain that consumes L-arabinose and produces ethanol. Appl. Environ. Microbiol. 69 (7), 4144–4150.
Beg, Q.K., Kapoor, M., Mahajan, L., Hoondal, G.S., 2001. Microbial xylanases and their industrial applications: a review. Appl. Microbiol. Biotechnol. 56 (3–4), 326–338.
Belkacemi, K., Turcotte, G., Savoie, P., 2002. Aqueous/steam-fractionated agricultural residues as substrates for ethanol production. Ind. Eng. Chem. Res. 41 (2), 173–179.
Bengtsson, O., Hahn-Hagerdal, B., Gorwa-Grauslund, M.F., 2009. Xylose reductase from *Pichia stipitis* with altered coenzyme preference improves ethanolic xylose fermentation by recombinant *Saccharomyces cerevisiae*. Biotechnol. Biofuels 2, 9.
Bengtsson, O., Jeppsson, M., Sonderegger, M., Parachin, N.S., Sauer, U., Hahn-Hagerdal, B., Gorwa-Grauslund, M.F., 2008. Identification of common traits in improved xylose-growing *Saccharomyces cerevisiae* for inverse metabolic engineering. Yeast 25 (11), 835–847.
Bertilsson, M., Andersson, J., Lidén, G., 2008. Modeling simultaneous glucose and xylose uptake in *Saccharomyces cerevisiae* from kinetics and gene expression of sugar transporters. Bioprocess Biosyst. Eng. 31 (4), 369–377.
Bettiga, M., Bengtsson, O., Hahn-Hägerdal, B., Gorwa-Grauslund, M.F., 2009. Arabinose and xylose fermentation by recombinant *Saccharomyces cerevisiae* expressing a fungal pentose utilization pathway. Microb. Cell Fact. 8, 40.
Bettiga, M., Hahn-Hägerdal, B., Gorwa-Grauslund, M.F., 2008. Comparing the xylose reductase/xylitol dehydrogenase and xylose isomerase pathways in arabinose and xylose fermenting *Saccharomyces cerevisiae* strains. Biotechnol. Biofuels 1 (1), 16.
Bjerre, A.B., Olesen, A.B., Fernqvist, T., Ploger, A., Schmidt, A.S., 1996. Pretreatment of wheat straw using combined wet oxidation and alkaline hydrolysis resulting in convertible cellulose and hemicellulose. Biotechnol. Bioeng. 49 (5), 568–577.
Blair, R. G., Hick, S. M., Truitt, J. H., 2009. Solid acid catalyzed hydrolysis of cellulosic materials. (WO/2009/061750).
Blumer-Schuette, S.E., Kataeva, I., Westpheling, J., Adams, M.W., Kelly, R.M., 2008. Extremely thermophilic microorganisms for biomass conversion: status and prospects. Curr. Opin. Biotechnol. 19 (3), 210–217.
Boles, E., Lehnert, W., Zimmermann, F.K., 1993. The role of the NAD-dependent glutamate dehydrogenase in restoring growth on glucose of a *Saccharomyces cerevisiae* phosphoglucose isomerase mutant. Eur. J. Biochem. 217 (1), 469–477.
Bothast, R.J., Saha, B.C., Flosenzier, A.V., Ingram, L.O., 1994. Fermentation of L-arabinose, D-xylose and D-glucose by ethanologenic recombinant *Klebsiella oxytoca* strain P2. Biotechnol. Lett. 16 (4), 401–406.
Boussaid, A., Cai, Y.J., Robinson, J., Gregg, D.J., Nguyen, Q., Saddler, J.N., 2001. Sugar recovery and fermentability of hemicellulose hydrolysates from steam-exploded softwoods containing bark. Biotechnol. Prog. 17 (5), 887–892.
Boussaid, A., Robinson, J., Cai, Y., Gregg, D.J., Saddler, J.N., 1999. Fermentability of the hemicellulose-derived sugars from steam-exploded softwood (Douglas fir). Biotechnol. Bioeng. 64 (3), 284–289.
Brillouet, J.M., Joseleau, J.P., Utille, J.P., Lelievre, D., 1982. Isolation, purification, and characterization of a complex heteroxylan from industrial wheat bran. J. Agri. Food Chem. 30 (3), 488–495.
Bruinenberg, P.M., Debot, P.H.M., van Dijken, J.P., Scheffers, W.A., 1983. The role of redox balances in the anaerobic fermentation of xylose by yeasts. Eur. J. Appl. Microbiol. Biotechnol. 18 (5), 287–292.
Bruinenberg, P.M., Debot, P.H.M., van Dijken, J.P., Scheffers, W.A., 1984a. NADH-linked aldose reductase: the key to anaerobic alcoholic fermentation of xylose by yeasts. Appl. Microbiol. Biotechnol. 19 (4), 256–260.

Bruinenberg, P.M., Jonker, R., van Dijken, J.P., Scheffers, W.A., 1985. Utilization of formate as an additional energy source by glucose-limited chemostat cultures of *Candida utilis* CBS 621 and *Saccharomyces cerevisiae* CBS 8066. Evidence for the absence of transhydrogenase activity in yeasts. Arch. Microbiol. 142 (3), 302–306.

Bruinenberg, P.M., van Dijken, J.P., Scheffers, W.A., 1984b. Production and consumption of NADPH and NADH during growth of *Candida utilis* on xylose. Antonie Van Leeuwenhoek 50 (1), 81–82.

Bura, R., Bothast, R.J., Mansfield, S.D., Saddler, J.N., 2003. Optimization of SO_2-catalyzed steam pretreatment of corn fiber for ethanol production. Appl. Biochem. Biotechnol. 105, 319–335.

Burma, D.P., Horecker, B.L., 1958a. Pentose fermentation by *Lactobacillus plantarum*. 3. Ribulokinase. J. Biol. Chem. 231 (2), 1039–1051.

Burma, D.P., Horecker, B.L., 1958b. Pentose fermentation by *Lactobacillus plantarum*. 4. L-ribulose-5-phosphate 4-epimerase. J. Biol. Chem. 231 (2), 1053–1064.

Camacho, F., González-Tello, P., Jurado, E., Robles, A., 1996. Microcrystalline-cellulose hydrolyisis with concentrated sulphuric acid. J. Chem. Technol. Biotechnol. 67, 350–356.

Cara, C., Ruiz, E., Ballesteros, M., Manzanares, P., Negro, M.J., Castro, E., 2008. Production of fuel ethanol from steam-explosion pretreated olive tree pruning. Fuel 87 (6), 692–700.

Cardona, C.A., Sanchez, O.J., 2007. Fuel ethanol production: process design trends and integration opportunities. Bioresour. Technol. 98 (12), 2415–2457.

Carpita, N.C., Gibeaut, D.M., 1993. Structural models of primary-cell walls in flowering plants – consistency of molecular-structure with the physical-properties of the walls during growth. Plant J. 3 (1), 1–30.

Carrasco, F., 1989. Fundamentos del faccionamiento de la biomassa. Afinidad 46, 425–429.

Carvalheiro, F., Duarte, L.C., Girio, F.M., 2008. Hemicellulose biorefineries: a review on biomass pretreatments. J. Sci. Ind. Res. 67 (11), 849–864.

Carvalheiro, F., Duarte, L.C., Lopes, S., Parajó, J.C., Pereira, H., Gírio, F.M., 2005. Evaluation of the detoxification of brewery's spent grain hydrolysate for xylitol production by *Debaryomyces hansenii* CCMI 941. Process Biochem. 40 (3–4), 1215–1223.

Carvalheiro, F., Duarte, L.C., Medeiros, R., Gírio, F.M., 2004a. Optimization of brewery's spent grain dilute-acid hydrolysis for the production of pentose-rich culture media. Appl. Biochem. Biotechnol. 113–116 (1–3), 1059–1072.

Carvalheiro, F., Esteves, M.P., Parajó, J.C., Pereira, H., Gírio, F.M., 2004b. Production of oligosaccharides by autohydrolysis of brewery's spent grain. Bioresour. Technol. 91 (1), 93–100.

Carvalheiro, F., Silva-Fernandes, T., Duarte, L.C., Gírio, F.M., 2009. Wheat straw autohydrolysis: process optimization and products characterization. Appl. Biochem. Biotechnol. 153 (1–3), 84–93.

Casey, G.P., Ingledew, W.M., 1986. Ethanol tolerance in yeasts. Crit. Rev. Microbiol. 13 (3), 219–280.

Chadha, B.S., Jaswinder, K., Rubinder, K., Saini, H.S., Singh, S., 1999. Xylanase production by *Thermomyces lanuginosus* wild and mutant strains. World J. Microbiol. Biotechnol. 15 (2), 217–221.

Chang, S.F., Ho, N.W.Y., 1988. Cloning the yeast xylulokinase gene for the improvement of xylose fermentation. Appl. Biochem. Biotechnol. 17, 313–318.

Chavez, R., Bull, P., Eyzaguirre, J., 2006. The xylanolytic enzyme system from the genus *Penicillium*. J. Biotechnol. 123 (4), 413–433.

Chen, K., Iverson, A.G., Garza, E.A., Grayburn, W.S., Zhou, S., 2010. Metabolic evolution of non-transgenic *Escherichia coli* SZ420 for enhanced homoethanol fermentation from xylose. Biotechnol. Lett. 32 (1), 87–96.

Chen, W.P., 1980. Glucose isomerase (a review). Proc. Biochem. 15 (5), 30–35.

Chiang, C., Knight, S.G., 1959. D-xylose metabolism by cell-free extracts of *Penicillium chrysogenum*. Biochim. Biophys. Acta 35 (2), 454–463.

Chiang, C., Knight, S.G., 1961. L-arabinose metabolism by cell-free extracts of *Penicillium chrysogenum*. Biochim. Biophys. Acta 46 (2), 271–278.

Cho, K.H., Jung, K.H., Pack, M.Y., 1995. Xylanase encoded by genetically engineered *Clostridium thermocellum* gene in *Bacillus subtilis*. Biotechnol. Lett. 17 (2), 157–160.

Chornet, E., Overend, R.P., 1988. Phenomenological kinetics and reaction engineering aspects of steam/aqueous treatments. In: Proceedings of the International Workshop on Steam Explosion Techniques: Fundamentals and Industrial Applications, pp. 21–58.

Christov, L.P., Prior, B.A., 1993. Esterases of xylan-degrading microorganisms: production, properties and significance. Enzyme Microb. Technol. 15 (6), 460–475.

Chung, B., Kim, C., Chung, I., Balagopal, V., Lee, Y., 1992. On fermentability of nafion catalyzed hemicellulose hydrolyzates. Appl. Biochem. Biotechnol. 34–35 (1), 125–129.

Cirillo, V.P., 1968. Galactose transport in *Saccharomyces cerevisiae*. I. Nonmetabolized sugars as substrates and inducers of the galactose transport system. J. Bacteriol. 95 (5), 1727–1731.

Collins, T., Gerday, C., Feller, G., 2005. Xylanases, xylanase families and extremophilic xylanases. FEMS Microbiol. Rev. 29 (1), 3–23.

Conner, A.H., 1984. Kinetic modeling of hardwood prehydrolysis. Part I. Xylan removal by water prehydrolysis. Wood Fiber Sci. 16 (2), 268–277.

Conner, A.H., Lorenz, L.F., 1986. Kinetic modeling of hardwood prehydrolysis. Part III. Water and dilute acetic-acid prehydrolysis of southern red oak. Wood Fiber Sci 18 (2), 248–263.

Coughlan, M.P., Hazlewood, G.P., 1993. β-1,4-D-xylan degrading enzyme systems – biochemistry, molecular biology and applications. Biotechnol. Appl. Biochem. 17, 259–289.

Dadi, A.P., Schall, C.A., Varanasi, S., 2007. Mitigation of cellulose recalcitrance to enzymatic hydrolysis by ionic liquid pretreatment. Appl. Biochem. Biotechnol. 137, 407–421.

Dalboge, H., 1997. Expression cloning of fungal enzyme genes; a novel approach for efficient isolation of enzyme genes of industrial relevance. FEMS Microbiol. Rev. 21 (1), 29–42.

de Vries, R.P., Visser, J., 2001. *Aspergillus* enzymes involved in degradation of plant cell wall polysaccharides. Microbiol. Mol. Biol Rev. 65 (4), 497.

De Wet, B.J.M., Matthew, M.K.A., Storbeck, K.H., van Zyl, W.H., Prior, B.A., 2008. Characterization of a family 54 α-L-arabinofuranosidase from *Aureobasidium pullulans*. Appl. Microbiol. Biotechnol. 77 (5), 975–983.

De Wet, B.J.M., van Zyl, W.H., Prior, B.A., 2006. Characterization of the *Aureobasidium pullulans* α-glucuronidase expressed in *Saccharomyces cerevisiae*. Enzyme Microb. Technol. 38 (5), 649–656.

Deanda, K., Zhang, M., Eddy, C., Picataggio, S., 1996. Development of an arabinose-fermenting *Zymomonas mobilis* strain by metabolic pathway engineering. Appl. Environ. Microbiol. 62 (12), 4465–4470.

Dekker, R.F.H., Wallis, A.F.A., 1983. Enzymic saccharification of sugarcane bagasse pretreated by autohydrolysis steam explosion. Biotechnol. Bioeng. 25 (12), 3027–3048.

Delgenes, J.P., Moletta, R., Navarro, J.M., 1986. The effect of aeration on D-xylose fermentation by *Pachysolen tannophilus*, *Pichia stipitis*, *Kluyveromyces marxianus* and *Candida shehatae*. Biotechnol. Lett. 8 (12), 897–900.

Delgenes, J.P., Moletta, R., Navarro, J.M., 1996. Effects of lignocellulose degradation products on ethanol fermentations of glucose and xylose by *Saccharomyces cerevisiae*, *Zymomonas mobilis*, *Pichia stipitis*, and *Candida shehatae*. Enzyme Microb. Technol. 19 (3), 220–225.

Demain, A.L., Newcomb, M., Wu, J.H., 2005. Cellulase, clostridia, and ethanol. Microbiol. Mol. Biol. Rev. 69 (1), 124–154.

Den Haan, R., Rose, S.H., Lynd, L.R., van Zyl, W.H., 2007. Hydrolysis and fermentation of amorphous cellulose by recombinant *Saccharomyces cerevisiae*. Metab. Eng. 9 (1), 87–94.

Den Haan, R., van Zyl, W.H., 2003. Enhanced xylan degradation and utilisation by *Pichia stipitis* overproducing fungal xylanolytic enzymes. Enzyme Microb. Technol. 33 (5), 620–628.

Dhawan, S., Kaur, J., 2007. Microbial mannanases: an overview of production and applications. Crit. Rev. Biotechnol. 27 (4), 197–216.

Dien, B., Iten, L., Bothast, R., 1999. Conversion of corn fiber to ethanol by recombinant *E. coli* strain FBR3. J. Ind. Microbiol. Biotechnol. 22 (6), 575–581.

Dien, B.S., Cotta, M.A., Jeffries, T.W., 2003. Bacteria engineered for fuel ethanol production: current status. Appl. Microbiol. Biotechnol. 63 (3), 258–266.

Dien, B.S., Kurtzman, C.P., Saha, B.C., Bothast, R.J., 1996. Screening for L-arabinose fermenting yeasts. Appl. Biochem. Biotechnol. 57–58, 233–242.

Dimarco, A.A., Romano, A.H., 1985. D-glucose transport system of *Zymomonas mobilis*. Appl. Environ. Microbiol. 49 (1), 151–157.

Does, A.L., Bisson, L.F., 1989. Characterization of xylose uptake in the yeasts *Pichia heedii* and *Pichia stipitis*. Appl. Environ. Microbiol. 55 (1), 159–164.

Domanska, U., Bogel-Lukasik, R., 2005. Physicochemical properties and solubility of alkyl-(2-hydroxyethyl)-dimethylammonium bromide. J. Phys. Chem. B 109 (24), 12124–12132.

Doran, J.B., Aldrich, H.C., Ingram, L.O., 1994. Saccharification and fermentation of sugar cane bagasse by *Klebsiella oxytoca* P2 containing chromosomally integrated genes encoding the *Zymomonas mobilis* ethanol pathway. Biotechnol. Bioeng. 44 (2), 240–247.

du Preez, J.C., 1994. Process parameters and environmental factors affecting D-xylose fermentation by yeasts. Enzyme Microb. Technol. 16, 944–956.

du Preez, J.C., van der Walt, J.P., 1983. Fermentation of D-xylose to ethanol by a strain of *Candida shehatae*. Biotechnol. Lett. 5 (5), 357–362.

du Preez, J.C., van Driessel, B., Prior, B.A., 1989. Fermentation of D-xylose by *Candida shehatae* and *Pichia stipitis* at low dissolved oxygen levels. Yeast 5, 129–130.

Duarte, L.C., Carvalheiro, F., Lopes, S., Marques, S., Parajó, J.C., Gírio, F.M., 2004. Comparison of two posthydrolysis processes of brewery's spent grain autohydrolysis liquor to produce a pentose-containing culture medium. Appl. Biochem. Biotechnol. 113–116 (1–3), 1041–1058.

Duarte, L.C., Silva-Fernandes, T., Carvalheiro, F., Gírio, F.M., 2009. Dilute acid hydrolysis of wheat straw oligosaccharides. Appl. Biochem. Biotechnol. 153 (1–3), 116–126.

Dwivedi, P., Vivekanand, V., Ganguly, R., Singh, R.P., 2009. *Parthenium* sp as a plant biomass for the production of alkalitolerant xylanase from mutant *Penicillium oxalicum* SAU(E)-3.510 in submerged fermentation. Biomass Bioenerg. 33 (4), 581–588.

Earle, M.J., Esperança, J.M.S.S., Gilea, M.A., Lopes, J.N.C., Rebelo, L.P.N., Magee, J.W., Seddon, K.R., Widegren, J.A., 2006. The distillation and volatility of ionic liquids. Nature 439 (7078), 831–834.

Ebringerová, A., Hromadkova, Z., Heinze, T., 2005. Hemicellulose. Adv. Polym. Sci. 186, 1–67.

Edye, L. A., Doherty, W. O. S., 2008. Fractionation of a lignocellulosic material. PCT Int. Appl. (PIXXD2WO 2008095252), 25.

Eliasson, A., Boles, E., Johansson, B., Österberg, M., Thevelein, J.M., Spencer-Martins, I., Juhnke, H., Hahn-Hägerdal, B., 2000a. Xylulose fermentation by mutant and wild-type strains of *Zygosaccharomyces* and *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. 53 (4), 376–382.

Eliasson, A., Christensson, C., Wahlbom, C.F., Hahn-Hägerdal, B., 2000b. Anaerobic xylose fermentation by recombinant *Saccharomyces cerevisiae* carrying XYL1, XYL2, and XKS1 in mineral medium chemostat cultures. Appl. Environ. Microbiol. 66 (8), 3381–3386.

Englesberg, E., 1961. Enzymatic characterization of 17 L-arabinose negative mutants of *Escherichia coli*. J Bacteriol 81, 996–1006.

Englesberg, E., Squires, C., Meronk Jr., F., 1969. The L-arabinose operon in *Escherichia coli* B-r: a genetic demonstration of two functional states of the product of a regulator gene. Proc. Natl. Acad. Sci. USA 62 (4), 1100–1107.

Ethier, N., Talbot, G., Sygusch, J., 1998. Gene cloning, DNA sequencing, and expression of thermostable beta-mannanase from *Bacillus stearothermophilus*. Appl. Environ. Microbiol. 64 (11), 4428–4432.

Farrell, A.E., Plevin, R.J., Turner, B.T., Jones, A.D., O'Hare, M., Kammen, D.M., 2006. Ethanol can contribute to energy and environmental goals. Science 311 (5760), 506–508.

Fengel, D., Wegener, G., 1983. Wood: Chemistry, Ultrastructure, Reactions. Walter de Gruyter and Co., Berlin.

Fernández-Bolaños, J., Rodríguez, G., Gómez, E., Guillén, R., Jimenez, A., Heredia, A., Rodríguez, R., 2004. Total recovery of the waste of two-phase olive oil processing: Isolation of added-value compounds. J. Agri. Food Chem. 52 (19), 5849–5855.

Ferrari, M.D., Neirotti, E., Albornoz, C., Saucedo, E., 1992. Ethanol production from eucalyptus wood hemicellulose hydrolysate by *Pichia stipitis*. Biotechnol. Bioeng. 40 (7), 753–759.

Fiaux, J., Cakar, Z.P., Sonderegger, M., Wuthrich, K., Szyperski, T., Sauer, U., 2003. Metabolic-flux profiling of the yeasts *Saccharomyces cerevisiae* and *Pichia stipitis*. Eukaryot. Cell 2 (1), 170–180.

Fonseca, C., Romão, R., Rodrigues de Sousa, H., Hahn-Hägerdal, B., Spencer-Martins, I., 2007a. L-arabinose transport and catabolism in yeast. FEBS J. 274 (14), 3589–3600.

Fonseca, C., Santos, S., Rodrigues de Sousa, H., Spencer-Martins, I., 2004. Interbreeding recombinant *Saccharomyces cerevisiae* strains for improved xylose fermentation. Congress Book. Eleventh International Congress on Yeasts (ICY2004). Rio de Janeiro, Brazil, 15–20 August 2004, p. 71.

Fonseca, C., Spencer-Martins, I., Hahn-Hägerdal, B., 2007b. L-arabinose metabolism in *Candida arabinofermentans* PYCC 5603^T and *Pichia guilliermondii* PYCC 3012: influence of sugar and oxygen on product formation. Appl. Microbiol. Biotechnol. 75 (2), 303–310.

Fort, D.A., Remsing, R.C., Swatloski, R.P., Moyna, P., Moyna, G., Rogers, R.D., 2007. Can ionic liquids dissolve wood? Processing and analysis of lignocellulosic materials with 1-n-butyl-3-methylimidazolium chloride. Green Chem. 9 (1), 63–69.

Fujita, Y., Ito, J., Ueda, M., Fukuda, H., Kondo, A., 2004. Synergistic saccharification, and direct fermentation to ethanol, of amorphous cellulose by use of an engineered yeast strain codisplaying three types of cellulolytic enzyme. Appl. Environ. Microbiol. 70 (2), 1207–1212.

Fukaya, Y., Sugimoto, A., Ohno, H., 2006. Superior solubility of polysaccharides in low viscosity, polar, and halogen-free 1,3-dialkylimidazolium formates. Biomacromolecules 7 (12), 3295–3297.

Galbe, M., Zacchi, G., 2007. Pretreatment of lignocellulosic materials for efficient bioethanol production. Adv. Biochem. Eng./Biotechnol. 108, 41–65.

Gancedo, J.M., 1998. Yeast carbon catabolite repression. Microbiol. Mol. Biol. Rev. 62 (2), 334–361.

Gárdonyi, M., Jeppsson, M., Lidén, G., Gorwa-Grauslund, M.F., Hahn-Hägerdal, B., 2003a. Control of xylose consumption by xylose transport in recombinant *Saccharomyces cerevisiae*. Biotechnol. Bioeng. 82 (7), 818–824.

Gárdonyi, M., Österberg, M., Rodrigues, C., Spencer-Martins, I., Hahn-Hägerdal, B., 2003b. High capacity xylose transport in *Candida intermedia* PYCC 4715. FEMS Yeast Res. 3 (1), 45–52.

Garrote, G., Domínguez, H., Parajó, J.C., 1999a. Hydrothermal processing of lignocellulosic materials. Holz Roh Werkst 57 (3), 191–202.

Garrote, G., Domínguez, H., Parajó, J.C., 1999b. Mild autohydrolysis: an environmentally friendly technology for xylooligosaccharide production from wood. J. Chem. Technol. Biotechnol. 74 (11), 1101–1109.

Garrote, G., Domínguez, H., Parajó, J.C., 2001a. Generation of xylose solutions from *Eucalyptus globulus* wood by autohydrolysis–posthydrolysis processes: posthydrolysis kinetics. Bioresour. Technol. 79 (2), 155–164.

Garrote, G., Domínguez, H., Parajó, J.C., 2001b. Kinetic modelling of corncob autohydrolysis. Process Biochem. 36 (6), 571–578.

Garrote, G., Domínguez, H., Parajó, J.C., 2001c. Manufacture of xylose-based fermentation media from corncobs by posthydrolysis of autohydrolysis liquors. Appl. Biochem. Biotechnol. 95 (3), 195–207.

Garrote, G., Parajó, J.C., 2002. Non-isothermal autohydrolysis of *Eucalyptus* wood. Wood Sci. Technol. 36 (2), 111–123.

Gírio, F.M., Roseiro, J.C., Sá-Machado, P., Duarte-Reis, A.R., Amaral-Collaço, M.T., 1994. Effect of oxygen transfer rate on levels of key enzymes of xylose metabolism in *Debaryomyces hansenii*. Enzyme Microb. Technol. 16 (12), 1074–1078.

Goldstein, I.S., 1983. Acid processes for cellulose hydrolysis and their mechanisms. Wood and agricultural residues. Academic Press Inc., New York. pp. 315–328.

Gominho, J., Fernandéz, J., Pereira, H., 2001. *Cynara cardunculus* L. – a new fibre crop for pulp and paper production. Ind. Crops Prod. 13 (1), 1–10.

Gorgens, J.F., van Zyl, W.H., Knoetze, J.H., Hahn-Hägerdal, B., 2001. The metabolic burden of the PGK1 and ADH2 promoter systems for heterologous xylanase production by *Saccharomyces cerevisiae* in defined medium. Biotechnol. Bioeng. 73 (3), 238–245.

Gray, K.A., Zhao, L.S., Emptage, M., 2006. Bioethanol. Curr. Opin. Chem. Biol. 10 (2), 141–146.

Grohmann, K., Torget, R., Himmel, M., 1985. Optimization of dilute acid pretreatment of biomass. Biotechnol. Bioeng. Symp. 15, 59–80.

Grotkjaer, T., Christakopoulos, P., Nielsen, J., Olsson, L., 2005. Comparative metabolic network analysis of two xylose fermenting recombinant *Saccharomyces cerevisiae* strains. Metab. Eng. 7 (5–6), 437–444.

Gunter, E.A., Kapustina, O.M., Popeyko, O.V., Chelpanova, T.I., Efimtseva, E.A., Ovodov, Y.S., 2008. Induction of β-1,3-glucanase in callus cultures in vitro. Biochemistry–Moscow 73 (7), 826–832.

Hahn-Hägerdal, B., Jeppsson, H., Skoog, K., Prior, B.A., 1994. Biochemistry and physiology of xylose fermentation by yeasts. Enzyme Microb. Technol. 16 (11), 933–943.

Hahn-Hägerdal, B., Karhumaa, K., Fonseca, C., Spencer-Martins, I., Gorwa-Grauslund, M.F., 2007a. Towards industrial pentose-fermenting yeast strains. Appl. Microbiol. Biotechnol. 74 (5), 937–953.

Hahn-Hägerdal, B., Karhumaa, K., Jeppsson, M., Gorwa-Grauslund, M.F., 2007b. Metabolic engineering for pentose utilization in *Saccharomyces cerevisiae*. Adv. Biochem. Eng./Biotechnol. 108, 147–177.

Hahn-Hägerdal, B., Karhumaa, K., Larsson, C., Gorwa-Grauslund, M., Görgens, J., van Zyl, W., 2005. Role of cultivation media in the development of yeast strains for large scale industrial use. Microb. Cell Fact. 4 (1), 31.

Hahn-Hägerdal, B., Skoog, K., Mattiason, B., 1984. Solid superacids for hydrolyzing oligosaccharides and polysaccharides. Ann. NY Acad. Sci. 434 (December), 161–163.

Hamacher, T., Becker, J., Gárdonyi, M., Hahn-Hägerdal, B., Boles, E., 2002. Characterization of the xylose-transporting properties of yeast hexose transporters and their influence on xylose utilization. Microbiology 148, 2783–2788.

Harhangi, H.R., Akhmanova, A.S., Emmens, R., van der Drift, C., de Laat, W.T., van Dijken, J.P., Jetten, M.S., Pronk, J.T., Op den Camp, H.J., 2003. Xylose metabolism in the anaerobic fungus *Piromyces* sp. strain E2 follows the bacterial pathway. Arch. Microbiol. 180 (2), 134–141.

Hatada, Y., Takeda, N., Hirasawa, K., Ohta, Y., Usami, R., Yoshida, Y., Grant, W.D., Ito, S., Horikoshi, K., 2005. Sequence of the gene for a high-alkaline mannanase from an alkaliphilic *Bacillus* sp. strain JAMB-750, its expression in *Bacillus subtilis* and characterization of the recombinant enzyme. Extremophiles 9 (6), 497–500.

Heath, E.C., Horecker, B.L., Smyrniotis, P.Z., Takagi, Y., 1958. Pentose fermentation by *Lactobacillus plantarum*. 2. L-arabinose isomerase. J. Biol. Chem. 231 (2), 1031–1037.

Hector, R.E., Qureshi, N., Hughes, S.R., Cotta, M.A., 2008. Expression of a heterologous xylose transporter in a *Saccharomyces cerevisiae* strain engineered to utilize xylose improves aerobic xylose consumption. Appl. Microbiol. Biotechnol. 80 (4), 675–684.

Heitz, M., Capek-Menard, E., Koeberle, P.G., Gagné, J., Chornet, E., Overend, R.P., Taylor, J.D., Yu, E., 1991. Fractionation of *Populus tremuloides* at the pilot-plant scale – optimization of steam pretreatment conditions using the stake-II technology. Bioresour. Technol. 35 (1), 23–32.

Heitz, M., Carrasco, F., Rubio, M., Chauvette, G., Chornet, E., Jaulin, L., Overend, R.P., 1986. Generalized correlations for the aqueous liquefaction of lignocellulosics. Can. J. Chem. Eng. 64 (4), 647–650.

Hill, J., Nelson, E., Tilman, D., Polasky, S., Tiffany, D., 2006. Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. PNAS 103 (30), 11206–11210.

Ho, N.W., Chen, Z., Brainard, A.P., 1998. Genetically engineered *Saccharomyces* yeast capable of effective cofermentation of glucose and xylose. Appl. Environ. Microbiol. 64 (5), 1852–1859.

Hohmann, S., Bell, W., Neves, M.J., Valckx, D., Thevelein, J.M., 1996. Evidence for trehalose-6-phosphate-dependent and -independent mechanisms in the control of sugar influx into yeast glycolysis. Mol. Microbiol. 20 (5), 981–991.

Hong, J., Tamaki, H., Yamamoto, K., Kumagai, H., 2003. Cloning of a gene encoding thermostable cellobiohydrolase from *Thermoascus aurantiacus* and its expression in yeast. Appl. Microbiol. Biotechnol. 63 (1), 42–50.

Horikoshi, K., 1996. Alkaliphiles – from an industrial point of view. FEMS Microbiol. Rev. 18 (2–3), 259–270.

Huber, G.W., Iborra, S., Corma, A., 2006. Synthesis of transportation fuels from biomass: Chemistry, catalysts, and engineering. Chem. Rev. 106 (9), 4044–4098.

Ingram, L.O., Conway, T., 1988. Expression of different levels of ethanologenic enzymes from *Zymomonas mobilis* in recombinant strains of *Escherichia coli*. Appl. Environ. Microbiol. 54 (2), 397–404.

Ingram, L.O., Conway, T., Clark, D.P., Sewell, G.W., Preston, J.F., 1987. Genetic engineering of ethanol production in *Escherichia coli*. Appl. Environ. Microbiol. 53 (10), 2420–2425.

Ishii, T., 1991. Acetylation at *O*-2 of arabinofuranose residues in feruloylated arabinoxylan from bamboo shoot cell-walls. Phytochemistry 30 (7), 2317–2320.

Jeffries, T.W., 2008. Engineering the *Pichia stipitis* genome for fermentation of hemicellulose hydrolysates. In: Wall, J.D., Hardwood, C.S., Demain, A. (Eds.), Bioenergy. ASM Press, Washington, DC, pp. 37–47.

Jeffries, T.W., Grigoriev, I.V., Grimwood, J., Laplaza, J.M., Aerts, A., Salamov, A., Schmutz, J., Lindquist, E., Dehal, P., Shapiro, H., Jin, Y.S., Passoth, V., Richardson, P.M., 2007. Genome sequence of the lignocellulose-bioconverting and xylose-fermenting yeast *Pichia stipitis*. Nat. Biotechnol. 25 (3), 319–326.

Jeffries, T.W., Van Vleet, J.R., 2009. *Pichia stipitis* genomics, transcriptomics, and gene clusters. FEMS Yeast Res. 9 (6), 793–807.

Jeppsson, M., Johansson, B., Hahn-Hägerdal, B., Gorwa-Grauslund, M.F., 2002. Reduced oxidative pentose phosphate pathway flux in recombinant xylose-utilizing *Saccharomyces cerevisiae* strains improves the ethanol yield from xylose. Appl. Environ. Microbiol. 68 (4), 1604–1609.

Jeppsson, M., Johansson, B., Jensen, P.R., Hahn-Hägerdal, B., Gorwa-Grauslund, M.F., 2003. The level of glucose-6-phosphate dehydrogenase activity strongly

influences xylose fermentation and inhibitor sensitivity in recombinant *Saccharomyces cerevisiae* strains. Yeast 20 (15), 1263–1272.
Jessop, P.G., Leitner, W.E., 1999. Introduction. In: Jessop, P.G., Leitner, W.E. (Eds.), Chemical Synthesis Using Supercritical Fluids. Wiley-VCH, Weinheim, pp. 1–66.
Jin, Y.S., Alper, H., Yang, Y.T., Stephanopoulos, G., 2005. Improvement of xylose uptake and ethanol production in recombinant *Saccharomyces cerevisiae* through an inverse metabolic engineering approach. Appl. Environ. Microbiol. 71 (12), 8249–8256.
Jin, Y.S., Jones, S., Shi, N.Q., Jeffries, T.W., 2002. Molecular cloning of *XYL3* (D-xylulokinase) from *Pichia stipitis* and characterization of its physiological function. Appl. Environ. Microbiol. 68 (3), 1232–1239.
Jin, Y.S., Laplaza, J.M., Jeffries, T.W., 2004. *Saccharomyces cerevisiae* engineered for xylose metabolism exhibits a respiratory response. Appl. Environ. Microbiol. 70 (11), 6816–6825.
Johansson, B., Christensson, C., Hobley, T., Hahn-Hägerdal, B., 2001. Xylulokinase overexpression in two strains of *Saccharomyces cerevisiae* also expressing xylose reductase and xylitol dehydrogenase and its effect on fermentation of xylose and lignocellulosic hydrolysate. Appl. Environ. Microbiol. 67 (9), 4249–4255.
Johansson, B., Hahn-Hägerdal, B., 2002. The non-oxidative pentose phosphate pathway controls the fermentation rate of xylulose but not of xylose in *Saccharomyces cerevisiae* TMB3001. FEMS Yeast Res. 2 (3), 277–282.
Kabel, M.A., Carvalheiro, F., Garrote, G., Avgerinos, E., Koukios, E., Parajó, J.C., Gírio, F.M., Schols, H.A., Voragen, A.G.J., 2002. Hydrothermally treated xylan rich by-products yield different classes of xylo-oligosaccharides. Carbohydr. Polym. 50 (1), 47–56.
Kanotra, S., Mathur, R.S., 1995. Isolation and partial characterization of a mutant of *Trichoderma reesei* and its application in solid-state fermentation of Paddy straw alone or in combination with *Pleurotus Sajor* Caju. J. Env. Sci. Health Part A 30 (6), 1339–1360.
Karhumaa, K., Hahn-Hägerdal, B., Gorwa-Grauslund, M.F., 2005. Investigation of limiting metabolic steps in the utilization of xylose by recombinant *Saccharomyces cerevisiae* using metabolic engineering. Yeast 22 (5), 359–368.
Karhumaa, K., Pahlman, A.K., Hahn-Hägerdal, B., Levander, F., Gorwa-Grauslund, M.F., 2009. Proteome analysis of the xylose-fermenting mutant yeast strain TMB 3400. Yeast 26 (7), 371–382.
Karhumaa, K., Wiedemann, B., Hahn-Hägerdal, B., Boles, E., Gorwa-Grauslund, M.F., 2006. Co-utilization of L-arabinose and D-xylose by laboratory and industrial *Saccharomyces cerevisiae* strains. Microb. Cell Fact. 5, 18–28.
Katahira, S., Fujita, Y., Mizuike, A., Fukuda, H., Kondo, A., 2004. Construction of a xylan-fermenting yeast strain through codisplay of xylanolytic enzymes on the surface of xylose-utilizing *Saccharomyces cerevisiae* cells. Appl. Environ. Microbiol. 70 (9), 5407–5414.
Katahira, S., Ito, M., Takema, H., Fujita, Y., Tanino, T., Tanaka, T., Fukuda, H., Kondo, A., 2008. Improvement of ethanol productivity during xylose and glucose co-fermentation by xylose-assimilating *S. cerevisiae* via expression of glucose transporter Sut1. Enzyme Microb. Technol. 43 (2), 115–119.
Kham, L., Le Bigot, Y., Delmas, M., Avignon, G., 2005. Delignification of wheat straw using a mixture of carboxylic acids and peroxoacids. Ind. Crops Prod. 21 (1), 9–15.
Khankal, R., Chin, J.W., Cirino, P.C., 2008. Role of xylose transporters in xylitol production from engineered *Escherichia coli*. J. Biotechnol. 134 (3–4), 246–252.
Khasin, A., Alchanati, I., Shoham, Y., 1993. Purification and characterization of a thermostable xylanase from *Bacillus stearothermophilus* T-6. Appl. Environ. Microbiol. 59 (6), 1725–1730.
Kilian, S.G., van Uden, N., 1988. Transport of xylose and glucose in the xylose-fermenting yeast *Pichia stipitis*. Appl. Microbiol. Biotechnol. 27 (5–6), 545–548.
Kim, J.S., Kim, H., Lee, J.S., Lee, J.P., Park, S.C., 2008. Pretreatment characteristics of waste oak wood by ammonia percolation. Appl. Biochem. Biotechnol. 148 (1–3), 15–22.
Kim, J.S., Lee, Y.Y., Park, S.C., 2000. Pretreatment of wastepaper and pulp mill sludge by aqueous ammonia and hydrogen peroxide. Appl. Biochem. Biotechnol. 84–6, 129–139.
Kim, K.H., Hong, J., 2001. Supercritical CO_2 pretreatment of lignocellulose enhances enzymatic cellulose hydrolysis. Bioresour. Technol. 77 (2), 139–144.
Kim, S.B., Lee, Y.Y., 1986. Hydrolysis of hemicellulose by solid superacid. Biotechnol. Bioeng. Symp. 15, 81–90.
Kim, T.H., Kim, J.S., Sunwoo, C., Lee, Y.Y., 2003. Pretreatment of corn stover by aqueous ammonia. Bioresour. Technol. 90 (1), 39–47.
Kim, T.H., Lee, Y.Y., 2005. Pretreatment and fractionation of corn stover by ammonia recycle percolation process. Bioresour. Technol. 96 (18), 2007–2013.
Kim, T.H., Lee, Y.Y., 2006. Fractionation of corn stover by hot-water and aqueous ammonia treatment. Bioresour. Technol. 97 (2), 224–232.
Kim, T.H., Lee, Y.Y., Sunwoo, C., Kim, J.S., 2006. Pretreatment of corn stover by low-liquid ammonia recycle percolation process. Appl. Biochem. Biotechnol. 133 (1), 41–57.
Kim, Y., Ingram, L.O., Shanmugam, K.T., 2007. Construction of an *Escherichia coli* K-12 mutant for homoethanologenic fermentation of glucose or xylose without foreign genes. Appl. Environ. Microbiol. 73 (6), 1766–1771.
Kin, Z., 1990. The acetolysis of beech wood. Tappi J. 73 (11), 237–238.
Kitano, M., Yamaguchi, D., Suganuma, S., Nakajima, K., Kato, H., Hayashi, S., Hara, M., 2009. Adsorption enhanced hydrolysis of beta-1,4-glucan on graphene-based Amorphous Carbon bearing SO_3H, COOH, and OH groups. Langmuir 25 (9), 5068–5075.
Klinke, H.B., Ahring, B.K., Schmidt, A.S., Thomsen, A.B., 2002. Characterization of degradation products from alkaline wet oxidation of wheat straw. Bioresour. Technol. 82 (1), 15–26.
Knoshaug, E., Franden, M., Stambuk, B., Zhang, M., Singh, A., 2009. Utilization and transport of L-arabinose by non-*Saccharomyces* yeasts. Cellulose 16 (4), 729–741.
Kokta, B.V., 1991. Steam explosion pulping. In: Focher, B., Marzetti, A., Crescenzi, V. (Eds.), Steam Explosion Techniques: Fundamentals and Applications. Breach Science Publishers, Philadelphia, pp. 163–206.
Kolodrubetz, D., Schleif, R., 1981. L-arabinose transport systems in *Escherichia coli* K-12. J. Bacteriol. 148 (2), 472–479.
Kötter, P., Ciriacy, M., 1993. Xylose fermentation by *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. 38 (6), 776–783.
Kotyk, A., 1967. Mobility of the free and of the loaded monosaccharide carrier in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta 135 (1), 112–119.
Krahulec, S., Klimacek, M., Nidetzky, B., 2009. Engineering of a matched pair of xylose reductase and xylitol dehydrogenase for xylose fermentation by *Saccharomyces cerevisiae*. Biotechnol. J. 4 (5), 684–694.
Krátký, Z., Biely, P., 1980. Inducible beta-xyloside permease as a constituent of the xylan-degrading enzyme system of the yeast *Cryptococcus albidus*. Eur. J. Biochem. 112 (2), 367–373.
Kuhn, A., van Zyl, C., van Tonder, A., Prior, B.A., 1995. Purification and partial characterization of an aldo-keto reductase from *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 61 (4), 1580–1585.
Kulkarni, N., Lakshmikumaran, M., Rao, M., 1999a. Xylanase II from an alkaliphilic thermophilic *Bacillus* with a distinctly different structure from other xylanases: Evolutionary relationship to alkaliphilic xylanases. Biochem. Biophys. Res. Commun. 263 (3), 640–645.
Kulkarni, N., Shendye, A., Rao, M., 1999b. Molecular and biotechnological aspects of xylanases. FEMS Microbiol. Rev. 23 (4), 411–456.
Kumar, K.S., Manimaran, A., Permaul, K., Singh, S., 2009. Production of β-xylanase by a *Thermomyces lanuginosus* MC 134 mutant on corn cobs and its application in biobleaching of bagasse pulp. J. Biosci. Bioeng. 107 (5), 494–498.
Kumar, R., Singh, R.P., 2001. Semi-solid-state fermentation of *Eicchornia crassipes* biomass as lignocellulosic biopolymer for cellulase and β-glucosidase production by cocultivation of *Aspergillus niger* RK3 and *Trichoderma reesei* MTCC164. Appl. Biochem. Biotechnol. 96 (1–3), 71–82.
Kumar, R., Singh, S., Singh, O.V., 2008. Bioconversion of lignocellulosic biomass: biochemical and molecular perspectives. J. Ind. Microbiol. Biotechnol. 35 (5), 377–391.
Kurtzman, C.P., Dien, B.S., 1998. *Candida arabinofermentans*, a new L-arabinose fermenting yeast. Antonie Van Leeuwenhoek 74 (4), 237–243.
Kuyper, M., Harhangi, H.R., Stave, A.K., Winkler, A.A., Jetten, M.S., de Laat, W.T., den Ridder, J.J., Op den Camp, H.J., van Dijken, J.P., Pronk, J.T., 2003. High-level functional expression of a fungal xylose isomerase: the key to efficient ethanolic fermentation of xylose by *Saccharomyces cerevisiae*? FEMS Yeast Res. 4 (1), 69–78.
Kuyper, M., Toirkens, M.J., Diderich, J.A., Winkler, A.A., van Dijken, J.P., Pronk, J.T., 2005. Evolutionary engineering of mixed-sugar utilization by a xylose-fermenting *Saccharomyces cerevisiae* strain. FEMS Yeast Res. 5 (10), 925–934.
La Grange, D.C., Pretorius, I.S., Claeyssens, M., van Zyl, W.H., 2001. Degradation of xylan to D-xylose by recombinant *Saccharomyces cerevisiae* coexpressing the *Aspergillus niger* beta-xylosidase (*xlnD*) and the *Trichoderma reesei* xylanase II (*xyn2*) genes. Appl. Environ. Microbiol. 67 (12), 5512–5519.
Lam, V.M., Daruwalla, K.R., Henderson, P.J., Jones-Mortimer, M.C., 1980. Proton-linked D-xylose transport in *Escherichia coli*. J. Bacteriol. 143 (1), 396–402.
Larsson, S., Cassland, P., Jönsson, L.J., 2001. Development of a *Saccharomyces cerevisiae* strain with enhanced resistance to phenolic fermentation inhibitors in lignocellulose hydrolysates by heterologous expression of laccase. Appl. Environ. Microbiol. 67 (3), 1163–1170.
Lau, M.W., Dale, B.E., 2009. Cellulosic ethanol production from AFEX-treated corn stover using *Saccharomyces cerevisiae* 424A(LNH-ST). Proc. Natl. Acad. Sci. USA 106 (5), 1368–1373.
Lawford, H.G., Rousseau, J.D., 1991. Fuel ethanol from hardwood hemicellulose hydrolysate by genetically engineered *Escherichia coli* B carrying genes from *Zymomonas mobilis*. Biotechnol. Lett. 13 (3), 191–196.
Lawford, H.G., Rousseau, J.D., Mohagheghi, A., McMillan, J.D., 1999. Fermentation performance characteristics of a prehydrolyzate-adapted xylose-fermenting recombinant *Zymomonas* in batch and continuous fermentations. Appl. Biochem. Biotechnol. 77–9, 191–204.
Leandro, M.J., Gonçalves, P., Spencer-Martins, I., 2006. Two glucose/xylose transporter genes from the yeast *Candida intermedia*: first molecular characterization of a yeast xylose-H^+ symporter. Biochem. J. 395 (3), 543–549.
Lee, Y.Y., Iyer, P., Torget, R.W., 1999a. In: Tsao, G.T. (Ed.), Dilute-acid Hydrolysis of Lignocellulosic Biomass, vol. 65. Springer-Verlag, Berlin, pp. 93–115.
Lee, J., 1997. Biological conversion of lignocellulosic biomass to ethanol. J. Biotechnol. 56 (1), 1–24.
Lee, S.H., Doherty, T.V., Linhardt, R.J., Dordick, J.S., 2009. Ionic liquid-mediated selective extraction of lignin from wood leading to enhanced enzymatic cellulose hydrolysis. Biotechnol. Bioeng. 102 (5), 1368–1376.
Lee, W.J., Kim, M.D., Ryu, Y.W., Bisson, L.F., Seo, J.H., 2002. Kinetic studies on glucose and xylose transport in *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. 60 (1–2), 186–191.
Lee, Y.Y., Iyer, P., Torget, R., 1999b. Dilute-acid hydrolysis of lignocellulosic biomass. Adv. Biochem. Eng./Biotechnol. 65, 93–115.
Li, H.M., Faury, D., Morosoli, R., 2006. Impact of amino acid changes in the signal peptide on the secretion of the Tat-dependent xylanase C from *Streptomyces lividans*. FEMS Microbiol. Lett. 255 (2), 268–274.

Li, K.C., Azadi, P., Collins, R., Tolan, J., Kim, J.S., Eriksson, K.E.L., 2000. Relationships between activities of xylanases and xylan structures. Enzyme Microb. Technol. 27 (1–2), 89–94.

Li, Y.Q., Chen, S.F., Cen, P.L., 2003. Mutagenesis and screening of high yield xylanase production strain of *Aspergillus usamii* by microwave irradiation. Chinese J. Chem. Eng. 11 (5), 594–597.

Ligthelm, M.E., Prior, B.A., du Preez, J.C., 1988. The oxygen requirements of yeasts for the fermentation of D-xylose and D-glucose to ethanol. Appl. Microbiol. Biotechnol. 28 (1), 63–68.

Lilly, M., Fierobe, H.P., van Zyl, W.H., Volschenk, H., 2009. Heterologous expression of a *Clostridium* minicellulosome in *Saccharomyces cerevisiae*. FEMS Yeast Res. in press.

Liu, Q.B., Janssen, M.H.A., van Rantwijk, F., Sheldon, R.A., 2005. Room-temperature ionic liquids that dissolve carbohydrates in high concentrations. Green Chem. 7 (1), 39–42.

Liu, Z.L., Ma, M., Song, M., 2009. Evolutionarily engineered ethanologenic yeast detoxifies lignocellulosic biomass conversion inhibitors by reprogrammed pathways. Mol. Genet. Genomics 282 (3), 233–244.

Lubomir, K., Peter, B., 1998. Disaccharides permeases: constituents of xylanolytic and mannanolytic systems of *Aureobasidium pullulans*. Biochim. Biophys. Acta 1425 (3), 560–566.

Lucas, C., van Uden, N., 1986. Transport of hemicellulose monomers in the xylose-fermenting yeast *Candida shehatae*. Appl. Microbiol. Biotechnol. 23 (6), 491–495.

Lynd, L.R., Laser, M.S., Bransby, D., Dale, B.E., Davison, B., Hamilton, R., Himmel, M., Keller, M., McMillan, J.D., Sheehan, J., Wyman, C.E., 2008. How biotech can transform biofuels. Nat. Biotechnol. 26 (2), 169–172.

Lynd, L.R., van Zyl, W.H., McBride, J.E., Laser, M., 2005. Consolidated bioprocessing of cellulosic biomass: an update. Curr. Opin. Biotechnol. 16 (5), 577–583.

Lynd, L.R., Weimer, P.J., van Zyl, W.H., Pretorius, I.S., 2002. Microbial cellulose utilization: fundamentals and biotechnology. Microbiol. Mol. Biol. Rev. 66 (3), 506–577.

Madhavan, A., Tamalampudi, S., Ushida, K., Kanai, D., Katahira, S., Srivastava, A., Fukuda, H., Bisaria, V.S., Kondo, A., 2009. Xylose isomerase from polycentric fungus *Orpinomyces*: gene sequencing, cloning, and expression in *Saccharomyces cerevisiae* for bioconversion of xylose to ethanol. Appl. Microbiol. Biotechnol. 82 (6), 1067–1078.

Magee, R.J., Kosaric, N., 1985. Bioconversion of hemicelluloses. Adv. Biochem. Eng./Biotechnol. 32, 61–93.

Maleszka, R., Schneider, H., 1982. Concurrent production and consumption of ethanol by cultures of *Pachysolen tannophilus* growing on D-xylose. Appl. Environ. Microbiol. 44 (4), 909–912.

Margeot, A., Hahn-Hägerdal, B., Edlund, M., Slade, R., Monot, F., 2009. New improvements for lignocellulosic ethanol. Curr. Opin. Biotechnol. 20 (3), 372–380.

Margolles-Clark, E., Tenkanen, M., Nakari-Setala, T., Penttilä, M., 1996. Cloning of genes encoding alpha-L-arabinofuranosidase and beta-xylosidase from *Trichoderma reesei* by expression in *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 62 (10), 3840–3846.

Marques, S., Alves, L., Roseiro, J.C., Girio, F.M., 2008. Conversion of recycled paper sludge to ethanol by SHF and SSF using *Pichia stipitis*. Biomass Bioenerg. 32 (5), 400–406.

Martín, C., Jönsson, L.J., 2003. Comparison of the resistance of industrial and laboratory strains of *Saccharomyces* and *Zygosaccharomyces* to lignocellulose-derived fermentation inhibitors. Enzyme Microb. Technol. 32 (3–4), 386–395.

Martín, C., Marcet, M., Thomsen, A.B., 2008. Comparison between wet oxidation and steam explosion as pretreatment methods for enzymatic hydrolysis of sugarcane bagasse. Bioresources 3 (3), 670–683.

Maruyama, K., Goto, C., Numata, M., Suzuki, T., Nakagawa, Y., Hoshino, T., Uchiyama, T., 1996. *O*-acetylated xyloglucan in extracellular polysaccharides from cell-suspension cultures of *Mentha*. Phytochemistry 41 (5), 1309–1314.

Marzialetti, T., Olarte, M.B.V., Sievers, C., Hoskins, T.J.C., Agrawal, P.K., Jones, C.W., 2008. Dilute acid hydrolysis of Loblolly pine: a comprehensive approach. Ind. Eng. Chem. Res. 47 (19), 7131–7140.

McMillan, J.D., Boynton, B.L., 1994. Arabinose utilization by xylose-fermenting yeasts and fungi. Appl. Biochem. Biotechnol. 45–46, 569–584.

McMillan, J.D., Newman, M.M., Templeton, D.W., Mohagheghi, A., 1999. Simultaneous saccharification and cofermentation of dilute-acid pretreated yellow poplar hardwood to ethanol using xylose-fermenting *Zymomonas mobilis*. Appl. Biochem. Biotechnol. 77–79, 649–665.

Meinander, N.Q., Hahn-Hagerdal, B., 1997. Fed-batch xylitol production with two recombinant *Saccharomyces cerevisiae* strains expressing *XYL1* at different levels, using glucose as a cosubstrate: a comparison of production parameters and strain stability. Biotechnol. Bioeng. 54 (4), 391–399.

Miranda, I., Pereira, H., 2002. Kinetics of ASAM and kraft pulping of eucalypt wood (*Eucalyptus globulus*). Holzforschung 56 (1), 85–90.

Miyafuji, H., Nakata, T., Ehara, K., Saka, S., 2005. Fermentability of water-soluble portion to ethanol obtained by supercritical water treatment of lignocellulosics. Appl. Biochem. Biotechnol. 121, 963–971.

Modig, T., Almeida, J.R., Gorwa-Grauslund, M.F., Lidén, G., 2008. Variability of the response of *Saccharomyces cerevisiae* strains to lignocellulose hydrolysate. Biotechnol. Bioeng. 100 (3), 423–429.

Mohagheghi, A., Dowe, N., Schell, D., Chou, Y.C., Eddy, C., Zhang, M., 2004. Performance of a newly developed integrant of *Zymomonas mobilis* for ethanol production on corn stover hydrolysate. Biotechnol. Lett. 26 (4), 321–325.

Mohagheghi, A., Evans, K., Chou, Y.C., Zhang, M., 2002. Cofermentation of glucose, xylose, and arabinose by genomic DNA-integrated xylose/arabinose fermenting strain of *Zymomonas mobilis* AX101. Appl. Biochem. Biotechnol. 98–100, 885–898.

Monavari, S., Galbe, M., Zacchi, G., 2009. The influence of solid/liquid separation techniques on the sugar yield in two-step dilute acid hydrolysis of softwood followed by enzymatic hydrolysis. Biotechnol. Biofuels 2 (1), 6.

Montané, D., Salvado, J., Farriol, X., 1994. Chemical analysis of partially hydrolyzed lignocellulosic biomass. Afinidad 51 (450), 109–116.

Morris, D.D., Reeves, R.A., Gibbs, M.D., Saul, D.J., Bergquist, P.L., 1995. Correction of the β-mannanase domain of the cell C pseudogene from *Caldocellulosiruptor saccharolyticus* and activity of the gene product on kraft pulp. Appl. Environ. Microbiol. 61 (6), 2262–2269.

Mosier, N., Wyman, C.E., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., Ladisch, M., 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour. Technol. 96 (6), 673–686.

Moura, P., Barata, R., Carvalheiro, F., Gírio, F.M., Loureiro-Dias, M.C., Esteves, M.P., 2007. *In vitro* fermentation of xylo-oligosaccharides from corn cobs autohydrolysis by *Bifidobacterium* and *Lactobacillus* strains. LWT 40 (6), 963–972.

Mussatto, S.I., Roberto, I.C., 2004. Kinetic behavior of *Candida guilliermondii* yeast during xylitol production from highly concentrated hydrolysate. Proc. Biochem. 39 (11), 1433–1439.

Nanavati, D.M., Thirangoon, K., Noll, K.M., 2006. Several archaeal homologs of putative oligopeptide-binding proteins encoded by *Thermotoga maritima* bind sugars. Appl. Environ. Microbiol. 72 (2), 1336–1345.

Neureiter, M., Danner, H., Thomasser, C., Saidi, B., Braun, R., 2002. Dilute-acid hydrolysis of sugarcane bagasse at varying conditions. Appl. Biochem. Biotechnol. 98–100, 49–58.

Ngo, H.L., LeCompte, K., Hargens, L., Mcewen, A.B., 2000. Thermal properties of imidazolium ionic liquids. Thermochim. Acta 357, 97–102.

Nigam, J.N., 2001a. Development of xylose-fermenting yeast *Pichia stipitis* for ethanol production through adaptation on hardwood hemicellulose acid prehydrolysate. J. Appl. Microbiol. 90 (2), 208–215.

Nigam, J.N., 2001b. Ethanol production from hardwood spent sulfite liquor using an adapted strain of *Pichia stipitis*. J. Ind. Microbiol. Biotechnol. 26 (3), 145–150.

Nigam, J.N., 2001c. Ethanol production from wheat straw hemicellulose hydrolysate by *Pichia stipitis*. J. Biotechnol. 87 (1), 17–27.

Nobre, A., Lucas, C., Leão, C., 1999. Transport and utilization of hexoses and pentoses in the halotolerant yeast *Debaryomyces hansenii*. Appl. Environ. Microbiol. 65 (8), 3594–3598.

Novick, N.J., Tyler, M.E., 1982. L-arabinose metabolism in *Azospirillum brasiliense*. J Bacteriol 149 (1), 364–367.

Novotny, C.P., Englesberg, E., 1966. The L-arabinose permease system in *Escherichia coli* B/r. Biochim. Biophys. Acta 117 (1), 217–230.

Nuyens, F., van Zyl, W.H., Iserentant, D., Verachtert, H., Michiels, C., 2001. Heterologous expression of the *Bacillus pumilus* endo-beta-xylanase (*xynA*) gene in the yeast *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. 56 (3–4), 431–434.

O'Brien, D.J., Senske, G.E., Kurantz, M.J., Craig Jr., J.C., 2004. Ethanol recovery from corn fiber hydrolysate fermentations by pervaporation. Bioresour. Technol. 92 (1), 15–19.

Ohara, H., Owaki, M., Sonomoto, K., 2006. Xylooligosaccharide fermentation with *Leuconostoc lactis*. J. Biosci. Bioeng. 101 (5), 415–420.

Öhgren, K., Bengtsson, O., Gorwa-Grauslund, M.F., Galbe, M., Hahn-Hägerdal, B., Zacchi, G., 2006. Simultaneous saccharification and co-fermentation of glucose and xylose in steam-pretreated corn stover at high fiber content with *Saccharomyces cerevisiae* TMB3400. J. Biotechnol. 126 (4), 488–498.

Ohno, H., Fukaya, Y., 2009. Task specific ionic liquids for cellulose technology. Chem. Lett. 38 (1), 2–7.

Ohta, K., Beall, D.S., Mejia, J.P., Shanmugam, K.T., Ingram, L.O., 1991a. Genetic improvement of *Escherichia coli* for ethanol production: chromosomal integration of *Zymomonas mobilis* genes encoding pyruvate decarboxylase and alcohol dehydrogenase II. Appl. Environ. Microbiol. 57 (4), 893–900.

Ohta, K., Beall, D.S., Mejia, J.P., Shanmugam, K.T., Ingram, L.O., 1991b. Metabolic engineering of *Klebsiella oxytoca* M5A1 for ethanol production from xylose and glucose. Appl. Environ. Microbiol. 57 (10), 2810–2815.

Okuhara, T., 2002. Water–tolerant solid acid catalysts. Chem. Rev. 102 (10), 3641–3665.

Olofsson, K., Bertilsson, M., Lidén, G., 2008a. A short review on SSF – an interesting process option for ethanol production from lignocellulosic feedstocks. Biotechnol. Biofuels 1 (1), 7.

Olofsson, K., Rudolf, A., Liden, G., 2008b. Designing simultaneous saccharification and fermentation for improved xylose conversion by a recombinant strain of *Saccharomyces cerevisiae*. J. Biotechnol. 134 (1–2), 112–120.

Olsson, L., Hahn-Hägerdal, B., 1996. Fermentation of lignocellulosic hydrolysates for ethanol production. Enzyme Microb. Technol. 18 (5), 312–331.

Olsson, L., Soerensen, H.R., Dam, B.P., Christensen, H., Krogh, K.M., Meyer, A.S., 2006. Separate and simultaneous enzymatic hydrolysis and fermentation of wheat hemicellulose with recombinant xylose utilizing *Saccharomyces cerevisiae*. Appl. Biochem. Biotechnol. 129–132, 117–129.

Onda, A., Ochi, T., Yanagisawa, K., 2009. Hydrolysis of cellulose selectively into glucose over sulfonated activated-carbon catalyst under hydrothermal conditions. Top. Catal. 52 (6–7), 801–807.

Osman, Y.A., Ingram, L.O., 1985. Mechanism of ethanol inhibition of fermentation in *Zymomonas mobilis* CP4. J. Bacteriol. 164 (1), 173–180.

Ozcan, S., Kotter, P., Ciriacy, M., 1991. Xylan-hydrolyzing enzymes of the yeast *Pichia stipitis*. Appl. Microbiol. Biotechnol. 36 (2), 190–195.

Pan, X.J., Arato, C., Gilkes, N., Gregg, D., Mabee, W., Pye, K., Xiao, Z.Z., Zhang, X., Saddler, J., 2005. Biorefining of softwoods using ethanol organosolv pulping: Preliminary evaluation of process streams for manufacture of fuel-grade ethanol and co-products. Biotechnol. Bioeng. 90 (4), 473–481.

Pan, X.J., Gilkes, N., Kadla, J., Pye, K., Saka, S., Gregg, D., Ehara, K., Xie, D., Lam, D., Saddler, J., 2006. Bioconversion of hybrid poplar to ethanol and co-products using an organosolv fractionation process: Optimization of process yields. Biotechnol. Bioeng. 94 (5), 851–861.

Panbangred, W., Fukusaki, E., Epifanio, E.C., Shinmyo, A., Okada, H., 1985. Expression of a xylanase gene of *Bacillus pumilus* in *Escherichia coli* and *Bacillus subtilis*. Appl. Microbiol. Biotechnol. 22 (4), 259–264.

Parker, C., Barnell, W.O., Snoep, J.L., Ingram, L.O., Conway, T., 1995. Characterization of the *Zymomonas mobilis* glucose facilitator gene product (*glf*) in recombinant *Escherichia coli*: examination of transport mechanism, kinetics and the role of glucokinase in glucose transport. Mol. Microbiol. 15 (5), 795–802.

Paulechka, Y.U., Kabo, G.J., Blokhin, A.V., Vydrov, O.A., Magee, J.W., Frenkel, M., 2003. Thermodynamic properties of 1-butyl-3-methylimidazolium hexafluorophosphate in the ideal gas state. J. Chem. Eng. Data 48 (3), 457–462.

Pereira, H., 1988. Variability in the chemical composition of plantation Eucalypts (*Eucalyptus globulus* Labill). Wood Fiber Sci. 20 (1), 82–90.

Pereira, H., Graça, J., Rodrigues, J.C., 2003. Wood chemistry in relation to quality. In: Barnett, J.R., Jeronimidis, G. (Eds.), Wood Quality and Its Biological Basis. Blackwell Publishing, Oxford, pp. 53–86.

Pérez-González, J.A., De Graaff, L.H., Visser, J., Ramón, D., 1996. Molecular cloning and expression in *Saccharomyces cerevisiae* of two *Aspergillus nidulans* xylanase genes. Appl. Environ. Microbiol. 62 (6), 2179–2182.

Persson, P., Barisic, Z., Cohen, A., Thörneby, L., Gorton, L., 2002a. Countercurrent supercritical fluid extraction of phenolic compounds from aqueous matrices. Anal. Chim. Acta 460 (1), 1–12.

Persson, P., Larsson, S., Jonsson, L.J., Nilvebrant, N.O., Sivik, B., Munteanu, F., Thorneby, L., Gorton, L., 2002b. Supercritical fluid extraction of a lignocellulosic hydrolysate of spruce for detoxification and to facilitate analysis of inhibitors. Biotechnol. Bioeng. 79 (6), 694–700.

Petersen, M.O., Larsen, J., Thomsen, M.H., 2009. Optimization of hydrothermal pretreatment of wheat straw for production of bioethanol at low water consumption without addition of chemicals. Biomass Bioenerg. 33 (5), 834–840.

Petersson, A., Almeida, J.R., Modig, T., Karhumaa, K., Hahn-Hägerdal, B., Gorwa-Grauslund, M.F., Lidén, G., 2006. A 5-hydroxymethyl furfural reducing enzyme encoded by the *Saccharomyces cerevisiae* ADH6 gene conveys HMF tolerance. Yeast 23 (6), 455–464.

Petschacher, B., Nidetzky, B., 2008. Altering the coenzyme preference of xylose reductase to favor utilization of NADH enhances ethanol yield from xylose in a metabolically engineered strain of *Saccharomyces cerevisiae*. Microb. Cell Fact. 7, 9.

Polizeli, M.L.T.M., Rizzatti, A.C.S., Monti, R., Terenzi, H.F., Jorge, J.A., Amorim, D.S., 2005. Xylanases from fungi: properties and industrial applications. Appl. Microbiol. Biotechnol. 67 (5), 577–591.

Puchart, V., Vrsanska, M., Svoboda, P., Pohl, J., Ogel, Z.B., Biely, P., 2004. Purification and characterization of two forms of endo-β-1,4-mannanase from a thermotolerant fungus, *Aspergillus fumigatus* IMI 385708 (formerly Thermomyces lanuginosus IMI 158749). BBA-Gen. Subj. 1674 (3), 239–250.

Puls, J., Poutanen, K., Körner, H.U., Viikari, L., 1985. Biotechnical utilization of wood carbohydrates after steaming pretreatment. Appl. Microbiol. Biotechnol. 22 (6), 416–423.

Qian, Y., Yomano, L.P., Preston, J.F., Aldrich, H.C., Ingram, L.O., 2003. Cloning, characterization, and functional expression of the *Klebsiella oxytoca* xylodextrin utilization operon (*xynTB*) in *Escherichia coli*. Appl. Environ. Microbiol. 69 (10), 5957–5967.

Rajoka, M.I., 2005. Double mutants of *Cellulomonas biazotea* for production of cellulases and hemicellulases following growth on straw of a perennial grass. World J. Microbiol. Biotechnol. 21 (6–7), 1063–1066.

Ranke, J., Stolte, S., Stormann, R., Arning, J., Jastorff, B., 2007. Design of sustainable chemical products – the example of ionic liquids. Chem. Rev. 107 (6), 2183–2206.

Ray, S.K., Rajeshwari, R., Sonti, R.V., 2000. Mutants of *Xanthomonas oryzae* pv. *oryzae* deficient in general secretory pathway are virulence deficient and unable to secrete xylanase. Mol. Plant Microbe. In 13 (4), 394–401.

Reifenberger, E., Boles, E., Ciriacy, M., 1997. Kinetic characterization of individual hexose transporters of *Saccharomyces cerevisiae* and their relation to the triggering mechanisms of glucose repression. Eur. J. Biochem. 245 (2), 324–333.

Ren, J.L., Sun, R.C., Liu, C.F., Cao, Z.N., Luo, W., 2007. Acetylation of wheat straw hemicelluloses in ionic liquid using iodine as a catalyst. Carbohydr. Polym. 70 (4), 406–414.

Richard, P., Londesborough, J., Putkonen, M., Kalkkinen, N., Penttilä, M., 2001. Cloning and expression of a fungal L-arabinitol 4-dehydrogenase gene. J. Biol. Chem. 276 (44), 40631–40637.

Richard, P., Putkonen, M., Vaananen, R., Londesborough, J., Penttilä, M., 2002. The missing link in the fungal L-arabinose catabolic pathway, identification of the L-xylulose reductase gene. Biochemistry 41 (20), 6432–6437.

Richard, P., Toivari, M.H., Penttilä, M., 1999. Evidence that the gene YLR070c of *Saccharomyces cerevisiae* encodes a xylitol dehydrogenase. FEBS Lett. 457 (1), 135–138.

Richard, P., Verho, R., Putkonen, M., Londesborough, J., Penttilä, M., 2003. Production of ethanol from L-arabinose by *Saccharomyces cerevisiae* containing a fungal L-arabinose pathway. FEMS Yeast Res. 3 (2), 185–189.

Rinaldi, R., Palkovits, R., Schuth, F., 2008. Depolymerization of cellulose using solid catalysts in ionic liquids. Angew. Chem. Int. Ed. Engl. 47 (42), 8047–8050.

Ritter, D.C., Campbell, A.G., 1991. Supercritical Carbon dioxide extraction of Southern Pine and Ponderosa Pine. Wood Fiber Sci. 23 (1), 98–113.

Rizzi, M., Erlemann, P., Buithanh, N.A., Dellweg, H., 1988. Xylose fermentation by yeasts.4. Purification and kinetic studies of xylose reductase from Pichia stipitis. Appl. Microbiol. Biotechnol. 29 (2–3), 148–154.

Ropars, M., Marchal, R., Pourquie, J., Vandecasteele, J.P., 1992. Large-scale enzymatic hydrolysis of agricultural lignocellulosic biomass. 1. Pretreatment procedures. Bioresour. Technol. 42 (3), 197–204.

Rubio, M., Tortosa, J.F., Quesada, J., Gomez, D., 1998. Fractionation of lignocellulosics. Solubilization of corn stalk hemicelluloses by autohydrolysis in aqueous medium. Biomass Bioenerg. 15 (6), 483–491.

Rudolf, A., Alkasrawi, M., Zacchi, G., Lidén, G., 2005. A comparison between batch and fed-batch simultaneous saccharification and fermentation of steam pretreated spruce. Enzyme Microb. Technol. 37 (2), 195–204.

Rudolf, A., Baudel, H., Zacchi, G., Hahn-Hägerdal, B., Lidén, G., 2008. Simultaneous saccharification and fermentation of steam-pretreated bagasse using *Saccharomyces cerevisiae* TMB3400 and *Pichia stipitis* CBS6054. Biotechnol. Bioeng. 99 (4), 783–790.

Ruiz, E., Cara, C., Manzanares, P., Ballesteros, M., Castro, E., 2008. Evaluation of steam explosion pre-treatment for enzymatic hydrolysis of sunflower stalks. Enzyme Microb. Technol. 42 (2), 160–166.

Runquist, D., Fonseca, C., Radstrom, P., Spencer-Martins, I., Hahn-Hägerdal, B., 2009. Expression of the Gxf1 transporter from *Candida intermedia* improves fermentation performance in recombinant xylose-utilizing *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. 82 (1), 123–130.

Ryabova, O., Vrsanska, M., Kaneko, S., van Zyl, W.H., Biely, P., 2009. A novel family of hemicellulolytic α-glucuronidase. FEBS Lett. 583 (9), 1457–1462.

Sá-Nogueira, I., de Lencastre, H., 1989. Cloning and characterization of *araA*, *araB*, and *araD*, the structural genes for L-arabinose utilization in *Bacillus subtilis*. J. Bacteriol. 171 (7), 4088–4091.

Sá-Nogueira, I., Nogueira, T.V., Soares, S., de Lencastre, H., 1997. The *Bacillus subtilis* L-arabinose (*ara*) operon: nucleotide sequence, genetic organization and expression. Microbiology 143 (Pt 3), 957–969.

Saha, B.C., Iten, L.B., Cotta, M.A., Wu, Y.V., 2005. Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol. Proc. Biochem. 40 (12), 3693–3700.

Saitoh, S., Tanaka, T., Kondo, A., 2008. Breeding of industrial diploid yeast strain with chromosomal integration of multiple beta-glucosidase genes. J. Biosci. Bioeng. 106 (6), 594–597.

Saloheimo, A., Rauta, J., Stasyk, O.V., Sibirny, A.A., Penttilä, M., Ruohonen, L., 2007. Xylose transport studies with xylose-utilizing *Saccharomyces cerevisiae* strains expressing heterologous and homologous permeases. Appl. Microbiol. Biotechnol. 74 (5), 1041–1052.

Salusjärvi, L., Kankainen, M., Soliymani, R., Pitkanen, J.P., Penttilä, M., Ruohonen, L., 2008. Regulation of xylose metabolism in recombinant *Saccharomyces cerevisiae*. Microb. Cell Fact. 7, 18.

Sasaki, M., Adschiri, T., Arai, K., 2003. Fractionation of sugarcane bagasse by hydrothermal treatment. Bioresour. Technol. 86 (3), 301–304.

Saska, M., Ozer, E., 1995. Aqueous extraction of sugarcane bagasse hemicellulose and production of xylose syrup. Biotechnol. Bioeng. 45 (6), 517–523.

Sassner, P., Galbe, M., Zacchi, G., 2004. Bioethanol from salix: optimization of the steam pretreatment step. In: 26th Symposium on Biotechnology for Fuels and Chemicals. NREL, Chattanooga, TN, p. 295.

Sauer, U., 2001. Evolutionary engineering of industrially important microbial phenotypes. Adv. Biochem. Eng./Biotechnol. 73, 129–169.

Scarborough, G.A., 1973. Transport in *Neurospora*. Int. Rev. Cytol. 34, 103–122.

Schacht, C., Zetzl, C., Brunner, G., 2008. From plant materials to ethanol by means of supercritical fluid technology. J. Supercrit. Fluids 46 (3), 299–321.

Schell, D.J., Ruth, M.F., Tucker, M.P., 1999. Modeling the enzymatic hydrolysis of dilute-acid pretreated Douglas fir. Appl. Biochem. Biotechnol. 77–79, 67–81.

Sedlak, M., Ho, N.W., 2001. Expression of *E. coli araBAD* operon encoding enzymes for metabolizing L-arabinose in *Saccharomyces cerevisiae*. Enzyme Microb. Technol. 28 (1), 16–24.

Sedlak, M., Ho, N.W., 2004. Characterization of the effectiveness of hexose transporters for transporting xylose during glucose and xylose co-fermentation by a recombinant *Saccharomyces* yeast. Yeast 21 (8), 671–684.

Senac, T., Hahn-Hägerdal, B., 1990. Intermediary metabolite concentrations in xylulose- and glucose-fermenting *Saccharomyces cerevisiae* cells. Appl. Environ. Microbiol. 56 (1), 120–126.

Setati, M.E., Ademark, P., van Zyl, W.H., Hahn-Hägerdal, B., Stalbrand, H., 2001. Expression of the *Aspergillus aculeatus* endo-beta-1,4-mannanase encoding gene (man1) in *Saccharomyces cerevisiae* and characterization of the recombinant enzyme. Protein Expr. Purif. 21 (1), 105–114.

Shallom, D., Shoham, Y., 2003. Microbial hemicellulases. Curr. Opin. Microbiol. 6 (3), 219–228.

Shevchenko, S.M., Chang, K., Robinson, J., Saddler, J.N., 2000. Optimization of monosaccharide recovery by post-hydrolysis of the water-soluble hemicellulose component after steam explosion of softwood chips. Bioresour. Technol. 72 (3), 207–211.

Shi, N.Q., Davis, B., Sherman, F., Cruz, J., Jeffries, T.W., 1999. Disruption of the cytochrome *c* gene in xylose-utilizing yeast *Pichia stipitis* leads to higher ethanol production. Yeast 15 (11), 1021–1030.

Shi, N.Q., Jeffries, T.W., 1998. Anaerobic growth and improved fermentation of *Pichia stipitis* bearing a *URA1* gene from *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. 50 (3), 339–345.

Shibuya, N., Iwasaki, T., 1985. Structural features of rice bran hemicellulose. Phytochemistry 24 (2), 285–289.

Sievers, C., Valenzuela-Olarte, M.B., Marzialetti, T., Musin, D., Agrawal, P.K., Jones, C.W., 2009. Ionic-liquid-phase hydrolysis of pine wood. Ind. Eng. Chem. Res. 48 (3), 1277–1286.

Silva, S.S., Vitolo, M., Pessoa, A., Felipe, M.G.A., 1996. Xylose reductase and xylitol dehydrogenase activities of D-xylose-xylitol-fermenting *Candida guilliermondii*. J. Basic Microbiol. 36 (3), 187–191.

Sims, I.M., Munro, S.L.A., Currie, G., Craik, D., Bacic, A., 1996. Structural characterisation of xyloglucan secreted by suspension-cultured cells of N*icotiana plumbaginifolia*. Carbohydr. Res. 293 (2), 147–172.

Singh, A., Kuhad, R.C., Kumar, M., 1995. Xylanase production by a hyperxylanolytic mutant of *Fusarium oxysporum*. Enzyme Microb. Technol. 17 (6), 551–553.

Singh, A., Kumar, P.K.R., Schügerl, K., 1992. Bioconversion of cellulosic materials to ethanol by filamentous fungi. Adv. Biochem. Eng./Biotechnol. 45, 29–55.

Skoog, K., Hahn-Hägerdal, B., 1990. Effect of oxygenation on xylose fermentation by *Pichia stipitis*. Appl. Environ. Microbiol. 56 (11), 3389–3394.

Skotnicki, M.L., Lee, K.J., Tribe, D.E., Rogers, P.L., 1981. Comparison of ethanol production by different *Zymomonas* strains. Appl. Environ. Microbiol. 41 (4), 889–893.

Söderström, J., Galbe, M., Zacchi, G., 2004. Effect of washing on yield in one- and two-step steam pretreatment of softwood for production of ethanol. Biotechnol. Prog. 20 (3), 744–749.

Söderström, J., Pilcher, L., Galbe, M., Zacchi, G., 2002. Two-step steam pretreatment of softwood with SO_2 impregnation for ethanol production. Appl. Biochem. Biotechnol. 98, 5–21.

Sonderegger, M., Jeppsson, M., Larsson, C., Gorwa-Grauslund, M.F., Boles, E., Olsson, L., Spencer-Martins, I., Hahn-Hägerdal, B., Sauer, U., 2004a. Fermentation performance of engineered and evolved xylose-fermenting *Saccharomyces cerevisiae* strains. Biotechnol. Bioeng. 87 (1), 90–98.

Sonderegger, M., Sauer, U., 2003. Evolutionary engineering of *Saccharomyces cerevisiae* for anaerobic growth on xylose. Appl. Environ. Microbiol. 69 (4), 1990–1998.

Sonderegger, M., Schumperli, M., Sauer, U., 2004b. Metabolic engineering of a phosphoketolase pathway for pentose catabolism in *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 70 (5), 2892–2897.

Souto-Maior, A.M., Runquist, D., Hahn-Hägerdal, B., 2009. Crabtree-negative characteristics of recombinant xylose-utilizing *Saccharomyces cerevisiae*. J. Biotechnol. 143 (2), 119–123.

Spencer-Martins, I., 1994. Transport of sugars in yeasts: implications in the fermentation of lignocellulosic materials. Bioresour. Technol. 50 (1), 51–57.

Stalbrand, H., Saloheimo, A., Vehmaanpera, J., Henrissat, B., Penttilä, M., 1995. Cloning and expression in *Saccharomyces cerevisiae* of a *Trichoderma reesei* beta-mannanase gene containing a cellulose binding domain. Appl. Environ. Microbiol. 61 (3), 1090–1097.

Stambuk, B.U., Franden, M.A., Singh, A., Zhang, M., 2003. D-xylose transport by *Candida succiphila* and *Kluyveromyces marxianus*. Appl. Biochem. Biotechnol. 105, 255–263.

Stephen, A.M., 1983. Other plant polysaccharides. In: Aspinall, G.O. (Ed.), The Polysaccharides. Academic Press, New York, pp. 97–194.

Stevis, P.E., Huang, J.J., Ho, N.W., 1987. Cloning of the *Pachysolen tannophilus* xylulokinase gene by complementation in *Escherichia coli*. Appl. Environ. Microbiol. 53 (12), 2975–2977.

Suganuma, S., Nakajima, K., Kitano, M., Yamaguchi, D., Kato, H., Hayashi, S., Michikazu, 2008. Hydrolysis of cellulose by amorphous carbon bearing SO_3H, COOH, and OH groups. J. Am. Chem. Soc. 130 (38), 12787–12793.

Sumiya, M., Davis, E.O., Packman, L.C., McDonald, T.P., Henderson, P.J., 1995. Molecular genetics of a receptor protein for D-xylose, encoded by the gene *xylF*, in *Escherichia coli*. Recept Channel 3 (2), 117–128.

Sun, N., Rahman, M., Qin, Y., Maxim, M.L., Rodriguez, H., Rogers, R.D., 2009. Complete dissolution and partial delignification of wood in the ionic liquid 1-ethyl-3-methylimidazolium acetate. Green Chem. 11 (5), 646–655.

Sunna, A., Antranikian, G., 1997. Xylanolytic enzymes from fungi and bacteria. Crit. Rev. Biotechnol. 17 (1), 39–67.

Sunna, A., Gibbs, M.D., Chin, C.W.J., Nelson, P.J., Bergquist, P.L., 2000. A gene encoding a novel multidomain β-1,4-mannanase from *Caldibacillus cellulovorans* and action of the recombinant enzyme on kraft pulp. Appl. Environ. Microbiol. 66 (2), 664–670.

Swatloski, R.P., Spear, S.K., Holbrey, J.D., Rogers, R.D., 2002. Dissolution of cellose with ionic liquids. J. Am. Chem. Soc. 124 (18), 4974–4975.

Taherzadeh, M.J., Eklund, R., Gustafsson, L., Niklasson, C., Lidén, G., 1997. Characterization and fermentation of dilute-acid hydrolyzates from wood. Ind. Eng. Chem. Res. 36 (11), 4659–4665.

Taherzadeh, M.J., Niklasson, C., Liden, G., 2000. On-line control of fed-batch fermentation of dilute-acid hydrolyzates. Biotechnol. Bioeng. 69 (3), 330–338.

Takada, G., Kawaguchi, T., Sumitani, J., Arai, M., 1998. Expression of *Aspergillus aculeatus* No. F-50 cellobiohydrolase I (*cbhI*) and beta-glucosidase 1 (*bgl1*) genes by *Saccharomyces cerevisiae*. Biosci. Biotechnol. Biochem. 62 (8), 1615–1618.

Talbot, G., Sygusch, J., 1990. Purification and characterization of thermostable β-mannanase and α-galactosidase from *Bacillus stearothermophilus*. Appl. Environ. Microbiol. 56 (11), 3505–3510.

Tantirungkij, M., Nakashima, N., Seki, T., Yoshida, T., 1993. Construction of xylose-assimilating *Saccharomyces cerevisiae*. J. Ferment. Bioeng. 75 (2), 83–88.

Tao, H., Gonzalez, R., Martinez, A., Rodriguez, M., Ingram, L.O., Preston, J.F., Shanmugam, K.T., 2001. Engineering a homo-ethanol pathway in *Escherichia coli*: increased glycolytic flux and levels of expression of glycolytic genes during xylose fermentation. J. Bacteriol. 183 (10), 2979–2988.

Tengborg, C., Stenberg, K., Galbe, M., Zacchi, G., Larsson, S., Palmqvist, E., Hahn-Hägerdal, B., 1998. Comparison of SO_2 and H_2SO_4 impregnation of softwood prior to steam pretreatment on ethanol production. Appl. Biochem. Biotechnol. 70–72, 3–15.

Timell, T.E., 1965. Wood hemicelluloses. Adv. Carbohydr. Chem. Biochem. 20, 409–483.

Timell, T.E., 1967. Recent progress in the chemistry of wood hemicelluloses. Wood Sci. Technol. 1, 45–70.

Tison, M.C., Andre-Leroux, G., Lafond, M., Georis, J., Juge, N., Berrin, J.G., 2009. Molecular determinants of substrate and inhibitor specificities of the *Penicillium griseofulvum* family 11 xylanases. Biochem. Biophys. Acta – Prot. Proteom. 1794 (3), 438–445.

Toivari, M.H., Aristidou, A., Ruohonen, L., Penttilä, M., 2001. Conversion of xylose to ethanol by recombinant *Saccharomyces cerevisiae*: importance of xylulokinase (*XKS1*) and oxygen availability. Metab. Eng. 3 (3), 236–249.

Toivola, A., Yarrow, D., van den Bosch, E., van Dijken, J.P., Scheffers, W.A., 1984. Alcoholic fermentation of D-xylose by yeasts. Appl. Environ. Microbiol. 47 (6), 1221–1223.

Tomás-Pejó, E., Garcia-Aparicio, M., Negro, M.J., Oliva, J.M., Ballesteros, M., 2009a. Effect of different cellulase dosages on cell viability and ethanol production by *Kluyveromyces marxianus* in SSF processes. Bioresour. Technol. 100 (2), 890–895.

Tomás-Pejó, E., Oliva, J.M., Ballesteros, M., Olsson, L., 2008. Comparison of SHF and SSF processes from steam-exploded wheat straw for ethanol production by xylose-fermenting and robust glucose-fermenting *Saccharomyces cerevisiae* strains. Biotechnol. Bioeng. 100 (6), 1122–1131.

Tomás-Pejó, E., Oliva, J.M., González, A., Ballesteros, I., Ballesteros, M., 2009b. Bioethanol production from wheat straw by the thermotolerant yeast *Kluyveromyces marxianus* CECT 10875 in a simultaneous saccharification and fermentation fed-batch process. Fuel 88 (11), 2142–2147.

Tomoyeda, M., Horitsu, H., 1964. Pentose metabolism by *Candida utilis* I. Xylose isomerase. Agric. Biol. Chem. 28 (3), 139.

Topakas, E., Vafiadi, C., Christakopoulos, P., 2007. Microbial production, characterization and applications of feruloyl esterases. Process Biochem. 42 (4), 497–509.

Torget, R., Hsu, T.A., 1994. Two temperature dilute-acid prehydrolysis of hardwood xylan using a percolation process. Appl. Biochem. Biotechnol. 45–46, 5–22.

Torget, R., Walter, P., Himmel, M., Grohmann, K., 1991. Dilute-acid pretreatment of corn residues and short-rotation woody crops. Appl. Biochem. Biotechnol. 28–29, 75–86.

Torget, R., Werdene, P., Himmel, M., Grohmann, K., 1990. Dilute acid pretreatment of short rotation woody and herbaceous crops. Appl. Biochem. Biotechnol. 24–25, 115–126.

Torget, R.W., Hayward, T.K., Elander, R., 1997. Total hydrolysis of lignocellulosic biomass using very dilute acid employing a novel shrinking bed reactor configuration. In: 19th Symposium on Biotechnology for Fuels and Chemicals. Colorado Springs, EUA, USA, Presentation no. 4.

Torget, R.W., Kim, J.S., Lee, Y.Y., 2000. Fundamental aspects of dilute acid hydrolysis/fractionation kinetics of hardwood carbohydrates. 1. Cellulose hydrolysis. Ind. Eng. Chem. Res. 39 (8), 2817–2825.

Torres, N.V., Riol-Cimas, J.M., Wolschek, M., Kubicek, C.P., 1996. Glucose transport by *Aspergillus niger*: The low-affinity carrier is only formed during growth on high glucose concentrations. Appl. Microbiol. Biotechnol. 44 (6), 790–794.

Träff, K.L., Otero Cordero, R.R., van Zyl, W.H., Hahn-Hägerdal, B., 2001. Deletion of the *GRE3* aldose reductase gene and its influence on xylose metabolism in recombinant strains of *Saccharomyces cerevisiae* expressing the *xylA* and *XKS1* genes. Appl. Environ. Microbiol. 67 (12), 5668–5674.

Tsujibo, H., Kosaka, M., Ikenishi, S., Sato, T., Miyamoto, K., Inamori, Y., 2004. Molecular characterization of a high-affinity xylobiose transporter of *Streptomyces thermoviolaceus* OPC-520 and its transcriptional regulation. J. Bacteriol. 186 (4), 1029–1037.

Van Rensburg, P., van Zyl, W.H., Pretorius, I.S., 1996. Co-expression of a Phanerochaete chrysosporium cellobiohydrolase gene and a Butyrivibrio fibrisolvens endo-beta-1,4-glucanase gene in *Saccharomyces cerevisiae*. Curr. Genet. 30 (3), 246–250.

van Rooyen, R., Hahn-Hägerdal, B., La Grange, D.C., van Zyl, W.H., 2005. Construction of cellobiose-growing and fermenting *Saccharomyces cerevisiae* strains. J. Biotechnol. 120 (3), 284–295.

Van Vleet, J.H., Jeffries, T.W., 2009. Yeast metabolic engineering for hemicellulosic ethanol production. Curr. Opin. Biotechnol. 20 (3), 300–306.

van Walsum, G.P., Allen, S.G., Spencer, M.J., Laser, M.S., Antal, M.J., Lynd, L.R., 1996. Conversion of lignocellulosics pretreated with liquid hot water to ethanol. Appl. Biochem. Biotechnol. 57–8, 157–170.

van Walsum, G.P., Shi, H., 2004. Carbonic acid enhancement of hydrolysis in aqueous pretreatment of corn stover. Bioresour. Technol. 93 (3), 217–226.

van Zyl, C., Prior, B., Du Preez, J., 1988. Production of ethanol from sugar cane bagasse hemicellulose hydrolyzate by *Pichia stipitis*. Appl. Biochem. Biotechnol. 17 (1), 357–369.

van Zyl, P.J., Moodley, V., Rose, S., Roth, R., van Zyl, W.H., 2009. Production of the *Aspergillus aculeatus* endo-1,4-β-mannanase in *A. Niger*. J. Ind. Microbiol. Biotechnol. 36 (4), 611–617.

van Zyl, W.H., Lynd, L.R., Den Haan, R., McBride, J.E., 2007. Consolidated bioprocessing for bioethanol production using *Saccharomyces cerevisiae*. Adv. Biochem. Eng./Biotechnol. 108, 205–235.

vanKuyk, P.A., Diderich, J.A., MacCabe, A.P., Hererro, O., Ruijter, G.J., Visser, J., 2004. *Aspergillus niger mstA* encodes a high-affinity sugar/H+ symporter which is regulated in response to extracellular pH. Biochem. J. 379 (Pt 2), 375–383.

Vázquez, D., Lage, M.A., Parajó, J.C., Vázquez, G., 1992. Fractionation of *Eucalyptus* Wood in acetic acid media. Bioresour. Technol. 40 (2), 131–136.

Vázquez, M.J., Alonso, J.L., Domínguez, H., Parajó, J.C., 2001. Production of xylose-containing fermentation media by enzymatic post-hydrolysis of oligomers produced by corn cob autohydrolysis. World J. Microbiol. Biotechnol. 17 (8), 817–822.

Vegas, R., Kabel, M., Schols, H.A., Alonso, J.L., Parajó, J.C., 2008. Hydrothermal processing of rice husks: effects of severity on product distribution. J. Chem. Technol. Biotechnol. 83 (7), 965–972.

Verduyn, C., Van Kleef, R., Frank, J., Schreuder, H., van Dijken, J.P., Scheffers, W.A., 1985. Properties of the NAD(P)H-dependent xylose reductase from the xylose-fermenting yeast *Pichia stipitis*. Biochem. J. 226 (3), 669–677.

Verho, R., Putkonen, M., Londesborough, J., Penttilä, M., Richard, P., 2004. A novel NADH-linked L-xylulose reductase in the L-arabinose catabolic pathway of yeast. J. Biol. Chem. 279 (15), 14746–14751.

Vila, C., Garrote, G., Domínguez, H., Parajó, J.C., 2002. Hydrolytic processing of rice husks in aqueous media: A kinetic assessment. Collect. Czechoslovak Chem. Commun. 67 (4), 509–530.

Vongsuvanlert, V., Tani, Y., 1988. Purification and characterization of xylose isomerase of a methanol yeast, *Candida boidinii*, which is involved in sorbitol production from glucose. Agric. Biol. Chem. 52 (7), 1817–1824.

Wahlbom, C.F., van Zyl, W.H., Jönsson, L.J., Hahn-Hägerdal, B., Otero, R.R.C., 2003. Generation of the improved recombinant xylose-utilizing *Saccharomyces cerevisiae* TMB 3400 by random mutagenesis and physiological comparison with *Pichia stipitis* CBS 6054. FEMS Yeast Res. 3 (3), 319–326.

Walfridsson, M., Anderlund, M., Bao, X., Hahn-Hägerdal, B., 1997. Expression of different levels of enzymes from the *Pichia stipitis XYL1* and *XYL2* genes in *Saccharomyces cerevisiae* and its effects on product formation during xylose utilisation. Appl. Environ. Microbiol. 48 (2), 218–224.

Walfridsson, M., Bao, X., Anderlund, M., Lilius, G., Bulow, L., Hahn-Hägerdal, B., 1996. Ethanolic fermentation of xylose with *Saccharomyces cerevisiae* harboring the *Thermus thermophilus xylA* gene, which expresses an active xylose (glucose) isomerase. Appl. Environ. Microbiol. 62 (12), 4648–4651.

Wang, P.Y., Johnson, B.F., Schneider, H., 1980. Fermentation of D-xylose by yeasts using glucose isomerase in the medium to convert D-xylose to D-xylulose. Biotechnol. Lett. 2 (6), 273–278.

Watanabe, S., Kodaki, T., Makino, K., 2005. Complete reversal of coenzyme specificity of xylitol dehydrogenase and increase of thermostability by the introduction of structural zinc. J. Biol. Chem. 280 (11), 10340–10349.

Watanabe, S., Kodaki, T., Makino, K., 2006. Cloning, expression, and characterization of bacterial L-arabinose 1-dehydrogenase involved in an alternative pathway of L-arabinose metabolism. J. Biol. Chem. 281 (5), 2612–2623.

Watanabe, S., Pack, S.P., Saleh, A.A., Annaluru, N., Kodaki, T., Makino, K., 2007. The positive effect of the decreased NADPH-preferring activity of xylose reductase from *Pichia stipitis* on ethanol production using xylose-fermenting recombinant *Saccharomyces cerevisiae*. Biosci. Biotechnol. Biochem. 71 (5), 1365–1369.

Weierstall, T., Hollenberg, C.P., Boles, E., 1999. Cloning and characterization of three genes (SUT1–3) encoding glucose transporters of the yeast Pichia stipitis. Mol. Microbiol. 31 (3), 871–883.

Weisser, P., Kramer, R., Sahm, H., Sprenger, G.A., 1995. Functional expression of the glucose transporter of *Zymomonas mobilis* leads to restoration of glucose and fructose uptake in *Escherichia coli* mutants and provides evidence for its facilitator action. J. Bacteriol. 177 (11), 3351–3354.

Weisser, P., Kramer, R., Sprenger, G.A., 1996. Expression of the *Escherichia coli* pmi gene, encoding phosphomannose-isomerase in *Zymomonas mobilis*, leads to utilization of mannose as a novel growth substrate, which can be used as a selective marker. Appl. Environ. Microbiol. 62 (11), 4155–4161.

Wende, G., Fry, S.C., 1997. *O*-feruloylated, *O*-acetylated oligosaccharides as side-chains of grass xylans. Phytochemistry 44 (6), 1011–1018.

Williamson, G., Faulds, C.B., Kroon, P.A., 1998. Specificity of ferulic acid (feruloyl) esterases. Biochem. Soc. Trans. 26 (2), 205–209.

Wingren, A., Galbe, M., Zacchi, G., 2003. Techno-economic evaluation of producing ethanol from softwood: comparison of SSF and SHF and identification of bottlenecks. Biotechnol. Prog. 19 (4), 1109–1117.

Wisselink, H.W., Toirkens, M.J., Rosario Franco, B.M., Winkler, A.A., van Dijken, J.P., Pronk, J.T., van Maris, A.J., 2007. Engineering of *Saccharomyces cerevisiae* for efficient anaerobic alcoholic fermentation of L-arabinose. Appl. Environ. Microbiol. 73 (15), 4881–4891.

Wisselink, H.W., Toirkens, M.J., Wu, Q., Pronk, J.T., van Maris, A.J.A., 2009. Novel evolutionary engineering approach for accelerated utilization of glucose, xylose, and arabinose mixtures by engineered *Saccharomyces cerevisiae* strains. Appl. Environ. Microbiol. 75 (4), 907–914.

Witteveen, C.F.B., Busink, R., van de Vondervoort, P., Dijkema, C., Swart, K., Visser, J., 1989. L-arabinose and D-xylose catabolism in *Aspergillus niger*. J. Gen. Microbiol. 135, 2163–2171.

Wong, K.K.Y., Tan, L.U.L., Saddler, J.N., 1988. Multiplicity of β-1,4-xylanase in microorganisms – functions and applications. Microbiol. Rev. 52 (3), 305–317.

Woodward, J., 1984. Xylanases: Functions, Properties and Applications. Ellis Horwood Ltd., Chichester.

Wyman, C.E., 1999. Biomass ethanol: technical progress, opportunities, and commercial challenges. Ann. Rev. Ener. Environ. 24, 189–226.

Wyman, C.E., 2003. Potential synergies and challenges in refining cellulosic biomass to fuels, chemicals, and power. Biotechnol. Prog. 19 (2), 254–262.

Xu, J., Thomsen, M.H., Thomsen, A.B., 2009. Enzymatic hydrolysis and fermentability of corn stover pretreated by lactic acid and/or acetic acid. J. Biotechnol. 139 (4), 300–305.

Xu, Z.H., Bai, Y.L., Xu, X., Shi, J.S., Tao, W.Y., 2005. Production of alkali-tolerant cellulase-free xylanase by *Pseudomonas* sp. WLUN024 with wheat bran as the main substrate. World J. Microbiol. Biotechnol. 21 (4), 575–581.

Yablochkova, E.N., Bolotnikova, O.I., Mikhailova, N.P., Nemova, N.N., Ginak, A.I., 2003. The activity of xylose reductase and xylitol dehydrogenase in yeasts. Microbiology 72 (4), 414–417.

Yamaguchi, D., Kitano, M., Suganuma, S., Nakajima, K., Kato, H., Hara, M., 2009. Hydrolysis of cellulose by a solid acid catalyst under optimal reaction conditions. J. Phys. Chem. C 113 (8), 3181–3188.

Yomano, L.P., York, S.W., Ingram, L.O., 1998. Isolation and characterization of ethanol-tolerant mutants of *Escherichia coli* KO11 for fuel ethanol production. J. Ind. Microbiol. Biotechnol. 20 (2), 132–138.

Yomano, L.P., York, S.W., Shanmugam, K.T., Ingram, L.O., 2009. Deletion of methylglyoxal synthase gene (mgsA) increased sugar co-metabolism in ethanol-producing *Escherichia coli*. Biotechnol. Lett. 31 (9), 1389–1398.

Yomano, L.P., York, S.W., Zhou, S., Shanmugam, K.T., Ingram, L.O., 2008. Re-engineering *Escherichia coli* for ethanol production. Biotechnol. Lett. 30 (12), 2097–2103.

Yoon, H.H., Wu, Z.W., Lee, Y.Y., 1995. Ammonia recycled percolation process for pretreatment of biomass feedstock. Appl. Biochem. Biotechnol. 51–2, 5–19.

Yourchisin, D.M., van Walsum, G.P., 2004. Comparison of microbial inhibition and enzymatic hydrolysis rates of liquid and solid fractions produced from pretreatment of biomass with carbonic acid and liquid hot water. Appl. Biochem. Biotechnol. 113–16, 1073–1086.

Zacchi, G., Axelsson, A., 1989. Economic evaluation of preconcentration in production of ethanol from dilute sugar solutions. Biotechnol. Bioeng. 34 (2), 223–233.

Zaldivar, J., Ingram, L.O., 1999. Effect of organic acids on the growth and fermentation of ethanologenic *Escherichia coli* LY01. Biotechnol. Bioeng. 66 (4), 203–210.

Zaldivar, J., Martinez, A., Ingram, L.O., 1999. Effect of selected aldehydes on the growth and fermentation of ethanologenic *Escherichia coli*. Biotechnol. Bioeng. 65 (1), 24–33.

Zaldivar, J., Martinez, A., Ingram, L.O., 2000. Effect of alcohol compounds found in hemicellulose hydrolysate on the growth and fermentation of ethanologenic *Escherichia coli*. Biotechnol. Bioeng. 68 (5), 524–530.

Zavrel, M., Bross, D., Funke, M., Buchs, J., Spiess, A.C., 2009. High-throughput screening for ionic liquids dissolving (ligno-)cellulose. Bioresour. Technol. 100 (9), 2580–2587.

Zhang, M., Eddy, C., Deanda, K., Finkelstein, M., Picataggio, S., 1995. Metabolic engineering of a pentose metabolism pathway in ethanologenic *Zymomonas mobilis*. Science 267 (5195), 240–243.

Zhang, Q., Yan, X., Zhang, L., Tang, W., 2006. Cloning, sequence analysis, and heterologous expression of a β-mannanase gene from *Bacillus subtilis* Z-2. Mol. Biol. 40 (3), 368–374.

Zhang, Y.H.P., Ding, S.Y., Mielenz, J.R., Cui, J.B., Elander, R., Laser, M., Himmel, M.E., McMillan, J.R., Lynd, L.R., 2007. Fractionating recalcitrant lignocellulose at modest reaction conditions. Biotechnol. Bioeng. 97, 214–223.

Zhang, Z., Zhao, Z.K., 2009. Solid acid and microwave-assisted hydrolysis of cellulose in ionic liquid. Carbohydr. Res. 344, 2069–2072.

Zhao, X.B., Cheng, K.K., Liu, D.H., 2009. Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. Appl. Microbiol. Biotechnol. 82 (5), 815–827.

Zheng, Y.Z., Lin, H.M., Tsao, G.T., 1998. Pretreatment for cellulose hydrolysis by carbon dioxide explosion. Biotechnol. Prog. 14 (6), 890–896.

Zhou, S., Iverson, A.G., Grayburn, W.S., 2008. Engineering a native homoethanol pathway in *Escherichia coli* B for ethanol production. Biotechnol. Lett. 30 (2), 335–342.

Zhou, S., Yomano, L.P., Shanmugam, K.T., Ingram, L.O., 2005. Fermentation of 10% (w/v) sugar to D(−)-lactate by engineered *Escherichia coli* B. Biotechnol. Lett. 27 (23), 1891–1896.

Zhu, Y., Kim, T.H., Lee, Y.Y., Chen, R., Elander, R.T., 2006. Enzymatic production of xylooligosaccharides from corn stover and corn cobs treated with aqueous ammonia. Appl. Biochem. Biotechnol. 129–132, 586–598.