Literature Review of Physical and Chemical Pretreatment Processes for Lignocellulosic Biomass

P.F.H. Harmsen[1]

W.J.J. Huijgen[2]

L.M. Bermúdez López[3]

R.R.C. Bakker[1]

[1]Wageningen University & Research centre - Food & Biobased Research (WUR-FBR, NL)
[2]Energy Research Centre of the Netherlands (ECN, NL)
[3]Abengoa Bioenergía Nuevas Tecnologías (ABNT, ES)

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Preface

This literature review was performed within the BioSynergy project (2007-2010). BioSynergy is a European Integrated Project supported through the Sixth Framework Programme for Research and Technological Development (038994-SES6). BioSynergy stands for "BIOmass for the market competitive and environmentally friendly SYNthesis of bio-products together with the production of secondary enERGY carriers through the biorefinery approach".

Within the BioSynergy project the overall goal of the pretreatment routes being developed is to convert raw lignocellulosic biomass into its composing sugars and lignin in a market competitive and environmentally sustainable way. This report reviews lignocellulose pretreatment in general as well as specific pretreatment technologies that are developed within the BioSynergy project including steam explosion (ABNT), mechanical/alkaline fractionation (WUR) and organosolv fractionation (ECN). In addition to these pretreatment technologies, other pretreatment technologies are studied within the BioSynergy project such as acetic/formic acid pretreatment and mild- and strong acid pretreatment.

Summary

Lignocellulose, the most abundant renewable biomass on earth, is composed mainly of cellulose, hemicellulose and lignin. Both the cellulose and hemicellulose fractions are polymers of sugars and thereby a potential source of fermentable sugars. Lignin can be used for the production of chemicals, combined heat and power or other purposes.

After initial biomass processing, the production of fermentable sugars from biomass is usually approached in two steps:

1. A pretreatment process in which the cellulose polymers are made accessible for further conversion. In this step hydrolysis of hemicellulose may occur, as well as separation of the lignin fraction, depending on the process applied.
2. Enzymatic cellulose hydrolysis to fermentable sugars using cellulase enzyme cocktails produced on location or acquired from enzyme manufacturers.

Obstacles in the existing pretreatment processes include insufficient separation of cellulose and lignin, formation of by-products that inhibit ethanol fermentation, high use of chemicals and/or energy, and considerable waste production. Research is focussed on converting biomass into its constituents in a market competitive and environmentally sustainable way.

Different pretreatment technologies published in public literature are described in terms of the mechanisms involved, advantages and disadvantages, and economic assessment. Pretreatment technologies for lignocellulosic biomass include biological, mechanical, chemical methods and various combinations thereof. The choice of the optimum pretreatment process depends very much on the objective of the biomass pretreatment, its economic assessment and environmental impact.

When fermentable sugars are produced, special attention must be paid to the formation of fermentation inhibitors. Especially the formation of phenolic compounds from lignin degradation should be prevented, as well as the formation of furfural and HMF from sugar degradation by keeping the process temperature and residence time as low and as short as possible.

Only a small number of pretreatment methods has been reported as being potentially cost-effective thus far. These include steam explosion, liquid hot water, concentrated acid hydrolysis and dilute acid pretreatments. At the moment the production of ethanol from lignocellulose is growing rapidly and by looking at the industrial activities in this field more knowledge can be gained on the applied pretreatment methods.

It is not possible to define the best pretreatment method as it depends on many factors such as type of lignocellulosic biomass and desired products. Pretreatments must improve the digestibility of lignocellulosic biomaterials, and each pretreatment has its own effect on the cellulose-, hemicellulose- and lignin fractions. Looking at industrial activities for the production of ethanol, acid-based pretreatment methods are preferred. In these processes lignin is left with the substrate and removed after the hydrolysis of the (hemi)cellulose or even after distillation. Research topics for these processes include amongst others minimization of sugar loss, increase of solids concentration and higher ethanol concentrations after fermentation. However, when lignin is removed from the biomass in an early stage of the process (i.e. after the pretreatment) it can be recovered as a co-product with potential high added value. Another advantage is that the enzymatic digestibility is strongly related to the lignin content, and that lignin removal greatly enhances enzymatic hydrolysis. In this case pretreatment methods that focus on lignin removal become more interesting.

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1. Introduction

Lignocellulose is the most abundant renewable biomass with a worldwide annual production of $1x10^{10}$ MT (Sánchez and Cardona, 2008). Lignocellulosic biomass is composed of cellulose, hemicellulose and lignin, as well as other minor components. Both the cellulose and hemicellulose fractions are polymers of sugars, and thereby a potential source of fermentable sugars, or other processes that convert sugars into products. Hemicellulose can be readily hydrolysed under mild acid or alkaline conditions. The cellulose fraction is more resistant and therefore requires more rigorous treatment.

After initial biomass processing by milling, the production of fermentable sugars is usually approached in two steps:

1. A pretreatment process in which the cellulose polymers are made accessible for further conversion. In this step hydrolysis of hemicellulose may occur (depending on the process conditions) as well as separation of the lignin fraction (for production of chemicals, combined heat and power production or other purposes);
2. Enzymatic cellulose hydrolysis, using cellulase enzyme cocktails produced on location or acquired from enzyme manufacturers.

Obstacles in the existing pretreatment processes include insufficient separation of cellulose and lignin (which reduces the effectiveness of subsequent enzymatic cellulose hydrolysis), formation of by-products that inhibit ethanol fermentation (e.g. acetic acid from hemicellulose, furans from sugars and phenolic compounds from the lignin fraction), high use of chemicals and/or energy, and considerable waste production. Research is focussed on converting biomass into its constituents in a market competitive and environmentally sustainable way. This literature review provides an overview of available pretreatment technologies.

The literature review is divided into three major chapters. In Chapter 2, physical and chemical properties of lignocellulose that are relevant to pretreatment are reviewed. Subsequently, Chapter 3 presents a general overview of different pretreatment pathways for lignocellulose. Finally, Chapter 4 describes in more detail two pretreatment processes that were evaluated during the BioSynergy project. Furthermore, a final chapter with concluding remarks and recommendations is included.

2. Physical and chemical characteristics of lignocellulosic biomass

2.1 Composition

The term "lignocellulosic biomass" is used when referring to higher plants, softwood or hardwood. The main components of the lignocellulosic materials are cellulose, hemicellulose and lignin.

Cellulose is a major structural component of cell walls, and it provides mechanical strength and chemical stability to plants. Solar energy is absorbed through the process of photosynthesis and stored in the form of cellulose. (Raven *et al.*,1992) Hemicellulose is a copolymer of different C5 and C6 sugars that also exist in the plant cell wall. Lignin is polymer of aromatic compounds produced through a biosynthetic process and forms a protective layer for the plant walls. In nature, the above substances grow and decay during the year. It has been estimated that around $7.5\text{x}10^{10}$ tonnes of cellulose are consumed and regenerated every year (Kirk-Otmer, 2001). It is thereby the most abundant organic compound in the world.
Apart from the three basic chemical compounds that lignocellulose consists of, water is also present in the complex. Furthermore, minor amounts of proteins, minerals and other components can be found in the lignocellulose composition as well.

The composition of lignocellulose highly depends on its source. There is a significant variation of the lignin and (hemi)cellulose content of lignocellulose depending on whether it is derived from hardwood, softwood, or grasses. 0 summarizes the composition of lignocellulose encountered in the most common sources of biomass.

Table 2.1 *Composition of lignocellulose in several sources on dry basis (Sun and Cheng, 2002)*

Lignocellulosic materials	**Cellulose (%)**	**Hemicellulose (%)**	**Lignin (%)**
Hardwoods stems	40–55	24–40	18–25
Softwood stems	45–50	25–35	25–35
Nut shells	25–30	25–30	30–40
Corn cobs	45	35	15
Grasses	25–40	35–50	10–30
Paper	85–99	0	0–15
Wheat straw	30	50	15
Sorted refuse	60	20	20
Leaves	15–20	80–85	0
Cotton seed hairs	80–95	5–20	0
Newspaper	40–55	25–40	18–30
Waste papers from chemical pulps	60–70	10–20	5–10
Primary wastewater solids	8–15	NA	24–29
Swine waste	6.0	28	NA
Solid cattle manure	1.6–4.7	1.4–3.3	2.7–5.7
Coastal Bermuda grass	25	35.7	6.4
Switchgrass	45	31.4	12.0

2.2 Internal structure - physical properties

Lignocellulosic biomass has a complex internal structure. It is comprised of a number of major components that have, in their turn, also complex structures. To obtain a clear picture of the material, an analysis of the structure of each main component is made in this section, concluding with the description of the structure of lignocellulose itself. Also addressed are the physical properties of each of the components of lignocellulose, and how each of these components contributes to the behaviour of the complex structure as a whole. The study is oriented towards breaking down the complex of lignocellulose and utilizing the components to produce sugars, and possibly, lignin, as this is one of the main goals of pretreatment.

2.2.1 Cellulose

Cellulose is the β-1,4-polyacetal of cellobiose (4-O-β-D-glucopyranosyl-D-glucose). Cellulose is more commonly considered as a polymer of glucose because cellobiose consists of two molecules of glucose. The chemical formula of cellulose is $(C_6H_{10}O_5)_n$ and the structure of one chain of the polymer is presented in 0.

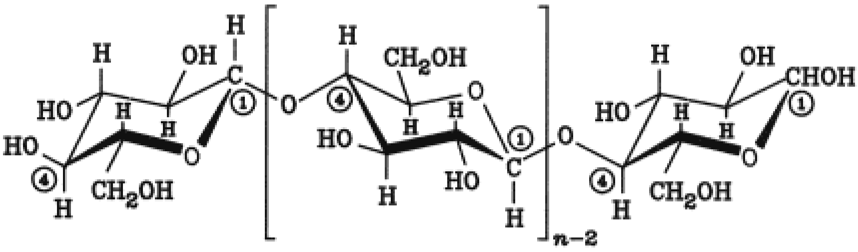

Figure 2.1 *Structure of single cellulose molecule*

Many properties of cellulose depend on its degree of polymerization (DP), i.e. the number of glucose units that make up one polymer molecule. The DP of cellulose can extend to a value of 17000, even though more commonly a number of 800-10000 units is encountered (Kirk-Otmer, 2001). For instance, cellulose from wood pulp has a DP between 300 and 1700.

The nature of bond between the glucose molecules (β-1,4 glucosidic) allows the polymer to be arranged in long straight chains. The latter arrangement of the molecule, together with the fact that the hydroxides are evenly distributed on both sides of the monomers, allows for the formation of hydrogen bonds between the molecules of cellulose. The hydrogen bonds in turn result in the formation of a compound that is comprised of several parallel chains attached to each other (Faulon *et al.*, 1994).

An illustration of the arrangement of the cellulose molecules in parallel chains and the accompanying hydrogen bonding is given in 0.

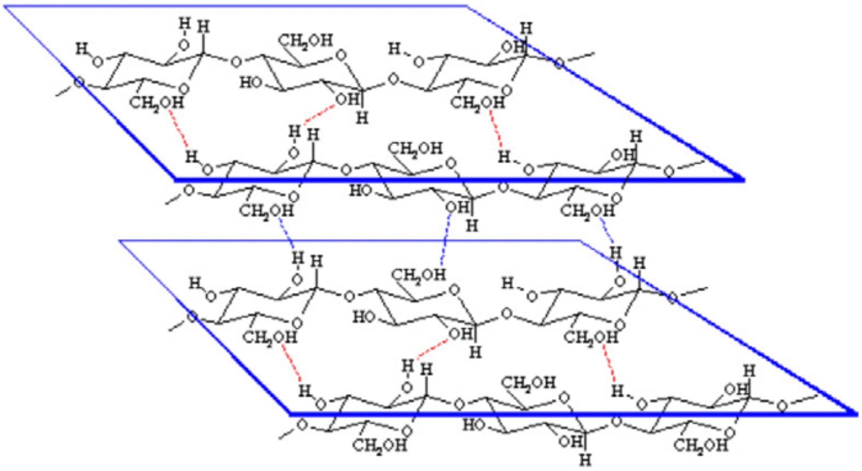

Figure 2.2 *Demonstration of the hydrogen bonding that allows the parallel arrangement of the cellulose polymer chains*

Cellulose is found in both the crystalline and the non-crystalline structure. The coalescence of several polymer chains leads to the formation of microfibrils, which in turn are united to form fibres. In this way cellulose can obtain a crystalline structure. 0 illustrates structure as well as the placement of cellulose in the cell wall.

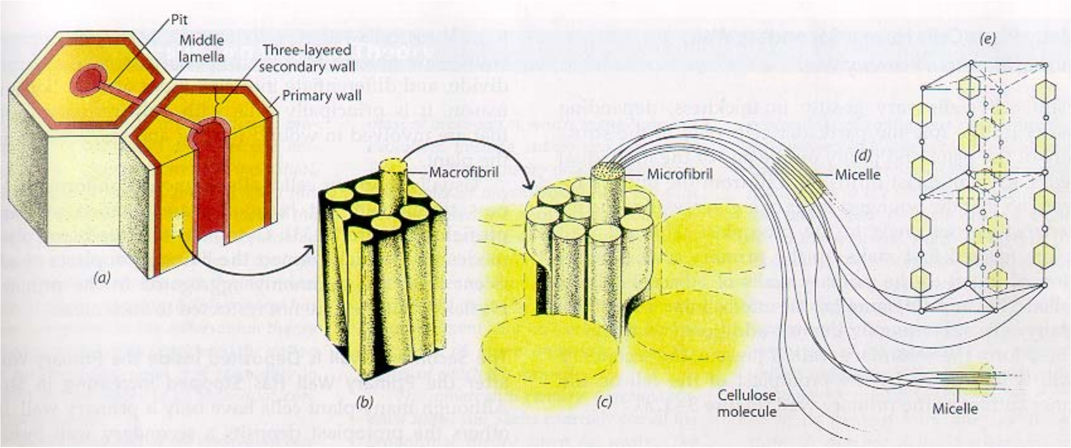

Figure 2.3 *Formation of micro- and macrofibrils (fibres) of cellulose and their position in the wall*

Cellulose is a relatively hygroscopic material absorbing 8-14% water under normal atmospheric conditions (20 °C, 60% relative humidity). Nevertheless, it is insoluble in water, where it swells. Cellulose is also insoluble in dilute acid solutions at low temperature. The solubility of the polymer is strongly related to the degree of hydrolysis achieved. As a result, factors that affect the hydrolysis rate of cellulose also affect its solubility that takes place, however, with the molecule being in a different form than the native one. At higher temperatures it becomes soluble, as the energy provided is enough to break the hydrogen bonds that hold the crystalline structure of the molecule. Cellulose is also soluble in concentrated acids, but severe degradation of the polymer by hydrolysis is caused. In alkaline solutions extensive swelling of cellulose takes place as well as dissolution of the low molecular weight fractions of the polymer (DP < 200) (Krassig and Schurz, 2002). Solvents of cellulose that have been applied in industrial or laboratory practice include uncommon and complex systems, such as cupriethylenediamine (cuen) hydroxide or the cadmium complex Cadoxen. Additionally, aqueous salt solutions, such as zinc chloride, dissolve limited amounts of cellulose (Kirk-Otmer, 2001). Cellulose does not melt with temperature, but its decomposition starts at 180^{o}C (Thermowoodhandbook, 2003).

2.2.2 Hemicellulose

The term hemicellulose is a collective term. It is used to represent a family of polysaccharides such as arabino-xylans, gluco-mannans, galactans, and others that are found in the plant cell wall and have different composition and structure depending on their source and the extraction method.

The most common type of polymers that belongs to the hemicellulose family of polysaccharides is xylan. As shown in 0, the molecule of a xylan involves 1->4 linkages of xylopyranosyl units with α-(4-O)-methyl-D-glucuronopyranosyl units attached to anhydroxylose units. The result is a branched polymer chain that is mainly composed of five carbon sugar monomers, xylose, and to a lesser extent six carbon sugar monomers such as glucose.

Important aspects of the structure and composition of hemicellulose are the lack of crystalline structure, mainly due to the highly branched structure, and the presence of acetyl groups connected to the polymer chain (Kirk-Otmer).

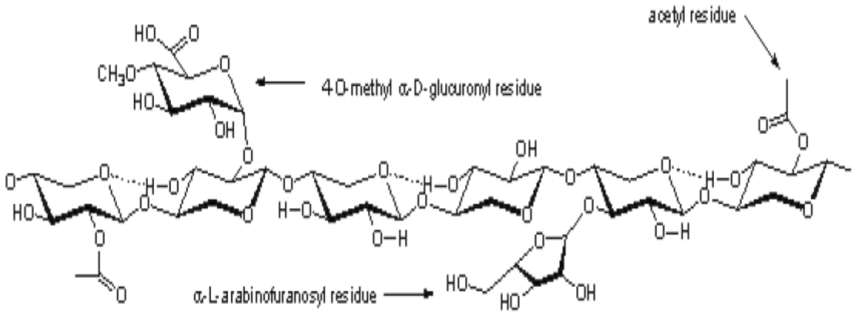

Figure 2.4 *A schematic representation of the hemicellulose backbone of arborescent plants*

Hemicellulose extracted from plants possesses a high degree of polydispersity, polydiversity and polymolecularity (a broad range of size, shape and mass characteristics). However, the degree of polymerization does not exceed the 200 units whereas the minimum limit can be around 150 monomers.

Hemicellulose is insoluble in water at low temperature. However, its hydrolysis starts at a temperature lower than that of cellulose, which renders it soluble at elevated temperatures (Thermowoodhandbook, 2003). The presence of acid highly improves the solubility of hemicellulose in water.

2.2.3 Lignin

Lignin is the most complex natural polymer. It is an amorphous three-dimensional polymer with phenylpropane units as the predominant building blocks. More specifically, p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (0) are the ones most commonly encountered.

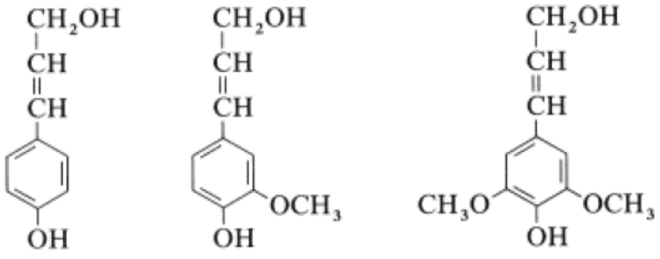

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Figure 2.5 *P-coumaryl- , coniferyl- and sinapyl alcohol: dominant building blocks of the three-dimensional polymer lignin*

Dividing higher plants into two categories, hardwood (angiosperm) and softwood (gymnosperm), it has been identified that lignin from softwood is made up of more than 90% of coniferyl alcohol with the remaining being mainly p-coumaryl alcohol units. Contrary to softwoods, lignin contained in hardwood is made up of varying ratios of coniferyl and sinapyl alcohol type of units (Kirk-Otmer, 2001).

The property of polydispersity, just as with hemicellulose, characterizes lignin as well. Different branching and bonding in otherwise similar molecules are encountered (Lin and Lin, 2002). 0 presents a model structure of lignin from spruce pine.

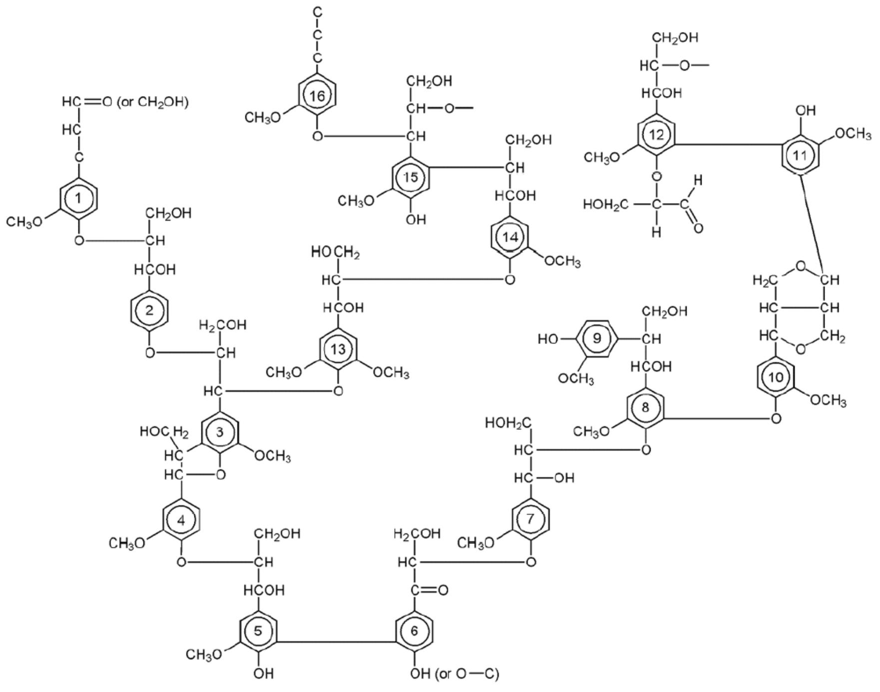


Figure 2.6 *Model structure of spruce lignin*

Lignin in wood behaves as an insoluble three-dimensional network. It plays an important role in the cell's endurance and development, as it affects the transport of water, nutrients and metabolites in the plant cell. It acts as binder between cells creating a composite material that has a remarkable resistance to impact, compression and bending.

Solvents that have been identified to significantly dissolve lignin include low molecular alcohols, dioxane, acetone, pyridine, and dimethyl sulfoxide. Furthermore, it has been observed that at elevated temperatures, thermal softening of lignin takes place, which allows depolymeristation reactions of acidic or alkaline nature to accelerate (O'Connor *et al.*, 2007).

2.2.4 Lignocellulose

As mentioned above, lignocellulose is a class of biomass that consists of three major compounds cellulose, hemicellulose and lignin. It also includes water and a small amount of proteins and other compounds, which do not participate significantly in forming the structure of the material (Raven *et al.*, 1992). Inside the lignocellulose complex, cellulose retains the crystalline fibrous structure and it appears to be the core of the complex. Hemicellulose is positioned both between the micro- and the macrofibrils of cellulose. Lignin provides a structural role of the matrix in which cellulose and hemicellulose is embedded (Faulon *et al.*, 1994).

Considering that cellulose is the main material of the plant cell walls, most of the lignin is found in the interfibrous area, whereas a smaller part can also be located on the cell surface (Kirk-Otmer, 2001).

2.3 Chemical interaction between components

There are four main types of bonds identified in the lignocellulose complex. Those are ether type of bonds, ester bonds, carbon-to-carbon bonds and hydrogen bonds. These four bonds are the main types of bonds that provide linkages within the individual components of lignocellulose (intrapolymer linkages), and connect the different components to form the complex (interpolymer linkages). The position and bonding function of the latter linkages is summarized in 0 (Faulon *et al*, 1994).

Table 2.2 *Overview of linkages between the monomer units that form the individual polymers lignin, cellulose and hemicellulose, and between the polymers to form lignocellulose*

Bonds within different components (intrapolymer linkages)	
Ether bond	Lignin, (hemi)cellulose
Carbon to carbon	Lignin
Hydrogen bond	Cellulose
Ester bond	Hemicellulose
Bonds connecting different components (interpolymer linkages)	
Ether bond	Cellulose-Lignin Hemicellulose lignin
Ester bond	Hemicellulose-lignin
Hydrogen bond	Cellulose-hemicellulose Hemicellulose-Lignin Cellulose-Lignin

2.3.1 Intrapolymer linkages

The main types of bonds that connect the building molecules within the lignin polymer are ether bonds and carbon-to-carbon bonds (Table 2.2). Ether bonds may appear between allylic and aryl carbon atoms, or between aryl and aryl carbon atoms, or even between two allylic carbon atoms. The total fraction of ether type bonds in the lignin molecule is around 70% of the total bonds between the monomer units. The carbon-to-carbon linkages form the remaining 30% of the total bonds between the units. They can also appear between two aryl carbon atoms or two allylic carbon atoms, or between one aryl and one allylic carbon atom (Kirk-Otmer, 2001).

The polymer of cellulose is formed on the basis of two main linkages:

1. The glucosidic linkage is the one that forms the initial polymer chain. More specifically, it is a 1-4 β D-glucosidic bond that connects the glucose units together. The glucosidic bond can also be considered as an ether bond, since it is in fact the connection of two carbon atoms with an elementary oxygen interfering (Solomon, 1988).
2. The hydrogen bond is considered to be responsible for the crystalline fibrous structure of cellulose. The arrangement of the polymer in long straight parallel chains together with the fact that the hydroxyl groups are evenly distributed in both sides of the glucose monomer (see 0 and 0), allow the formation of hydrogen bond between two hydroxyl groups of different polymer chains (Faulon *et al.*, 1994).

It has been identified that carboxyl groups are also present in cellulose in a fraction of 1 carboxyl per 100 or 1000 monomer units of glucose (Krassig and Schurz, 2002), although this does not appear obvious from the main structure of cellulose.

As already mentioned, hemicellulose consists of polysaccharides other than cellulose. Its structure (see 0) reveals that ether type of bonds, such as the fructosic and glucosidic bonds, is the main one that forms its molecule. The main difference with cellulose is that the hydrogen bonds are absent and that there is significant amount of carboxyl groups. The carboxyl groups can be present as carboxyl or as esters or even as salts in the molecule (Kirk-Otmer, 2001).

2.3.2 Interpolymer linkages

In order to determine the linkages that connect the different polymers of the lignocellulose complex, lignocellulose is broken down and the individual components are separated. However, their separation is commonly achieved by methods that result in alteration of their original structure. As a consequence, the conclusions on the connecting linkages between the polymers are not definite.

However, it has been identified that there are hydrogen bonds connecting lignin with cellulose and with hemicellulose, respectively. Furthermore, the existence of covalent bonds between lignin and polysaccharides is identified. More specifically, it is certain that hemicellulose connects to lignin via ester bonds. It is also known that there are ether bonds between lignin and the polysaccharides. It is still not clear though whether the ether bonds are formed between lignin and cellulose, or hemicellulose.

Hydrogen bonding between hemicellulose and cellulose is also identified. However, this linkage is not expected to be strong due to the fact that hemicellulose lacks of primary alcohol functional group external to the pyranoside ring (Faulon *et al.*, 1994).

2.3.3 Functional groups and chemical properties of lignocellulose components

From the aspect of producing sugar monomers from lignocellulose and ultimately ethanol or other chemical building blocks, the functional groups that are of interest are:

1. Functional groups that are involved in the hydrolysis of the polysaccharides to their monomers and the possible subsequent degradation reactions of these monomers (e.g. to furfural).
2. Functional groups that are involved in the (partial) depolymerisation of lignin (into fragments or phenolic compounds) so that the cellulose fraction becomes more accessible for enzymes.

The functional groups of all three components are summarized in 0.

Table 2.3 *Functional groups in components of lignocellulose*

Functional Group	Lignin	Cellulose	Hemicellulose
Aromatic ring	X		
Hydroxyl group	X		
Carbon to carbon linkage	X		
Ether (glucosidic) linkage	X	X	X
Ester bond			X
Hydrogen bond*		X	X

** The hydrogen bond is not a functional group, as its reaction does not lead to chemical change of the molecule. However, it changes the solubility of the molecule, though and it is therefore important for the breakdown of lignocellulose*

The lignin polymer contains most different functional groups involved in its depolymerisation and degradation, eventually to derivatives that are soluble in water. Concerning the cellulose polymer, the main interest is focused on breaking the glucosidic (ether) bond that would lead to production of sugar monomers. Following there is a description of reactions that can take place utilizing the functional groups of 0.

Aromatic ring

Chlorination and nitration are reactions that take place via the mechanism of electrophilic substitution and ultimately substitute the aromatic ring of the lignin polymer with chlorine or nitro groups. The substitution in this case is not achieved in a uniform manner.

By means of oxidation using oxidants such as chlorine, chlorine dioxide and oxygen the aromatic ring can be converted to cyclic structures and ultimately to smaller molecules such as mono- and dicarboxylic acids. Oxidants can also break the side chain of the monomer units of lignin, leading to fragments of three, two or one carbon atoms (Kirk-Otmer, 2001).

Hydroxyl group

The hydroxyl group initiates substitution reactions as well. Acidic conditions lead to transformation of the hydroxyl group to an aryl or allylic ether and ultimately the ether is substituted with an acid group (e.g. sulfonic acid). The benefit of the latter reaction is that the presence of the acid group in the molecule of lignin renders the polymer soluble in water (i.e., so-called lignosulfonates) (Kirk-Otmer, 2001).

Ether bond

The ether bond appears to be the most interesting among the functional groups described above:

1. It is the ether bond that holds the glucose monomers in a polymer chain (glucosidic linkage)
2. It is by far the most predominant bond in the lignin polymer.

Therefore, the cleavage of the ether bond can lead to separation of lignin from the polysaccharides matrix and degradation of the polymers to monomer sugars and lignin fragments.

The cleavage of the ether bond occurs through solvolytic reactions. It can take place under acidic or alkaline conditions via different mechanisms. In the case of lignin, under acidic conditions the ether bond is converted into hydroxyl and then converted to carbonyl or carboxyl before it is finally fragmented into C_3 or C_2 molecules. Under alkaline conditions the mechanism is different and the end result is not fragmentation of the side chain, but separation of the aromatic rings. An example of the cleavage of the ether bond in alkaline media is presented in 0. The cleavage of the ether bond might be enhanced by the addition of hydrosulfide.

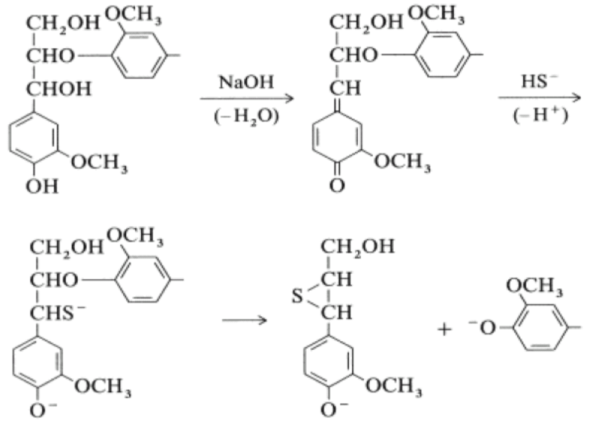


Figure 2.7 *Cleavage of ether bond of lignin in alkaline solution (Lin and Lin, 2002)*

In the case of cellulose the cleavage of ether bonds can proceed both in acidic and alkaline media. When acidic media are used, the acid acts as a catalyst protonating the oxygen atom. The charged group leaves the polymer chain and is replaced by the hydroxyl group of water. The reaction is shown in 0. The reaction can happen either homogeneously or heterogeneously. In both cases the reaction is of first order.

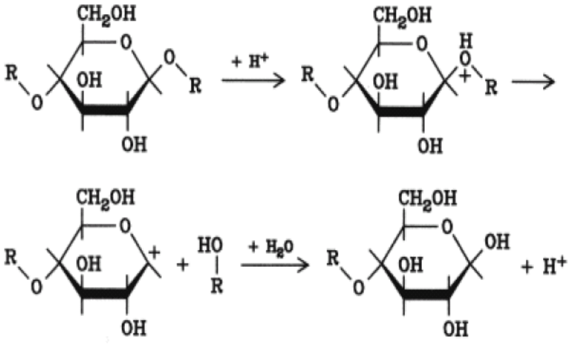


Figure 2.8 *Hydrolysis of cellulose in acidic media (Krassig and Schurz, 2002)*

In the case of alkaline media, the mechanism most probably involves the intermediate formation of 1,2-anhydro configuration as shown in 0. The intermediate form is a type of epoxide that, due to the ring that is formed between the two carbon atoms and oxygen, allows via the S_N2 mechanism the nucleophilic substitution of hydrogen (Solomon, 1988). The use of a strong base and a minimum temperature of 150°C are needed for a sufficient reaction rate.

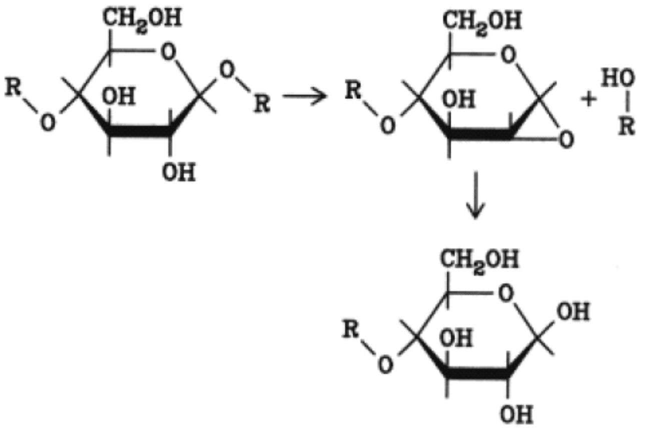


Figure 2.9 *Cellulose hydrolysis in alkaline media (Krassig and Schurz, 2002)*

Ester bond
Ester bonds are identified between lignin and polysaccharides as well as within the hemicellulose polymer. In the latter case it is the acetyl group that forms ester bond with a hydroxyl of the main chain of the polysaccharides. However, with respect to the linkage of lignin with polysaccharides there is no definite conclusion whether the ester bond lies between lignin and cellulose or lignin and hemicellulose, or between lignin and both cellulose and hemicellulose (Faulon *et al.*, 1994).

In general, hydrolysis is performed to break the ester bond and result in the corresponding carboxyl and hydroxyl groups. The reaction is essentially reversible and endothermic. The equilibrium is favoured by excess of water and high temperature. It is common application though, to use catalysts to increase the rate of the reaction. Either acid or alkaline catalysts can be used leading to different mechanisms. If alkaline solution is used the reaction is known as saponification. The most prominent difference with the acid catalysed reaction route is that it leads to irreversible hydrolysis of the ester (Solomon, 1988).

Hydrogen bond
The presence of hydrogen bonds is identified between the cellulose polymer chains. As shown in 0, hydrogen bonds are formed between the hydrogen atom of one hydroxyl group of a glucose monomer and the oxygen atom of a hydroxyl of another glucose monomer in the parallel polymer chain of cellulose. The formation of the cellulose fibres and the fact that it is insoluble in water is essentially a result of hydrogen bonds.

It has also been identified that hydrogen bonds exist in the polymer of hemicellulose as well. However, because of the absence of primary alcohol functional groups outside the pyranoside ring, the capacity of hemicellulose to form hydrogen bonds is limited. Therefore, hemicellulose is not expected to be strongly connected to the cellulose molecule. However, connection of hemicellulose to cellulose has been noted with the orientation of the two molecules being parallel to each other.

Breaking of hydrogen bonding can be accomplished by applying high temperatures to the solution and/or by substituting the molecule that forms the bond with hydrogen. Considering the latter, there are in general two ways of breaking the hydrogen bond:

1. Introduction of groups that form hydrogen bonds of higher energy than the ones formed in cellulose (>21 kJ/mol of cellulose).
2. Altering the structure of cellulose so that the hydrogen bonds that still exist in the polymer are of lower energy than that of the hydrogen bonds formed by the molecules of water. This can be

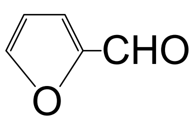
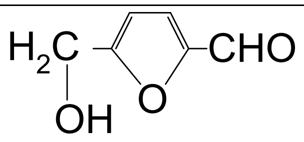
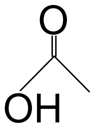
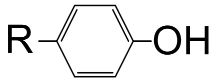
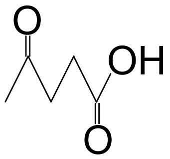
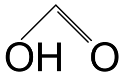
achieved by a physical destruction of the cellulose molecule or by chemically producing a cellulose derivative such as cellulose acetate (Bochek, 2003).

2.4 Formation of fermentation inhibitors

Pretreatment of lignocellulosic biomass may produce degradation products with an inhibitory effect on the fermentation process. These inhibitors have toxic effects on the fermenting organisms, thus reducing the ethanol yield and productivity. The level of toxicity depends in part on fermentation variables including cell physiological conditions, dissolved oxygen concentration and pH of the medium. In addition, the fermenting organisms may, to some extent, be resistant to inhibitors or may become gradually adapted to their presence. However, the optimal approach is to prevent the formation of inhibitors as much as possible through (adaptation of) the pretreatment process conditions or other measures.

The major types of inhibitors are discussed below and summarized in 0. The inhibitory effect of these compounds is higher when they are present together due to a synergistic effect (Mussatto and Roberto, 2004).

Table 2.4 *Major types of inhibitors and their chemical structure (Mussatto and Roberto, 2004)*

		
Furfural	5-hydroxymethylfurfural (HMF) (furfurylalcohol)	Acetic acid
		
Phenols	Levulinic acid	Formic acid

The relative toxicity of the various inhibitors for ethanol fermentation can be summarized as phenolic compounds>furfural>HMF>acetic acid>extractives.

2.4.1 Sugar degradation products

Subsequent to hemicellulose hydrolysis, pentose sugar monomers may dehydrate to the inhibitor furfural. Similarly. hexose sugars (e.g. glucose) may degrade to the toxic hydroxymethyl-furfural (HMF). Furfural and HMF affect cell growth and respiration. HMF is considered less toxic than furfural and its concentration in (hemi)cellulose hydrolysates is usually low.

It is clear that extensive degradation of (hemi)cellulose is responsible for the formation of the latter inhibitor compounds. Kinetic studies have shown that the production of furfural strongly increases with temperature and reaction time. Temperatures higher than 160 ^{o}C and residence time of acid pretreatment longer than 4 hours have been reported to be sufficient for furfural or HMF to form, with their formation being more significant at higher temperature or longer residence times (McKillip and Collin, 2002).

2.4.2 Lignin degradation products

A variety of compounds (e.g. aromatic, polyaromatic, phenolic and aldehydic) may be released from the lignin fraction. Phenolic compounds have a considerable inhibitory effect and are more toxic (even at low concentrations) than furfural and HMF. Low molecular weight phenolics are the most toxic. Phenolic compounds cause partition and loss of integrity of cell membranes of the fermenting organisms reducing cell growth and sugar assimilation. The main factors influencing formation are process temperature and residence time. At temperatures lower than 180°C lignin degradation is negligible, if no strong acid or alkaline conditions are present.

2.4.3 Acetic acid

Acetic acid is derived from the acetyl groups in hemicellulose. At low pH in the fermentation medium the acetic acid (pKa=4.75) is in the undissociated form, is liposoluble and diffuses into the cells. In the cell (pH=7.4) the acid dissociates causing a lowering of cell pH that inhibits cell activity. The toxicity varies according to the fermentation conditions. Since the formation of acetic acid is inherent to hemicellulose hydrolysis, its formation cannot be prevented. However, a higher fermentation pH can reduce this effect or the acid can be neutralized before fermentation.

2.4.4 Inhibitory extractives

Extractives are derived from the lignocellulose structure and include acidic resins, tanninic and terpene acids. These extractives are less toxic than lignin breakdown products or acetic acid.

2.4.5 Heavy metal ions

Heavy metal ions (e.g. Fe, Cr, Ni and Cu) may originate from corrosion of process equipment. Their toxicity may inhibit enzymes in the fermenting organisms metabolism.

2.4.6 Summary

The conclusion is that especially formation of phenolic compounds from lignin degradation should be prevented and (secondly) the formation of furfural and HMF by keeping the process temperature and residence time as low and as short as possible. Furthermore, the use of e.g. a (strong) acid should be restricted in time or concentration. The formation of acetic acid and extractives is difficult to prevent because they originate directly from the feedstock. However, it may be possible to remove them or reduce the toxic effect through specific measures, such as a raise in pH directly after pretreatment or by converting them to non-toxic products.

0 summarizes the various inhibitors and gives an indicative value for a “maximum allowable concentration”. It must be noted however that these concentrations are no more than an indication because toxicity depends on various factors including the sugar concentration, the type of organism and fermentation variables, the number and concentration of inhibitors and their synergistic effects.

Table 2.5 *Origin of various inhibitors and indication of maximum allowable concentration for ethanol fermentation (Klinke et al. 2004)*

Component	Inhibitor	Maximum concentration [g/l]	Remarks
Hemicellulose >> Xylose >> Mannose, galactose, glucose	Acetic acid Furfural HMF	3 <0.25 0.25	Inhibition of acetic acid depends on pH during fermentation
Cellulose >> Glucose	HMF	0.25	HMF may further degrade to formic acid and

			levulinic acid
Lignin	Aromatics Poly-aromatics Aldehydes Phenolic compounds	< 0.1	Vanillic acid, caproic acid, caprylic acid, pelargonic acid, palmitic acid

3. Overview pretreatment routes

3.1 Introduction

Pretreatment is a crucial process step for the biochemical conversion of lignocellulosic biomass into e.g. bioethanol. It is required to alter the structure of cellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars (Mosier *et al.,* 2005). Pretreatment has been recognised as one of the most expensive processing steps in cellulosic biomass-to-fermentable sugars conversion and several recent review articles provide a general overview of the field (Alvira *et al.* 2009; Carvalheiro *et al.,* 2008; Hendriks and Zeeman, 2008; Taherzadeh and Karimi, 2008).

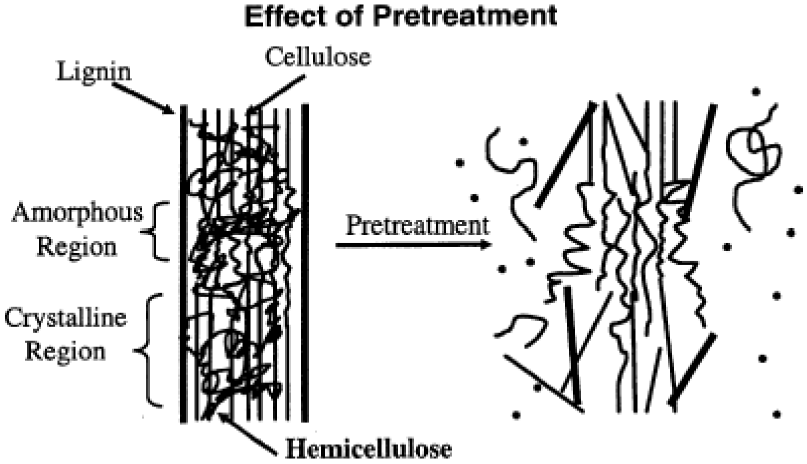


Figure 1 Schematic presentation of effects of pretreatment on lignocellulosic biomass (Hsu et al, 1980)

Pretreatment involves the alteration of biomass so that (enzymatic) hydrolysis of cellulose and hemicellulose can be achieved more rapidly and with greater yields. Possible goals include the removal of lignin and disruption of the crystalline structure of cellulose (Figure 2.1). The following criteria lead to an improvement in (enzymatic) hydrolysis of lignocellulosic material:

- Increasing of the surface area and porosity
- Modification of lignin structure
- Removal of lignin
- (Partial) depolymerization of hemicellulose
- Removal of hemicellulose
- Reducing the crystallinity of cellulose

In an ideal case the pretreatment employed leads to a limited formation of degradation products that inhibit enzymatic hydrolysis and fermentation, and is also cost effective. However, these are actually the most important challenges of current pretreatment technologies. In the following sections the most common pretreatment techniques of biomass are described.

3.2 Mechanical pretreatment

3.2.1 Milling

Reduction of particle size is often needed to make material handling easier and to increase surface/volume ratio. This can be done by chipping, milling or grinding. Mechanical pretreatment is usually carried out before a following processing step, and the desired particle size is dependent on these subsequent steps. For mechanical pretreatment factors like capital costs, operating costs, scale-up possibilities and depreciation of equipment are very important.

3.2.2 Ultrasonic pretreatment

The method of ultrasonication for lignocellulosic biomass is investigated at laboratory scale; it is a well known technique for treatment of sludge from waste water treatment plants. The experiments show the effect of the pretreatment of pure cellulose on its enzymatic hydrolysis, using a model compound (Carboxyl Methyl Cellulose, CMC). The issue of lignocellulose pretreatment is not addressed in the experiments. The experimental results showed that when a suspension of cellulose is provided with energy by irradiation, the reaction rate of the subsequent enzymatic hydrolysis is increased by approximately 200% (Imai *et al.*, 2004). The mode of action is not investigated. Presumably, it is the hydrogen bonds of the cellulose crystalline structure that break if treated with enough energy. However, the energy provided in this case is 130 kJ/g CMC, which is significantly higher than the energy of the hydrogen bond 0.12 kJ/g cellulose (21 kJ/mol cellulose (Bochek, 2003)).

3.3 Chemical pretreatment

To this group belong the pretreatments that are purely initiated by chemical reactions for disruption of the biomass structure. Combinations of chemical pretreatment and other types of pretreatment will be dealt with in 3.4

3.3.1 Liquid hot water

Liquid hot water (LHW) processes are biomass pretreatments with water at high temperature and pressure. Other terms are hydrothermolysis, hydrothermal pretreatment, aqueous fractionation, solvolysis or aquasolv (Mosier *et al.*, 2005). Solvolysis by hot compressed water contacts water with biomass for up to 15 min at temperatures of 200–230 °C. Between 40% and 60% of the total biomass is dissolved in the process, with 4–22% of the cellulose, 35–60% of the lignin and all of the hemicellulose being removed. Over 90% of the hemicellulose is recovered as monomeric sugars when acid was used to hydrolyze the resulting liquid. In addition, acetic acid is formed during the treatment and acts as a catalyst for polysaccharide hydrolysis. This results in the formation of monomeric sugars that may further decompose to furfural (inhibitor of fermentation). Variability in results was related to the biomass type with high lignin solubilization impeding recovery of hemicellulose sugars (Mok and Antal Jr, 1992).

3.3.2 Weak acid hydrolysis

Dilute acid treatment is one of the most effective pretreatment methods for lignocellulosic biomass. In general there are two types of weak acid hydrolysis:

1. High temperature and continuous flow process for low-solids loading (T> 160 °C, 5-10 wt% substrate concentration).

2. Low temperature and batch process for high-solids loading (T≤160 °C, 10-40% substrate concentration).

Dilute (mostly sulphuric) acid is sprayed onto the raw material and the mixture is held at 160-220 °C for short periods up to a few minutes. Hydrolysis of hemicellulose then occurs, releasing monomeric sugars and soluble oligomers from the cell wall matrix into the hydrolysate. Hemicellulose removal increases porosity and improves enzymatic digestibility, with maximum enzymatic digestibility usually coinciding with complete hemicellulose removal (Chen *et al.*, 2007). As an alternative to inorganic acids, organic acids (e.g. maleic acid, fumaric acid) can be used for dilute acid pretreatment (Kootstra et al, 2009).

The treatment offers good performance in terms of recovering hemicellulose sugars but there are also some drawbacks. The hemicellulose sugars might be further degraded to furfural and hydroxymethyl furfural, strong inhibitors to microbial fermentation. Furthermore, acids can be corrosive and neutralization results in the formation of solid waste. The method is especially suitable for biomass with low lignin content, as almost no lignin is removed from the biomass.

3.3.3 Strong acid hydrolysis

Concentrated strong acids such as H_2SO_4 and HCl have been widely used for treating lignocellulosic materials because they are powerful agents for cellulose hydrolysis (Sun and Cheng, 2002), and no enzymes are needed subsequent to the acid hydrolysis. Advantages of concentrated acid hydrolysis are the flexibility in terms of feedstock choice, high monomeric sugar yield as well as mild temperature conditions that are needed. Drawbacks of using concentrated acids are corrosive nature of the reaction and the need to recycle acids in order to lower cost. To date, several companies are in the process of commercialising strong acid hydrolysis of lignocellulosic biomass for microbial fermentation purposes (BlueFire Ethanol, 2010; Biosulfurol, 2010).

3.3.4 Alkaline hydrolysis

The major effect of alkaline pretreatment is the removal of lignin from the biomass, thus improving the reactivity of the remaining polysaccharides. In addition, alkali pretreatments remove acetyl and the various uronic acid substitutions on hemicellulose that lower the accessibility of the enzyme to the hemicellulose and cellulose surface (Chang and Holtzapple, 2000). It is reported that alkaline hydrolysis mechanism is based on saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components such as lignin (Sun and Cheng, 2002).

Calcium or sodium hydroxide

Usually lime (calcium hydroxide) or sodium hydroxide is used. By using these components salts are formed that may be incorporated in the biomass and need to be removed or recycled (González *et al.*, 1986). Process conditions are relatively mild but reaction times can be long. These mild conditions prevent condensation of lignin, resulting in a high lignin solubility, especially for biomass with a low lignin content such as softwood and grasses. Due to the mild conditions, degradation of sugars to furfural, HMF and organic acids is limited. The addition of air or oxygen to the reaction mixture greatly improves the delignification, especially highly lignified materials (Chang and Holtzapple, 2000).

Ammonia

Pretreatment of biomass with aqueous ammonia at elevated temperatures reduces lignin content and removes some hemicellulose while decrystallising cellulose. Ammonia pretreatment techniques include the ammonia fibre explosion-method (AFEX), ammonia recycle percolation (ARP) and soaking in aqueous ammonia (SAA).

With 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 (e.g. 2.3 MPa) (Kim *et al.*, 2003; Kim and Lee, 2005). After reaction the solid fraction, rich in cellulose and hemicellulose, is separated from the liquid. This liquid fraction is sent into a steam-heated evaporator for ammonia recovery and lignin and other sugar separation. Ammonia is then recycled to the reactor inlet whereas the separated fraction is sent into a crystallizer. After crystallization a washing step is carried out in order to extract the sugars that have been retained in the solid matrix.

Soaking in aqueous ammonia (SAA) at low temperature removes efficiently the lignin in the raw material by minimizing the interaction with hemicellulose. As a result an increase of surface area and pore size is achieved. Thus, retained hemicellulose and cellulose can be hydrolyzed to fermentable sugars by most commercial xylanase and cellulase mixtures. Kim et al. 2008 evaluated SAA as a pretreatment method of biomass (Kim *et al.*, 2008). In their study, destarched barley hull was treated with 15-30% aqueous ammonia, at 30-75ºC for 12h to 77days with no agitation and a solid-to-liquid ratio 1:12. After soaking, the solids were recovered by filtrating, washed and analyzed. As result, they obtained 66% of lignin solubilization and observed saccharification yields of 83% for glucan and 63% for xylan when treating biomass with 15% aqueous ammonia at 75ºC during 48h.

The cost of ammonia and especially of ammonia recovery drives the cost of the pre-treatment (Holtzapple *et al.*, 1991 & 1994). However, biomass pretreatment economics are also strongly influenced by total sugar yields achieved.

See also Annex A for examples of process conditions and pretreatment results.

3.3.5 Organosolv

Organosolv processes use an organic solvent or mixtures of organic solvents with water for removal of lignin before enzymatic hydrolysis of the cellulose fraction. In addition to lignin removal, hemicellulose hydrolysis occurs leading to improved enzymatic digestibility of the cellulose fraction. Common solvents for the process include ethanol, methanol, acetone, and ethylene glycol. Temperatures used for the process can be as high as 200 °C, but lower temperatures can be sufficient depending on e.g. the type of biomass and the use of a catalyst (Ghose *et al.*, 1983). Possible catalysts include inorganic or organic acids (Sun and Cheng, 2002).

The solvent itself can be an inhibitor for the enzymatic hydrolysis and fermentation step. Therefore, the solvent must be (partly) removed prior to fermentation. Removal and recovery of the solvent is required for reducing its cost and environmental impact as well.

Benefits of organosolv pretreatment include:
- The production of a high-quality lignin, which might facilitate higher-value applications of lignin such as production of (platform) chemicals.
- Potentially lowering the enzyme costs by separation of lignin before the enzymatic hydrolysis of the cellulose fraction. In addition to improved accessibility of the cellulose fibres, also absorption of cellulase enzymes to lignin is minimized by actual removal of lignin beforehand.

For a more elaborate discussion on this pretreatment route see Annex B. In Annex A examples of process conditions and results described in public literature are given.

3.3.6 Oxidative delignification

Delignification of lignocellulose can also be achieved by treatment with an oxidising agent such as hydrogen peroxide, ozone, oxygen or air. The effectiveness in delignification can be attributed, as discussed earlier in the document, to the high reactivity of oxidising chemicals with the aromatic ring. Thus, the lignin polymer will be converted into e.g. carboxylic acids. Since these acids formed will act as inhibitors in the fermentation step, they have to be neutralized or removed. In addition to an effect on lignin, oxidative treatment also affects the hemicellulose fraction of the lignocellulose complex. A substantial part of the hemicellulose might be degraded and can no longer be used for sugar production.

Hydrogen peroxide

An oxidative compound commonly used is hydrogen peroxide (H_2O_2). Dissolution of about 50% of lignin and most of the hemicellulose has been achieved in a solution of 2% H_2O_2 at 30 °C. The yield of enzymatic hydrolysis followed can be as high as 95%.

Ozonolysis

Ozone treatment focuses on lignin degradation by attacking and cleavage of aromatic rings structures, while hemicellulose and cellulose are hardly decomposed. It can be used to disrupt the structure of many different lignocellulosic materials, such as wheat straw, bagasse, pine, peanut, cotton straw and poplar sawdust (Sun and Cheng, 2002).

Wet oxidation

Wet oxidation operates with oxygen or air in combination with water at elevated temperature and pressure (McGinnis *et al.*, 1983). It was presented as an alternative to steam explosion which had become the most widely used pretreatment method (Ahring *et al*, 1999). Industrially, wet air oxidation processes have been used for the treatment of wastes with a high organic matter by oxidation of soluble or suspended materials by using oxygen in aqueous phase at high temperatures (150-350 °C) and high pressure (5-20 MPa) (Jorgensen *et al.*, 2007).

Wet oxidation has been successfully applied for the treatment of wheat straw and hardwood (Schmidt *et al.*, 1996; Schmidt and Thomsen 1998). In recent studies on alkaline wet oxidation of wheat straw, the main degradation products found from hemicellulose and lignin were carboxylic acids, CO_2, and H_2O. Compared to other pretreatment processes, wet oxidation has been proven to be efficient for treating lignocellulosic materials because the crystalline structure of cellulose is opened during the process (Panagiotou and Olsson, 2007).

About a 65% degree of delignification could be achieved with wheat straw (Klinke *et al.*, 2002). Wet oxidation of wood material has been shown to dissolve mainly the hemicellulose. One reported advantage of the wet oxidation process is the lower production of furfural and 5-hydroxymethylfurfural, which are potential inhibitors in the fermentation step.

3.3.7 Room Temperature Ionic Liquids (RTIL)

Room Temperature Ionic Liquids (RTIL) are salts that are in the liquid phase at temperature as low as room temperature. There is a vast variety of different RTIL, however, they share a common characteristic, which is that they are usually comprised of an inorganic anion and an organic cation of very heterogeneous molecular structure. The difference in the molecular structure renders the bonding of the ions weak enough for the salt to appear as liquid at room temperature (Van Rantwijk, 2003).

As of yet, there is no industrial application employing RTIL. Furthermore, very limited information exists in literature to describe their action with lignocellulose (Imai *et al.*, 2004). However, there is indication that mainly due to their polarity and in general their unique properties, they can function as selective solvents of lignin or cellulose. That would result in separation of lignin and increase of cellulose accessibility under ambient conditions and with no use of acid or alkaline solution. The formation of inhibitor compounds could also be avoided.

Despite the potential this method appears to have, there are several uncertainties due to lack of experience. Among the most important ones are the ability to recover the RTIL used, the toxicity of the compounds, and the combination of water with RTIL.

3.4 Combined chemical and mechanical pretreatment

These methods combine mechanical and chemical action. To this group belong the following pretreatments.

3.4.1 Steam explosion

Steam explosion (uncatalysed or catalysed) is one of the most applied pretreatment processes owing to its low use of chemicals and limited energy consumption. With this method high-pressure saturated steam is injected into a batch or continuous reactor filled with biomass. During the steam injection, the temperature rises to 160-260 ºC. Subsequently, pressure is suddenly reduced and the biomass undergoes an explosive decompression with hemicellulose degradation and lignin matrix disruption as result.

Results of steam-explosion pretreatment depend on residence time, temperature, particle size and moisture content (Sun and Cheng, 2002). Studies have been carried out to try to improve the results of steam explosion by addition of chemicals such as acid or alkali (Cara *et al.*, 2008, Stenberg *et al.*, 1998, Zimbardi *et al.*, 2007). Limitations of steam explosion include the formation of degradation products that may inhibit downstream processes (Garcia-Aparicio *et al.,* 2006).

In Annex A, examples of process conditions and results described in public literature are presented.

3.4.2 Ammonia fibre explosion (AFEX)

In the AFEX process, biomass is treated with liquid ammonia at high temperature and pressure (Teymouri *et al.*, 2005). After a few seconds, pressure is swiftly reduced. A typical AFEX process is carried out with 1-2 kg ammonia/kg dry biomass at 90 °C during 30 min. It reduces the lignin content and removes some hemicellulose while decrystallising cellulose. The cost of ammonia and especially of ammonia recovery drives the cost of the pre-treatment (Holtzapple *et al.*, 1991 & 1994), although ammonia is easily recovered due to its volatility.

3.4.3 CO_2 explosion

This method is similar to steam and ammonia fibre explosion; high pressure CO_2 is injected into the batch reactor and then liberated by an explosive decompression. It is believed that CO_2 reacts to carbonic acid (carbon dioxide in water), thereby improving the hydrolysis rate. Yields of CO_2 explosion are lower than those obtained with steam or ammonia explosion, but they are higher than those reached with enzymatic hydrolysis without pretreatment (Sun and Cheng, 2002).

Carbonic acid may offer the benefits of acid catalysts without the use of an acid like sulphuric acid. The pH of carbonic acid is determined by the partial pressure of CO_2 in water, and can be neutralized by releasing the reactor pressure. Studies indicate that combined capital and operating costs of the carbonic acid system are slightly higher than a sulphuric acid-based system and highly sensitive to reactor pressure and solids concentration (Jayawardhana and Van Walsum, 2004). In case of a bioprocessing plant that produces carbon dioxide, carbonic acid may be a viable reagent for promoting hydrolysis without mineral acids (Van Walsum, 2001).

An alternative use of CO_2 in pretreatment is extraction with supercritical CO_2. Carbon dioxide becomes supercritical under relatively mild conditions (T_c=304K and P_c=73 bar). Supercritical carbon dioxide (SC-CO_2) has been widely used as an extraction solvent. Recently, SC-CO_2 extraction is being considered as possible pretreatment route for lignocellulosic material.

Sahle reported the enhanced permeability of Douglas-fir by SC-CO2 (Sahle Demessie *et al.,* 1995). Kim and Hong (2001) investigated SC-CO_2 as possible pretreatment for enzymatic hydrolysis of hardwood and softwood. A positive effect was found for lignocellulosic material with a high moisture content (>40%), but the pretreatment was not effective enough to compensate for the high capital costs for high-pressure equipment. Other studies showed no significant change in microscopic morphology of wood after extraction of pine wood with SC-CO_2 (Ritter and Campbell, 1986; Ritter and Campbell, 1991), and SC-CO_2 was considered not an effective tool for lignocellulosic treatment.

3.4.4 Mechanical/alkaline pretreatment

Combined mechanical/alkaline pretreatment consists of a continuous mechanical pretreatment (e.g. milling, extrusion, refining) of lignocellulosic biomass with the aid of an alkali. The resulting fractions consist of a soluble fraction (containing lignin, hemicellulose and inorganic components) and a cellulose-enriched solid fraction.

As opposed to the acid-catalysed methods, the general principle behind alkaline pretreatment methods is the removal of lignin whereas cellulose and part of the hemicelluloses remain in the solid fraction. The solid fraction is submitted to enzymatic hydrolysis for the production of C6- and C5 sugars and this pretreatment method is especially suitable in combination with fermentation routes in which both C6- and C5 sugars can be converted to products.

By performing extrusion and chemical pretreatment in one step the accessibility of cellulose for enzymes is improved, resulting in higher delignification values and improved enzymatic hydrolysis. In addition, the moderate operation temperatures of this process prevent the formation of degradation and oxidation products.

The combination of alkaline pretreatment with mechanical action increases the efficiency of the pretreatment compared to alkaline pretreatment, but the use of expensive chemicals remain necessary, and recycling and waste treatment is an important issue.

For more information on this pretreatment route see Annexes A and C.

3.5 Biological pretreatment

In this group of pretreatments microorganisms such as white, brown and soft rot-fungi are employed to degrade hemicellulose and lignin. Advantages of biological pretreatments are low energy requirement and mild operation conditions. Nevertheless, the rate of biological hydrolysis is usually very low, so this pretreatment requires long residence times (Cardona and Sanchez, 2007; Sun and Cheng, 2002; Tengerdy and Szakacs, 2003).

3.6 Economic analysis of pretreatment routes

An economic assessment of the biomass pretreatment methods available in literature must be made in order to determine their feasibility when designing an industrial biomass-based process. A quantitative economic analysis of some pretreatment methods is given by Eggeman and Elander (2005). Within this work, five different biomass pretreatment technologies were evaluated in order to compare them in terms of capital costs: dilute acid, hot water, ammonia fibre explosion (AFEX), ammonia recycle percolation (ARP), and lime. As biomass corn stover was used. Two additional pretreatment cases were considered as reference: 'No Pretreatment' involved the dilution of the biomass to 20 wt% solids prior to enzymatic hydrolysis. For the 'Ideal Pretreatment', the biomass was diluted to 20 wt% prior to hydrolysis, and the yield of glucose and xylose sugars after enzymatic hydrolysis were assumed to be 100% of the theoretical value.

The starting point was an Aspen Plus simulation model of a full bioethanol production facility into which every pretreatment was integrated. Results obtained from mass and energy balances were employed to estimate the variable operating costs and to size the major pieces of equipment. The capital cost was based on a factored estimate and the fixed operating costs were assumed to be similar to those corresponding to bioethanol production facilities of similar capacity. 0 summarizes the capital cost for each case.

Table 3.1 *Capital costs for liberation of sugars from corn stover for each pretreatment technology (Eggeman and Elander, 2005)*

	Pretreatment direct fixed capital, $MM	**Pretreatment breakdown, % Reactor/% other**	**Total fixed capital, $MM**	**Ethanol production, MMgal/yr**	**Total fixed capital, $/gal annual capacity**
Dilute acid	25.0	64/36	208.6	56.1	3.72
Hot water	4.5	100/0	200.9	44.0	4.57
AFEX	25.7	26/74	211.5	56.8	3.72
ARP	28.3	25/75	210.9	46.3	4.56
Lime	22.3	19/81	163.6	48.9	3.35
No pretreatment	0	–	200.3	9.0	22.26
Ideal pretreatment	0	–	162.5	64.7	2.51

The pretreatment area direct fixed capital is roughly the same for dilute acid, AFEX, ARP and lime pretreatment. Although the contribution of the reactor to the pretreatment area cost is very important in case of dilute acid pretreatment, other pieces of equipment are dominant for the rest of technologies. As a result, the total fixed capital is similar in all cases, except for lime and ideal pretreatment, therefore it can be concluded that low cost reactors in some pretreatments are counterbalanced by higher cost associated with chemical recovery. These results serve as a guide but they should not be taken as a basis, because no differentiation for variation in the state of development of each technology was made.

For other available pretreatment methods, no quantitative data were found in public literature. Most of the work described in this review offers only a qualitative vision of this aspect, which could be useful to get a first idea of which pretreatment technology could be interesting to evaluate. For instance, ozonolysis requires high amount of O_3, which is expensive, so this pretreatment method would be less cost-effective than other ones. Another example is strong acid, which requires special materials that are resistant to corrosion and this increases its capital cost. Nevertheless, a quantitative analysis must be carried out in order to evaluate correctly every technology.

3.7 Conclusions

Different pretreatment technologies published in public literature were described in terms of the mechanisms involved, advantages and disadvantages, and economic assessment. 0 gives a summary of the main pretreatment routes and their major characteristics for a number of criteria. The choice of the optimum pretreatment process depends very much on the objective of the biomass pretreatment since different products are yielded. In addition, the choice of a pretreatment method should not only be based only on its potential yield but also on other important parameters such as its economic assessment and environmental impact.

Table 3.2 *Main pretreatment routes compared*

Pretreatment	**Mode of action (in addition to increasing the surface area)**	**Potential sugar yield**	**Inhibitor formation**	**Residue formation**	**Need for recycling chemicals**	**Low investment costs**	**Low operational costs**	**Applicable to various biomass**	**Proven at pilot scale**	**Additional remarks**
Mechanical		--	++	++	++	+	-	+	+	
Liquid hot water	• Removal of hemicellulose	++	-	++	++	+			++	
Weak acid	• Removal of hemicellulose (major) • Alteration lignin structure (minor)	++	-	-	-	+/-	+	+	++	Specially suitable for biomass with low lignin content
Strong acid	• Hydrolysis of cellulose and hemicellulose	++	-	-	-	-	+/-	++	++	Strong acid is hazardous, toxic and corrosive.
Alkaline	• Removal of lignin (major) and hemicellulose (minor)	++	++	-	-	++		+/-	+/-	
Organosolv	• Removal of lignin (major) • Removal of hemicellulose (minor), depending on solvent used	++	++	+	-	-	-	+	++	High quality lignin Solvent used may be inhibitor for cell growth
Wet oxidation	• Removal of lignin (major) • Dissolve hemicelluloses • Decrystallization cellulose	+/-	++	+	++	+			-	
Steam explosion	• Removal hemicellulose (major) • Alteration lignin structure (minor)	+	-	+	++	+	+	+/-	++	Low environmental impact
AFEX	• Removal of lignin (major) and hemicellulose (minor) • Decrystallization cellulose	++	++		-			-		No need for small particle size for efficacy
CO_2 explosion	• Removal of hemicellulose • Decrystallization cellulose	+	+	++	++	-			-	More cost effective than AFEX
Combined mechanical/ alkaline	• Removal of lignin (major) and hemicellulose (minor)	++	++	-	-	+/-	+/-	+	+	

+ = positive characteristic: E.g. high yield of fermentable sugars, no or low fermentation inhibitors, no residue formation, no or low need for recycling of chemicals, low investment costs, high applicability to different biomass types, proven at pilot scale, low operational costs

- = negative characteristic: E.g. low yield of fermentable sugars, high amount of fermentation inhibitors, high residue formation, need for recycling of chemicals, high in vestment costs, low applicability to different biomass types, not (yet) proven at pilot scale, high operational costs

4. Concluding remarks and future perspectives

Pretreatment technologies for lignocellulosic biomass include biological, mechanical, chemical methods and various combinations thereof. Biological pretreatments employ microorganisms which degrade lignin and hemicellulose. The main disadvantage here is that fungi primarily attack cellulose and that the hydrolysis rate is often very low. Mechanical methods such as chipping, grinding and milling (often referred to as physical methods) reduce crystallinity but more importantly give reduction of particle size, make material handling easier and increase surface/volume ratio. These processes often have high energy and capital costs. Chemical pretreatments that use chemicals like alkalis, ozone, peroxide, or organic solvents are largely focussed on lignin removal, which in turn leads to an enhanced enzymatic degradability of cellulose. Chemical pretreatments with acids result in the hydrolysis of (hemi)cellulose to sugars. Often, the pretreatment is a combination of mechanical and chemical action and moderate amounts of chemicals are used. To this group belong methods like steam explosion and mechanical/alkaline pretreatment.

Only a small number of pretreatment methods has been reported as being potentially cost-effective. These include steam explosion, liquid hot water, concentrated acid hydrolysis (Groenestijn et al.), and dilute acid pretreatments (Mosier *et al*., 2005). At the moment the production of ethanol from lignocellulose is growing rapidly, and by looking at the industrial activities in this field more knowledge can be gained on the applied pretreatment methods. The International Energy Agency (IEA) has a Bioenergy Task 39 entitled 'Commercializing 1st and 2nd generation Liquid Biofuels from Biomass'. An overview by Bacovsky shows demonstration plants worldwide for the production of biofuels and applied pretreatment methods (Bacovsky, 2009). Two main pathways are defined: biochemical conversion and thermochemical conversion (e.g. gasification and combined pathways).

For the biochemical pathway (i.e. biomass-pretreatment-hydrolysis-fermentation-distillation-ethanol) 33 projects were identified of which 13 are currently operational. In Table 8 six projects are described with special attention to the applied pretreatment method and fate of the lignin. As shown by this overview pretreatment methods using acids are preferred. As a result the lignin is recovered as a low added-value residue that is mainly used to generate steam or electricity.

It is not possible to define the best pretreatment method as it depends on many factors such as type of lignocellulosic biomass and desired products. Pretreatments must improve the digestibility of lignocellulosic biomaterials, and each pretreatment has its own effect on the cellulose-, hemicellulose- and lignin fractions. Looking at industrial activities for the production of ethanol, acid-based pretreatment methods are preferred. In these processes lignin is left with the substrate and removed after the hydrolysis of the (hemi)cellulose or even after distillation. Research topics for these processes include amongst others minimization of sugar loss, increase of solids concentration and higher ethanol concentrations after fermentation.

When lignin is removed from the biomass in an early stage of the process (i.e. after the pretreatment) it can be recovered as a co-product with potential high added value. Another advantage is that the enzymatic digestibility is strongly related to the lignin content, and that lignin removal greatly enhances enzymatic hydrolysis. In this case pretreatment methods that focus on lignin removal become more interesting and recycling of chemicals becomes one of the most important issues to resolve.

Table 4.1 *Worldwide production of bioethanol; 6 different technologies (adapted from bio-fuels.abc-energy.at/demoplants)*

Process	Location	Products	Status	Raw material	Pretreatment	Fate of lignin
Abengoa	Spain	4.000 t/a EtOH	Demo facility, start-up 2009[a]	Wheat straw	Acid catalysed steam explosion	• As co-product • Recovered after distillation
Inbicon	Denmark	• 4.000 t/a EtOH • C5-molasses • Solid biofuel	Demo-facility, start-up 2009[b]	Wheat straw	Liquid hot water (hydrothermal, auto catalysed)	• Solid biofuel for powerplant • Recovered after distillation
Iogen	Canada	70.000 t/a EtOH	Commercial facility, start-up 2011[c]	Straw (wheat, barley, oat)	Modified steam explosion	• For steam and electricity generation, • Recovered after enzymatic hydrolysis
KL Energy	USA	4.500 t/a EtOH	Demo facility, operational since 2007	Wood waste, cardboard and paper	Thermo mechanical	• For steam or electricity generation, or as wood pellet co-product • Recovered after distillation
SEKAB	Sweden	4.500 t/a EtOH	Demo facility, start-up 2011[d]	Wood chips or sugarcane bagasse	Acid pretreatment	• For energy production or other uses • Recovered after (enzymatic) hydrolysis
Verenium process	USA	4.200 t/a EtOH	Demo facility, operational since 2009[e]	Sugarcane bagasse, energy crops, wood products and switchgrass	Mild acid hydrolysis and steam explosion	• Lignin-rich residue burned for steam generation • Recovered after distillation

[a] Pilot facility with corn stover operational since 2007, commercial facility with corn-stover start-up 2011 (USA)

[b] 2 Pilot facilities operational since 2003 and 2004

[c] Demo facility operational since 2004

[d] Pilot facility operational since 2004, another demo facility of 50.000 t/a EtOH planned to start-up in 2014 and commercial facility of 120.000 t/a EtOH planned to start-up in 2016

[e] Pilot facility operational since 2007

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Appendix A Overview literature studied pretreatment technologies evaluated

Steam Explosion												
Reference	**Biomass**	**Handling**	**Chemicals**	**Concentration**	**Biomass loading**	**Temperature**	**Pressure**	**Residence time**	**Recovery of WIS**[1]	**EH**[2] **cellulose conversion**	**EH Yield**	**Overall sugar yield**
(Linde *et al.*, 2008)	Wheat straw	Cutting and sieving 2-10 mm	H_2SO_4	0.2 wt%	60g dry matter	190 °C	-	10 min	64%	37.8 glucose/100 g dry wheat	-	61.2 glucose &xylose/100g dry matter
(Cara *et al.*, 2008)	Olive tree pruning	-	H_2SO_4	1 wt%	-	230 °C	-	-	-	-	-	-
(Ruiz *et al.*, 2008)	Sunflower stalks	Air drying at RT, milling	-	-	150 g dry matter	220 °C	-	5 min	66%	-	72%	21 g sugars/100 g raw material
(Zimbardi et al., 2007)	Corn stover	Chopping and grounding to 0.2 mm	H_2SO_4	1.5 %	500 g	190 °C	-	5 min	67.3%	65.2%	-	65.9% glucose 63.1% xylose
(Sun *et al.*, 2004; 2005a; 2005b)	Wheat straw	Drying and cutting	-	-	500 g dry matter	220 °C	22 bar	3 min	40 %	-	-	-
(Stenberg *et al.*, 1998)	Wood chips (spruce and pine)	Chipping and sieving	SO_2	3.5% (w/w)	200g dry matter	210 °C	-	5.5 min	-	-	28.9 g glucose/g wood	42.1% (glucose and mannose)

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[1] *Water insoluble solids*

[2] *Enzymatic hydrolysis*

Organosolv process												
Reference	**Biomass**	**Remarks**	**Organic solvent**	**Catalyst**	**L/S (% w/w)**	**Temperature (°C)**	**Pressure (bar)**	**Cooking time (h)**	**Pulp yield (%)**	**Lignin removal (%)**	**Cellulose recovery (%)**	**Hemicellulose removal**
(Lee *et al.*, 1986)	Corn stover	Pre-treated with dilute H2SO4.	Methanol, butanol, aromatic alcohols	H_2SO_4	5	160		1		>90		
(Zhang *et al.*, 2007)	Corn stover	Knife-milled/ screened.	H3PO4 / acetone			50	Atm	0.5 - 1		50	95	79
(O'Connor *et al.*, 2007)	Corn stover	Chopped, pre-soaked.	Ethanol	H_2SO_4	6	170		0.5	40	85	92	91
(Carioca *et al.*, 1985)	Elephant grass		Ethanol		3 – 14	180		1 – 3		70	95	90
(Ibrahim *et al.*, 1999)	Oak (red)	After steam pre-treatment	Acetic acid		11	60		1		~60		
(Hasegawa *et al.*, 2004)	Oil palm shell wastes / Apricot tree		Acetone			200				High		High
(Black *et al.*, 1994)	Poplar	Chips	Ethanol / MIBK	H_2SO_4	9	140		1	64			
(Pan *et al.*, 2006)	Poplar	Chopped	Ethanol	H_2SO_4		180		1		74	88	
(Ghose *et al.*, 1983)	Rice straw	Chopped <1cm	Butanol	Organic catalysts		120		2	54	83		
(Kiran *et al.*, 1994)	Spruce (red)	Chipped <7mm, flow-through reactor	Acetic acid			180	250	3	51	93		
(Gonçalves *et al.*, 2003)	Sugarcane bagasse		Ethanol		10			1-3	44-52	>75		
(Arora *et al.*, 1990)	Sugarcane bagasse / elephant grass		Ethanol	Various catalysts		180						
(Pasquini *et al.*, 2005)	Sugarcane bagasse / Pine (P. taeda)		Ethanol, acetic acid, methanol, dioxane	sc CO_2		142 - 198	147 – 232	0.5 - 2.5	33 - 44	88 - 93		
(Papatheofanous *et al.*, 1995)	Wheat straw	Pre-treated with acid hydrolysis.	Ethanol	H_2SO_4		81		1.5	63	>70	>98	50
(Sun *et al.*, 2007)	Wheat straw		Glycerol		15	240	Atm	4		>70	95	>90
(Arato *et al.*, 2005)	Woody biomass		Ethanol		9 - 20	180-195	30	0.5 - 1.5				

Organosolv process (continued)												
Reference	**Biomass**	**Remarks**	**Organic solvent**	**Catalyst**	**L/S (% w/w)**	**Temperature (°C)**	**Pressure (bar)**	**Cooking time (h)**	**Pulp yield (%)**	**Lignin removal (%)**	**Cellulose recovery (%)**	**Hemicellulose removal (%)**
(Lee *et al.*, 1986)	Corn stover	Pre-treated with dilute H2SO4.	Methanol, butanol, aromatic alcohols	H_2SO_4	5	160		1		>90		
(Zhang *et al.*, 2007)	Corn stover	Knife-milled and screened.	H3PO4 / acetone			50	Atm	0.5 - 1		50	95	79
(O'Connor *et al.*, 2007)	Corn stover	Chopped, pre-soaked.	Ethanol	H_2SO_4	6	170		0.5	40	85	92	91
(Carioca *et al.*, 1985)	Elephant grass		Ethanol		3 – 14	180		1 – 3		70	95	90
(Ibrahim *et al.*, 1999)	Oak (red)	After steam pre-treatment	Acetic acid		11	60		1		~60		
(Hasegawa *et al.*, 2004)	Oil palm shell wastes / Apricot tree		Acetone			200				High		High

Alkaline pretreatment

Reference	Biomass	Handling	Chemicals	Conc.	L/S %w/w	Temperature (°C)	Pressure (bar)	Time	Cellulose yield (%)	Hemicellulose removal (%)	Lignin removal (%)	EH cellulose conversion (%)
(Zhao *et al.*, 2008)	Spruce chips	Refining	NaOH NaOH/urea	1-12% 1-7%/12%	20	-15/23/60		2-24 h	91	40	19	70
(Saha and Cotta, 2007)	Wheat straw	Drying, hammer mill	$Ca(OH)_2$	100 mg/g straw	12	121	autoclave	1 h				
(Chen *et al.*, 2007)	Barley and wheat straw	milling (<1 mm)	NaOH	0.5-2.0	9	121	15 psi	1 h	70-90	7-40	20-85	
(Zhu *et al.*, 2006)	wheat straw	Cutting (1-2 cm)	NaOH	1 %	8	100		25 min at 700W 60 min boiling				Conv alkali 65% Microwave alkali 70%
(Saha and Cotta, 2006)	Wheat straw	Hammer mill	H_2O_2 NaOH	0-4.3	12	25-35		3-24 h				56% total sugars
(Sun *et al.*, 2004; 2005a; 2005b)	Wheat straw	Cutting (2-3 cm) Steam explosion	H_2O_2 NaOH	2%	20	50		2.5h	31-40	8-13	14-15	
(Carrillo *et al.*, 2005)	Wheat straw	Milling (<6 mm) washing/drying	NaOH	0.5 M	20	80		6 h				
(Kim *et al.*, 2003)	Corn stover	Grounding	Aqueous NH_3 (ARP)	15 wt%	15 g	170 °C	-	20 min	>95%	40-60%	70-85%	-

Alkaline/mechanical pretreatment

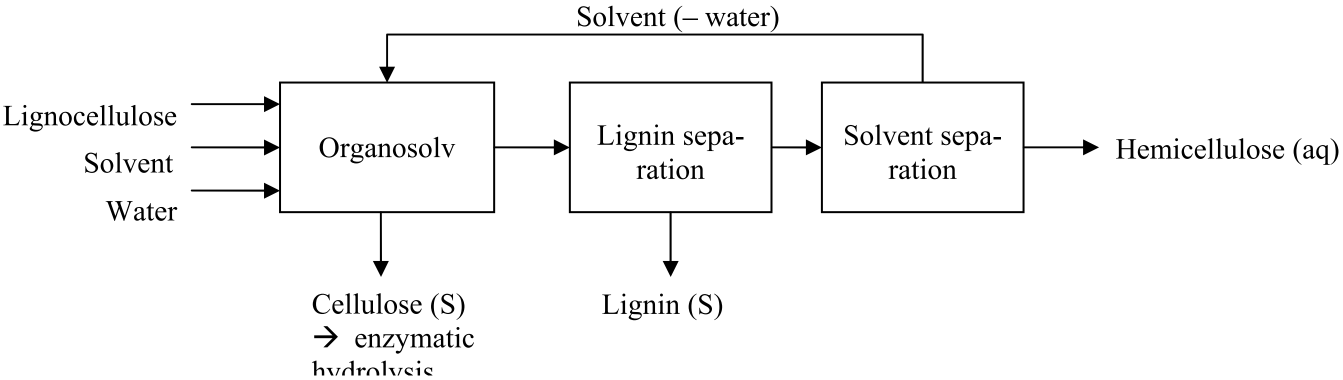
Reference	Biomass	Handling	Chemicals	Conc.	L/S (%w/w)	Temperature (°C)	Pressure	Time	Cellulose yield (%)	Hemicellulose removal (%)	Lignin removal (%)	EH Cellulose conversion (%)
(Vrije de *et al.*, 2002)	*Miscanthus*	Milling (<1 mm)	NaOH	12%	6	70 °C		various	>95%	44%	77%	69%
(Riddlestone and Harris 2006; Riddlestone *et al.*, 2007a; 2007b; Harris *et al.*, 2008)	*Wheat straw*	Hammer mill	NaOH	12%								

Appendix B Organosolv

B.1 Introduction

The organosolv process was originally developed as an alternative pulping process for paper making (Kleinert *et al.*, 1931; Pye and Lora, 1991). In the process lignocellulosic biomass is treated with an organic solvent, such as alcohols and organic acids, in order to extract the lignin from the biomass matrix. The process is usually performed at elevated temperature and pressure in the presence of water as a co-solvent. This is required to thermochemically break the bonds within the lignin-cellulose-complex (LCC). During the process, also hemicellulose is hydrolysed. The part that remains of the biomass after organosolv pulping is cellulose fibres that can be used for e.g. paper production.

In addition to papermaking, organosolv can also be applied for biomass fractionation/pre-treatment. First, the lignocellulosic biomass is separated into its main three constituents (cellulose, hemicellulose, lignin). Second, by fractionation and the treatment during organosolv the cellulose fraction can be made more susceptible for enzymatic hydrolysis to fermentable sugars. For more information on organosolv we refer to reviews written on this subject (Aziz *et al.*, 1989; Muurinen, 2000; Varshney *et al.*, 1988; Zhao *et al.*, 2009). The chemistry of the organosolv process has been discussed extensively by McDonough (McDonough, 1992).



Figuur B.1 *Simplified scheme of a possible organsolv fractionation process*

B.2 Literature on organosolv

The amount of literature on organosolv is very large, and the list in the tables in Annex A is not intended to be complete but it gives an idea about the organosolv process. In case multiple experimental results are given, the experimental result with the highest fractionation efficiency was chosen to be included.

In addition to biomass pretreatment, organic solvent based processes have also been studied for the total liquefaction of biomass. In case of the latter, the idea is to dissolve the biomass totally i.e. including the cellulose. This is not the intention of the biomass pretreatment process within the BioSynergy biorefinery concept. However, at less severe conditions (lower reaction temperature, less catalyst, etc) these organosolv processes usually become a biomass fractionation process. Therefore, also a number of total liquefaction processes are listed in the tables.

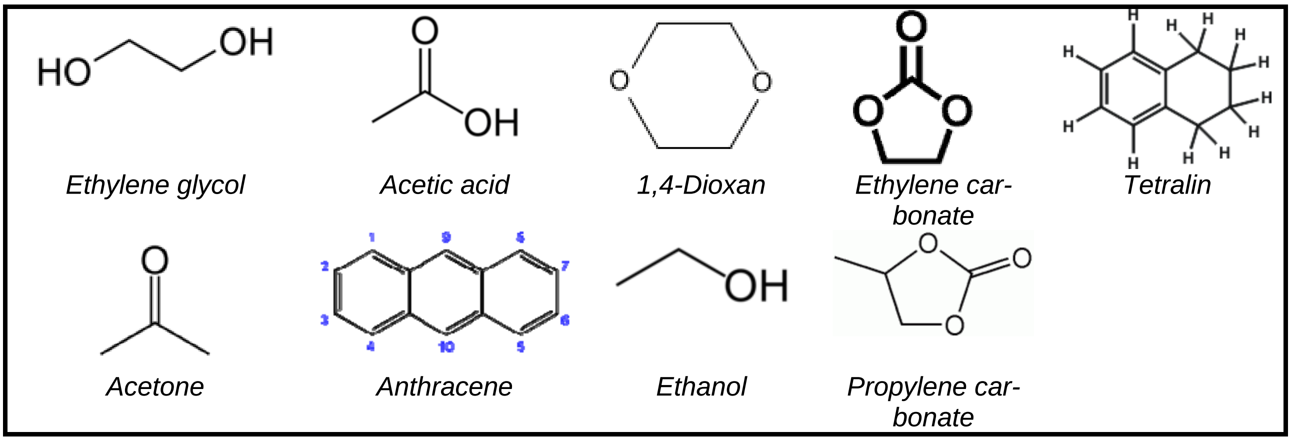
B.3 Organic solvents

As can be observed from the tables above, many different organic solvent have been applied for the organosolv process. Muurinen has given an extensive overview of possible organic solvents for organosolv pulping in her PhD thesis (Muurinen, 2000). Furthermore in the review by Aziz on organosolv pulping many solvents are discussed (Aziz *et al*., 1989).

Tabel B.1 *Organic solvents studied in literature as a co-solvent for water in organosolv processes together with example references (list not intended to be complete)*

Alcohols	Aliphatic	Methanol	(Solár *et al.*, 2001)
		Ethanol	(Pye *et al.*, 1991; Girard *et al.*, 2000; Pan *et al.*, 2006; Arato *et al.*, 2005; Carioca *et al.*, 1985; Gonçalves *et al.*, 2003)
		Butanol	(Ghose *et al.*, 1983; Jiménez *et al.*, 1999)
		Other alcohols (e.g., propanol)	
		Tetrahydrofurfuryl alcohol (THF)	
	Diols	1,3-Butane diol	(Kishimoto *et al.*, 2001)
		Ethylene Glycol	(Jiménez *et al.*, 2004)
		Glycerol	(Adeeb, 2004)
		Polyethylene glycol	(Guo *et al.*, 2003)
	Aromatic	Phenol	
		Cresols	
Organic acids		Formic acid	
		Acetic acid	(Ibrahim *et al.*, 1999; Kiran *et al.*, 1994)
		Peroxyacetic acid	(Nada *et al.*, 1999)
		Thioacetic acid	
		Chloroacetic acid	
Ethyl acetate			(Varshney *et al.*, 1988)
Amines		E.g., ethylene diamine	(Heitz *et al.*, 1994)
		Hexamethylene diamine	(Aziz *et al.*, 1989)
Ketones		Acetone	(Alcaide *et al.*, 2003; Hasegawa *et al.*, 2004)
		γ-Lactone	(Petrus, 2006)
Ethers	Linear		
	Cyclic	1,4-Dioxane	(Mun *et al.*, 2004a)
		Dioxolane	
Aromatics		Anthracene oil	(Crofcheck *et al.*, 2005)
		Ethylene & propylene carbonate	(Yamada et al., 2006)
		Pyridine	(Schuerch, 1952)

In Figuur B.2 some structural formulas of solvents used for biomass processing are given:



Figuur B.2 *Examples of organic solvents used for biomass processing*

Ethanol has most widely been applied as solvent for the organosolv process. It has been used for the delignification of various types of biomass as well as in the pre-commercial Alcell process (Pye *et al*., 1991). (Semi) commercial processes have also been developed with methanol, ethanol and acetone, e.g.:

- Acid Catalyzed Organosolv Saccharification (ACOS)
  - Paszner, UBC, Canada. Acetone with acid, 200 °C, 40 bar, 0.5 hour.
  - Dedini, Brasil. Ethanol (Oliverio *et al*., 2004).
- Alcell, Canada. Three stages: ethanol-water (Pye *et al*., 1991).
- Organocell, Germany. First stage: methanol-water (50/50), 190°C, 20-40 min. Second stage: methanol-water-NaOH (35/25/20), 170°C, 40-60 min.

B.4 Process conditions

Although the required process conditions depend largely on the type of biomass processed, some general conclusions can be made based on Table 2.1 and Table 2.2. Generally, a process temperature of 120-200 °C is required for effective biomass fractionation. At higher temperatures, also the cellulose fraction of the biomass will be depolymerised/degraded. Cooking times vary between 0.5 and several hours for biomass fractionation. At these process conditions, organosolv is well suitable to hydrolyse hemicellulose, recover cellulose and make the cellulose fraction more susceptible for enzymatic hydrolysis (not included in the tables). Also substantial delignification of biomass is realized, although (virtually) complete delignification seems hard to achieve.

Organosolv for biomass pretreatment can be either carried out non-catalytically or catalytically. The most applied catalyst is sulphuric acid. In general, it can be stated that uncatalysed organosolv at standard conditions can pulp only low-density hardwoods and agricultural residues. For the pulping of softwoods or high-density hardwoods, more severe process conditions (e.g. in the form of a catalyst) are required (Paszner *et al*., 1989).

B.5 Conclusions

Compared to other pretreatment technologies, organosolv seems to be the most suitable process if one wants to produce lignin in a sufficient quality for the production of chemicals. In most other pretreatment technologies lignin remains with the cellulose fractions throughout the whole biomass conversion process. Generally, this leads to an impure lignin product containing unhydrolysed sugar polymers as well as other organics. In the organosolv process, lignin is extracted and re-precipitated in the beginning of the process which leads to a relatively pure lignin.

Potential advantages of organosolv process include:

- Maximum valorisation of all main biomass fractions including lignin. Organosolv lignin has a high purity, which is beneficial for applications like conversion into chemicals (applications with a higher value than combined heat and power).
- No large amounts of waste produced in the form of e.g. gypsum due to neutralisation of (sulphuric) acid used.
- The removal of lignin prior to enzymatic hydrolysis as well as the (partial) hydrolysis of hemicellulose during organosolv pre-treatment reduces the amount of enzymes required and/or the residence time of the enzymatic hydrolysis stage.
- Compared to acid hydrolysis (being the industrially most applied pretreatment route), relatively low formation of inhibitor compounds due to the absence of a strongly acidic environment (if at all, only catalytic amounts of e.g. H_2SO_4 are used).

Potential drawbacks of the organosolv process include:

- The use of organic solvents leads to relatively high costs, both operational costs and investment costs (extra equipment required for recycling of the solvent). These extra costs have to be compensated by e.g. the extra earnings resulting from the lignin-based chemicals or lower enzyme costs.
- An extra environmental burden due to losses of organic chemicals to e.g. the atmosphere cannot be precluded beforehand. For this reason as well as process economics, recycling of the organic solvent deserves full attention.

Appendix C Mechanical/alkaline pretreatment

C.1 Introduction

Various pretreatment methods are described in literature that promote the accessibility of polysaccharides in a lignocellulosic material for enzymatic hydrolysis. Many methods require harsh conditions such as high temperatures and chemical loads, resulting in the formation of degradation products that may act as inhibitors during fermentation. An alternative pretreatment method is the combination of mechanical action (e.g. milling, extrusion, refining) and the use of moderate amounts of alkaline chemicals. As opposed to the acid-catalysed methods, the general principle behind alkaline pretreatment methods is the removal of lignin whereas cellulose and a part of the hemicelluloses remain in the solid material. The combination of alkaline pretreatment with mechanical action increases the efficiency of this pretreatment.

C.2 Literature on mechanical/alkaline pretreatment

The amount of literature on mechanical/alkaline pretreatment is limited. For the pre treatment of Miscanthus for hydrogen production de Vrije combined extrusion with sodium hydroxide (Vrije de *et al.*, 2002). This study showed an inverse relationship between lignin content and efficiency of enzymatic hydrolysis of polysaccharides. High delignification values were obtained by the combination of mechanical and chemical pretreatment. An optimized process consisted of a one-step extrusion/sodium hydroxide pretreatment at moderate temperature (70 °C). This pretreatment resulted in 77% delignification, 95% cellulose yield and 44% hydrolysis of hemicellulose. After enzymatic hydrolysis, 69% and 38% of the cellulose and hemicellulose fraction was converted to sugars. Mechanical action can consist of chopping, milling, extruding or refining of the material. In the case of Miscanthus, sodium hydroxide treatment of chopped material was less effective, while the milled material (<1 mm) was delignified for 70%. However, the highest degree of delignification was obtained when the material was treated with sodium hydroxide in combination with extrusion. In the study by de Vrije a corotating twin-screw extruder was used. In this extruder, the biomass is transported via transport screws to a reversed screw element (RSE). This results in accumulation and compression of the material in the space between the transport screws and the RSE. High compression and shear forces cause fibrillation and shortening of the fibres in the biomass. Besides extrusion also refining can cause fibrillation and shortening of the fibres. The result of refining is highly dependent on the temperature applied. At low temperatures, a mixture of intact fibres, conglomerates of fibres and broken parts of fibres are formed. Refining at higher temperatures results in more intact fibres with a more lignin rich surface.

C.3 Chemicals

Chemicals used in alkaline pretreatment include sodium hydroxide, lime and ammonia. Each chemical has its own advantages and disadvantages:

- Sodium hydroxide causes the disruption of H-bonding in cellulose and hemicellulose, breakage of ester linkages between lignin and xylan, and deprotonation of phenolic groups. As a result, swelling of cellulose and the partial solubilization of hemicellulose and lignin occurs (Chen *et al.,* 2007). This treatment is especially suitable for wheat or barley straw. A disadvantage is the high costs of use of sodiumhydroxide.

- Lime has the benefits of low costs and high safety, and being recoverable from water as insoluble calcium carbonate by reaction with carbon dioxide. The action of lime is slower compared to ammonia or sodium hydroxide.
- Aqueous ammonia causes swelling of cellulose and change of the crystal structure. Treatment with ammonia reduces lignin and removes hemicelluloses to a lesser extent while decrystallizing cellulose. The cost and recovery of ammonia drives the cost of pretreatment with ammonia.

C.4 Conclusions

Extrusion and/or refining are viable techniques to increase alkaline pretreatment efficiency and enzymatic degradability of lignocellulosic biomass. High compression and shear forces lead to extensive defibration, fibrillation and shortening of fibres in the biomass and thereby increasing accessibility of carbohydrates to enzymatic attack. Due to the mild process conditions, the formation of inhibitors is limited.

As shown by the two references in the literature overview in Annex A, the area of combined mechanical/alkaline pretreatment is rather innovative. As stated in the review of Alvira (Alvira *et al.,* 2009), the extrusion process is a novel and promising physical pretreatment method for biomass conversion. With extrusion the materials are subjected to heating, mixing and shearing, resulting in physical and chemical modifications during the passage through the extruder. Besides alkaline chemicals also enzymes can be applied during the extrusion process and this is being considered as a promising technology for ethanol production (Karunanithy *et al*., 2008).