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Delignification Process of Agro-Industrial Wastes an Alternative to Obtain Fermentable Carbohydrates for Producing Fuel

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1. Introduction

Fossil fuels, mainly petroleum, coal, and natural gas, were the main energy sources for most industries during the 20th century, and are still the most important feedstocks to produce energy in the world. "Currently, the world energy market worth around 1.5 trillion dollars is dominated by fossil fuels" (Goldemberg, 2006). However, these sources are not longer regarded sustainable, and their availability is much lower. Shafiee and Topal (2009) predicted that oil, coal and gas reserves will last around 35, 107 and 37 years for petroleum, coal and natural gas, respectively. In addition their combustion involve environmental issues such as global warming due to greenhouse gas emissions (Naik et al., 2010). Therefore, the interest for questing sustainable and environmental energy sources has risen in the last two decades; giving origin to the production of fuels from renewable feedstocks, such as biomass. These feedstocks are commonly divided in three categories: wood, residues from agricultural, industrial or domestic origin, and energy crops from dedicated farming (Bringezu et al., 2007).

The use of these renewable resources to produce fuel has created two different generations in biofuel production. The first biofuel generation is based on grain or food sources, and is constituted mainly by ethanol, fatty acid methyl ester (FAME), and pure plant oil (PPO) (Bringezu et al., 2007). However, environmental impacts, energy efficiency, and eutrophycation have limited the production of these first generation biofuels. In addition, land competition of energy crops with food crops has arisen a fuel-versus-food debate exacerbated by the increase of food prices, particularly those of maize, wheat, sugar beet, cassava, sweet sorghum, sugarcane, oilseed rape, soybean and oil palm (Börjesson & Tufvesson, 2011; Vries et al., 2010). For example, the United States Department of Energy reported that in 2005 the bioethanol production reached about 15 billion litters for which nearly 36 MMt of maize, 13% of total US maize crops, were used. These values were estimated to double in 2010 by the Renewable Fuel Association (Cassman & Liska, 2007).

The second generation of biofuels is produced from non-grain and non-food sources such as lignocellulosic sources and algae biomass (Naik et al., 2010; Simmons et al., 2008).

Lignocellulosic feedstocks include agro-industrial by-products, perennial grasses, vegetable and wood residues. They can be burned to produce heat and electricity and also be used to obtain liquid fuels (Naik et al., 2010). Wastes and by-products from agro-industrial processes such as coconut shells, rice husks, sugarcane bagasse, corncob and corn stover among many others, are abundantly produced in the world daily and have modest if any applications. These wastes and by-products are rich sources of cellulose and hemicellulose, which constitute important substrates in fermentative processes directed to biofuel production. However, as opposed to sugarcane juice or maize starch, these substrates are not readily available. The structural carbohydrates in the plant cell wall are wrapped up in lignin, which is an inert polymer that protects the plant and consequently constitutes an important barrier to fermentation. Therefore, a very effective way —but not the only option— to significantly increase biomass digestibility is lignin degradation or separation (delignification). The operation is aimed to increase the digestibility of constituent sugars through increment in gross material pore size (Sierra et al., 2008). It is challenging due to the recalcitrance of lignin and may require expensive chemicals and relatively high temperatures and pressures for acceptable reaction rates. Otherwise, at mild conditions (i.e. use of microorganisms or purified enzymes) it takes long times. Other ways to increase lignocelluloses digestibility include partial to total solubilization of hemicelluloses, and separation acetyl groups that link hemicellulose and lignin (Zhu et al., 2008). Crystallinity reduction of cellulose fibrils is sought because low crystallinity results in more reactivity; however, after delignification and sugar degradation with chemicals, an increased crystallinity is usually observed (Chang & Holtzapple, 2000). This increase is attributed to a preferential degradation of amorphous cellulose and less ordered crystalline forms during chemical pretreatment. A common method to obtain a significant reduction in crystallinity is sudden release of reactor vapor pressure. This operation is known as steam explosion.

The operations aimed to turn lignocellulose digestible through either of the mechanisms described above are widely known as “pretreatments”. They typically start with size reduction by chipping and grinding. In addition to being a rate-limiting step, a chemical pretreatment increases the cost of bioethanol production due to the high-energy requirements of heating and mechanical size reduction. Energy consumption during size reduction of wood may surpass 0.1—0.4 MJ/kg, which is the required energy consumption to achieve sensible net energy output from wood to ethanol production (Kumar et al., 2009).

Chemical separation of lignin and carbohydrates can be achieved through the use of acids, alkalies, and solvents, which promote selective solubilisation of either component. If acidic, carbohydrates solubilise; if alkaline, lignin degrades and solubilises (Mosier et al., 2005); if with solvents –widely known as organosolv pretreatment– carbohydrates solubilise (Zhao et al., 2009). Chemical processes may not be as selective as biological processes but may represent advantages related to required time, scalability, and process control.

Biological delignification can be conducted using either microorganisms, which produce a set of enzymes that work synergically, or purified enzymes. The most widely used microorganisms are fungi from the *Basidiomysetes* family. Nevertheless, bacteria from *Pseudomonas*, *Flavobacteria*, *Xanthomonas*, *Bacillus*, *Aeromonas* and *Cellulomonas* strains can also decompose lignin and its derivatives. Biological lignin degradation can be conducted by culturing the microorganism in submerged, semisolid or solid cultures where enzymes such as lignin peroxidase, xylanase, laccase, and manganese peroxidase (among others) perform selective lignin degradation.

In this chapter, the reactions that lead to delignification of feedstocks in several different choices of chemical and biological pretreatments are discussed. In addition, other compositional changes during pretreatment are briefly reviewed. Although some basic chemical aspects related to lignocelluloses compositional analysis is presented here, it is out of the scope of this chapter to make a deep chemical discussion on this subject. All throughout the text, the reader is referred to several recent reviews and key articles for more detailed discussions.

2. Generalities of lignocellulosic compounds

Lignocellulose is the major renewable organic matter with an estimated production of about 200 x 10^9 tons per year (Reddy & Yang, 2005). This is mainly constituted by lignin and cell wall polysaccharides such as cellulose, pectins, and hemicelluloses, which may be valuable substrates for biofuels production. Lignin is found in the cell wall and sometimes within woody tissue in quantities that vary widely depending on the type of plant, the part of the plant, and its age. Klason lignin is reported to range between 8 and 22% for herbaceous crops and between 19 to 30% for woody crops (Hatakeyama & Hatakeyama, 2005). Lignins are complex natural polymers resulting from oxidative coupling of, primarily, 4-hydroxyphenylpropanoids. The monomers conforming lignin are p-coumaril, conyferil, and sinapyl alcohols. They differ from each other in the degree of methoxylation (Fig. 1). The proportion in which they are present in lignin varies widely depending on the type of plant. These monomers produce p-hydroxyphenyl, guaiacyl, and syringyl phenylpropanoid units (Fig. 1), which are capable of generating electron delocalized radicals that couple at various sites (Boerjan et al., 2003). These monomers can form under different chemical routes dimmers or participate in lignifications. Some basic linkages between lignin monomers are illustrated in Fig. 1. For a deeper discussion refer to Boerjan et al. (2003).

Cellulose is constituted entirely by glucan chains linked by β(1→4) bonds which interact with each other via hydrogen bonds (Keegstra, 2010). Hemicelluloses on the other hand, are constituted by backbones of glucose β(1→4) linked, mannose, and xylose with an equatorial configuration. Consequently, hemicelluloses include xylans (mainly), glucans, mannans, glucomannans, and xyloglucans (Scheller & Ulvskov, 2010).

2.1 Physic-chemical properties of lignin

Complete studies and reviews of the chemistry and physic-chemical properties of lignin were reported in the ACS symposium 397 (Glasser & Sarkanen, 1989), and by few authors (Agarwal & Atalla, 2010; Hatakeyama & Hatakeyama, 2010a; 2010b; Hatakeyama & Hatakeyama, 2005; Ralph & Landucci, 2010; Schmidt, 2010). A brief summary of these revisions is presented here. Readers seeking a greater detail should consult the aforementioned references.

Lignin molecular mass ranges from 10^3 and 10^5 depending on plant species and variations. The reported values may also be influenced by extracting or processing methods and analytic techniques. Soluble lignin presents a discrete maximum absorbance at 205 or 280 nm, with an extinction coefficient (ε) varying according to the lignin source and the used solvent. It also presents a characteristic fluorescence with a broad maximum emission ranging from 400 to 500 nm, depending on the excitation wavelength. Lignin is an amorphous polymer and presents a broad distribution of molecular arrangements, with

intra- and intermolecular distances that can vary from 0.40 to 0.98 nm. The glass transition of lignin in solid state does not present first order thermodynamic transitions; and at temperatures lower than the decomposition temperature, lignin can adopt a glassy or rubbery state. It has been shown that the heat capacity and intermolecular distance increase at the glass transition temperature (T_g). Also, T_g increases linearly with increments in lignin molecular weight, and strongly depends on the thermal history of the lignin sample. For example, T_g in kraft lignin is close to 100 °C, while it varies between 70 to 85 °C for hydrolysis lignin. Since the solidification rate affects the enthalpy of glassy lignin, enthalpy of the glassy state decreases when a lignin sample is slowly cooled from the melt. On the other hand, if the sample is quenched, the enthalpy increases since molecular chains are more randomly frozen than those of slowly glassified samples. Then, in samples below T_g the measured enthalpy of glassy polymers decreases as a function of time. This is called enthalpy relaxation, which is monitored through heat capacity change at glass transition.

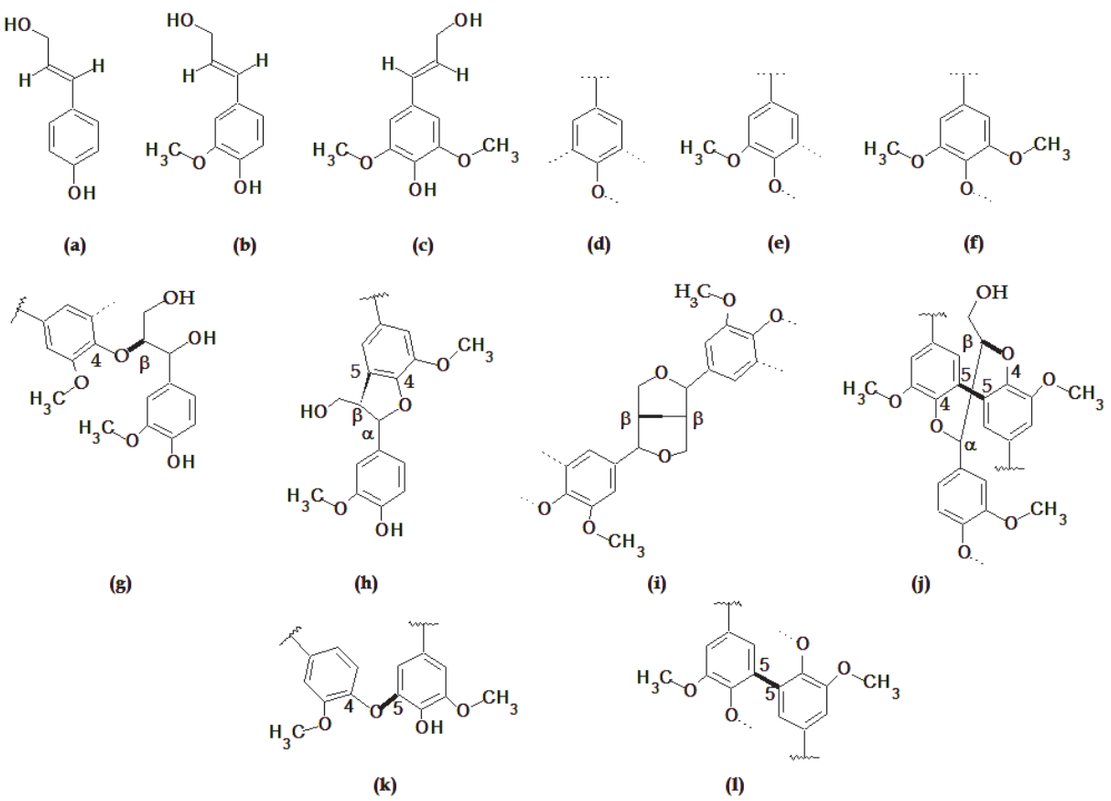

Fig. 1. Lignin monomers p-coumaril (a), conyferil (b) and sinapyl (c) alcohols, and their units inside lignin p-hydroxyphenyl (d), guaiacyl (e), and syringyl (f). Major structural units of lignin and presence in softwood (sw) and hardwood (hw), (g) β-O-4 unit (β- aryl ether), sw: 50 % and hw: 60 %; (h) β-5 (α-O-4) unit (phenylcoumaran), sw: 11% and hw 6 %; (i) β-β unit (resinol), sw and hw ~ 2 %; (j) [5-5/β-O-4(α-O-4)] unit (dibenzodioxocin); (k) 4-O-5 unit (biphenyl ether), sw and hw ~ 5 %; (l) 5-5 unit (biphenyl), sw: 18 % and hw: 10 % (Boerjan et al., 2003; Dimmel, 2010).

2.2 Chemical composition of agro-industrial wastes and by-products

Knowledge of chemical components of agro-industrial wastes produced worldwide should precede any attempt for fuel production. Although it is difficult to establish the major agro-industrial crops and their by-products generated worldwide, this work presents in Table 1 a selection of agro-industrial by-products based on their use for bioethanol production, and major crops reported by the FAO (2008) for the most populated countries in the word (China, India, US, Indonesia, Brazil, Bangladesh, Nigeria, Russia and Japan). This is according to the Population Reference Bureau (PRB, 2010).

3. Biological delignification

The production of alcohols from lignocellulosic materials involve two steps: 1) the hydrolysis of macromolecules (cellulose, hemicellulose, lignin and xylose) to release the carbohydrates that are further depolymerized to produce free sugars, and 2) the fermentation of these free sugars (Martinez et al., 2009; Perez et al., 2002). Among the lignocellulose constituent macromolecules, lignin is the most recalcitrant due to its amorphous hydrophobic heteropolymeric nature (Martinez et al., 2009). Although chemical/thermal processes allow good delignification levels, biodelignification has mild reaction conditions, higher product yields, few side reactions, less energy demand, and less reactor requirements to resist pressure and corrosion, which makes the bio-delignification a promissory pre-treatment for the production of biofuels. However, bio-delignification takes much longer than chemical or thermal processes, usually 8-12 weeks (Yu et al., 2010a), showing the importance of exploring new microorganisms, and the improving of culture conditions. Tables 2 and 3 summarize some fungi and bacteria used for bio-delignification of lingocellulosic materials.

3.1 Delignifying enzymes

Bio-delignification can be produced by the action of lignin peroxidase (LiP), manganese peroxidase (MnP), laccase, and versatile peroxidase (VP). Although up to now it has not been described a microorganism producing all the ligninolytic enzymes, the presence of two or more enzymes have been associated with higher delignification levels than those observed with the isolated enzymes showing the synergistic action of this set of enzymes (Costa et al., 2002; Gonçalves et al., 1998; Kannan et al., 1990).

3.1.1 Lignin peroxidase

LiP [EC 1.11.1.14] has been mainly isolated from *Phanerochaete chrysosporium*. Nevertheless, LiPs have been also found in other white- and brown-rot fungi strains, *Aspergilllus* strains, and bacteria such as *Acinetobacter calcoaceticus*, *Streptomyces viridosporu*s and *Streptomyces lividans* (Crawford et al., 1993; Ghodake et al., 2009; Zerbini et al., 1999). LiPs are glycosilated enzymes of about 340 amino acids with a molecular weight between 38 and 50 kDa, a single heme, and two calcium ions (Hammel & Cullen, 2008; Sinclair et al., 1992). LiPs isozymes of *P. chrysosporium* are encoded by a family of 10 closely related and well-characterized genes (Hammel & Cullen, 2008), while in *Trametes versicolor* the LiP gene is arranged into a gene cluster encoding for two LiP and one MnP (Johansson & Nyman, 1996), and bacterial LiP seems to be encoded only for one gene (Wang et al., 1990).

By-product	Country	L[c]	G	X	A	C	H	Reference
Corn storver	US	13.3	31.9	18.9	2.8			(Templeton et al., 2009)
Cotton stalk	China	19.75				35.60	21.88	(Zhang et al., 2010)
Sugarcane baggase	Brazil	22.8	43.4	24.4	2.0			(Carrasco et al., 2010; Guo et al., 2009)
Oil palm (empty fruit bunches)	Colombia	4.15				46.77	17.92	(Piarpuzán et al., 2011)
Silvergrass	Europe [a,b]	7.6-11.5				43.0-52.2	24.8-33.9	(Hodgson et al., 2010)
Switchgrass (*Panicum sp.*)	US	21.4	35.0	21.8	3.5			(Garlock et al., 2011)
Rice straw	China	7.2-12.8				30.3-38.2	19.8-31.6	(Jin & Chen, 2007)
Wheat straw	Canada	18.15	38.27	18.75	1.5			(Tamaki & Mazza, 2010)
Oat straw	India	15.5				36.0	48.5	(Pandey & Pandey, 2002)
Barley straw	Russia	22.2				41.3	19.5	(Kocheva et al., 2008)
Rye straw	Russia	20.5				49.0	17.4	(Kocheva et al., 2008)
Soya stalks	Russia	25.4				37.6	17.3	(Torgashov et al., 2010)
Coconut husk	Malaysia	32.8				44.2	12.1	(Khalil et al., 2006)
Eucalyptus wood	China	26.2	44.9	11.4				(Yu et al., 2010c)
Sunflower stalks	India	17.5				38.5	33.5	(Sharma et al., 2002)
Sorghum stover	China	14.3	27.3	13.1	1.4			(Li et al., 2010)
Cassava baggase	Thailand	2.2	19.1	4.2	1.4			(Kosugi et al., 2009)
Olive wood	Spain	20.4				34.4	20.3	(Ruiz et al., 2006)
Eucalyptus wood	Brazil China	27.1 26.2	51.49 44.9	13.11 11.4	0.47			(Brito et al., 2008) (Yu et al., 2010c)
Construction wood waste	Korea	22.7-25.3		6.3-9		45.4-51.2		(Cho et al., 2011)
Pinus wood	US	26.8		6.6	1.6	43.6		(Frederick Jr et al., 2008)

[a] Samples were taken in three countries: Germany, Denmark and Sweden. [b] Variations are due to the *Miscanthus* species and harvest season. [c] Lignin composition refers to total acid- soluble and insoluble lignin. N.D: Not determined.

Table 1. Composition of major agro-industrial by-products use in delignification processes to obtain fermentable carbohydrates (L, lignin; G, glucan; X, xylan; A, arabian or arabinosyl substituents; C, cellulose, and H, hemicellulose).

Strain	Culture Conditions			L* (%)	Substrate	Remarks	Reference
	Process	Temp. (°C)	Time (d)				
Aspergillus niger	SS	55	0.08	20	Cellulose pulp	Crude extract containing xylanases	(Betini et al., 2009)
Aspergillus niveus	SS	55	0.08	20	Cellulose pulp	Crude extract containing xylanases	(Betini et al., 2009)
Aspergillus ochraceus	SS	55	0.08	20	Cellulose pulp	Crude extract containing xylanases	(Betini et al., 2009)
Ceriporiopsis subvermispora	SS	27	30	20	Sugarcane bagasse	Xylanase and MnP production	(Costa et al., 2005)
	SS	25	60	N.S.	Paddy straw	Cellulose lost.	(Sharma & Arora, 2010)
	SS	28	42	32	Corn stover	Improvement in sugar hydrolysis	(Wan & Li, 2010)
Echinodontium taxodii	Sm	25	30	42	Corn straw	Enhanced efficiency of alkaline/oxidative treatment	(Yu et al., 2010b)
Euc-1	SS	28	35	55	Wheat straw	Laccase, MnP and LiP production	(Dias et al., 2010)
Gleophyllum trabeum	SS	27	12	0	Wood chips	68% of carbohydrate degradation	(Fissore et al., 2010)
Gonoderma lacidum	Sm	25	30	55	Corn straw	Enhanced efficiency of alkaline/oxidative treatment	(Yu et al., 2010b)
Irpex lacteus	SS	28	35	55	Wheat straw	MnP and LiP production	(Dias et al., 2010)
	SS	28	15	12	Corn stalks	Combination with mild alkaline pretreatment allowed 80% lignin reduction	(Yu et al., 2010a)
Oxysporus sp.	Sm	25	60	69	Pomace from olive oil	Laccase production	(Haddadin et al., 2002)
Panos tigrinus strains	SS	30	10	4 to 7	Sugarcane bagasse	Expression of MnP, LiP and lacasse	(Gonçalves et al., 1998)
Penicillium oxalicum + *Pleurotus osteatus*	SS	N.S.	0.125	21	Wood pulp	Synergistic action of crude extract containing xylanase and laccase	(Dwivedi et al., 2010)
Phanerochaete chrysosporium	Sm	39	14	34	Cotton stalks	Improvement in carbohydrate availability	(Shi et al., 2009)
	SS	39	10	21	Cotton stalks	Improvement in carbohydrate availability	(Shi et al., 2009)
Phlebia brevispora	SS	25	60	N.S.	Paddy straw	Cellulose lost.	(Sharma & Arora, 2010)

Strain	Culture Conditions			L* (%)	Substrate	Remarks	Reference
	Process	Temp. (°C)	Time (d)				
Phlebia floridensis	SS	25	60	N.S.	Paddy straw	Cellulose lost.	(Sharma & Arora, 2010)
Phlebia radiate	SS	25	60	N.S.	Paddy straw	Cellulose lost.	(Sharma & Arora, 2010)
Pycnoporus cinnabarinus	SS	24	30	12	Sugarcane bagasse	No cellulose degradation	(Meza et al., 2006)
Pycnoporus sanguineus	Sm	30	21	25	Wheat straw		(Lu et al., 2010)
	Sm	28	1	71	Wheat straw	Native and recombinant laccase	(Lu et al., 2010)
	Sm	30	21	27	Corn strover		(Lu et al., 2010)
	Sm	28	1	57	Corn strover	Native and recombinant laccase	(Lu et al., 2010)
Pynnoporus cinnabarinus	SS	30	15	12	*Prosopis juliflora*	20% increment in sugar release	(Gupta et al., 2010)
	SS	30	15	8	*Lantana camara*	20% increment in sugar release	(Gupta et al., 2010)
Trametes versicolor	Sm	25	30	52	Corn straw	Enhanced efficiency of alkaline/oxidative treatment	(Yu et al., 2010b)

Table 2. Fungus strains used for the delignification of lignocelullose materials. L* = Lignin Lost, Sm = Submerged Fermentation, SS = Solid-state fermentation, MnP = Manganese peroxidase, LnP = Lignin peroxidase , N.S. = Non specified.

Strain	Culture Conditions			L (%)	Substrate	Remarks	Reference
	Process	Temp. (°C)	Time (d)				
Bacillus macerans	SS	28	45	50	Cotton saw		(Singh et al., 2008)
Bacillus pamilus	Sm	55	2	8	Kraft-pulp	Cellulase-free	(Kaur et al., 2010)
Bacillus sp.	Sm	35	2	56	Pulp and paper mill effluent	Xylanase and MnP production	(Mishra & Thakur, 2010)
Cellulomonas cartae	SS	28	45	50	Cotton saw		(Singh et al., 2008)
Cellulomonas uda	SS	28	45	50	Cotton saw		(Singh et al., 2008)
Zymomonas mobilis	SS	28	45	33	Cotton saw		(Singh et al., 2008)

Table 3. Bacteria strains used for the delignification of lignocelullose materials. L* = Lignin Lost, Sm = Submerged Fermentation, SS = Solid-state fermentation, MnP = Manganese peroxidase, LnP = Lignin peroxidase , N.S. = Non specified.

Due to the complex nature of lignin, the delignification process must be carried out extracellulary, and the LiP is found in all the peripheral regions of the fungal cell cytoplasm in association with the cell membrane, fungal cell wall, and extracellular slime materials (Daniel et al., 1989). In addition, in solid wood LiP is detected in low concentrations associated with both superficial and degradation zones within secondary cell walls undergoing fungal attack, while in liquid cultures (i.e. submerged-state fermentation) a much greater level of extracellular peroxidase activity is associated with wood fragments degraded by fungus. Although LiP is able to oxidize aromatic compounds with a high redox potentials by single electron abstraction and is the most efficient lignin-degrading enzyme (Piontek et al., 2001), *in vitro* the majority of lignin-derived preparations actually experience overall polymerization after LiP exposure (Sarkanen et al., 1991). LiP activity is controlled by the ionization degree suggesting that specifics amino acids residues play a role in lignin binding, and that this enzyme is capable of oxidizing lignin directly at the protein surface by a long-range electron transfer process (Johjima et al., 1999).

LiPs degrade lignin through H_2O_2 using a mechanism that resembles that used for other peroxidades (Hammel & Cullen, 2008; Martinez et al., 2005; Wong, 2009). The first step involves the formation of a high redox potential oxo-ferryl intermediate (compound I, LiP-I) as result of the reaction of the heme cofactor with H_2O_2. In a second step two consecutive $1e^-$ reductions are carried out (Fig. 2): (i) a $1e^-$ reduction of LiP by a reducing substrate yields to compound II (LiP-II) and a substrate radical cation, and (ii) a $1e^-$ reduction that returns the enzyme to the ferric oxidation state, completing the catalytic cycle (Wong, 2009). The radical cation of the substrate produced in this cycle undergoes rearrangements and non-enzymatic degradations (Fig. 2), which in turn leads to a set of reactions that results in lignin depolymerization (Wong, 2009). Mutagenesis studies have shown that LiP has two distinct substrate interaction sites: (i) the classical heme edge, which degrades lignin-derived compounds and dyes, and (ii) the glutamine 146 site, which is implicated in catalysis of lignin-derived compounds.

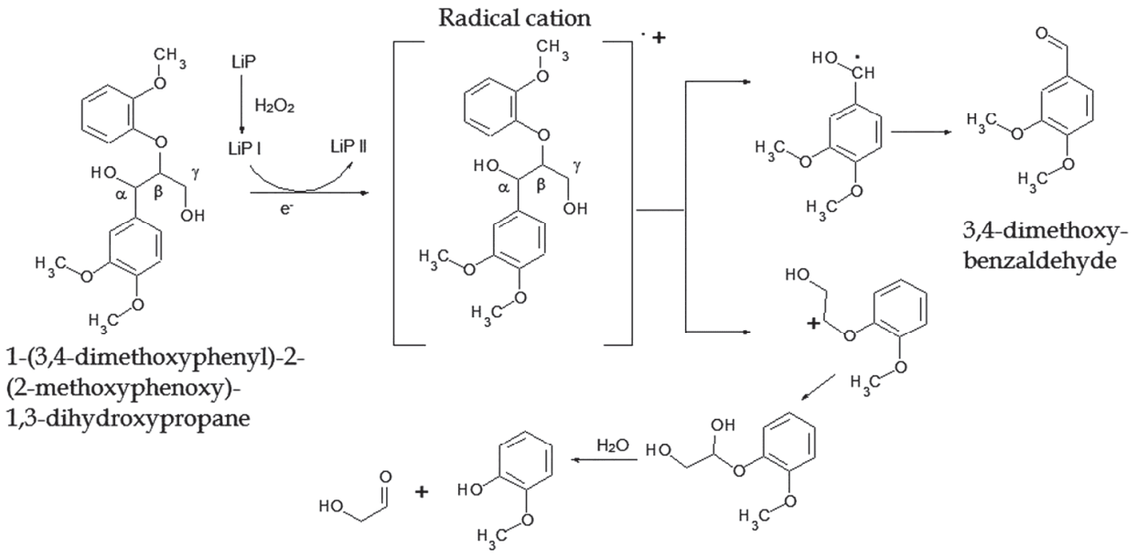

Fig. 2. LiP-catalyzed oxidation of non-phenolic β-O-4 lignin model compound. Modified from Wong (2009).

LiP genes have been cloned and expressed in *Phanerochaete sordida*, *Trichoderma reesei*, *Pichia pastoris*, *Pichia methanolica* and *Escherichia coli*, obtaining recombinant enzymes with similar properties to those observed in native enzymes (Nie et al., 1998; Saloheimo et al., 1989; Sugiura et al., 2009; Wang & Wen, 2009). A recent work showed that by coupling a directed evolution technique with a rapid colorimetric screening method, it was possible to obtain a recombinant LiP with improved H_2O_2 stability, polychlorinated phenol degradability, and kinetic properties (Ryu et al., 2008).

3.1.2 Manganese peroxidase

The MnPs [EC 1.11.1.13] share many of the characteristics previously discussed for LiPs. They are glycosilated, extracellular heme-containing enzymes of about 350 amino acids, a molecular weight around 40 kDa, and the same peroxidase catalytic cycle of LiP (Martinez et al., 2009; Martinez et al., 2005). MnP has been isolated mainly from basidiomycetes including *P. chrysosporium*, *Schizophyllum sp.*, *Ceriporiopsis subvermispora*, *Panus tigrinus*, *Lentinula edodes*, *Nematoloma frowardii*, *Bjerkandera adusta*, *T. versicolor*, and *Dichomitus squalens*, among others. The presence of MnP has been also reported in an *Aspergillus terreus* strain (Kanayama et al., 2002). The enzyme production is specie- and strain-dependent, with an important role of the carbon source, the lignocellulosic substrate and the presence of aromatic compounds (Elisashvili & Kachlishvili, 2009). Catalytic cycle of MnP is similar to that of LiP (Fig. 3): (i) compound I (MnP-I), a Fe(IV)-oxo-porphyrin radical cation, is produced by reaction of enzyme and H_2O_2, (ii) Mn^{2+} reduces compound I to compound II, producing Mn^{3+}, and (iii) the resting enzyme is regenerated (Wong, 2009). This Mn^{3+} oxidizes phenolic substrates in a second-order reaction, producing phenoxy-radicals, which in turn led to a set of reactions that result in lignin depolymerization (Perez et al., 2002) (Fig. 3).

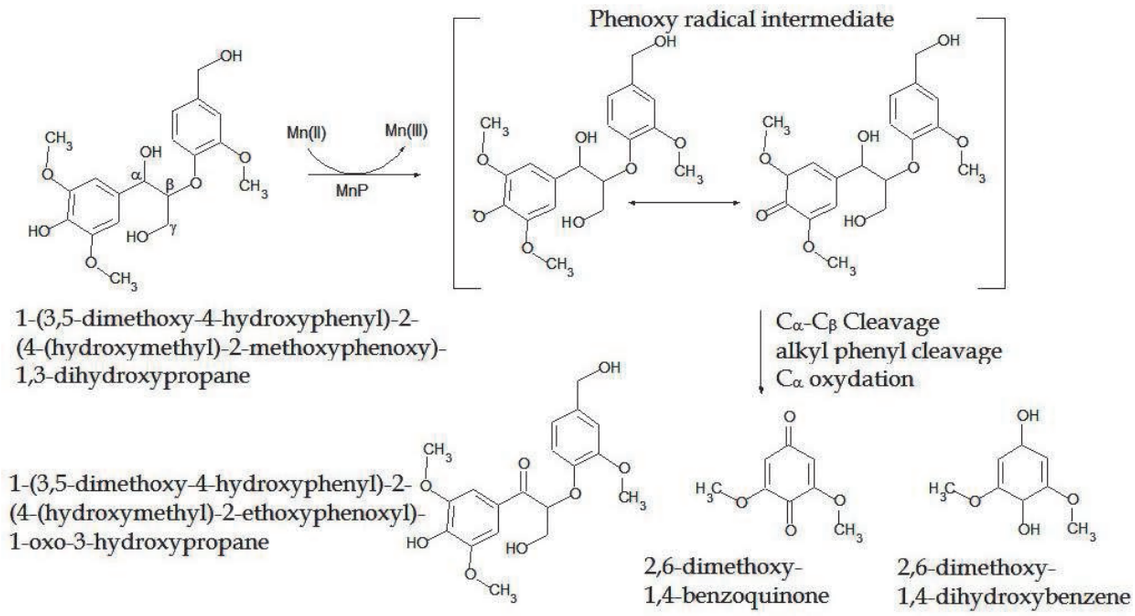

Fig. 3. MnP-catalyzed oxidation of phenolic aryglycerol β-aryl ether lignin model compound. Modified from Wong (2009).

The MnP has a single binding site for Mn^{2+} near the heme, from which two Mn^{3+} equivalents are obtained at the expense of one H_2O_2 equivalent in a reaction requiring oxalate or another appropriate manganese chelator (Timofeevski & Aust, 1997; Wariishi et al., 1992). However, MnP can also catalyze a manganese-dependent disproportionation of H_2O_2 in the absence of manganese chelator, which may protect the enzyme from inactivation by H_2O_2 under limited free oxalate conditions (Timofeevski & Aust, 1997). Reaction of Mn^{3+} with H_2O_2 is catalyzed by Cu^{2+}, which explains the inhibition of MnP by Cu^{2+} (Aitken & Irvine, 1990). Active site and crystal structure analysis of *P. chrysosporium* MnP, the most studied MnP enzyme, have shown that arginine 42 and acidic amino acids (i.e glutamic or aspartic acid) at positions 35, 45, 39 and 179 are involved in Mn^{2+} binding (Sundaramoorthy et al., 2010; Whitwam et al., 1997), while serine 172 seems to been involved in heme binding (Ambert-Balay et al., 2000). MnP encoding genes have been cloned from several white-rot fungi strains, where up to three different genes have been reported each one encoding for a specific isoform (Alvarez et al., 2009; Martinez et al., 2009; Martinez et al., 2005). The expression of MnP genes is mainly regulated by Mn via a growth-stage-specific and concentration-dependent mechanism (Brown et al., 1991). Cu^{2+}, Zn^{2+}, Ag^{+}, Cd^{2+}, Se, H_2O_2, ethanol, sodium arsenite, and 2,4-dichlorophenol, as well as heat shock regulate the MnP gene expression, while the co-administration with Mn significantly enhances the MnP production (Alvarez et al., 2009; Catal et al., 2008).
MnP genes have been homo- or heterologous expressed in *P. chrysosporium*, *P. ostreatus*, *P. pastoris*, *Aspergillus oryzae*, and *Zea mays*, producing recombinant enzymes with similar kinetic and stabilities properties to those observed for native enzymes (Clough et al., 2006; Gu et al., 2003; Jiang et al., 2008).

3.1.3 Laccase

Laccases [EC 1.10.3.2] are glycosilated multicopper enzymes, with wide molecular weights ranging from 50 to 140 kDa (Gaitan et al., 2011; Wong, 2009). Unlike LiP and MnP, laccases are widely distributed in nature and can be found in plants, fungi, bacteria and insects (Dwivedi et al., 2011; Riva, 2006). Although transgenic plants over-expressing laccase genes have been used for energy production, phytoremediation, and alteration in phenolic metabolism (Riva, 2006), most of the delignification process have been carried out by using microorganisms. In fungi, where they play a role in the host-pathogen interaction during the first step of infection (Punelli et al., 2009), laccases have been found in *Trametes versicolor, Trametes pubescens, Pycnoporus cinnabarinus, Myceliophthora thermophila, Pleurotus eryngii, Pleurotus ferulae, Pleurotus astreatus, Cerrena unicolor, Cyathus bulleri, Lentinula edodedes*, and *Agaricus blazei,* among others. In bacteria the enzyme has been reported in *Azospirrullum lipoferum*, *Marinomonas medierranea, Streptomyces griseus, Haloferax volcanii,* and *Bacillus subtilis*. Laccases are monomeric or homopolymeric enzymes (Riva, 2006; Wong, 2009), and the cellular localization depends on the organism, with plant and fungal laccases as extracellular enzymes, while an intracellular localization is observed in most of the bacterial laccases (Diamantidis et al., 2000; Riva, 2006).
Laccase catalyze the monoelectric oxidation of phenolic and nonphenolic substrates to their corresponding reactive radicals in a reaction mediated by the four Cu atoms located at the catalytic core. During the substrate oxidation there is a reduction of one oxygen molecule to two water molecules and four radicals (Arora & Sharma, 2010). The four copper atoms are arranged in three different groups: Type-1 or blue Cu, Type-2 or normal Cu and Type-3 or

coupled binuclear Cu centers. These Cu atoms produce the lignin degradation in a three-step reaction: (i) the Type-1 Cu is reduced by oxidizing lignin, (ii) the electron is transferred from Type-1 Cu to Type-2 and Type-3 Cu cluster, and (iii) oxygen is reduced to water at Type-2 and Type-3 Cu centres (Dwivedi et al., 2011; Riva, 2006). Laccases can oxidize a wide range of substrates including polyphenols, methoxysubstituted phenols, and aromatic diamines, among others, through Cα-Cβ cleavage, alkyl-aryl cleavage or C_α oxidation (Fig. 4) (Arora & Sharma, 2010; Wong, 2009). Since laccases use oxygen instead of the H_2O_2 uses for peroxidases, the toxic effect of this compound in cell viability can be avoided giving to the laccases an important role in early stages of delignification (Sterjiades et al., 1993). Due to the large size of laccases and the highly complex structure of lignin, in most cases it cannot be degraded directly, requiring the presence of intermediate substrates (i.e. chemical mediators), which oxidized radicals are able to induce the depolymerization of complex substrates (Riva, 2006).

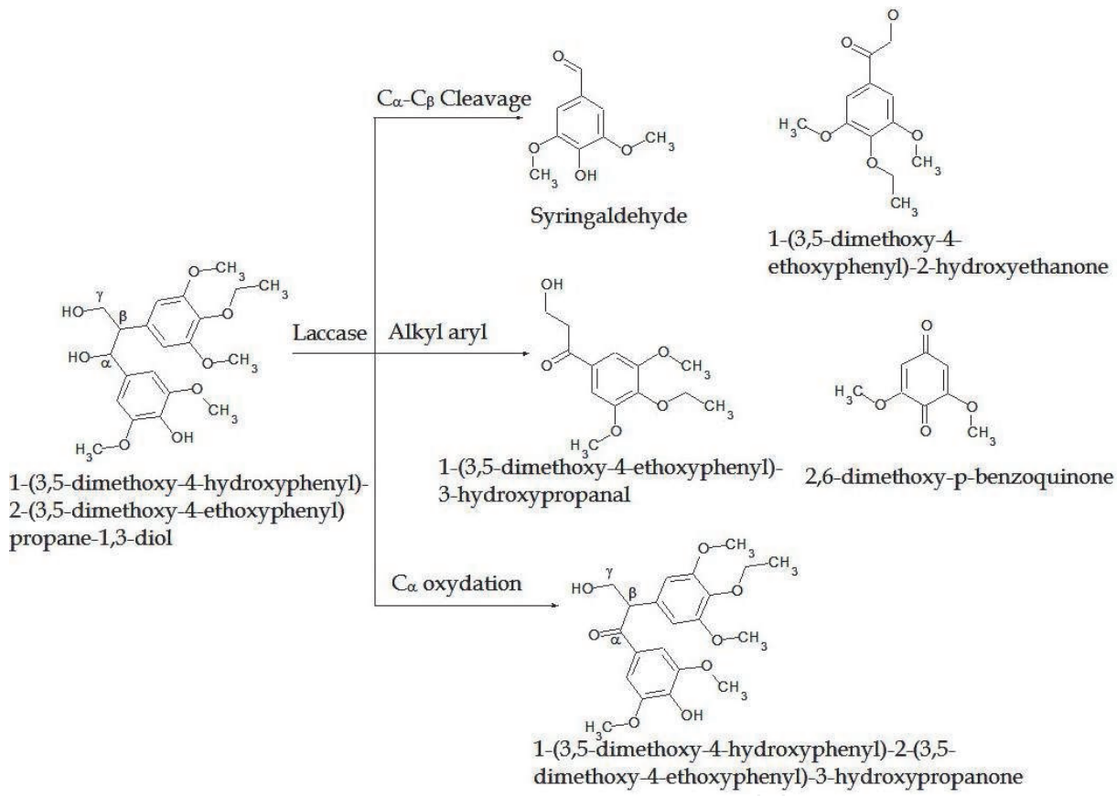

Fig. 4. Laccase-catalyzed oxidation of phenolic β-1 lignin model compound. Modified from Wong (2009).

Laccases are presented as up to six isoenzymes, with different molecular weights, expression profiles, stability, substrate affinity, and encoded by different genes (Dwivedi et al., 2011; Gaitan et al., 2011; Xiao et al., 2006). Laccase gene expression is mainly up regulated by Cu^{2+} addition, although other potent inducers are Mn^{2+}, Fe^{3+}, heavy metals, 2,6-dimethoxy-1,4-benzoquinone, H_2O_2, caffeine, amphotericin B, syringic acid, tannic acid, Tween 80, soybean oil, aromatic compounds, and microclimatic changes (i.e. lower temperature and osmotic pressure) (Dekker et al., 2007; Galhaup et al., 2002; Xiao et al.,

2006). On the other hand, laccase production can be down-regulated or inhibited by proline, urea, glucose Hg^{2+}, Mg^{2+}, Ca^{2+}, Sn^{2+}, Ba^{2+}, Co^{2+}, Cd^{2+}, Mn^{2+}, and Zn^{2+}, fatty acids, sulfydryeagents, hydroxyglycine, kojic acid, EDTA, l-cysteine, dithiothreitol, glutathione, thiourea, and cationic quaternary ammonium detergents; by chelating the Cu atoms, by modifying amino acid residues or by causing conformational changes in the glycoprotein (Dekker et al., 2007; Dwivedi et al., 2011; Galhaup et al., 2002).

Laccase genes have been cloned and expressed in *Pichia pastoris*, *P. methanolic*, *Kluyveromyces lactis*, *Aspergillus niger*, *Coriolus versicolor*, and *E. coli*, with significantly higher levels and similar or even better kinetic and stability profiles than those observed for native enzymes (Guo et al., 2006; Hong et al., 2007; Ranieri et al., 2009; Rodriguez et al., 2008; Salony et al., 2008).

3.2 Fungi delignification

During the last two decades white-rot fungi strains have shown to produce the more efficient delignification (Martinez et al., 2009; Vicuña, 2000; Wong, 2009) *Phanerochaete chrysosporium* is the most studied white-rot fungi used in biodelignification, with lignin reduction levels ranging from 11 to 73% and incubation times between 10 to 60 days. However, the differences among the substrates (i.e. rice straw, cotton stalks, mustard straw and sugarcane bagasse) and the culture conditions (e.g. solid-state fermentation-SSF, submerged fermentation-SmF, temperature and time), limit the comparison between the studies.

Shi et al. (2009), showed that higher lignin lost was observed in SmF than in SSF delignification of cotton stalks, while a 73% lignin lost of kraft pulp was observed with SmF while no delignification was observed in SSF (Pellinen et al., 1989). Wheat straw and sugarcane bagasse, two important substrates for biofuels production (Kaparaju et al., 2009; Vasquez et al., 2007), showed low lignin degradation by *P. chrysosporium* strains (Dorado et al., 1999; Li et al., 2002). Similar results were observed for sugarcane bagasse delignification with *Panos tigrinus* (Gonçalves et al., 1998), *Phanerochaete sordida* (Li et al., 2002) and *Pycnoporus cinnabarinus* (Meza et al., 2006), showing the recalcitrant nature of this substrate for delignification. However, delignification levels above 50% were observed with *Irpex lacteus* for wheat straw (Dias et al., 2010) and *Phlebia sp*. for sugarcane bagasse (Li et al., 2002; Osono & Takeda, 2006). Significant delignification results have also been reported for *Coriolus versicolor* (Tripathi et al., 2008), *Echinodontium taxodii* (Yu et al., 2010b), *Euc-1* (Dias et al., 2010), *Gonoderma* (Bourbonnais et al., 1997; Haddadin et al., 2002; Tripathi et al., 2008; Yu et al., 2010b), *Oxysporus sp.* (Haddadin et al., 2002), *Phlebia sp.* (Li et al., 2002), *Trametes versicolor* (Yu et al., 2010b) and *Trichoderma reesei* (Singh et al., 2008), with reduction of lignin levels ranging from 40% to 60%. Recent studies have shown that actinomycetes strains (e.g. *Aspergillus*) can induce lignin degradation levels of up to 92%, with similar culture conditions to those used by basidiomycetes (Singh et al., 2008).

Since carbohydrates are needed for the second stage of biofuel production (i.e. sugar fermentation), the degradation during the delignification stage is a side reaction that affects the yield of biofuel production. Hence, a selection of a microorganism with a low cellulose activity is an important task. *Phanerochaete chrysosporium* (Shi et al., 2009), *Pycnoporus cinnabarinus* (Meza et al., 2006), *Echinodontium taxodii* (Yu et al., 2010b), *Euc-1* (Dias et al., 2010), *Irpex lacteus* (Dias et al., 2010; Yu et al., 2010a), and *Pycnoporus sanguineus* (Lu et al., 2010), have shown high lignin degradation specificity, while significant levels of

carbohydrate degradation have been reported for *Ceriporiopsis subvermispora* (Wan & Li, 2010), *Gleophyllum trabeum* (Fissore et al., 2010), *Phlebia brevispora* (Sharma & Arora, 2010), *Phlebia floridensis* (Sharma & Arora, 2010), *Phlebia radiata* (Sharma & Arora, 2010), and *Pleurotus sajor-caju* (Kannan et al., 1990). This carbohydrates degradation during biodelignification could also depend on the substrate, since fungi with high lignin selectivity can show carbohydrate lost with certain lignocellulosic materials, as observed for paddy straw that presented cellulose lost with all the white-rot fungi used in the delignification (Sharma & Arora, 2010).

Biodelignification has a direct effect on the availability of sugars for ethanol production, which significantly improves the yield of biofuel production. Although this beneficial effect of delignification is not evaluated in all the studies, the results show that there is no need to produce a 100% delignification since increases above 20% in carbohydrates availability can be obtained with just 8% delignification (Gupta et al., 2010; Shi et al., 2009; Wan & Li, 2010). In addition, the biodelignification has been used before chemical pretreatments (i.e. alkaline/oxidative or mild alkaline) allowing up to 80% lignin reduction (Yu et al., 2010a; Yu et al., 2010b). These results strongly suggest that combination of biological and chemical/physical delignification methods might be a feasible alternative to improves delignification levels and reduces the volume and conditions of the chemical/physical pretreatments, promoting the generation of eco-friendly processes for paper and biofuels companies.

Finally, the use of crude extracts from ligninolytic fungi has shown to be a promissory alternative for delignification. Crude extracts from *Aspergillus niger*, *A. niveus*, and *A. ochraceus,* containging xylanases, allowed a 20% delignification of cellulase pulp in 2 h (Betini et al., 2009), while a crude extract of *Penicillium oxalicum* and *Pleurotus osteatus*, containg xylanase and laccase enzymes, produced a 21% delignification of wood pulp in 3 h (Dwivedi et al., 2010).

3.3 Bacterial delignification

Bacteria are less used microorganism in delignification of lignocellulose materials. Most of the studies use *Bacillus* strains (Table 3), although *Cellulomonas* and *Zymomonas* have been recently reported as promissory ligninolytic bacteria (Singh et al., 2008).

The loss of lignin ranges from 8% to 56%, with similar temperatures and times to those used in fungi-mediated delignification. Bacteria delignification is mainly mediated by extracellular xylanases, although a synergistic effect has been observed by the addition of MnP, pectinase or α-L-arabinofuranosidase (Bezalel et al., 1993; Kaur et al., 2010). However, these high lignin removal levels might be accompanied with high levels of cellulose degradation, as observed for *B. macerans*, *C. cartae*, *C. uda*, and *Z. mobilis* with cellulose reductions ranging from 31% to 51%. One strategy to overcome this issue is the use of cellulase-free extracts (Kaur et al., 2010) or purified enzymes (Bezalel et al., 1993), that allows up to 20% delignification levels within shorter incubations times than those observed with the whole microorganisms.

Even though bacteria have shown significant delignification levels, the main application is in the paper industry on pre-bleaching steps or to reduce toxic compounds from paper mill effluents. However, biodelignification has a direct impact in pulp properties, free-sugars, and reduction in effluent toxicity and in the use of chloride compounds (Kaur et al., 2010; Mishra & Thakur, 2010; Singh et al., 2008).

4. Chemical separation of Lignin and carbohydrates

This section establishes the basis of three types of chemical pretreatment: acidic, alkaline and with solvents. For each, lignin and carbohydrate solubilisation (or degradation) are discussed. It is clear that different types of chemical pretreatments affect biomass differently not only because of differences in the solubilized component (Harmsen et al., 2010), but also because the accessibility attained is different and changes differently as subsequent enzymatic hydrolysis occurs (Kumar & Wyman, 2009).

A few reports giving meaningful comparisons between different types of chemical pretreatment have been obtained (Wyman et al., 2005a; 2005b; Wyman et al., 2009). A more complete coverage is impractical because different authors present important differences in selected substrates, analytical procedures, and/or method to report results. Abatzoglou et al. (1992) discuss the application of a severity factor or severity parameter to account for the effect of acid on lignocelluloses composition and the extent of reaction if acid pretreatment is used. They state that due to the complexity of reactions, kinetic parameters lack mechanistic meaning and are functions of the ranges of experimental conditions used. Severity factors combine into a single parameter the effect of the different operational variables and have been adapted to other types of chemical pretreatments. One widely adopted defining equation (slightly or importantly modified in some later studies) is as follows:

$$R_0 = e^{\left[(T-100)/14.5\right]t} \tag{1}$$

where R_0 is the reaction ordinate, T is the pretreatment temperature in ^{o}C, and t is the pretreatment time in min. R_0 is meant to provide a zonal indication of the predominant reactions taking place in lignocelluloses. This end result was empirically motivated.

4.1 Acidic

Two main configurations of acid pretreatment are diluted and concentrated. During dilute acid pretreatment structural carbohydrates are efficiently solubilized and possibly degraded to an extent that depends on the pretreatment conditions. Here, partial hemicelluloses solubilisation is obtained, therefore increasing pore size. Hydrolysis of the remaining hemicelluloses and cellulose is frequently achieved through a subsequent enzymatic hydrolysis stage that uses enzyme cocktails containing cellulases and xylanases (Mosier et al., 2005; Wyman & Lloyd, 2005). The acidic reagent may be liquid hot water (pH of water decreases with temperature), dilute or concentrated hydrochloric, sulfuric, phosphoric, peracetic, oxalic, and maleic acid among others. Virtually any acid (either mineral or organic) may be used, but sulfuric acid is widely preferred, even though recent studies have shown better hemicelluloses yields with other acids (Lee & Jeffries, 2011). Pretreatment time (accounted from the moment the biomass reaches the desired pretreatment temperature) ranges between 1 min and 180 h. Reactor configurations include but are not limited to flow-through, continuous, and batch. Temperatures range between 25 and 200°C at pressures between 1 atm and 15 atm. Initial acid concentration may range between 0.1 and 6%, more typically between 0.7 and 4% (Kumar et al., 2009; Sierra et al., 2008). In batch reactors, total pressure corresponds to the saturation pressure of the mixture at the pretreatment temperature. Typically, the highest temperatures require less time and result in a more extensive cellulose degradation. The dilute sulfuric acid pretreatment can achieve high

reaction rates and significantly improves cellulose hydrolysis (Esteghlalian et al., 1997). Its cost is similar or higher than for other pretreatment options (Eggeman & Elander, 2005).
Neutralization of pH is necessary for the downstream enzymatic hydrolysis or fermentation processes. Dilute-acid pretreatment is also known to have a negative influence on the enzymatic hydrolysis of biomass. For example, recent studies report the formation of spherical droplets of lignin and/or lignin and carbohydrate complexes on the cellulose surface. These droplets, preferentially formed at temperatures greater than 130°C, have an important negative impact on biomass subsequent enzymatic digestibility (Selig et al., 2007). Some other researches argue that materials that have been subjected to acid hydrolysis can be harder to ferment because of the presence of toxic substances including but not limited to furfural and hydroxymethylfurfural (Galbe & Zacchi, 2002). Furthermore, acid pretreatment results in costly materials of construction, high pressures, neutralization and conditioning of hydrolysate prior to biological steps, slow cellulose digestion by enzymes, and nonproductive binding of enzymes to lignin (Wyman et al., 2005b).
The concentrated acid pretreatment is more widely known as concentrated acid hydrolyisis because it results in complete solubilisation of carbohydrates. Clearly, subsequent enzymatic hydrolysis is not required. This type of pretreatment has a long story (1883 for hydrolysis of cellulose in cotton) with the first industrial plant built in Germany in 1937 using hydrochloric acid. The Arkenol's process, claimed to reduce sugar loss to less than 3%, separating 98% of the acid (Farone & Cuzens, 1996), starts mixing lignocelluloses waste (less than 10% moisture content) with 70%-77% sulfuric acid added at a ratio of 1.25:1 (acid : cellulose+hemicellulose), the temperature is controlled at less than 50°C. Afterwards, water is added to dilute the acid to 20%-30 and hydrolysis at 100°C takes place for an hour. This results in a gel, which is pressed to remove an acid/sugar product stream. Residual solids are subjected to a second hydrolysis step. A chromatographic column is used to separate acid and sugars (Nanguneri & Hester, 1990). The fermentation converts both the xylose and the glucose to ethanol at theoretical yields of 85% and 92%, respectively. A triple effect evaporator is required to reconcentrate the acid (Yancey & Kadam, 1997).
The industrially used concentrated pretreatment must not be confused with the complete solubilisation of carbohydrates widely used for quantification of structural lignin and carbohydrates. The corresponding protocol uses sulfuric acid reagent in two stages, one concentrated at mild temperature (72% w/w, 30°C) and the other diluted at high temperature (4% w/w, 121°C) (Sluiter et al., 2004).

4.1.1 Reactions during acid pretreatment

During acid pretreatment a rather severe solubilisation (and some degradation of monomers) of hemicelluloses occur. Concurrently, a change in the lignin structure takes place. Wood lignin submitted to diluted acid pretreatment (Sannigrahi et al., 2008), as well as wood submitted to steam explosion (Li et al., 2007), and switchgrass submitted to diluted acid pretreatment (Pingali et al., 2010) presented a decrease in the β-O-4 linkages in lignin, which are fragmented during high temperature acid-catalyzed reactions. The extent at which these reactions occur is illustrated in Fig. 5 (Li et al., 2007). Other effects of acid pretreatment on the structure of wood lignin include a decrease in the protonated and oxygenated aromatic carbons per aromatic ring (Sannigrahi et al., 2008).
As a result of the substantial cleavage of β-O-4 linkages, the molecular weight distribution of lignin would be expected to decrease; however, this is not the case. A comprehensive repolymerization (condensation reactions) resulting in an increase in molecular size and a

more heterogeneous lignin structure is obtained (Li et al., 2007) (Fig. 6). The simultaneous depolymerization and repolymerization of lignin during an acid pretreatment are undesirable reactions since they will lead to an increase in the heterogeneity of the resulting lignin. In addition, solubility and reactivity properties will be negatively affected. Reactions, shifting the pH-conditions of the steam treatment towards the alkaline side could be one way to reduce the formation of carbonium ions and inhibit severe structural changes in the lignin polymer. Alternatively, the addition of inhibitors for the repolimerization reactions (i.e., reactive phenol, 2-naphthol (Li et al., 2007)) lead to an important delignification with the production of lignin with uniform structure.

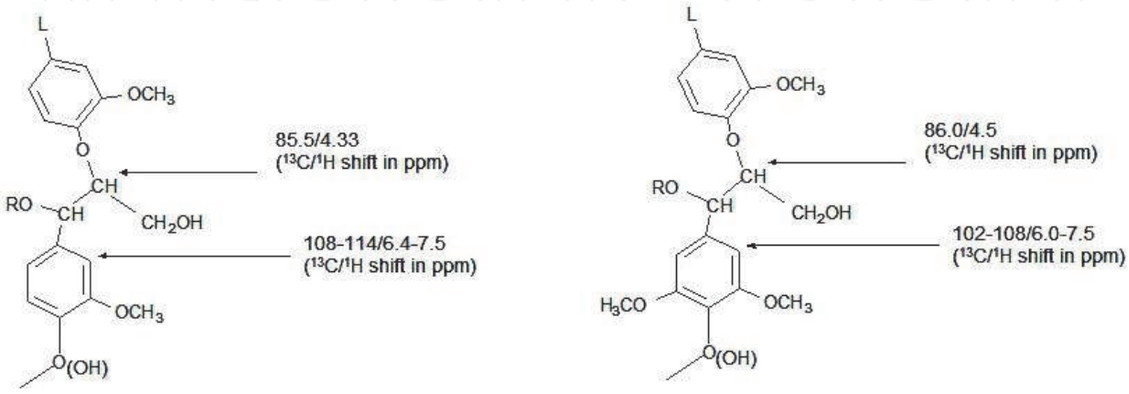

Fig. 5. The β-O-4 structure in lignin together with $^{13}C/^{1}H$ shift values for CH-groups.

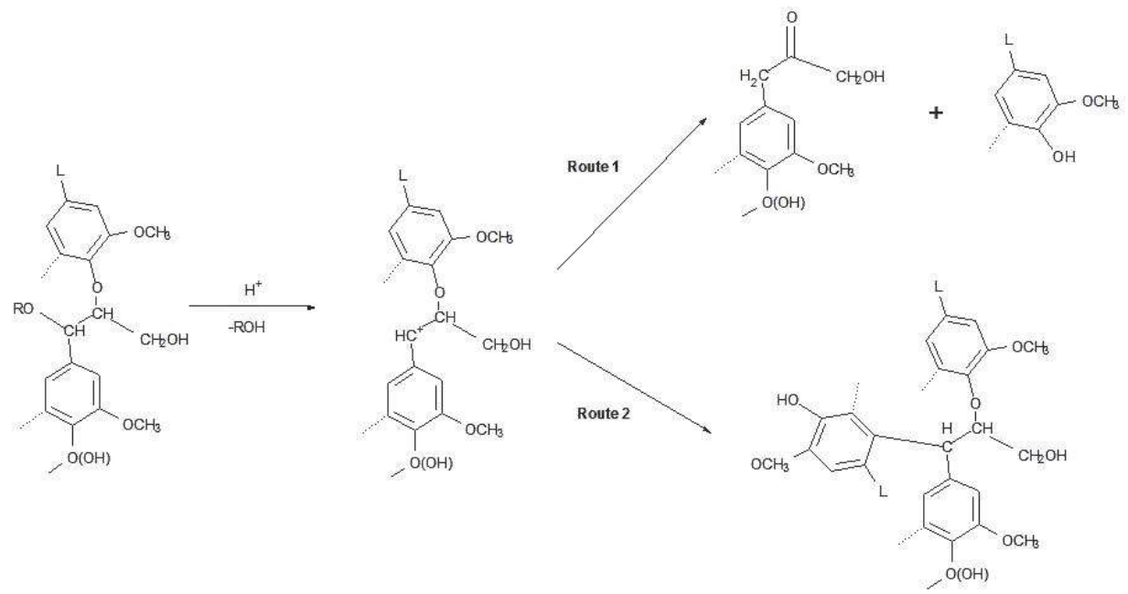

Fig. 6. Competition between depolimerization (route 1) and repolimerization (route 2) reactions in lignin during acid pretreatment (Li et al., 2007).

On the other hand, depolymerization of polysaccharides resulting from steam treatment through cleavage of glucosidic linkages is desirable for conversion of carbohydrates through fermentation processes. However, if monomeric carbohydrates are further degraded into

HMF (from hexoses) and furfural (from pentoses), a true yield loss occurs. Maximum loss of glucose and xylose in HFM and furfural formation have been reported as ~35% and ~5% of original content respectively depending on pretreatment conditions (Farone & Cuzens, 1996). While the carbohydrate content decreases following pretreatment, the proportion of hexose sugars within the carbohydrate fraction increases. Li et al (Li et al., 2005) reported that only about half this fraction was true lignin while the rest comprised "pseudo-lignin" material. This lignin like material is formed by dehydration and repolymerization of polysaccharide degradation products such as furfural, which are formed during high temperature pretreatments.

The discussion presented here is not comprehensive but only fundamental. The presence of products from lignin, glucose and xylose degradation reactions including but not limited to organic acids, phenols and aromatic aldehydes (Chen et al., 2006) is an indication of this fact. In depth studies to elucidate the mechanisms through which these reactions occur is important because all of the degradation products are inhibitors of fermentation and result in an undesired carbohydrates yield loss.

4.1.2 Kinetic modeling acid pretreatment

As discussed in the previous section, all of the main cell wall constituents of lignocelluloses react during acid pretreatment. However, kinetic studies are not available for lignin reactions. The main focus of kinetic modeling work has been hemicelluloses hydrolysis with its consequent degradation. Because xylose is often the main constituent in hemicelluloses, comprising up to 90% of the total hemicellulose dry weight depending on the feedstock (McMillan, 1992), hemicelluloses degradation modeling only accounts for the production of xylose monomers. Three main mechanisms have been proposed and are depicted in Fig. 7. The most adapted mechanism (Fig. 7b) uses a four-step pseudo-first-order irreversible reactions with Arrhenius type constants.

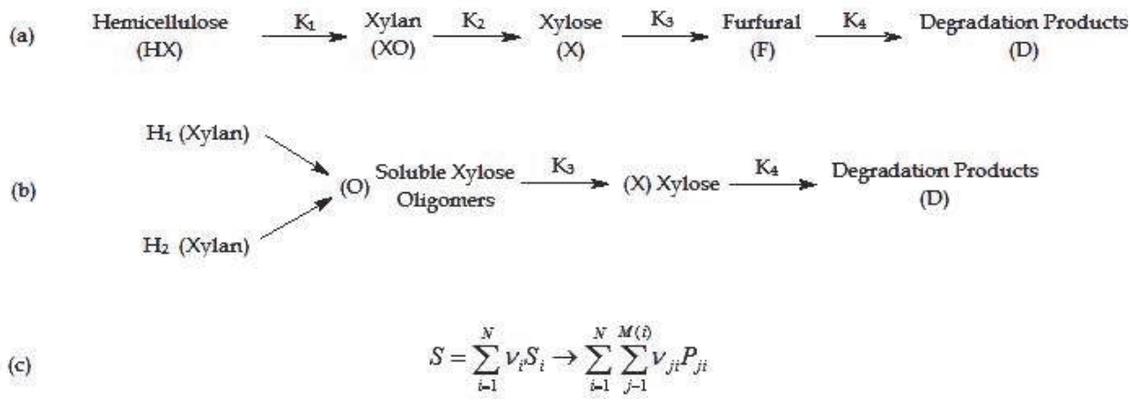

Fig. 7. Mechanisms for degradation of hemicelluloses during acid pretreatment. (a) Accounts for the formation of furfural and from there other degradation products (Morinelly et al., 2009) (b) Accounts for different hemicelluloses hydrolysis reactivities (fast and slow)(Chen et al., 1996; Esteghlalian et al., 1997; Grohmann et al., 1985; Lee et al., 2000; Schell et al., 2003) (c) lumped system approach. A reaction system (S) is composed of N subsystems producing M final products P. The reaction stoichiometric coefficients are ν (Abatzoglou et al., 1992).

Some work also accounts for cellulose degradation reactions as shown in the following equation (Lee et al., 2000):

$$\text{Cellulose} \xrightarrow{k_1} \text{Glucan} \xrightarrow{k_2} \text{Glucose} \xrightarrow{k_3} \text{Degradatio n products} \quad (2)$$

Rate equations are then obtained for the different reactors and reactions considered. For example, for reactions in Fig. 7a the equations are:

$$\frac{dXH(t)}{dt} = -k_1 XH(t); \frac{dXO(t)}{dt} = -k_1 XH(t) - k_2 XO(t); \frac{dX(t)}{dt} = -k_2 XO(t) - k_3 X(t); \frac{dF(t)}{dt} = -k_3 X(t) - k_4 F(t) \quad (3)$$

For models in Fig. 7b, reported activation energies in k_1 and k_2 varied between 50 and 250 kJ kmol^{-1} depending on the reactor employed and the feedstock used. The highest values were reported for continuous reactors at pilot plant scale using corn stover (Schell et al., 2003) and the lowest for wheat straw in a batch reactor (Grohmann et al., 1985). High activation energies may serve as an indication that the system follows a chemical reaction-limited kinetics rather than a mass transport limited kinetics. Some interesting conclusions from the diverse dilute sulfuric acid kinetic studies are: (i) xylose yields are favored at high temperatures and short times (Chen et al., 1996; Esteghlalian et al., 1997; Grohmann et al., 1985; Lee et al., 2000; Morinelly et al., 2009; Schell et al., 2003); (ii) selectivity defined in terms of either Arrhenius constants ratios or activation energies ratios shows that high temperatures have an enhancing effect on the hemicelluloses breakdown to oligomers (i.e., in Fig. 7a, $E_1/E_2 > 1$), however, the hydrolysis of oligomers is less favored than the formation of degradation products (i.e., in Fig 7a, $E_2/E_3 < 1$) (Morinelly et al., 2009); (iii) high solids concentration is desirable because in addition to help the process economics, less degradation of xylose to furfural is obtained, and these solids act as a barrier that protects monomers from degradation, though the main drawback of is slow reaction rates (Morinelly et al., 2009); (iv) total carbohydrates yields are lower at pilot scale than they are in laboratory scale, demonstrating the importance of pilot plant scale measurements in the scaling up of the process (Schell et al., 2003); (v) countercurrent shrinking bed reactors result in solubilisation of both cellulose and hemicellulase, but selectivity of desired monomers is better than with other reactors because of lower residence times (Lee et al., 2000); and (vi) no direct comparisons among different studies can be made due to important differences in reaction conditions.

4.2 Alkaline

Alkali delignification of lignocellulosic biomass is widely applied at industrial scale. The process is known as soda and kraft pulping and its chemistry has been extensively discussed (Alen, 2000; Klinke et al., 2002; Sjöström, 1981). Frequently, an oxidative agent is applied at high temperatures obtaining an important enhancement of the pretreatment effects. Sodium, potassium, calcium, and ammonium hydroxides are suitable for this type of pretreatment. Among these, calcium hydroxide is the least expensive and can be easily regenerated using the lime Kiln technology; however, at industrial scale, sodium hydroxide is widely preferred (Sanchez, 2007). Lime pretreatment has proven to be a useful method for selectively reducing the lignin content of lignocellulosic biomass without significant loss in carbohydrates, thus realizing an important increase in biodigestibility (Chang et al., 1997; Kim & Holtzapple, 2005; Sierra, 2005; Sierra et al., 2009a; 2009b). With ammonium

hydroxide two main processes have been developed: Ammonia Freeze Explosion (AFEX) and Ammonia Recycle Percolation (ARP). In AFEX, biomass is mixed with liquid ammonia and then treated at high-pressure. When pretreatment time is elapsed, the pressure is suddenly released. The combined chemical and physical effects of this pretreatment increase lignocelluloses pore size making it digestible (Shao et al., 2010). There are many adjustable parameters in the AFEX process: ammonia loading, water loading, temperature, time, blow down pressure, and number of treatments (Holtzapple et al., 1991). In ARP the biomass is pretreated with aqueous ammonia in a flow-through column reactor. The liquid flows at high temperature through the reactor column, which has been previously packed with biomass. To prevent flash evaporation the reactor system must be slightly pressurized (Kim et al., 2003). After reaction the solid fraction, rich in cellulose and hemicellulose, is separated from the liquid.

Another pretreatment ranked within this category is referred to as *wet alkaline oxidation* or *catalytic wet oxidation*. The alkali agents reported are NaOH and Na_2CO_3. This is not to be confused with *wet oxidation* pretreatment which does not use an alkali. The oxidative agent may be oxygen, air, or hydrogen peroxide (Klinke et al., 2002). Alkaline wet oxidation uses temperatures above 180°C, pressures in the range 1.4 – 1.6 MPa, and reaction times up to 30 min.

4.2.1 Reactions during alkaline pretreatment

The major effect of alkaline pretreatment is delignification. Alkaline pretreatments successfully increase lignocelluloses digestibility without the production of furfural and methylfurfural (Harmsen et al., 2010; Sierra et al., 2008). Concurrently, acetyl removal is obtained, which is advantageous because acetyl groups inhibit fermentation (Wyman et al., 2009). Alkaline hydrolysis mechanism is based on saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and lignin (Sun & Cheng, 2002).

Although lignin degradation has been the subject of much study over many years, it is far from being completely understood. Complete biomass delignification is difficult because of its location within the deep cell wall, hydrophobicity, physical stiffness, strong polyring bonds of C–O–C, C–C, and the tendency of recondensation (Kim et al., 2003). Fortunately, complete delignification is not required to make biomass fully digestible (Sierra et al., 2009b; Sierra et al., 2010).

Alkaline depolymerization of lignin mostly depends on the cleavage of two types of aryl ether bonds: $C_{aliphatic}$—O—$C_{aromatic}$ and $C_{aromatic}$—O—$C_{aromatic}$ (ordered from least to most stable), which frequently correspond to α- and β-aryl ether bonds (50-70% in wood). Examples of these typical delignification reactions (only OH^- anions involved) are presented in Fig. 8. Oxidative agents importantly enhance the effects of alkaline pretreatments. Oxygen is relatively unreactive; however, in alkaline media it is reduced through the reaction with phenolic hydroxyl groups to superoxide radical ($-O_2\bullet$). The production of these groups requires very basic conditions (pH >12). Reactions involved in alkaline oxidative pretreatments are primarily single-electron (radical) reactions. Delignification reactions involve the formation of several different acids that introduce hydrophilic groups into the lignin structure. Nucleophilic attack also occurs in some extend causing ring opening, which promotes further degradation and solubilization. Condensation products may leave remaining lignin unreactive in the oxidative alkaline media.

Because lignin fractions contain reactive groups, undesirable condensation reactions may occur between lignin entities retarding delignification. This is known to occur mostly in terminal phases of delignification processes and at the unoccupied C-5 position of phenolic units (Sjöström, 1981).

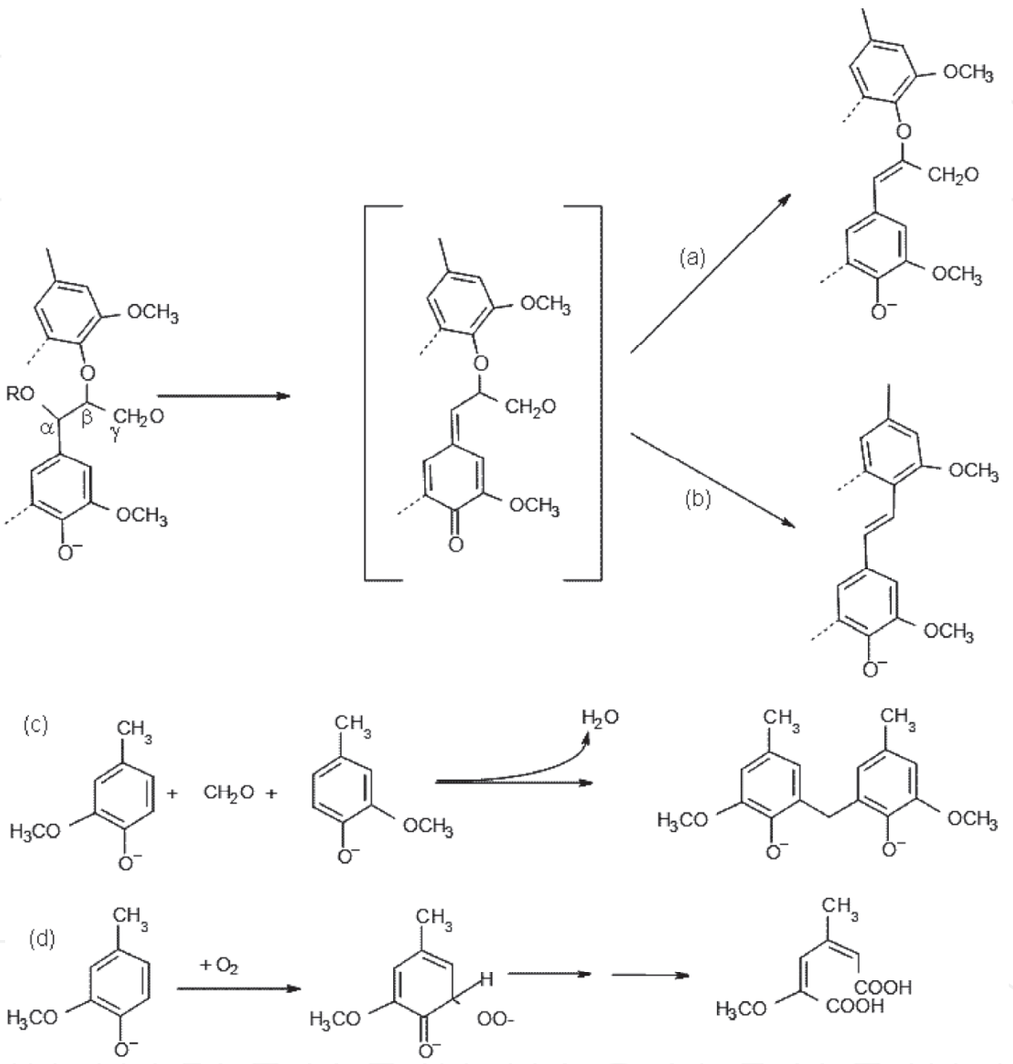

Fig. 8. Lignin degradation reactions in alkaline conditions involving α- and β-aryl ether linkages (a) cleavage of α-aryl ether linkage (b) cleavage of CH_2O group (c) example of a possible condensation reaction. (d) Example of alkaline oxygen degradation of lignin (Gierer & Stockholm, 1985; Guay, 2000).

Unfortunately, alkaline delignification is not completely selective. A fraction of carbohydrates in the presence of alkali and oxygen undergo both oxidation and alkaline degradation generating a complex mixture of products. A wide variety of organic acids are produced as a result of carbohydrates degradation during alkaline pretreatment (Klinke et al., 2002). Due to this fact, an important decrease in pH may take place during pretreatment, particularly if initial alkali concentration is not high. If pretreatment starts with a high initial

alkali concentration, pH is approximately constant and first order reaction rates of cellobiose degradation are obtained (Bonn et al., 1985).

4.3.2 Kinetic modeling alkaline pretreatment

Kinetic models applied to oxygen bleaching of paper pulp focus on the degradation of polymers, either lignin or carbohydrates. Even though there is complex modeling (Kopelman, 1988), most models separate different moieties that degrade at three different rates: rapid, medium, and slow (de Groot et al., 1994; Susilo & Bennington, 2007). These models were successfully applied to lignin and carbohydrate degradation of lime pretreatment of sugar cane bagasse (Granda, 2005), corn stover (Kim & Holtzapple, 2006), and poplar wood (Sierra et al., 2011a; Sierra et al., 2011b).

Two parallel, first-order reactions accurately represent delignification and carbohydrate degradation. They consider differing reactivities for each biomass component (lignin, cellulose, and hemicellulose). This model successfully represents literature data (Sierra et al., 2011a).

$$-\frac{dY_i}{dt} = k_{if} M_{O_2}{}^{\beta if} Y_{if} + k_{is} M_{O_2}{}^{\beta is} Y_{is} \tag{4}$$

Where

$$k_{ij} = a_{ij} \exp\left(-\frac{E_{ij}}{RT}\right) \tag{5}$$

and

k^{ij} = rate constant for component i (L, G o X) and moiety j (f, s) (min^{-1})

a^{ij} = frequency factor for component i (L, G o X) and moiety j (f, s) (min^{-1})

E^{ij} = activation energy for component i (L, G o X) and moiety j (f, s) (kJ/mol)

R = ideal gas constant (8.314×10^{-3} kJ/(mol·K))

T = absolute temperature (K)

M^{O2} = initial oxygen charge (kg initial oxygen/kg initial dry biomass)

β^{ij} = exponent for component i (L, G o X) and moiety j (f, s) (dimensionless)

The models allow the calculation of selectivity, defined for glucan with equation 6. Glucan and xylan selectivity measure the ability of pretreatment to remove lignin while retaining carbohydrates.

$$S_{dG} \equiv \frac{dY_L/dt}{dY_G/dt} = \frac{a_{Lf} \exp\left(-\frac{E_{Lf}}{RT}\right) M_{O2}{}^{\beta Lf} Y_{Lf} + a_{Ls} \exp\left(-\frac{E_{Ls}}{RT}\right) M_{O2}{}^{\beta Ls} Y_{Ls}}{a_{Gf} \exp\left(-\frac{E_{Gf}}{RT}\right) M_{O2}{}^{\beta Gf} Y_{Gf} + a_{Gs} \exp\left(-\frac{E_{Gs}}{RT}\right) M_{O2}{}^{\beta Gs} Y_{Gs}} \tag{6}$$

Using this definition it is possible to calculate the pretreatment conditions that result in a desired biomass composition.

4.3 Organosolv

Organosolv pretreatment is similar to organosolv pulping. The main purpose is lignin separation from the carbohydrates matrix. This pretreatment is effective removing biomass recalcitrance because it achieves almost complete separation of biomass components (Zhao et al., 2009). Three separate fractions are obtained: solid lignin, an aqueous stream, and high purity cellulose. Lignin is recovered unaltered; thus, it has high potential for other applications. In the aqueous phase hemicelluloses are either hydrolysed to xylose monomers or degraded (Johanson et al., 1987).

Some of the most commonly used solvents are low boiling point methanol, ethanol, and acetone. Both alcohols are low cost and miscible in water. At 180 ºC, the addition of a catalyst is required (at ~0.2% concentration). Both mineral acids (i.e., hydrochloric, sulfuric and phosphoric) and organic acids (i.e., formic, oxalic, acetylsalicylic, and salicylic) may be used as catalysts to accelerate delignification and xylan degradation (Zhao et al., 2009); however, acids are not required if the pretreatment is conducted at temperatures in the range 190 to 210 ºC because liquid hot water is acidic (Duf & Murray, 1996). In alcohol organosolv, the solvent concentration is 50-80% with the highest concentration for hard to pretreat biomass. Usually, pretreatment temperatures for methanol are lower than for ethanol, but ethanol is safer due to its lower toxicity. The Lignol technology uses ethanol as solvent and converts a range of cellulosic feedstocks into several valuable organic chemicals and fuels (Arato et al., 2005). Using low boiling point solvents, such as ethanol, methanol and acetone, organosolv pretreatment requires high pressures because pretreatment runs at high temperatures. To perform at atmospheric pressure, a variety of high boiling point alcohols may be used including but not limited to ethylene glycol, glycerol, and tetrahydrofurfuryl. Organosolv pretreatment with low boiling point alcohols has successfully run at 240 ºC for 4 h. Drawbacks include high-energy consumption for solvents recovery and high costs of solvents (Aziz and Sarkanen 1989).

Other kind of organic compounds such as formic, acetic, and peracetic acids, dimethylsulfoxide, ethers, ketone, and phenols are widely used (Thring et al., 1990). When the solvents are organic acids, subsequent enzymatic hydrolysis may not be as successful as with other solvents, probably due to inhibition caused by acetyl groups (Pan et al., 2006). If peracetic acid is used, pretreatment can be conducted at ambient or mild temperatures (e.g., 80ºC) for long times and the addition of NaOH may cause biomass swelling. Compared to acid and alkaline pretreatment at the same conditions, peracetic acid is more effective (Zhao et al., 2008).

Due to important advantages obtained through efficient separation of components, organosolv pre-treatments are the most promising options for a biorefinery. Nevertheless, these type of pre-treatments are still very undeveloped because they require extensive and cumbersome washing steps in order to obtain complete solvents removal, and they are energy intensive if high solvents recovery is to be achieved (Zhao et al., 2009). Removal of solvents is not only required for the process economics but also because their presence may be inhibitory for enzymatic hydrolysis and fermentation (Holtzapple & Humphrey, 1984). If high pressures must be applied, the equipment costs increase substantially.

4.3.1 Reactions during organosolv pretreatment

In organosolv pretreatment, the reactions that take place highly depend on the solvent choice. For example, if alcohols are used, OH^- attacks the lignin-hemicellulose acid ester

bonds at the α carbons of the lignin monomers as well as the ether and 4-O-methylglucoronic bonds. Of these, cleavages of ether linkages are primarily responsible for lignin breakdown. Also, hydrolysis of the glycosidic bonds in hemicelluloses occurs. If an acid catalyst is employed, degradation of lignin and monosaccharides may happen (Chum et al., 1990).

On the other hand, if peracetic acid is used, the hydroxonium ion is present (OH^+) generated from peracetic acid in acidic media. This ion attacks lignin by ring hydroxylation. Peracetic acid is a powerful oxidizing agent; thus, oxidative demethylation and ring opening may occur. Other reactions are displacement of side chains and cleavage of β-arylether bonds.

5. Biotransformation of lignocellulosic material into alternative fuels

The main interest in the use of agro-industrial by-products and agro-waste streams is the release of the fermentable carbohydrates contained into the lignocellulosic matrix. The cost of lignocellulosic feedstocks is currently appealing because it is lower than any other energy sources. For example, crude oil price ranges from $40 to $80 per barrel, generating an energy price that ranges from $7.1 to $14.2 GJ^{-1}, while the energy price from lignocellulosic ranges from $0 to $3 GJ^{-1} (Zhang, 2008). Nevertheless, the actual cost of fuel production from lignocellulosic feedstock can be higher than conventional processes (e.g. ethanol from sugarcane or corn) generating an expensive fuel (Ferris & Joshi, 2010). This can be caused by increased lignocelluloses prices due to higher demand, biomass conditioning and transportation costs, and the cost and yields of the different process stages like pretreatment, saccharification, detoxification and fermentation (Aden et al., 2002). The National Renewable Energy Laboratory (NREL), in its economical evaluation of biotransforming corn stover to ethanol in a plant that uses simultaneous saccharification and fermentation technology, calculated an ethanol selling price close to $1.07 per gallon (+$0.12/-$0.05) in a plant with an installed capacity of 2000 metric tons of lignocellulose per day. They considered the total project investment, the variable operating cost and fixed operating cost for a plant life of 20 years and an ethanol production of 69.5 millions of gallons per year. They showed that the most expensive item in the process is the feedstock cost followed by the pretreatment cost. Further details can be consulted in the NREL report (Aden et al., 2002). Technological alternatives to Simultaneous Saccharification and Fermentation may even result in more favorable prices.

Transformation of highly oxygenated compounds like carbohydrates allows the production of alcohols, carboxylic acids, and esters. Compounds like fragrances solvents, and lubricants may be produced from lignocellulosic feedstocks, and bio-polymers such as polylactic acid and poly(trimethylene terephthalate) that use lactic acid and 1,3-propanediol as precursors, respectively, can be obtained through carbohydrates fermentations (Ragauskas et al., 2006). Among the different chemical compounds that can be used as fuels, alcohols are of a particular interest due to characteristics like a high octane number, broad flammability limits, high flame speeds, and high vaporization heats. These properties have allowed blending ethanol, one of the most important alcohols, with gasoline in mixtures that contain up to 85% ethanol (E85)(Turner et al., 2011). Currently, ethanol and methanol are the most important alcohols used in internal engine combustion. Although these alcohols are obtained after fermentation of the free sugars that result from the depolymerization of carbohydrate polymers, alcohols also can be obtained by direct fermentation of the released

carbohydrate polymers. Fig. 9 summarizes the process stages in the widely studied Simultaneous Saccharification and Fermentation Technology.

During pretreatment toxic compounds are released in a concentration that may vary widely. These compounds have been grouped in four categories: (i) sugar degradation products, (ii) lignin degradation products, (iii) compounds derived from cellulose structure, and (iv) heavy metal ions released by corrosion of the hydrolysis equipments. These compounds include but are not limited to furfural and hydroxymethyl furfural formed by the degradation of pentoses and hexoses; acetic, formic, galacturonic and glucuronic acids formed during the hydrolysis of hemicellulose; and aromatic and phenolic compounds, like cinnamaldehyde, p-hydroxybenzaldehyde, and syringaldehyde (Cardona et al., 2010; Mussatto & Roberto, 2004; Sánchez & Cardona, 2008).

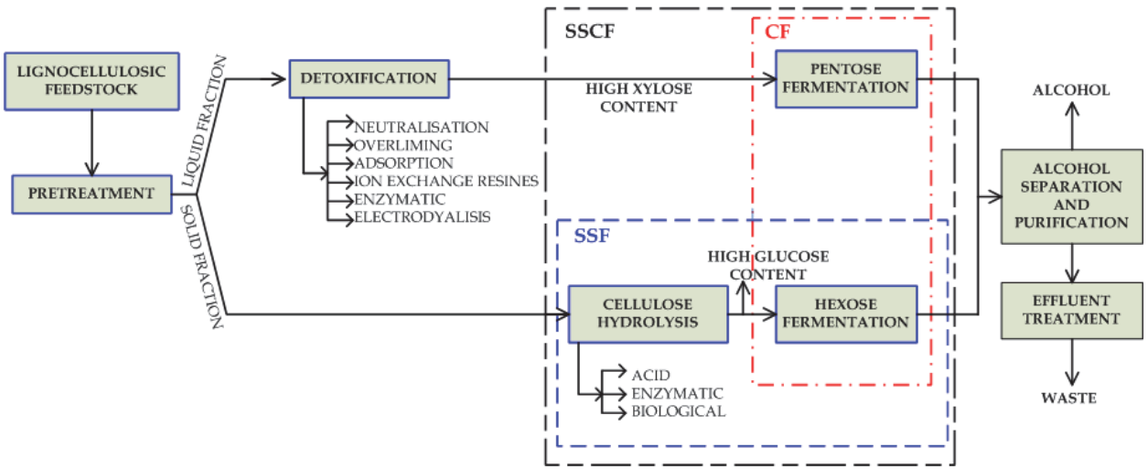

Fig. 9. Process scheme of the lignocellulosic biomass transformation to liquid fuels, mainly alcohols. SSF: simultaneous saccharification and fermentation, CF: co-fermentation, and SSCF: simultaneous saccharification and co-fermentation (Adapted from Cardona et al. (2010))

The detoxification stage, in which most of the inhibitory compounds formed in the pre-treatment stage are removed, is important for the further fermentation stage. This process can be conducted by physical and chemical or biological means. The biological detoxification (BD) may be advantageous because of minimal generation of waste streams, chemical inputs are not required, possible recharging of adsorption resins is not needed, and it is suitable for solid-liquid mixtures (Nichols et al., 2010). The participation of peroxidases and laccases in BD processes has been evaluated. Lacasses have completely removed phenol compounds of low molecular weight by an oxidative polymerization mechanism without varying the acetic acid and furans composition in the hydrolyzate (Chandel et al., 2007). The use of microorganisms like the fungi *Thrichoderma reesei* and *Aspergillus nidulas* to remove acetic and formic acids, furfural and hydroxymethyl furfural, and acid benzoic derivatives has been also reported (Palmqvist & Hahn-Hägerdal, 2000; Yu et al., 2011). Finally, another important but not widely reported method is the adaptation of microorganisms to the hydrolyzate. This method is based in the re-use of the microorganism in successive hydrolizate treatments where the microorganism of one assay is used as inoculum for the next one (Silva & Roberto, 2001). Classical physical-chemical processes are based on toxic compound precipitation, adsorption onto charcoal, diatomaceous earth, ion-exchange resins,

and pH adjustment of the extractive (Mussatto & Roberto, 2004). Moroever, new alternatives that use membranes have emerged. One example is the use of reactive membrane extraction in which is avoid the dispersion of one phase in the other reducing the presence of organic compounds, or solvents, in the aqueous phase or hydrolyzate (Simonne et al., 1997).

Carbohydrates depolymerization widely known as hydrolysis or saccharification is carried out by using cellulolytic enzymes complexes constituted by endoglucanases, exoglucanases or cellobiohydrolases, and cellobiases (Bansal et al., 2009; Lee, 1997). Endoglucanases (EC 3.2.1.4, endo-1,4-D-glucanohydrolase) create free chain-ends attacking low crystallinity regions in the cellulose fiber by cleaving α-1,4-glycosidic bonds. Exoglucanases (EC 3.2.1.91, 1,4-b-D-glucan cellobiohydrolase) remove cellobiose units from the free chain-ends providing a further hydrolysis of the molecule. Finally, the cellobiase or β-glucosidase (EC 3.2.1.21) hydrolyses the cellobiose to glucose. Bansal et al. (2009) reviewed the mechanism and mathematical modelling of cellulose hydrolysis by cellulases where the mechanism main steps are: (i) cellulose adsorption onto the substrate via the binding domain, (ii) location of a suitable bond to hydrolysis on the substrate surface, usually the end of the chain or cleavable bond if cellobiohydrolase or endoglucanase is presented, respectively, (iii) formation of enzyme–substrate complex to initiate hydrolysis, (iv) hydrolysis of the α-glycosidic bond and simultaneous forward sliding of the enzyme along the cellulose chain, (v) desorption of cellulases from the substrate or repetition of step iv or steps ii and iii if the catalytic domain detaches from chain, vi) hydrolysis of cellobiose to glucose if α-glucosidase. The main product of the cellulose hydrolysis is glucose, which can be further fermented or chemically transformed. In this stage, the most important parameters to be controlled are reaction time, temperature, pH, enzyme dosage and substrate load (Sánchez & Cardona, 2008).

The last biological stage is fermentation. Streams from the detoxification and/or saccharification stages have a high xylose and glucose concentration. These sugars might be bio-converted to fuels by several microorganisms, including *Candida albicans*, *Candida shehatae*, *Kluveromyces fragilis*, *Kluveromyces marxianus*, *Pichia stipitis, Saccharomyces cerevisiae, Bacillus subtilis*, *Bacillus stearothermophilus*, *Clostridium cellulolyticum*, *Clostridium beijerinckii*, *Escherichia coli*, and *Zymomonas mobilis*, which can produce compounds like ethanol, glycerol, butanol, and acetate, among others depending on their metabolic networks. Nevertheless, important differences in the metabolic transformation of xylose a C-5 carbohydrate and glucose a C-6 carbohydrate between bacteria and fungi cause that not all native microorganisms are capable of metabolizing both carbohydrates to ethanol.

It is known that bacteria can transform xylose to xylulose by using xylose isomerase, while most yeasts, fungi, plants, and animals use aldose (xylose) reductase and xylitol dehydrogenase with xylitol as an intermediate (Chiang & Knight, 1960). Nevertheless, if the first reaction step uses NADPH as a cofactor, the reaction is tied to NADPH production, with a subsequent NAD^+ reduction, which can cause a cofactor imbalance under oxygen limitation (Bruinenberg et al., 1983). For example, Toivari (2004) reported an endogenous xylose metabolic pathway in *Saccharomyces cerevisiae* which in aerobic conditions generated a xylitol accumulation into cells caused by lack of NAD^+, but when xylitol is released to the medium the ethanol production yield decreases (Eliasson et al., 2000; Eliasson et al., 2001). Due to these metabolic drawbacks, genetically modified microorganisms capable to ferment both C-5 and C-6 carbohydrates are the focus of attention to improve ethanol production yields. Extensive literature use *S. cerevisiae* as biological model due to its tolerance to media

up to 20 $\%^{v}/_{v}$ in ethanol among other toxic compounds released during its growth. Also, *Z. mobilis* is amply considered since it has been genetically modified to obtain ethanol by co-fermentation of several substrates like glucose, xylose and arabinose. This topics have been reviewed elsewhere (Kambam & Henson, 2010; Vinuselvi et al., 2011).

For the production of liquid biofuels, mainly ethanol, sequential or simultaneous operational configurations between the hydrolysis or saccharification and fermentation stages have been adopted. Simultaneous saccharification-fermentation (SSF) processes present better ethanol production yields and less energy consumption than sequential hydrolysis-fermentation (SHF) (Sánchez & Cardona, 2008), fermentation time is shorter than SHF, contamination risk with external microorganism is reduced, and presents an important cost reduction since cellulose hydrolysis occurs during glucose fermentation that decreases the inhibition caused by sugars on cellulases. The most important disadvantages of SSF are the difference of optimal temperature for the hydrolysis (45-50°C) and fermentation (28-35°C) stages, the inhibition of the fermenting microorganism and even the cellulose activity caused by the ethanol and the toxic compounds than can be eventually produced during the lignocellulosic biomass pretreatment, and low final concentration of products due to the use of dilute media to obtain suitable rheological properties (Sánchez & Cardona, 2008; Szczodrak & Fiedurek, 1996). The most important advantage of SHF is that hydrolysis and fermentation stages can be carried out at their optimal condition.

The search of new strains or genetically engineered strains that can produce ethanol from xylose allows that through independent fermentations (co-fermentation, CF) of pentoses and hexoses, the ethanol production yield increases (Fig. 9). Nevertheless, the fact that most of the genetically engineered strains to metabolize xylose produce ethanol from glucose has risen in the inclusion of the co-fermentation process to the SSF, which is known as simultaneous saccharification and co-fermentation (SSCF) (Fig. 9). This configuration integrates in only one unit the hydrolysis of cellulose and the fermentation of pentoses. Although economical benefits are evident by using CF and SSCF, challenges emerge because most of xylose-fermenting yeasts (XFY) are not ethanol tolerant; thus, they are prompt to inhibition. New ethanol-tolerant strains should be selected for the CF process, otherwise, joining ethanol removal processes to the SSCF process can help incrementing the ethanol production yield (Chen, 2010).

The direct microbial conversion (DMC) of lignocellulosic biomass to ethanol has also been studied. In DMC, the biodelignification, hydrolysis, and fermentation are done in the same process unit by either one microorganism or a consortium of microorganisms. DMC seems to be an economical process since all the necessary stages for lignocellulosic bioconversion are interconnected. Nevertheless, DMC are slow, and undesired by-products mainly acetic and lactic acids may result when bacteria are used. Another obstacle in DMC is that hydrolysis products such as glucose and cellobiose, can cause inhibition of the cellulase complex limiting the rate of saccharification and final concentration of sugars which result in low ethanol production yields (Mielenz, 2001; Szczodrak & Fiedurek, 1996).

Although most of alternative fuel investigations have focused on biotechnology processes, mainly ethanol production, other chemical compounds of interest useful as fuel, like esters hydrogen, and hydrocarbons can be obtained. In addition, chemical technologies have been rising up as a feasible alternative in which the main goal is to obtain oxygenated hydrocarbons, by removing oxygen from carbohydrates, which can be subsequently transformed to an alternative fuel (Ragauskas et al., 2006).

Despite the fact that biological agents can be involved in all the lignocellulosic transformation stages, few processes employ biological agents in all the stages. More commonly, a combination of biological, chemical, and thermo-chemical processes is employed along the different stages. This results in the production of methanol, butanol, hydrogen, and methane, as important fuel alternatives. Methanol production can be accomplished by (i) biochemical conversion of sugars, (ii) thermo-chemical conversion of biomass by using heat energy and chemical catalyst, (iii) production of syngas from biomass gasification at high temperature and an oxygen-starved environment, and (iv) biomass pyrolysis at high temperature under an oxygen-free environment (Dolan, 2010). Hydrogen production from biomass can be achieved by (i) syngas production and its later transformation by water shift reaction, (ii) autothermal reforming of bio-oils produced by pyrolysis, (iii) aqueous phase reforming of sugars and sugar alcohols, and (iv) biological means, enzymes and microorganisms, in dark fermentations (Tanksale et al., 2010). Methane is produced mainly by anaerobic digestion of the biomass (Li et al., 2011). The catalytic thermochemical processes do not require a delignification stage of biomass, but this is a necessary step to improve the amount of fermentable carbohydrates if the fuel is to be produced by reforming the gasses produced by anaerobic fermentation of these sugars or by any biological means. Thermochemical and chemical catalyst production of these fuels has been reviewed elsewhere (Kalinci et al., 2009; Tanksale et al., 2010).

6. Conclusions

An efficient biorefinery must be capable of using all the components in lignocellulosic wastes resourcefully for the production of fuels in addition to other chemicals and products. Valuable substances contained in these resources must be extracted first. Then, polysaccharides and lignin must be used to produce fuels and other valuable chemicals. Some carbohydrates derived products are: ethanol, carboxylic acids, levulinic acid, glycerol and sorbitol. Lignin, on the other hand, may be used as an energy source through combustion or can be transformed into syngas or bio-oil.

If polysaccharides are to be biologically processed, the lignocelluloses digestibility must be importantly improved. This is achieved by pretreatment, which separate lignin from carbohydrates through chemical or biological means. In some instances, this is achieved by partial delignification or lignin degradation.

Full biological processes are advantageous due to high selectivity and mild reaction conditions, but they are not available yet. Recent studies promote the production of genetically engineered multitask microorganisms able not only to lignocelluloses delignification but also saccharification and fermentation. Additionally, these microorganisms should be marginally inhibited by substrate and product concentration. While this technological dream becomes a scalable economic reality, pretreatments may be carried out by chemical means. In this sense, there is a wide range of possibilities. Advantages and disadvantages of each have been briefly discussed above.

Notable efforts have been conducted to improve ethanol production yields by using lignocellulosic wastes and crops, some of which have been submitted to genetic modifications in the lignin composition. Concurrently, highly desirable integrated processes for non-liquid alternative fuels such as hydrogen are in a very early stage of development. Thus, liquid fuels and products from lignocelluloses can be considered as a bridge between

the current highly contaminating petroleum age and the future more sophisticated and clean hydrogen combustion age.

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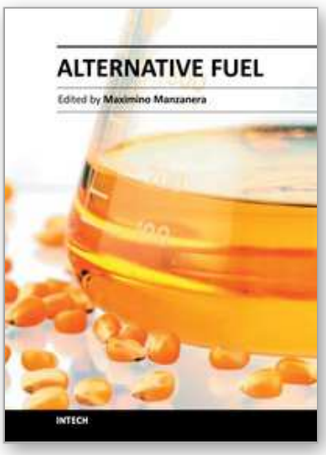

Alternative Fuel

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ISBN 978-953-307-372-9
Hard cover, 346 pages
Publisher InTech
Published online 09, August, 2011
Published in print edition August, 2011

Renewable energy sources such as biodiesel, bioethanol, biomethane, biomass from wastes or hydrogen are subject of great interest in the current energy scene. These fuels contribute to the reduction of prices and dependence on fossil fuels. In addition, energy sources such as these could partially replace the use of what is considered as the major factor responsible for global warming and the main source of local environmental pollution. For these reasons they are known as alternative fuels. There is an urgent need to find and optimise the use of alternative fuels to provide a net energy gain, to be economically competitive and to be producible in large quantities without compromising food resources.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Oscar Sánchez, Roció Sierra and Carlos J. Alméciga-Díaz (2011). Delignification Process of Agro-Industrial Wastes an Alternative to Obtain Fermentable Carbohydrates for Producing Fuel, Alternative Fuel, Dr. Maximino Manzanera (Ed.), ISBN: 978-953-307-372-9, InTech, Available from: http://www.intechopen.com/books/alternative-fuel/delignification-process-of-agro-industrial-wastes-an-alternative-to-obtain-fermentable-carbohydrates

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