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# Pretreatment of lignocellulose for biotechnological production of lactic acid

Research review

Paulien Harmsen, Steef Lips, Rob Bakker
February 2013

Rapport 1384, public version

# Colophon


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|---|---|
| Title | Pretreatment of lignocellulose for biotechnological production of lactic acid; Research review |
| Author(s) | Paulien Harmsen, Steef Lips, Rob Bakker |
| Number | 1384 |
| ISBN-number | 978-94-6173-607-9 |
| Date of publication | February 2013 |
| Confidentiality | No |
| OPD code | OPD code |
| Approved by | |



Wageningen UR Food & Biobased Research
P.O. Box 17
NL-6700 AA Wageningen
Tel: +31 (0)317 480 084
E-mail: info.fbr@wur.nl
Internet: www.wur.nl

# Content

 

# 1 Introduction

The use of lignocellulosic feedstocks for biofuels has gained much interest the last few decades but also chemical building blocks from renewable resources form a huge potential and in particular lactic acid for the production of the biodegradable plastic polylactic acid (PLA). To date, lactic acid is mainly produced from starch originating from corn and sugarcane, but a sustainable and cost-effective production process at a scale meeting future demands for PLA requires the use of second generation biomass such as lignocellulosics.

Lignocellulosic biomass is mainly composed of cellulose, hemicellulose and lignin. Cellulose and hemicellulose are a potential source of fermentable sugars for lactic acid, while lignin can be converted into solid biofuel or higher added-value products. Obtaining fermentable sugars from lignocellulosic biomass usually requires two steps: first a pretreatment in which the cellulose fraction is isolated by hydrolysis of hemicellulose and/or delignification, followed by the enzymatic hydrolysis of cellulose to sugars. Both pentose and hexose sugars can be used for lactic acid fermentation.

The breakdown of biomass in pretreatment facilitates enzymatic hydrolysis by disrupting cell wall structures, driving lignin into solution or modification of the lignin structure, and reducing cellulose crystallinity and chain length, while preventing hydrolysis of cellulose. Hemicellulose is converted to soluble sugars and acetyl groups in the hemicellulose are liberated as acetic acid [1]. The nature and extent of such changes are highly dependent on the pretreatment chemistry and reaction severity (e.g. residence time, temperature, catalyst loading), but also on the nature of the biomass. Sugar degradation products such as furfural and 5-hydroxymethyl furfural (HMF) can also be formed and can have adverse effects on the fermenting organisms in sufficiently high concentrations. Milder pretreatment prevents the formation of significant amounts of degradation products but is less efficient in breakdown of the lignocellulose, making it less susceptible for enzymatic hydrolysis.

In an ideal situation the pretreatment leads to high yields of fermentable sugars with a limited formation of degradation products that inhibit enzymatic hydrolysis and fermentation to lactic acid, while remaining cost effective. This review deals with these challenges by providing information on available pretreatment technologies in general (chapter 3), and more specific on pretreatment of the model feedstock sugarcane bagasse (chapter 4). Techno economic studies are described in chapter 5 with the NREL study from 2011 as benchmark. This review starts with characteristics of lignocellulosic biomass in relation to compostion and formation of inhibitors.

# 2 Physical-chemical characteristics of lignocellulosic biomass

## 2.1 Composition

Lignocellulosic biomass is mainly composed of cellulose (insoluble fibers of β-1,4-glucan), hemicellulose (non-cellulosic polysaccharides including xylan, arabinan, mannan and glucan), and lignin (a complex polyphenolic structure). Cellulose, hemicellulose and lignin form ~90% of the total dry matter. Lignocellulose also contains lesser amounts of minerals, oils, and other components [2, 3].

The structural and chemical composition of lignocellulosic material has varying amounts of these components because of genetic and environmental influences and their interactions. The proportion of biomass constituents varies between species and there are distinct differences. For example, the total content of cellulose and hemicellulose is higher in hardwoods than in softwoods, but the total content of lignin is higher in softwoods than in hardwoods.

### *2.1.1 Cellulose*

Cellulose, the major component of plant biomass (30–60% of total feedstock dry matter), is a homopolysaccharide composed of β-glucopyranose units, linked by β-(1→4)-glycosidic bonds. The orientation of the linkages and additional hydrogen bonding make the polymer rigid and difficult to break.

Cellulose has a strong physic-chemical interaction with hemicellulose and lignin. Native cellulose has about 10.000 units in the cellulose chain that form fibrils which are stabilized by strong intermolecular hydrogen bonds between hydroxyl groups of the adjacent molecules. Cellulose has crystalline domains separated by less ordered (amorphous) regions, potential points for chemical and enzymatic attack. Cellulose is degraded by acid or cellulases to its monomer glucose, which can be further fermented to enthanol, butanol, lactic acid, succinic acid etc.

### *2.1.2 Hemicellulose*

Hemicellulose (20–40% of total feedstock dry matter) is a short, highly branched heterogeneous polymer consisting of pentose (xylose and arabinose), hexose (galactose, glucose, and mannose), and acid sugars [4]. Mannose is the dominant hemicellulose sugar in softwoods, while xylose is dominant in hardwoods and agricultural residues [5]. Hemicellulose is more readily hydrolyzed compared to cellulose because of its branched and amorphous nature. The pentose and hexose sugars form a loose, very hydrophilic structure acting as a glue between cellulose and lignin.

### *2.1.3 Lignin*

Lignin (15–25% of total feedstock dry matter) is an aromatic polymer synthesized from phenylpropanoid precursors. The phenylpropane units of lignin (primarily syringyl, guaiacyl, and phydroxy phenol) are bonded together by a set of linkages to form a very complex matrix. Lignin is hydrophobic and highly resistant to chemical and biological degradation. It is present in the middle lamella and acts as cement between the plant cells. It is also located in the layers of the

 

cell walls, forming, together with hemicelluloses, an amorphous matrix in which the cellulose fibrils are embedded and protected against biodegradation.
Processing of lignocellulose will make lignin available for conversion into value added products rather than its fuel value, e.g. for use as substitutes for phenol-formaldehyde resins, polyurethane foams, adhesives etc.

## 2.2 Chemical interaction between components

### *2.2.1 Introduction*

Information in this section is adapted from an earlier review on pretreatment of lignocellulosic biomass [6]. 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 Table 1.

Table 1: Overview of linkages between the monomer units that form the individual polymers (lignin, cellulose and hemicellulose) and between the polymers to form lignocellulose [7]

| | |
|---|---|
| **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<br>Hemicellulose lignin |
| Ester bond | Hemicellulose-lignin |
| Hydrogen bond | Cellulose-hemicellulose<br>Hemicellulose-Lignin<br>Cellulose-Lignin |

### *2.2.2 Intrapolymer linkages*

The main types of bonds that connect the building molecules within the lignin polymer are ether bonds and carbon-to-carbon bonds. 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 [8].

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 [9].
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, allowing the formation of a hydrogen bond between two hydroxyl groups of different polymer chains [7].

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 , although this does not appear obvious from the main structure of cellulose.

As already mentioned, hemicellulose consists of polysaccharides other than cellulose. Its structure reveals that ether type of bonds, such as the fructosic and glucosidic bonds,are the main bonds that form the 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 [8].

### *2.2.3 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.

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 [7].

 

### *2.2.4 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 Table 2.

Table 2: 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 Table 2.

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 [8].

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) [8].

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. 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 Figure 1. The cleavage of the ether bond might be enhanced by the addition of hydrosulfide.

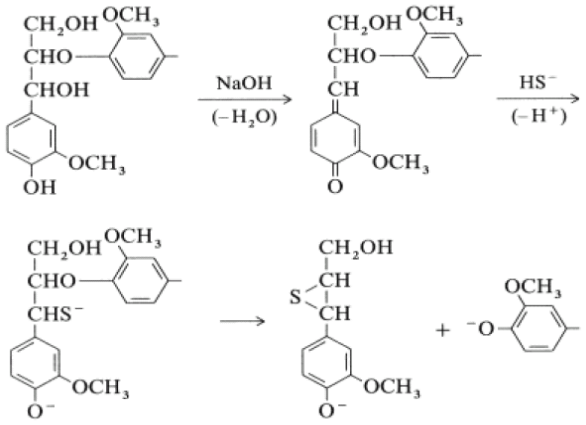


Figure 1: Cleavage of ether bond of lignin in alkaline solution [10]

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 Figure 2. The reaction can happen either homogeneously or heterogeneously. In both cases the reaction is of first order.

 

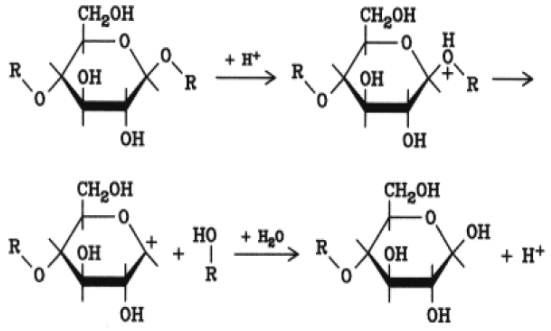

Figure 2: Hydrolysis of cellulose in acidic media [11]

In the case of alkaline media, the mechanism most probably involves the intermediate formation of 1,2-anhydro configuration as shown in Figure 3. 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 [9]. The use of a strong base and a minimum temperature of 150°C are needed for a sufficient reaction rate.

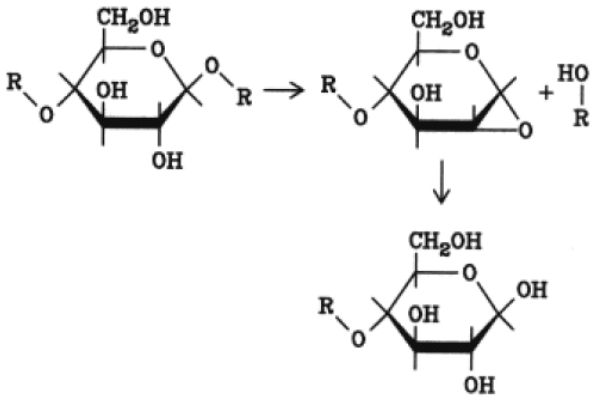

Figure 3: Cellulose hydrolysis in alkaline media [11]

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 [7].

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 [9].

Hydrogen bond

The presence of hydrogen bonds is identified between the cellulose polymer chains. 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 [12].

## 2.3 Inhibiting compounds

### *2.3.1 Introduction*

The pretreatment of lignocellulose does not only acquire fermentable sugars, but a whole range of other compounds [13]. Some of these compounds are known to become inhibiting or even toxic at higher concentrations for micro-organisms during the fermentation to lactic acid, or for the enzymes during cellulose hydrolysis to monomeric sugars. For most compounds however, although inhibition is expected based on their properties and structure, inhibition is not yet proven. Furthermore, the interaction between the different compounds is not well understood. Also, some compounds might only be inhibitory to certain types of micro-organisms. The potential inhibitory compounds can be divided in three different categories, based on their origin, as described in the following sections.

 

### *2.3.2 Compounds present in lignocellulose structure*

The first category consists of molecules which are already present in the lignocellulose structure, but inside the polymeric structure, mainly lignin. During pretreatment, the polymers can be degraded to monomers, thereby being released to substrate. Lignin degradation will result in the release of a range of potential inhibiting aldehyde and phenolic compounds (see [14] for a complete overview of all phenolic and alhehyde compounds). Acids present in the hemicellulose structure, such as acetic acid, glucuronic and galacturonic acid, can also become inhibiting at high concentrations.

### *2.3.3 Degraded compounds*

The second category consists of molecules which are packed as non-inhibiting molecules inside the lignin structure, but are degraded due to the harsh conditions during the pretreatment. The most studied example is pentose and hexose sugar. Under the influence of a high temperature and an acidic environment, pentoses can be degraded to furfural (also known as furan-2-carbaldehyde) , and hexoses to 5-hydroxymethyl furfural (5-HMF) [15]. These compounds can be further degraded to its corresponding alcohol and acid, but also to smaller acids. HMF can degrade to levulinic acid and formic acid, while furfural can degrade to furoic acid and formic acid. These compounds are considered to be less toxic, although further investigation is required.

### *2.3.4 Non-lignocellulosic inhibitory factors*

The last category consists of non-lignocellulose related compounds present in the feedstock. When a feedstock is acquired from a polluted area, nitrogen and sulphuric containing compounds can be found in the feedstock, which can affect the micro-organisms. Furthermore, heavy metals can pose a threat to the fermentation and enzyme hydrolysis. Pesticides used in agriculture can also cause problems when agricultural residues are used. The addition of chemicals during the pretreatment is also able to cause inhibition, mainly due to the addition of osmotic pressure on the cells.

# 3 Pretreatment technology

## 3.1 Introduction

Pretreatment is a crucial process step in the biochemical conversion of lignocellulosic biomass to fermentable sugars and finally to products like e.g. lactic acid. 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 [16]. Pretreatment has been recognised as one of the most expensive processing steps in cellulosic biomass-to-fermentable sugars conversion and several review articles provide a general overview of the field [5, 17-19].

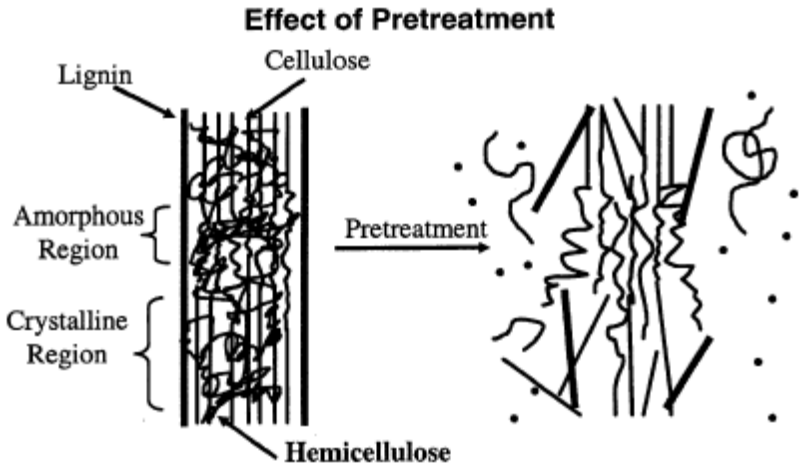


Figure 4: Schematic presentation of effects of pretreatment on lignocellulosic biomass [20]

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 4). 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 goals are actually the most important challenges of current pretreatment technologies. In the following sections the most common pretreatment techniques of biomass are described. Part of this information is adapted from an earlier review on pretreatment of lignocellulosic biomass [6].

 

This chapter is completed with an overview of worldwide industrial activities on pretreatment of lignocellulosic biomass. A table is composed (Table 3), listing companies that produce alcohols, fermentable sugars or other chemical building blocks from 2nd generation biomass. Explanation of the most common pretreatment techniques in the next paragraph is illustrated by a description of industrial activities of these companies.

## 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*

Ultrasonic pretreatment is a well-known technique for treatment of sludge from waste water treatment plants. The method of ultrasonication for lignocellulosic biomass was investigated at laboratory scale by Imai and coworkers [21].The experiments showed the effect of the pretreatment of pure cellulose on its enzymatic hydrolysis using a model compound (Carboxyl Methyl Cellulose, CMC). The experimental results showed that when a suspension of cellulose was provided with energy by irradiation, the reaction rate of the subsequent enzymatic hydrolysis was increased by approximately 200% [21]. 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 was 130 kJ/g CMC, which is significantly higher than the energy of the hydrogen bond 0.12 kJ/g cellulose (21 kJ/mol cellulose [12]).

## 3.3 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 [22-24].

## 3.4 Chemical pretreatment

To this group belong the pretreatments that are initiated by chemical reactions for disruption of the biomass structure.

### *3.4.1 Liquid hot water*

Liquid hot water (LHW) processes are biomass pretreatments with water at high temperature and pressure. Other terms are hydrothermolysis, autohydrolysis, hydrothermal pretreatment, aqueous fractionation, solvolysis or aquasolv [25]. Hot compressed water is in contact 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 is related to the biomass type with high lignin solubilization impeding recovery of hemicellulose sugars [26].

**Inbicon** in Denmark produces ethanol from straw by autohydrolysis on demonstration scale. Advantages of this process include the absence of chemicals and the low water use as they operate at high dry matter content (>30 wt%).

### *3.4.2 Supercritical fluids*

A supercritical fluid is any substance at a temperature and pressure above its critical point where distinct liquid and gas phases not exist. Supercritical fluid behaves like a liquid with the viscosity of a gas: it can diffuse through solids like a gas and dissolves material like a liquid. Carbon dioxide and water are the most commonly used supercritical fluids being used for various food and non-food applications. $CO_2$ becomes supercritical above 31 °C and 73 bar, water above 374 °C and 218 bar [27].

Supercritical $CO_2$

Supercritical carbon dioxide (SC-$CO_2$) is widely used as an extraction solvent for various applications. SC-$CO_2$ extraction is also being considered as possible pretreatment route for lignocellulosic material.

Sahle reported the enhanced permeability of Douglas-fir by SC-$CO_2$ [28]. Kim and Hong [29] 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-$CO_2$ [30, 31], and SC-$CO_2$ was considered not an effective tool for lignocellulosic treatment.

Supercritical water

Supercritical water can potentially be applied for decomposition of biomass. The company **Renmatix** is currently commercialising the Plantrose pretreatment technology [32]. To our knowledge there are no public papers available on the efficiency of the Plantrose technology. Further description of the Plantrose process can be found in the patent literature [33]. In here, methods are disclosed for the continuous treatment of biomass comprising a

 

1. Pretreatment step, where biomass is contacted with a first supercritical, near-critical, or sub-critical fluid (including water, with optional 10% $CO_2$) to form a solid matrix (cellulose and lignin) and a first liquid fraction (C5 sugars)
2. A second hydrolysis step under more severe conditions where the solid matrix formed in the first pretreatment step is contacted with a second supercritical or near-supercritical fluid to produce a second liquid fraction and an insoluble lignin-containing fraction

After both steps a separation is included. According to the claims, the first pretreatment step is carried out at temperatures ranging from 150 to 300 °C and at pressures of 50 to 115 bar, with pretreatment times ranging from 1-5 min. The second pretreatment step, which is carried out after soluble hemicellulose-derived sugars are removed, is carried out at temperatures ranging from 220-320 °C and at pressures ranging from 35 to 85 bars. This second pretreatment is carried out with water containing 1% acid as a catalyst (under these conditions the supercritical state of water is not reached). The relative ease of hydrolysis of the hemicelluloses compared to the recalcitrant cellulose necessitates this two-step process in order to preserve the C5 sugars that would be rapidly destroyed under the more severe conditions necessary for cellulose dissolution.

The benefits of the Plantrose technology, according to Renmatix, include very high throughput capacities (i.e. low residence times), no requirement for enzymes (although oligomer hydrolysis is needed), high conversion efficiencies and low capital costs. Renmatix is not only focussing on producing bioethanol from sugars but on a range of products including furfural and xylitol from xylose, glycolic acid and ethylene glycol from cellulose as well as lignin products.

### *3.4.3 Dilute acid hydrolysis*

Dilute or weak acid hydrolysis is one of the most effective pretreatment methods for lignocellulosic biomass. In general there are two types of dilute 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 wt% substrate concentration)

Acid (sulphuric acid, sulphur dioxide, carbonic acid) is added to the raw material and the mixture is held at elevated T for short period of time. 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 [34]. As an alternative to inorganic acids, organic acids (e.g. maleic acid, fumaric acid) can be used for dilute acid pretreatment [35].

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.

Severity factor

The severity of acid hydrolysis can be described by the severity factor $R_0$, it is equal to the P-factor originally used for isothermal cooking of wood in the Kraft process [36]. The calculation involves the reaction temperature T (°C) and reaction time t (minutes) and is described by the following formula:

$$R_0 = t * e^{\frac{T-100}{14.75}}$$

However, this formula does not include the catalytic effect of applied or released acids. Therefore, several authors [36-38] used an extended severity factor that includes the effect of pH, the so-called combined severity factor $R'_0$. With this factor the severity of the treatment can be expressed under different process conditions:

$$R'_0 = [H^+]R_0 = (10^{-pH})\left(t * e^{\frac{T-100}{14.75}}\right)$$

Concentrations of xylose, furfural, HMF and hydrolysis yield can also be related to the severity factor. This severity factor can be used to predict the results of experiments done under various conditions.

Possible process routes

In case of acid pretreatment of biomass there are a number of different process routes optional. The choice of the final route will be influenced by research results and economic considerations. Figure 5 gives an schematic overview of the possible process routes in which the blue striped lines are the most straight forward route with the lowest costs.

 

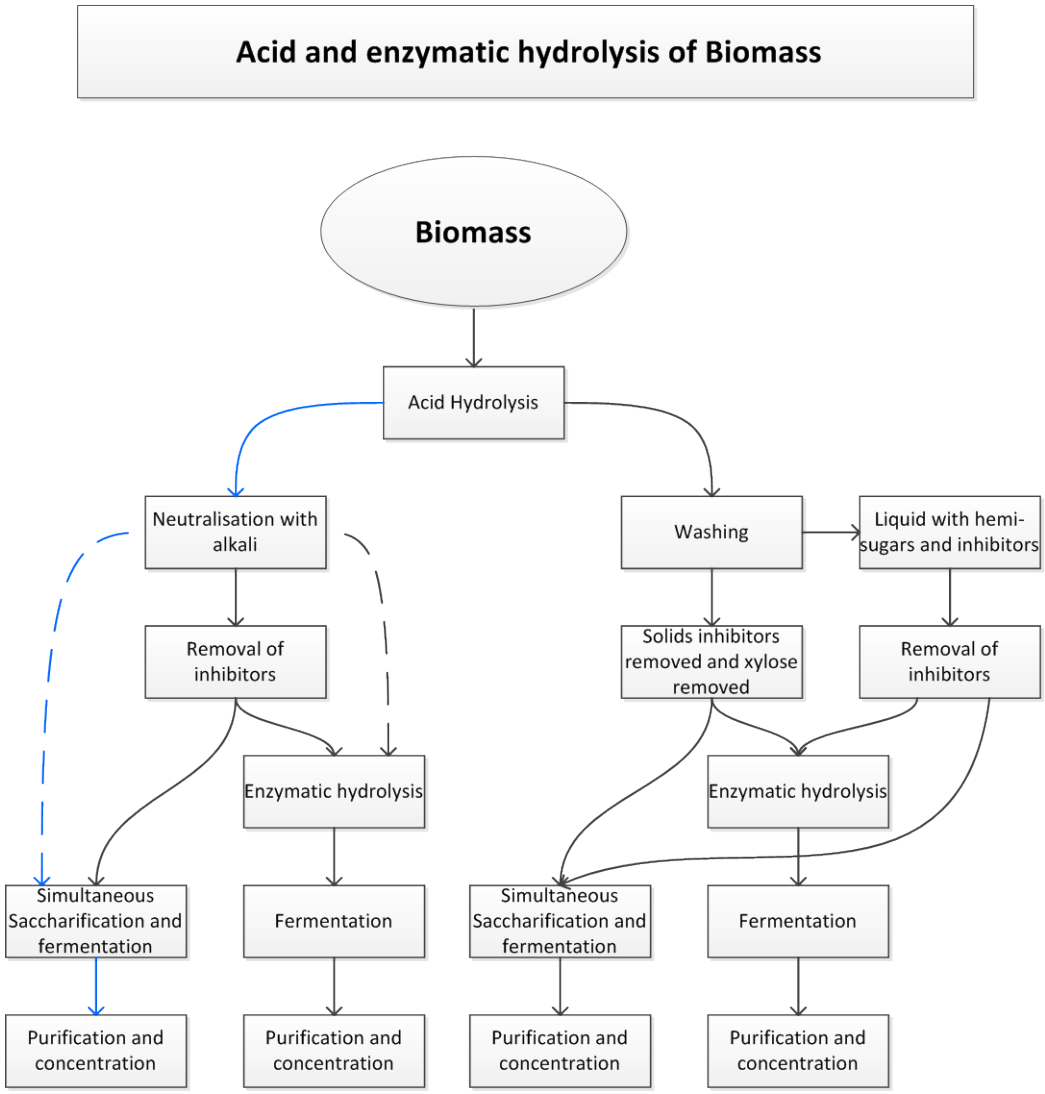

Figure 5: Possible process routes with dilute acid pretreatment

Purification and concentrating must be technically- and economically viable. To avoid the removal of inhibitors, the severity of the acid hydrolysis must be below certain limits (depends on raw material) but this will lead to lower yields for enzymatic hydrolysis and xylose yield in the hydrolysis liquid. High sugar concentrations are important for the economics of the process so hydrolysates with a high concentration of substrate are required, but then product inhibition is unavoidable. Simultaneous saccharification and fermentation will solve this problem of product inhibition, but another possibility is washing of the solids. This does not only remove inhibitors but also other dissolved organic and inorganic components that might interfere with the following process steps and it probably will make purification easier. A washing step can be advantageous, however also necessary nutrients for fermentation might be removed in such amounts that extra supply of these nutrients is needed. Removal of components by washing will lead to an extra liquid stream that has to be processed too.

This raises the question whether inhibitors are easier to remove from the wash stream than from the total hydrolysate and if this side stream can be combined with the main stream again, or the side stream may be used in anaerobic digestion without removal of inhibitors. This illustrates the complexity of the matter and the route to choose will have to be indicated by the constrains given by the final steps of fermentation and purification.

Industrial activities

Several companies use dilute acid as pretreatment method for fractionation of lignocellulosic biomass. **Blue Sugars** in the US has a demonstration plant for the production of ethanol from sugarcane bagasse. They combine dilute acid with mechanical action and co-ferment the C5 and C6-sugars. **Cobalt Technologies,** in cooperation with Rhodia and Andritz, are building a demonstration plant in Brazil for the production of butanol from sugarcane bagasse. They combine dilute acid hydrolysis with ABE-fermentation and claim that enzymatic hydrolysis is not necessary in their process. **POET-DSM** is building at the moment a commercial cellulosic ethanol plant in the US with an expected start-up in 2013. POET is the largest ethanol producer from corn in the US and the new plant will use corn cobs and/or corn stover as biomass for their process. The pretreatment technology is dilute acid or acid catalysed steam explosion followed by enzymatic hydrolysis with enzymes provided by DSM. In Europe the Swedish company **Sekab** is producing ethanol on demonstration scale from softwood, straw and sugarcane bagasse. The lignin fraction is dewatered to 50% dry matter and is used as solid biofuel.

### *3.4.4 Concentrated acid hydrolysis*

Concentrated acids such as $H_2SO_4$ and HCl have been widely used for treating lignocellulosic materials because they are powerful agents for cellulose hydrolysis [23] and no enzymes are needed subsequent to the acid hydrolysis. Advantages of concentrated acid hydrolysis are the flexibility in terms of feedstock choice, low concentration of inhibitors, high monomeric sugar yield as well as mild temperature conditions that are needed (cooling might be needed). Drawbacks of using concentrated acids are corrosive nature of the reaction and the need to recycle acids in order to lower cost. For recovery of acid used it is essential that the biomass has a high dry matter content, otherwise the biomass stream is too much diluted.

To date, several companies are in the process of commercialising strong acid hydrolysis of lignocellulosic biomass. **Blue Fire Renewables** (US) acquired the rights from Arkenol for the production of sugars and ethanol from biomass. They run a production plant in Japan where they treat wood chips with concentrated $H_2SO_4$. The lignin that is obtained as by-product is used as energy source. **Virdia** (formerly known as HCL Cleantech) produces sugars from lignocellulosic biomass by using concentrated HCl. Their CASE™ process is demonstrated at pilot scale at the moment and samples of cellulosic sugars and lignin are being produced for commercial application testing. In Europe the Norwegian company **Weyland** is producing sugars and lignin on pilot scale since 2010. They mainly use wood and agricultural residues as biomass source.

 

### *3.4.5 Alkaline hydrolysis*

The major effect of alkaline pretreatment is the removal of lignin from biomass, thereby improving the reactivity of the remaining polysaccharides, and decrystallisation of cellulose. 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 [39]. Depending on the severity it also removes substantial amounts of hemicellulose. It is reported that the alkaline hydrolysis mechanism is based on saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components such as lignin [23].

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. For alkaline hydrolysis calcium hydroxide, sodium hydroxide or ammonia can be used.

Calcium or sodium hydroxide

By using calcium hydroxide (lime) or sodium hydroxide during the pretreatment salts are formed that may be incorporated in the biomass and need to be removed or recycled [40]. Process conditions are relatively mild but reaction times can be long. These mild conditions prevent recondensation of lignin, resulting in a high lignin solubility, especially for biomass with a low lignin content such as hardwood 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 delignification, especially highly lignified materials [39].

For a more efficient process the alkaline pretreatment can be combined with mechanical action like milling, extrusion or refining. The resulting fractions consist of a soluble fraction (containing lignin, hemicellulose and inorganic components) and a cellulose-enriched solid fraction.
By performing extrusion and alkaline 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, but the use of expensive chemicals remain necessary, and recycling and waste treatment is an important issue.

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, see 3.5), 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 packed with biomass. To prevent flash evaporation the reactor system must be slightly pressurized (e.g. 2.3 MPa) [41]. 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.

On industrial scale only **DuPont Danisco** applies alkaline conditions for their biomass pretreatment. The pilot plant in the US produces ethanol from lignocellulosic biomass (switchgrass, corn cobs, corn stover) by dilute ammonia hydrolysis followed by enzymatic hydrolysis to produce the fermentable sugars. They have plans for production on commercial scale in 2014.

### *3.4.6 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 may occur leading to improved enzymatic digestibility of the cellulose fraction. Common solvents for the process include ethanol, methanol, acetone, ethylene glycol, formic acid and acetic acid. 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 [42]. Possible catalysts include inorganic or organic acids [23].
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 costs 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.
- Easy recovery of solvents by distillation (depending on the solvent)
- 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.

 

Organosolv originates from the pulp and paper industry where it was developed as an alternative for kraft pulping. To date several companies use the organosolv technology for the fractionation of biomass. **Chempolis** in Finland uses a mixture of formic acid and acetic acid in water as pulping liquid. The Formico Biorefinery Technology processes non-wood biomass on demonstration scale. From the cellulose fraction ethanol and paper pulp is obtained, from the hemicellulose fraction ethanol, furfural, acetic acid and formic acid, and the lignin is used to generate power and steam.
Also **CIMV** in France uses formic acid and acetic acid for their organosolv process. The pilot plant is running since 2006 and processes wheat straw into a variety of intermediar products: paper pulp and glucose from cellulose, C5-sugars from hemicellulose, and lignin for the chemical industry (not as fuel).
**Lignol** in Canada uses ethanol as solvent in their Alcell process. Ethanol has a big advantage over other organic solvents as it has a low boiling point and can easily be recovered by distillation. The cellulose and hemicellulose fractions are hydrolysed by enzymes for the production of ethanol and other biochemicals. The high purity lignin is considered as a new class of valuable-added renewable aromatic chemicals. Lignol has a running pilot plant since 2010.

### *3.4.7 Oxidative delignification*

Delignification of lignocellulose can 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. However, oxidation might not be very selective, leading to high chemical use and high processing costs.
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.

<u>Hydrogen peroxide</u>

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%.

<u>Ozonolysis</u>

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.

Wet oxidation

Wet oxidation operates with oxygen or air in combination with water at elevated temperature and pressure [43]. It was presented as an alternative to steam explosion which had become the most widely used pretreatment method [44]. 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) [45]. However, high pressure equipment is expensive and continuous operation is difficult.

Wet oxidation has been successfully applied for the pretreatment of wheat straw and hardwood [46, 47]. 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 [48]. One reported advantage of the wet oxidation process is the lower production of furfural and 5-hydroxymethylfurfural, potential inhibitors in fermentation. About 65% degree of delignification could be achieved with wheat straw [49]. Wet oxidation of wood material has been shown to dissolve mainly the hemicellulose.

**BioGasol** in Denmark combines wet oxidation with steam explosion for the production of ethanol from agricultural residues. The process is called 'wet explosion' and the use of oxygen and pressure release at high temperature (170-200 °C) are combined. All by-products are further converted to energy carriers (e.g. ethanol, hydrogen, methane and solid biofuel). A demonstration plant is running in Denmark since 2011.

### *3.4.8 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, but they share a common characteristic in 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 [50].

As of yet, there is no industrial application employing RTIL. There are indications 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. In addition, RTIL are expensive solvents and research is done on cheaper alternatives.

 

## 3.5 Explosion processes

### *3.5.1 Introduction*

Explosion processes are characterised by high T and P treatment at short residence times (few minutes). At pressure release the biomass undergoes explosive decompression with hemicellulose degradation and lignin matrix disruption as result. Explosion processes are conducted with water (uncatalysed or catalysed by e.g. sulphuric acid), ammonia or $CO_2$.

### *3.5.2 Steam explosion*

Steam explosion 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 steam injection the temperature rises to 160-260 ºC. Subsequently, pressure is suddenly reduced and the biomass undergoes an explosive decompression with.

Results of steam-explosion pretreatment depend on residence time, temperature, particle size and moisture content [23]. Studies have been carried out to try to improve the results of steam explosion by addition of a catalyst such as sulphuric acid [51-53]. Limitations of steam explosion include the formation of degradation products that may inhibit downstream processes [54].

Steam explosion is by far the most applied pretreatment technology by industrial companies.
**Abengoa** (a large ethanol producer from cereals) produces ethanol from wheat straw or corn stover in demonstration plants in Spain and the US by sulphuric acid-catalysed steam explosion. All by-products, including lignin residues, are used for energy applications.
**BetaRenewables** is building a commercial plant in Italy for the production of ethanol from Arundo Donax (giant cane) and wheat straw. The pretreatment applied is uncatalysed steam explosion. Sugars are further converted by SSF to ethanol, and residual lignin is used as energy source. The start-up was planned for 2012 but this will probably become 2013. Their technology is called PROESA and is licensed to other companies.
In the US a demonstration plant is operated by **BP Biofuels** for the production of ethanol. The pretreatment is acid-catalysed steam explosion followed by enzymatic hydrolysis (with enzymes provided by **Verenium**). C5 and C6 sugars are separately fermented to ethanol, and lignin is burned for steam generation.
Another big player is **Iogen** (a biotech company and enzyme producer) in Canada. The demonstration plant is running since 2004 and produces ethanol from wheat straw. The pretreatment is a 'modified' steam explosion process followed by enzymatic hydrolysis. Iogen and Shell had plans for commercial activities on this field but the plans were cancelled. Now Iogen is cooperating with the Raizen Group (producer of sugarcane ethanol) for the development of a cellulosic ethanol plant in Brazil. For this process sugarcane bagasse will be used as biomass source.

### *3.5.3 Steam explosion with $SO_2$*

It is known that impregnation of softwood with $SO_2$ (sulphur dioxide) prior to steam explosion improves the enzymatic saccharification [55]. Steam explosion pretreatment with $SO_2$ is related to a well-known technology known as sulphite pulping (see 3.6.3).

Pretreatment with $SO_2$ is an acid-based process. The pretreatment converts most of the hemicellulose carbohydrates in the feedstock to soluble sugars by hydrolysis reactions similar to dilute acid pretreatment. Most glucan remains in an insoluble form that requires subsequent enzymatic hydrolysis to produce fermentable products. $SO_2$ pretreatment enhances glucose and xylose yields in a way similar to dilute acid pretreatment, but the purchased costs of $SO_2$ is relatively high due to costs of shipping $SO_2$ safely. Onsite production is believed to be more cost effective in large scale (cellulosic ethanol) processes [56].

### *3.5.4 Ammonia fibre explosion (AFEX)*

In the AFEX process, biomass is treated with liquid ammonia at high temperature and pressure. 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 pretreatment [57, 58], although ammonia is easily recovered due to its volatility, but like $SO_2$ shipping of $NH_3$ will be costly due to safety reasons. In a recent conceptual design for AFEX treatment of lignocellulose, the pretreatment is carried out in a series of packed beds that are equipped with steam stripping of ammonia, which allows for recovery of 90% of ammonia [59].

### *3.5.5 $CO_2$-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 ($H_2CO_3$, carbon dioxide in water), thereby improving the hydrolysis rate. Yields of $CO_2$ explosion are in general lower than those obtained with steam or ammonia explosion [23].

Carbonic acid may offer the benefits of an 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. The efficiency of the treatment is highly sensitive to reactor pressure and solids concentration [60]. In case of a bioprocessing plant or a power plant nearby that produces carbon dioxide, carbonic acid may be a viable reagent for promoting hydrolysis without acids [61].

 

## 3.6 Techniques related to the paper and pulp industry

### *3.6.1 Introduction*

Cellulose pulp that is used for papermaking seems to be a good source for producing sugars by enzymatic treatment as the amount of inhibiting lignin is low due to the pulping process. The cell walls of the fibres have improved accessibility for enzymes by the removal of lignin as well as a substantial part of the hemicellulose. The cell wall structure changes from a dense polymeric structure into a more open structure with more and bigger pores giving access to the cellulase. Conventional processes for the production of pulp from wood are based on sulphur containing pulping, followed by (in most cases) chlorine based bleaching. Two processes can be distinguished, the sulphate or kraft process (alkaline) and the sulphite process (acid).

### *3.6.2 Kraft pulping*

The kraft process, invented by C.F. Dahl, is the most applied pulping process. The kraft process made it possible to produce a pulp from wood that gives strong (=kraft in German) paper. It was patented in 1884 and first used commercially in Sweden in 1885.

The cooking liquor (also called *white liquor*) contains the active cooking chemicals sodium hydroxide (NaOH) and sodium sulphide ($Na_2S$, about 30% sulphidity). After cooking at 170 °C during 3-4 hours the fibres and cooking liquid are separated. The very dark cooking liquor called *black liquor* contains around 80% of the lignin, degraded and dissolved. After evaporation and incineration of the black liquor the furnace smelt is dissolved in water to give the *green liquor* which contains sodium carbonate ($Na_2CO_3$) and sodium sulphide. With lime ($Ca(OH)_2$) the green liquor is transformed in *white liquor* for the next cycle.

The sulphide accelerates the delignification and produces sulphated lignin. The use of sodium sulphate ($Na_2SO_4$) as the makeup chemical for the lost sulphide gives the process its alternative name of sulphate process. The makeup sulphate is reduced in the recovery furnace to sodium sulphide. The total process of pulping, incineration and recovery combined with a low yield results in a costly pulp with an average sales price of 700 $/ton. This price varies strongly depending on the market situation. For printing and writing papers the residual lignin is removed by bleaching which will increase the price with 100 $/ton.

Besides removal of the majority of the lignin also about 50% of the hemicellulose and 10% of the cellulose is removed. These components are all dissolved in the black liquor which is burned in the recovery system. In a biorefinery system, lignin and a part of the hemicellulose can be recovered from the black liquor. The lignin from the kraft process is sulphated which narrows the possible applications compared to non-sulphated lignin.

Non-wood biomass generally contains less lignin and requires less severe conditions in the pulping process compared to wood and then the soda process is most common. This process is the precursor of the kraft process and invented in 1854. The active chemical is sodium hydroxide (NaOH) and the makeup chemical for the used chemical is sodium carbonate ($Na_2CO_3$).

The polysaccharides in kraft pulps can be enzymatically converted to monomeric sugars with high yields. Eucalyptus pretreated with the kraft process at 155 and 165 °C and 15 to 20% active alkali during 15-60 minutes showed glucan recoveries of 77% and 90%. The enzymatic hydrolysis of kraft pulps with cellulose presented a rapid glucan conversion rate to glucose with values over 90% [62]. Soda and kraft cooking of Japanese cedar showed good saccharification [63]. With lignin contents of the cooked cedar up to 10%, the yields of sugar to wood were almost constant (40%) whether the pretreatment was soda or kraft cooking. Soda cooking was suitable as a pretreatment method for saccharification of softwood.

In spite of high sugar yields the price per unit of sugars is high due to the expensive kraft process. A more interesting approach is the repurpose of kraft pulp mills that have to shut down due to falling demands in the pulp and paper industry. These mills can be repurposed to ethanol production or other fermentation products. Various pretreatments were studied for such a mill [64] and green liquor (sodium carbonate and sodium sulphide) pretreatment appeared to be the most effective as this ensures chemical recovery using the proven technology of the kraft process. A patent for this green liquor pretreatment has been applied by the N.C. State University. With corn stover as raw material about 70% of the original polysaccharides were converted into fermentable sugars [65], while with hardwood a yield of 77% was reached [66]. These pretreatments are much milder than normally used for kraft pulp needed for paper production.

### *3.6.3 Sulphite process*

Another important pulping process is the sulphite process, which was one of the first chemical pulping methods and already used in the 1860's [67]. It was more common up to the late 1940's, but has declined since then. The first mill was built in Sweden in 1874. In the US no more sulphite pulping mills have been built since the 1960's.
In the sulphite process a mixture of sulphurous acid ($H_2SO_3$) and bisulphite ion ($HSO_3^-$) is used to degrade lignin. Acid sulphite is pulping with an excess of sulphurous acid at pH 1-2 while bisulphite pulping is applied at pH 3-5.
The sulphite process is sensitive to wood species. It works well for softwoods like spruce and fir and several hardwoods, but resinous softwoods and tannin rich hardwoods are difficult to handle, furthermore there is an intolerance to bark [68]. These sensitivities and relative weaker papers compared to kraft papers resulted in a strong decline of the share of sulphite pulp in the total pulp production. In 1961 the ratio of the total world production of sulphite was 37% of that of kraft pulp and in 2011 this had been changed to 4% [69].
Another disadvantage of the process was the lack of an efficient chemical recovery, in the past resulting in discharging of spent liquor and environmental problems. This was often solved by evaporation of the liquid and production of lignosulphonates for use in industrial applications like plasticizers in making concrete and dispersing agent.

 

In the sulphite process the acidity of the cooking liquid results not only in hydrolysis and dissolution of the sulphonated lignin but also in hydrolysis of a substantial part of the hemicelluloses. In case of softwoods primarily hexoses are formed but with hardwood pulping the sugars are mainly pentoses. Already in 1909 the first mill used this pentose-rich spent liquor to produce ethanol. In Sweden there have been 33 mills producing ethanol on this basis but only the mill of Domsjö is still producing ethanol [70].

In Norway only **Borregaard** is still producing pulp and ethanol. The mill has become a biorefinery from which several totally different products leave the mill gates. Borregaard decided already 50 years ago to maximize the output of products from the processed wood resulting in the production of different type of cellulose fibre products, lignosulphonates, bioethanol, vanillin and even bark beetle pheromones.
Borregaard adapted their sulphite pulping process to a patented pretreatment process for biomass conversion into sugars or fermentation products called the BALI process [67, 70]. The patent covers the sulphite treatment and the production of different products from various streams after the sulphite treatment. The claimed advantages of this process are the production of water soluble lignin that can be converted into valuable products, no enzymatic inhibition of enzymes by the residual lignin in the lignocellulose, yields up to 90% of the theoretical amount of fermentable sugars and more than 80% of the biomass to be converted in marketable products. As the remaining lignin does not absorb the enzymes, recycling of enzymes becomes possible, resulting in less process costs. A demonstration plant with a budget of 16.7 million € [70] was built that had its start-up in June 2012. Borregaard expects to have enough results to assess whether to move forward with full-scale production toward the end of 2013 or early 2014 [71].

The sulphite pulping technology is also applied in the patented SPORL process (Sulphite Pretreatment to Overcome Recalcitrance of Lignocellulose) for enzymatic saccharification [72]. The process can be used for woodchips which are reduced in size by disk refining after the treatment. A treatment with 8-10% bisulphite and 1.8–3.7% sulfuric acid on oven dry wood treatment at 180 °C for 30 min resulted in more than 90% cellulose conversion and low inhibitor amounts. In comparison of SPORL with dilute acid (DA) treatment of spruce, the amount of known inhibitors was 65% lower and about 32% of the lignin was dissolved as lignosulfonate. The enzymatic treatment resulted in cellulose to glucose conversion yields of 91% at 24 h for the SPORL substrate and 55% at 48 h for the DA substrate, respectively [73]. In a comparison between SPORL, dilute acid and alkaline processes with switch grass as raw material SPORL showed better enzymatic digestibility hydrolysis than the other two processes. The residual hemicellulose content after the SPORL treatment had more effect on the digestibility than the residual lignin [74].

### *3.6.4 Kraft and sulphite compared*

For the paper pulp producer, the advantages of Kraft over Sulphite pulping are stronger paper, the possibility to use more wood species and a good recovery of chemicals and energy from burning the dissolved organic fraction in the pulping liquor. The acid Sulphite process degrades cellulose more than the Kraft process leading to weaker papers. Neutral and Alkaline Sulphite pulping was mainly used for semi-chemical pulping (still substantial amounts of lignin present). Alkaline Sulphite pulping was used for raw materials that contained high silica contents like rice straw. The strong alkali dissolves the silica which would otherwise cause severe wearing of the paper machine parts. In most cases in the past the pulping liquids were just discarded which of course is not possible nowadays.

In papermaking other demands are set to a process than for a pre-treatment of lignocellulose before enzymatic treatment. As many different types of paper are made the specifications of the pulps can be very different. For wrapping paper a strong paper is required which means good bonding between strong fibres. For writing papers a low lignin content and of course strength is required, which means severe process conditions to ensure low lignin levels. In many cases these pulping processes are more severe than required for pre-treatments sugar productions for which accessibility of the cellulose for enzymes is the main issue. In this case a mild soda (NaOH without Sulfide) or sulphite process can be enough.

Borregaard adapted its sulphite pulping process into a biomass conversion process (BALI process) in which besides sugars also a more valuable lignin than from the Kraft process can be produced. A real biorefinery concept which is now being tested in a demonstration plant.

Detailed production costs of the different pulping processes are not available to us at the moment.[1]

[1] *Within the ISPT pre-project "Bio based feed for bulk chemicals", an attempt is made to estimate costs of various pulping processes*

 

### 3.7 Status and IP worldwide production 2nd generation biofuels and chemicals

Nowadays the interest for second generation biofuels and chemicals is enormous. In the table below industrial activities worldwide are listed. The table is partly based on a report by D. Sanchez from Purac [75] and information found on the internet [76]. This table provides information on the products, raw metarial, process and current status. Also IP is included, but with a focus on pretreatment processes; the number of patents is much larger when topics such as enzymatic hydrolysis, detoxification and fermentation are incorporated. Remarkable is the enormous increase of patent applications during the last few years. From the table some trends can be distinguished, categorized to products, raw material, process and current status:

Products

Majority of the companies aim for biofuels; ethanol is still the largest one but also other alcohols like butanol and isobutanol are gaining more interest. Retrofit of existing ethanol plants to produce other alcohols is taking place.Lignin is in most processes applied as fuel but some companies like Weyland , CIMV, Borregaard and Lignol believe that lignin has a higher added-value.

Raw material

Activities concentrate at areas where large amounts of biomass are available. This is e.g. corn residues in the US, sugarcane bagasse in Brazil and wood in (Northern) Europe. Processing of municipal solid waste (MSW) to valuable products is gaining more interest.

Process

Most of the companies are already mentioned in the previous paragraphs to elucidate the various pretreatment methods. The applied processes can roughly be divided in 'sugar-removing methods' or 'lignin-removing methods':

- In the pulp and paper industry the general principle of the pulping methods is removal of lignin. Examples are kraft, soda, sulphide and organosolv. These processes result in relatively pure polymeric sugar streams with lignin degradation by-products.
- For the acid–related methods like dilute acid, steam explosion or liquid hot water the (mainly hemicellulose) sugars are removed (=dissolved) and lignin and sugar degradation products are formed as by-products.

Techniques like organosolv, sulphide pulping and concentrated acid often produce other (multiple) products like sugars or (high quality) lignin. Concentrated acid is one of the few techniques that does not need enzymatic hydrolysis for the production of monomeric sugars. Often companies are not clear about their pretreatment technology, in that case the patent literature listed in the table may provide some additional information.

Current status

Pretreatment techniques that are being developed at minimal pilot scale are listed below, with steam explosion and dilute acid as most applied techniques for the production of alcohols (main product) and energy:

- Steam explosion (catalysed or uncatalysed) combined with enzymatic hydrolysis
- Dilute acid combined with enzymatic hydrolysis
- Liquid hot water combined with enzymatic hydrolysis
- Organosolv combined with enzymatic hydrolysis
- Sulphide pulping combined with enzymatic hydrolysis
- Concentrated acid
- Mild alkaline ($NH_3$) combined with enzymatic hydrolysis
- Consolidated bioprocessing (singl;e step hydrolysis and fermentation)

Many companies report on the pilot or demonstration scale of their process. A large number of plans are announced (especially for 2013) but it remains to be seen whether these plans will become reality; one has to distinguish between serious plans and hot air. The number of working demonstration plants is still scarce.

### 3.8 Pretreatment for lactic acid production

Lactic acid is gaining more interest as an industrially important product, mainly for the production of poly lactic acid (PLA), a green substitute for petrochemical plastics. Currently, optically pure lactic acid is produced from 1$^{st}$ generation biomass (e.g. corn starch) since the (commercial) use of lignocellulose is still problematic. This chapter has illustrated the industrial developments in the field of 2$^{nd}$ generation biomass conversion to biofuels and building blocks. Biofuels like ethanol will be the first products generated from lignocellulose, but also fermentable sugars as input for fermentative processes are gaining more interest. The next chapter describes the developments of sugarcane bagasse conversion at R&D-level, with emphasis on lactic acid production.

 

Table 3: Worldwide activities on products from second generation biomass

| Company | Location | Products | Status | Raw material | Process | Remarks | IP |
|---|---|---|---|---|---|---|---|
| **Aemetis** | US | EtOH 500 t/a<br>Isoprene<br>Glycerin | Pilot, operational | Switchgrass, grass seed and straw | Ambient Temperature Starch/Cellulose Hydrolysis (ATSCH)<br>Consolidated Bioprocessing (CBP) | CBP from Mascoma<br>Adipic acid (pipeline)<br>Monoterpenes (pipeline) | |
| **Abengoa** | US, Spain | EtOH | Demo, operational since 2008 | Wheat straw | Steam explosion (acid catalysed), EH | Commercial plans? | [77-84] |
| **American Process Inc** | US | Pulp<br>Board products<br>Ethanol | Started up in 2012 | Wood | Modified $SO_2$-proces, EH | GreenPower+®<br>AVAP™ | [85-90] |
| **Andritz** | Austria | Technology | | | | | [91-94] |
| **Beta Renewables**<br>**(Chemtex, Gruppo Mossi & Ghisolfi)** | Italy | EtOH 40.000 t/a | Commercial, planned start up 2012 | Arundo Donax and wheat straw | Steam explosion, EH | PROESA™ process<br>Licensees: GraalBio, Genomatica, Gevo, Colbiocel, Amyris, Codexis | [95, 96] |
| **BioGasol**<br>**[97, 98]** | Denmark | Ethanol (main)<br>Biogas<br>Hydrogen<br>Lignin for fuel | Demo plant operational since 2011<br>Commercial planned in 2013 | Switch grass, corn stover, bagasse | Wet explosion; a combination of steam explosion and wet oxidation, EH | Carbofrac™ pretreatment<br>Pentoferm™( C5 fermentation)<br>Separate xylose and glucose fermentation. | [99-105] |
| **BlueFire Renewables** | US | Ethanol<br>Sugars | Production plant in Japan since 2002<br>Plant in Fulton (US) under construction. | Wood wastes, urban trash, rice and wheat straws and other agricultural residues | Hydrolysis in concentrated $H_2SO_4$ | Arkenol patented process | [106-112] |
| **Blue Sugars (former KL Energy)** | US | EtOH | Demo plant in Upton (WY) since 2009.<br>Up scaling in Brazil in progress | Various types, including bagasse | Dilute acid combined with mechanical treatment, EH, C5-C6-cofermentation | Petrobras as partner | [113] |
| **Borregaard** | Norway | Cellulose pulp<br>Ethanol<br>Lignin,vanillin<br>Chemicals | Commercial since 1930<br>Status BALI pilot? | Spruce wood | Acidic calcium bisulfite (sulfite pulping process) | | [67, 114-116] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **BP Biofuels Verenium** | US | Ethanol | Demonstration plant in Jennings (2009) | Straw, wood, bagasse | Steam explosion, EH | Separate fermentation of C6 and C5 to EtOH Lignin-rich residue burned for steam generation | [117, 118] |
| **Catchlight Energy (Chevron and Weyerhaeuser)** | US | Biofuel | Unknown | Perennials, short rotation trees, crops and residuals | Modified sulphite process, EH | | [119-124] |
| **Chempolis** | Oulu, Finland | Ethanol Biochemicals Paper fibres | Demo, operational since 2009. Processing of 25kt/a of biomass. | non-wood biomasses like straw, bagasse, corn stover and bamboo | Organosolv with formic acid/acetic acid (FormicoBio) | Expanding to China | [125-134] |
| **CIMV** | France | Cellulose (pulp, glucose) Biolignin Sugars (C5) | Pilot since 2006<br><br>Production plant in Marne in progress | Wheat straw, wood, bagasse | Organosolv with formic acid/acetic acid | | [135-138] |
| **Colusa Biomass Energy** | US | Ethanol | ? | Rice straw | Carbonic acid (weak acid) | ? | [139] |
| **Cobalt Technologies & Rhodia & Andritz** | US | Butanol | Demo plant (Brazil) start building 2012 Status? | Sugarcane bagasse | Dilute acid, ABE-fermentation (no EH) | | [140] |
| **Comet Biorefining** | Canada | Sugars | ? | ? | Semi-alkaline steam explosion, EH | | [141, 142] |
| **DuPont Danisco** | US | Ethanol | Pilot since 2009 | Switchgrass, corn cobs/stover | Alkaline (dilute ammonia), EH | Commercial in 2014? | [143-151] |
| **Edeniq** | US | Ethanol | ? | Various | Mechanical (colloid mill) and enzymes, EH | | [152-155] |
| **Ethtec** | Australia | Ethanol | Pilot, status? | Wood, sugarcane bagasse, agr residues | Concentrated acid | Technology licenced from Apace Research | [156-158] |
| **Fiberight** | US | Ethanol and energy | Pilot 2008-2009 Demonstration scale early 2013. | MSW (municipal solid waste) | Mechanical, pulping, EH | Novozymes as partner. Former dry-mill corn ethanol plant to retrofit. | [159, 160] |
| **Genahol** | US | Ethanol | ? | MSW | Conversion technology? | | - |
| **Genesyst** | US | Ethanol | ? | MSW | Gravity pressure vessel | Waste-to-ethanol | See webpage |

 

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | (GPV) for wet-air oxidation. Dilute acid hydrolysis.[161] | plant in Malta planned | |
| **Greenfield Ethanol** | Canada | Ethanol | small-scale pilot facility | Corn cobs | Steam explosion, EH | Also work on thermochemical process (gasification to syngas, conversion to liquid alcohol ) | [162-171] |
| **Helios Scientific**<br>**Helios Sunburst** | US | Sugars | Pilot runs at NREL | ? | ? | | - |
| **ICM** | US | Technology provider | Plans for scale up unclear | switchgrass, sorghum, and corn fiber | ? | | |
| **Inbicon** | Denmark | Ethanol | Demo plant since 2009 | Wheat straw Corn stover, barley and rice straws, bagasse, EFB, garden and household wastes. | Liquid hot water, EH | Plans in US (corn cobs/stover) and Malaysia (Sime Darby, EFB) | [172-180] |
| **Iogen Energy Corp** | Canada | Ethanol | Demo since 2004 | Wheat straw | Modified steam explosion, EH | Iogen and Shell scrap plans commercial plant (April 2012) Iogen and Raizen (Br) for commercial production from bagasse (Oct 2012) | 42 patents! [123, 181] |
| **Lignol** | Canada | Ethanol Lignin | Pilot since 2010 | Lignocellulose (wood) | Organosolv (Alcell, ethanol pulping), EH | Commercial activities? | [182-188] |
| **MetsaBoard**<br>**(former M-real)** | Finland | paper | | | | | - |
| **Mascoma** | US | Ethanol | Demo since 2008 Commercial scale Hardwood CBP Facility planned | Hardwood | CBP (single step hydrolysis/fermentation) | | [189-192] +24 other |
| **Novozymes/ COFCO-Sinopec** | China | Ethanol | Pilot since 2007 Plans for commercial plant (50 MT) in 2013 | Corn cobs, corn stover | Steam explosion? | | [193-197] |
| **POET-DSM Advanced Biofuels** | US | Ethanol | Pilot since 2008? Commercial scale planned for 2013 | Corn-related biomass | Steam explosion/dilute acid?, EH | Project Liberty: | [198-205] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Praj Matrix | India | Ethanol | | | | | - |
| Queensland University of Technology | Australia | Ethanol | Pilot | Sugarcane bagasse | Acid/alkaline/steam explosion, EH | | - |
| REAC Fuel | Sweden | Sugars | ? | Lignocellulose | Thermochemical | | |
| Renmatix | US | sugars | Pilot since 2010? | Lignocellulose (wood, switchgrass, corn cobs/stover) | Plantrose™ process: 2-step hydrolysis (supercritical water) | ? | [206-213] |
| SEKAB | Sweden | Ethanol | Demo since 2004<br>Further plans? | Softwood, straw, bagasse | Dilute acid (200 °C), EH | | [214-216] |
| **Shengquan** | China | | | | | | - |
| Clariant (Sud-Chemie) | Germany | Ethanol | Pilot since 2009 (max 2 ton EtOH/y)<br>Demo planned begin 2012 | ? | Sunliquid® process (pretreatment, EH) | | - |
| Sweetwater Energy | US | Separate C5 and C6 sugars | Pilot<br>Demo planned 2013 | Agricultural residues | Dilute Acid and EH<br>Ensiling?? | | [217] |
| Terrabon | US | Ketones to alcohols to biofuel | Demo since 2009<br>Biorefinery operational in 2013 | MSW, sludge, forest residues | MixAlco® (biorefining technology)<br>Fermentation and several chemical conversion steps | | [218-221] |
| Trenton Fuels Works | US | ? | | MSW | High T acid hydrolysis | Patented and demonstrated process | ?? |
| Tsukishima Kikai | Japan | Ethanol | Pilot since 2001 (in US) | Sugarcane bagasse, various waste material | Hydrolysis<br>Fermentation of C5 and C6 with bacteria and yeast | | [222] and 32 patents in Japanese |
| Virdia<br>**(former HCl CleanTech)** | US | Sugars | | | CASE™ process, concentrated HCl | | [223, 224] |
| Weyland | Norway | Sugars and lignin | Pilot since 2010 | Wood and agricultural waste | Hydrolysis with concentrated acid | | [225-227] |

 

# 4 Pretreatment of sugarcane bagasse

## 4.1 Introduction

Sugarcane bagasse (SCB) is the fibrous residue obtained after extraction the juice from sugarcane in the sugar production process. It is one of the major lignocellulosic materials found in great quantities in tropical countries. It is produced in large quantities by the sugar and alcohol industries in Brazil, India, Cuba, China, Mexico, Indonesia and Colombia.
Worldwide production of sugarcane is 540 Mtons per year (dry biomass), and 1 ton of sugarcane generates 280 kg of bagasse. This makes the annual production of SCB around 150 Mton. About 50% of this residue is used in distillery plants as a source of energy, the remainder is stockpiled (75 Mton). There is great interest in developing methods for the biological production of fuel and chemicals that offer economic, environmental and strategic advantages [228]. This by-product of the sugar industry has the advantage over other agricultural residues like straw of already been transported to the mill site and free of additional logistic costs.

## 4.2 Structure, composition and morphology of sugarcane bagasse

### *4.2.1 Chemical composition*

SCB is primarily composed of lignin (20-25%), cellulose (40–45%) and hemicelluloses (25-30%). The average chemical composition of SCB found in the reviewed articles described in this report is presented in Table 4. Not always every component is given in these articles so the different averages are based on different numbers of analysis. In the second part of the table the results of a study by Rocha [229] are given.

Table 4: Chemical composition of SCB

| | Cellulose | Hemicellulose | Lignin | Extractives | Ash |
|---|---|---|---|---|---|
| | | *This review* | | | |
| Wt% DM | 42 ± 6 | 28 ± 5 | 20 ± 5 | 7 ± 4 | 3 ± 1 |
| n | 10 | 10 | 14 | 12 | 13 |
| | | *Rocha [229]* | | | |
| Wt% DM | 43 ± 1 | 25 ± 2 | 23 ± 1 | 4 ± 2 | 3 ± 1 |
| n | 20 | 20 | 20 | 20 | 20 |

Rocha studied the steam explosion process and the variety of the chemical composition in sugar cane bagasse. In that study, the analytical results of 20 samples of most diverse varieties and origins of natural sugarcane bagasse considering planting soils, planting periods and weather show no significant chemical differences. The standard deviations of Rocha's analysis were much lower than the standard deviations in this literature study. In Rocha's study all the samples were analysed in the same laboratory, which excludes differences introduced by differences in laboratory procedures. Table 4 shows that the average composition of the reviewed articles is very close to the values found by Rocha, except for the extractives where the averages have a relative high difference. In some of the reviewed articles the chemical composition of cellulose

and hemicellulose is presented as their individual polysaccharides (Table 5). These data correspond well with the data in Table 4.

Table 5: Polysaccharides, lignin and acetyl groups in SCB (references in this review)

| | Arabinan | Galactan | Glucan | Xylan | Mannan | AIL | ASL | Acetyl |
|---|---|---|---|---|---|---|---|---|
| Wt% DM | 2.1 ± 0.6 | 1 ± 1 | 41 ± 3 | 24 ± 3 | 0.3 ± 0.0 | 21 ± 2 | 2.9 ± 0.0 | 3.0 ±0.6 |
| n | 12 | 3 | 15 | 15 | 2 | 6 | 2 | 2 |

#### *4.2.2 Structure and morphology*

Work on structure and surface characterization of SCB has not been done extensively. A study by Cardona showed that milled SCB-fibres had smooth surface layers and characteristic elongations with lengths over 200 μm; XRD analysis showed that crust and marrow bagasse exhibit different structures and crystallinity [228]. Most of the developments in SCB transformation to sugars and ethanol have the common scientific basis with other lignocellulosic materials because there are not considerable qualitative differences in composition and structure. Similarly to other plant cell walls, SCB is mainly formed by two carbohydrate fractions (cellulose and hemicellulose) embedded in a lignin matrix.

### 4.3 Pretreatment for lactic acid production

In a review by Abdel-Rahman [2] 'conventional' processes for producing lactic acid from lignocellulosic materials with lactic acid bacteria are described. The hydrolysate of a lignocellulosic biomass is a mixture of hexoses and pentoses; lignin cannot be used for lactic acid fermentation. In order to achieve maximum lactic acid yield and productivity, a large number of studies have investigated lactic acid fermentation by lactic acid bacteria (LAB) from lignocellulosic biomass in the field of microbial technology (see also Table 6). Main problems encountered in the efficient conversion of lignocellulosic biomass to lactic acid were:

- Resistant nature of biomass (e.g. resistance towards hydrolysis by crystalline structure of cellulose and lignin acting as physical barrier)
- High costs of enzymes and inhibition by hydrolysed sugars
- Formation of by-products due to fermentation of pentose sugars (co-production of acetic acid)
- Carbon catabolite repression caused by the heterogeneity of hydrolysate-sugar composition (*i.e.* sequential utilization of mixed sugars in fermentation). For maximum product yield all sugars from lignocellulose must be utilized.

 

Table 6: Lactic acid production from lignocellulosic biomass materials and lignocellulose-derived sugars by lactic acid bacteria [2]

| Microorganism | Substrate | Ferment. process | $C_{LA}$[a] (g/L) | $Y_{LA}$[b] (g/g) | $P_{LA}$[c] ($gL^{-1}h^{-1}$) | Reference |
|---|---|---|---|---|---|---|
| *E. mundtii* QU 25 | Cellobiose | Batch | 119 | 0.83 | 1.12 | Abdel-Rahman et al. (2011a) |
| | Xylose | Batch | 86.7 | 0.84 | 0.90 | Abdel-Rahman et al. (2010a, 2011b) |
| | Glucose/cellobiose | Batch | 35.1 | 0.91 | 2.99 | Abdel-Rahman et al. (2010b, 2011a) |
| | Glucose/xylose | Batch | – | 0.83 | 3.6[d] | Abdel-Rahman et al. (2010b) |
| | Glucose/xylose/cellobiose | Batch | – | 0.79 | 2.6[d] | Abdel-Rahman et al. (2010b) |
| *E. faecalis* RKY1 | Wood hydrolysate | Batch | 93.0 | 0.93 | 1.7 | Wee et al. (2004) |
| *E. casseliflavus* and *Lb. casei* | Xylose and glucose | Batch | 95.0 | – | – | Taniguchi et al., 2004 |
| *Lb. bifermentans* DSM 20003 | Wheat bran hydrolysate | Batch with cell immobilization | 62.8 | 0.83 | 1.17 | Givry et al. (2008) |
| *Lb. brevis* | Corncob | Batch | 39.1 | 0.70 | 0.81 | Guo et al. (2010) |
| *Lb. brevis* and *Lb. pentosus* | Wheat straw hemicellulose | Batch | 7.1 | 0.95 | – | Garde et al. (2002) |
| *Lb. casei* NCIMB 3254 | Cassava bagasse | Batch SSF | 83.8 | 0.96 | 1.40 | John et al. (2006a) |
| *Lb. casei* subsp *rhamnosus* | Soft wood | Batch | 21.1–23.75 | 0.74–0.83 | 0.15–0.23 | Iyer et al. (2000) |
| *Lb. coryniformis* ATCC 25600 | Cellulose | SSF | 54.0 | 0.89 | 0.5 | Yanez et al. (2003) |
| *Lb. coryniformis* spp. *torquens* ATCC 25600 | Pretreated cardboard | Batch SSF | 23.4 | 0.56 | 0.48 | Yanez et al. (2005) |
| *Lb. delbrueckii* | Alfalfa fibers | SSF | 35.4 | 0.35 | 0.75 | Sreenath et al. (2001) |
| *Lb. delbrueckii* NRRL-B445 | Cellulose | SSF | 65.0 | 0.18 | – | Iyer and Lee (1999a,b) |
| *Lb. delbrueckii* IFO 3202 | Defatted rice bran | SSF | 28.0 | 0.28 | 0.77 | Tanaka et al. (2006) |
| *Lb. delbrueckii* mutant Uc-3 | Cellobiose | Batch | 90.0 | 0.90 | 2.25 | Adsul et al. (2007b) |
| | Molasses | Batch | 166 | 0.95 | 4.15 | Dumbrepatil et al. (2008) |
| *Lb. delbrueckii* NCIM 2025 | Cassava bagasse | Batch SSF | 81.9 | 0.94 | 1.36 | John et al. (2006a) |
| *Lb. delbrueckii* subsp. *delbrueckii* Mutant Uc-3 | Sugar cane bagasse | Batch SSF | 67.0 | 0.83 | 0.93 | Adsul et al. (2007a) |
| *Lb. delbrueckii* UFV H2B20 | Brewer's spent grain | Batch | 35.5 | 0.99 | 0.59 | Mussatto et al. (2008) |
| *Lb. delbrueckii* ZU-S2 | Corn cob residue | Batch/continuous | 48.7/44.2 | 0.95/0.92 | 1.01/5.7 | Shen and Xia (2006) |
| *Lb. casei* and *Lb. lactis* | Date juice | Batch | 60.3 | – | 3.2[d] | Nancib et al., 2009 |
| *Lb. lactis* RM 2-24 | Cellobiose | Batch | 80.0 | 0.8 | 1.66 | Singhvi et al. (2010) |
| | α-Cellulose | SSF | 73.0 | 0.73 | 1.52 | Singhvi et al. (2010) |
| *Lb. pentosus* | Vine shoots | Batch | 24.0 | 0.76 | 0.51 | Moldes et al. (2006) |
| | Barley bran husks hydrolysates | Batch | 33.0 | 0.57 | 0.60 | Moldes et al. (2006) |
| | Corncob | Batch | 26.0 | 0.53 | 0.34 | Moldes et al. (2006) |
| *Lb. pentosus* ATCC 8041 | Vine-trimming wastes | Batch | | 0.77 | 0.84 | Bustos et al. (2004) |
| | Corn stover | Fed-batch SSF | 74.8 | 0.65 | – | Zhu et al. (2007) |
| *Lb. planlarum* | Alfalfa fibers | SSF | 46.4 | 0.46 | 0.64 | Sreenath et al. (2001) |
| *Lb. plantarum* (Recombinant) | β-Glucan/cellopentaose/cellohexaose | Batch | 1.47/ | – | – | Okano et al. (2010a) |
| | | | 1.27/ | – | – | |
| | | | 1.27 | – | – | |
| *Lb. plantarum* (Recombinant) | Arabinose | Batch | 38.6 | 0.82 | 3.78[d] | Okano et al. (2009a) |
| *Lb. plantarum* (Recombinant) | Xylose | Batch | 41.2 | 0.89 | 1.6[d] | Okano et al. (2009b) |
| *Lb. rhamnosus* and *Lb. brevis* | Corn stover | SSF | 20.95 | 0.70 | 0.58 | Cui et al. (2011) |
| *Lb. rhamnosus* ATCC 7469 | Paper sludge | Batch SSF | 73.0 | 0.97 | 2.9 | Marques et al. (2008) |
| *Lb. rhamnosus* ATCC 9595 (CECT288) | Apple pomace | Batch | 32.5 | 0.88 | 5.41 | Gullon et al. (2008) |
| | Cellulosic biosludge | SSF | 39.4 | 0.36 | 0.82 | Romani et al. (2008) |
| *Lactobacillus* sp. RKY2 | Rice and wheat bran | Batch | 129 | 0.95 | 2.9 | Yun et al. (2004) |
| | Lignocellulosic hydrolysates | Continuous with cell-recycle | 27.0 | 0.9 | 6.7 | Wee and Ryu (2009) |
| *Lc. lactis* IO-1 | Xylose | Batch | 33.26 | 0.68 | – | Tanaka et al. (2002) |
| | Sugar cane baggase | Batch | 10.9 | 0.36 | 0.17 | Laopaiboon et al. (2010) |
| *Leuconostoc lactis* SHO-47 and SHO-54 | Hydrolyzed xylan (Xylooligosaccharides) | Batch | 2.3 | – | – | Ohara et al. (2006) |

*E*, *Enterococcus*; *Lb*, *Lactobacillus*; *Lc*, *Lactococcus*; SSF, simultaneous saccharification and fermentation.

[a] Lactic acid concentration (g/L).

[b] Yield of lactic acid produced (g) to substrate consumed (g).

[c] Lactic acid productivity.

[d] Maximum volumetric productivity.

Abdel-Rahman *et al.* described many studies and the findings of lactic acid fermentation by LAB from lignocellulosic materials and also compared the features of LAB with other microorganisms. They concluded that industrial lactic acid production from lignocellulosic materials has not been sufficiently profitable. One of the reasons for this was the high cost of hydrolytic enzymes for the saccharification of cellulose and hemicellulose. To address this problem, attempts to isolate LAB that can ferment cellulose or xylan directly to lactic acid, and the development of genetically modified LAB that have hydrolytic enzymes with high activity should be continued. Designed biomass studies using those LAB would facilitate the industrial production of lactic acid.

## 4.4 Dilute acid hydrolysis

### *4.4.1 Overview*

In most dilute acid treatments sulphuric acid is chosen as catalyst, in some cases hydrochloric acid or phosphoric acid is used. $H_2SO_4$ concentrations ranged from 0.06 to 10% (w/v or w/w) and liquid/solid ratios (L/S) were mostly 10 or higher. Some studies used steam explosion combined with $SO_2$ treatment, and treatment temperatures ranged from 110-200 °C. In Table 7 all the results are summarized, and more detailed information is given in Appendix 1.

### *4.4.2 C5-sugars in hydrolysates*

In a substantial part of the literature the authors aimed at high xylose yields in the hydrolysates of acid-treated SCB. Yields on total sugars varied between 29 and 37 wt% of dry SCB and maximum xylose yields varied between 74 and 86%. Sugar concentrations in the hydrolysates ranged from 17 to 47 g/l depending on the L/S ratio.

Vargas Betancur was able to reach a concentration of 82 g/l of xylose with an extremely low L/S ratio of 1.7 [230]. This means that there is no free liquid and al the dissolved sugars have to be extracted from the material by washing. This will take diffusion time and results in lower concentrations again. The result of working with such a low L/S ratio is not only high sugar concentrations but also high inhibitor concentrations. Furfural concentrations went up to 1.3 to 2% and probably about 1g/l will inhibit fermentation strongly.

Cheng tried to increase the sugar concentration by recycling the liquid phase for next batches [231]. By doing so the concentration of total sugars was enriched from 28 g/l in the first cycle to 64 g/l in the third cycle. However, also furfural was enriched to 2 g/l. This was not only caused by recycling the liquid and with that concentrating dissolved components, but also by exposing the xylose longer to higher temperatures (three times versus once).
At higher severity of the acid treatment, xylose yields will drop due to degradation of xylose into products that can inhibit subsequent fermentation. Detoxification of these products appeared best with ion exchange [232]. In milder treatments post hydrolysis can increase the xylose concentration with the risk of increasing inhibitors.

 

Table 7: Overview dilute acid pre-treatment of sugar cane bagasse

| Ref | Product | Particle size | Chemicals and L/S ratio | T (°C) | Time | Results (Yield/EH/fermentation) |
|---|---|---|---|---|---|---|
| [230] | Xylose | | $H_2SO_4$ 0.5-1.75% w/v L/S=1.7-3.3 | 121 | 27-93 min | Max xylose yield 74% (68 g/l) and furfural (2 g/l). Maximal concentration 82 g/l at 60% yield and furfural 1 .3 g/l. |
| [231] | EtOH | 0.45-0.9 mm | $H_2SO_4$, 1.25 w/w% (11.25% w/w SCB) L/S=9 | 121 | 2h | Ethanol from hydrolysate. Detox by boiling and electrodialysis. Three cycles of acidic treatments increased reducing sugar from 28 to 64 g/l. and 52% of xylose yield. Furfural 2 g/l, acetic acid 8.4 g/l. 88% recovery of $H_2SO_4$ with ED. |
| [232] | EtOH | 2-10 mm | HCl (0.5-3.5 %v/v) L/S=10 | 140 | 30 min | Max yield at 2.5% HCl, 30 g/l total sugars, 22 g/l xylose. Furans 1.9 g/l and phenolics 2.8 g/l. Reduction by treatment with anion exchange resin for furans 63% and phenolics 76%. Final result 0.48 g EtOH/g sugar |
| [233] | EtOH | 0.15-1.68 mm | HCl, 0.6 % w/v L/S 10 to 30 | 121 | 4h | Yield reducing sugars 37 wt% for depithed bagasse and 35 wt% for pith bagasse (based on dry SCB). In acid hydrolysate, no enzyme treatment. Xylose 35 g/l and glucose 10 g/l |
| [234] | Xylitol | | $H_2SO_4$ 0.5% w/v L/S=10 | 121 | 10 min | Xylan release 48% into monomers at 18 g/l; post hydrolysis increase to 24 g/l. Furfural and HMF 0.08 g/l, acetic acid 1.5 g/l and phenolics 3.8 g/l. |
| [235] | Xylose | 0.4-0.6 mm | $H_2SO_4$ 0.06-0.34% w/v L/S=10 | 170-200 | 8-22 min | Max xylose yield 79 and 76% at 170 and 200 °C. Optimum at 170 °C , 0.24% H2SO4 and 15 min. |
| [236] | Xylose | <0.5 mm | $H_2SO_4$ 2-6% w/v L/S=10 | 100-128 | 6-300 min | Optimum 2% $H_2SO_4$ and 122 °C during 24 minutes: 90% hemicellulose hydrolysis: xylose 22 g/l, glucose 3 g/l and furfural 0.5 g/l. |
| [237] | Xylose | | $H_2SO_4$ 10% w/w | 121-130 | | Max xylose yield 83% with 2 g/l furfural.. At H-factor 5.45 (see chapter 5.3.2 ) a xylose yield of 74% at 19 g/l xylose and 23 g/l total sugars. Low furfural 0.08 g/l, HMF 0.007 g/l and phenolic compounds 0.3 g/l |
| [238] | Hydrogen | 0.5 mm | $H_2SO_4$, 0.25-7 v/v%. L/S=15 | 121 | 15-240 min | Rind removed. Optimal conditions 0.5% $H_2SO_4$ for 60 min, yielding 25 g/l total sugar in hemicellulose hydrolysate (glucose 11 g/l; xylose 11 g/l; arabinose 2 g/l; acetic acid 2.5 g/l, furfural 0.12 g/l). |
| [239] | Single cell protein | <0.75 mm | $H_2SO_4$, 7% w/w L/S=5 | 125 | 120 | Xylose 47 g/l, total sugars 58 g/l, furfural 0.8 g/l and acetic acid 11 g/l |
| [240] | Sugars | 20 mesh | $H_2SO_4$ (0-3%) w/v) L/S ratio 6.7 | 113-158 | 5-35 min | EH 24h max. cellulose saccharification 34% at 20 min. 135 °C and 3% at EH 72 h max. is 45% cellulose saccharification. Order of effect T>conc>t |
| [241] | EtOH | <1.5 mm | $H_2SO_4$ 2% v/v L/S ratio 9 | 134 | 60 min | 72% sugar recovery after acid and enzymatic treatment at 48 g/l. Ethanol yield in next step 84% of theoretical maximum. |
| [242] | EtOH | < 2mm | $H_2SO_4$ 2% v/v L/S ratio 10 | 122 | 20-60 | 60 min xylan conversion 81% at 19 g/l (total sugars 26 g/l). Enzymatic treatment ->convertibility 66%, total conversion 40% of cellulose in raw bagasse in enzymatic hydrolysate |
| [243] | sugars | | $H_2SO_4$ 1.7% w/w | 150 | 30 min | Effect of enzyme load and Tween, effect solid load not significant –same conversion level but higher conc. Max cellulose conv 65%, sugar conc 27 g/l |

| Ref | Product | Particle size | Chemicals and L/S ratio | T (°C) | Time | Results (Yield/EH/fermentation) |
|---|---|---|---|---|---|---|
| [244] | EtOH | 16-60 mesh | 1% $H_2SO_4$ + 1% HAc w/v | 190 | 10 min | Hemicellulose dissolution >90% and glucan dissolution 16%.<br>76% cellulose conversion after enzymatic hydrolysis |
| [245] | Treated solids | 40 mesh | $H_2SO_4$ 0.2M (= 39% w/w SCB) L/S=20 | 130-190 | 5-10 min | At 190 °C strong degradation of hemi-cellulose, strong degradation in structure and crystallinity-decrease in particle size. |
| [246] | EtOH | | $H_2SO_4$ 0-0.8M | Probably room | 24h | 0.8 M most effective and ethanol production was 2.8 times as high than with alkaline peroxide presented in the same article |

 

High xylose concentrations require low L/S ratios. However, for high hydrolysis yields the acid concentration is the most important factor and high L/S ratios are needed to get high yields. An overview of the effect of different process parameters by Vargas Betancur is presented in Table 8 [230]. In contrary with traditional models in which yield is independent of solid concentration also Jacobsen found higher yields at lower solid concentrations [247]. Canilha describes dilute acid treatments with sulphuric acid and found within the tested variable window the following order of effect: temperature> acid concentration > residence time [240].

Table 8: Effect of different process parameters [230]

| **Response variables for the hydrolysis process** | **Optimization criteria** | **Appropriate level** | | |
|---|---|---|---|---|
| | | Acid concentration | L/S ratio | Time of exposure |
| Xylose concentration | Maximize | ↑↑ | ↓↓ | ● |
| Hydrolysis yield | Maximize | ↑↑ | ↑↑ | ● |
| Acetic acid concentration | Minimize | ↓↓ | ↑↑ | ↓↓ |
| Furfural concentration | Minimize | ↓↓ | ↑↑ | ↓↓ |
| Hydroxymethylfurfural concentration | Minimize | ↓↓ | ↑↑ | ↓↓ |
| Phenolic compounds concentration | Minimize | ● | ↑↑ | ● |
| ↑↑ High levels, ↓↓ Low levels, ●No statistical significance | | | | |

In enzymatic hydrolysis of acid pretreated SCB, Martin found an overall cellulose conversion of only 40% based on cellulose in SCB [242]. Other authors found cellulose conversion of pre-treated SCB between 45% and 76% based on the cellulose in the pre-treated SCB. The surfactant Tween 20 was found to increase the sugar yield in the enzymatic hydrolysis. Herbaceous crops or by-products show much higher sugar yields than woody biomass. SCB and wheat straw gave the highest yields in the experiments of Jeon [241]. Martin found that SCB had better sugar yields than rice hulls, peanut shells and cassava stalks.

## 4.5 Steam (explosion) and liquid hot water

### *4.5.1 Overview*

Often steam treatment is a dilute acid assisted hydrolysis and $SO_2$ is frequently used as a catalyst. In this paragraph literature is described of SCB-pretreatment with steam or steam explosion, including liquid hot water (LHW). The data is summarized in Table 9 and more detailed information is given in Appendix 1.

Non-catalysed hydrolysis at 200 $^{o}$C resulted in xylose yields in the hydrolysate of 75 to 80 % [247]. These yields were equal or higher compared to dilute acid pretreatment. So when the aim is to produce high xylose yields in the hydrolysate then the use of acid is not necessary. However, Jacobsen did not apply enzymatic hydrolysis on the solid residue and the effect on cellulose conversion of the pretreatment is not known. Enzymatic hydrolysis of this type of pretreatment was investigated by Silva who found highest cellulose conversion after an extra alkaline step,

resulting in a very high cellulose conversion [248]. However, overall glucose yield was only 49% due to losses in the preceding steps.

Pereira used non-catalysed steam and obtained cellulose conversion of 45% at a glucose concentration of 22 g/l [249]. Laser found better results for LHW compared to steam with an Simultaneous Saccharification and Fermentation (SSF) yield of 83% [250]. Soares followed another route and washed steam pretreated SCB with an alkaline solution and found 37% of glucose on dry SCB after enzymatic hydrolysis. Milling to 6 or 20 mesh did not have a significant effect [251].

Carrasco applied $SO_2$-catalysed steam and this resulted in high polysaccharide conversions of 92% for cellulose and 82% for xylan (mainly in the acid hydrolysate). Due the end-product inhibition these high conversions were only reached at high L/S ratios, maximum yield was 12 g/l of total sugars [252]. With $SO_2$-impregnated steam exploded SCB Ewanick also reached over 90% of cellulose conversion and subsequent SSF showed over 80% of the theoretical ethanol yield. The sugar concentration after enzymatic hydrolysis was 23 g/l, mainly glucose [253]. Martin found a glucose yield of 35% on dry SCB after enzymatic hydrolysis of $SO_2$-impregnated and steam exploded SCB, corresponding to over 80% of the maximum theoretical yield, and a total sugar concentration of 25 g/l. However, non-catalysed steam explosion yielded only 8% less glucose compared to catalysed steam explosion. This yield loss is probably more than compensated by the cost reduction of omitting $SO_2$ and the additional safety measurements [254]. In another non-catalysed steam explosion experiment Martin found a cellulose conversion yield of 40% at a concentration of 4 g/l.

Geddes used phosphoric acid catalysed steam explosion on SCB. This treatment was followed by an initial liquefaction step before fermentation with a hydrolysate resistant *Eschrichia coli* strain. Without liquid/solid separation an ethanol yield of 207 kg/ton SCB was reached, corresponding to 57% of the maximum theoretical ethanol yield [255].

Rocha found an overall cellulose recovery of 69% after steam explosion followed by an alkaline treatment at 100 °C. The average SCB composition after treatment was 87% cellulose, 4% hemicellulose and 6% lignin [229].
Dias carried out a simulation of integration of first and second-generation bioethanol production. The second-generation part included different pretreatment methods of surplus bagasse and 50% recovery of trash from the field. An economic risk analysis showed the best results for steam explosion treatment in combination with enzymatic hydrolysis at high solids loading for 24-48 hours [256].

 

Table 9: Overview pretreatment of sugar cane bagasse with steam or LHW

| Ref | Product | Pretreatment | Particle size | Chemicals | T (°C) | Time | Results (Yield/EH/fermentation) |
|---|---|---|---|---|---|---|---|
| [229] | Cellulosic pulp | Steam explosion + alkaline delignification | 16-60 mesh | 1 % NaOH w/v (delignification) L/S =10 | 190 °C and 100 °C (NaOH) | 15 min 1 hour (NaOH) | After the two treatments pulps haven an average cellulose content of 87 %. |
| [247] | | Non-catalysed hydrolysis | 1 mm | None L/S = 9-199 | 200 | 1-20 min | Max xylose yields at 10 min. Yields are between 75 and 80% of theoretical max except for L/S ratio 199 with a yield of 86%. Xylose oligomer fraction was about 90%. |
| [248] | EtOH | Hydrothermal (Non-catalysed hydrolysis) | | None L/S ratio 10 | 185-195 | 10 min | Highest ethanol yields reached after subsequent alkaline delignification of thermal treated SCB at 195 °C. Overall glucose yield 49% of theoretical max. |
| [249] | Sugars | steam | < 2mm | None | 200 | 7 min | 45% cellulose conversion at 22 g/l |
| [250] | EtOH | Liquid hot water or steam | +14 mesh | None | 170-230 | 1-46 min | Strong inhibition at 0.15 g/l furfural or higher. LHW results in higher xylan recovery. Average of 4 best LHW runs→SSF conversion of 83% at 20 g/l cellulose. Xylan recovery 84%. |
| [252] | EtOH | Washed and $SO_2$ steam | As such from mill | 6% w/w | 180-205 | 5-10 min | Best condition 190 °C for 5 minutes: EH 72h conversion glucan 92%, xylan 82%, overall 87% at a total sugar concentration of about 12 g/l. At EH 24h about 80% of these yields |
| [253] | EtOH | $SO_2$ soaking steam explosion | 2.5-5 mm | $SO_2$ 3% w/w SCB | 205 | 10 min. | Cellulose recovery after steam explosion 84-88%. Cellulose conversion of residue over 90% at about 23 g/l of sugars (22 g/l glucose). SSF showed over 80% of theoretical ethanol yield. |
| [254] | EtOH | Steam explosion | 2.2-10 mm | None, $SO_2$ , $H_2SO_4$ 1% w/dry SCB | 205 | 10 min | $H_2SO_4$ already dissolves glucose and produces too much inhibitors for fermentation. $SO_2$ and not-impregnated SCB gave 53% and 47 % sugar on dry bagasse after enzymatic hydrolysis at a sugar concentration of 25 g/l. |
| [255] | EtOH | Dilute acid + steam explosion | | Phosphoric acid 1% w/w SCB | 160-190 | 10 min | At 180 and 190 °C recovered sugars in pretreated bagasse slurry is 70% of the bagasse dry weight and about 98% of total sugars. Inhibitor conc is higher at 190 °C. Sugar concentration 50 g/l. |
| [257] | EtOH | Steam explosion | <2 mm | None | 205 | 10 min | 56% cellulose yield in steam explosion residue. 482 g/kg cellulose in SSF. Overall cellulose conversion 27% |
| [258] | Sugars | Steam explosion | < 2mm | None | 205 | 10 min | 81% cellulose recovery in residue; 49% enzymatic cellulose conversion. Overall cellulose conversion is 40% |

### *4.5.2 Summary*

From the literature described above it appears that $SO_2$-catalysed steam explosion results in 10-50% higher yields compared to non-catalysed steam explosion. It also seems to give higher cellulose conversion yields than dilute acid treatment.
Steam treatment/explosion shares the same issues as dilute acid treatments. Higher yields or concentrations will result in more inhibitors making removal of inhibitors by a choice of removal methods or washing necessary. Applying severe conditions to get high yields can lead to uneconomic processes. Most of the sugar concentrations in the literature were 20 to 25 g/l with one exception of 50 g/l (Geddes).

## 4.6 Alkaline hydrolysis

### *4.6.1 Overview*

Studies are focussed on the effect of the pretreatment on the subsequent enzymatic hydrolysis of pretreated SCB and fermentation of released sugars. Data of the alkaline pretreatments are given in Table 10 and more detailed information can be found in Appendix 1.

Lime ($Ca(OH)_2$) pretreatments seem to have a maximum effect at 10% concentration on dry matter. Authors reported maximum cellulose conversions between 46 to 60%. Rabelo used lime in combination with an extremely high dose of hydrogen peroxide ( 25 to 180 % on dry SCB), resulting in a maximum of 76% cellulose conversion [259]. Beukes found $NH_4OH$ and NaOH more effective than $Ca(OH)_2$, especially the ammonium treatment [260, 261]. In general, the lime-pretreated SCB was washed to remove the dissolved lignin fragments and undissolved lime and to bring the pH closer to a neutral pH. Enzymatic hydrolysis was mostly performed at high L/S ratio resulting in very low sugar concentrations of less than 2 g/l.

With NaOH-treatment Hernandez found about 50% reducing sugars/g bagasse released with an optimal enzyme cocktail at a concentration of about 70 g sugars/l. The total enzyme dose was very high (0.57 ml/g SCB) [233]. Microwave assisted NaOH treatment was found to yield 0.67 g reducing sugars/g pretreated bagasse and an additional acid microwave treatment increased this yield to 83% [262].

Using a combination of NaOH and $H_2O_2$, Cheng found equally high cellulose conversion of 78% as Rabelo did with a combination of $Ca(OH)_2$ and $H_2O_2$. However, the NaOH and $H_2O_2$ loads were much lower and recycling of NaOH seemed possible [263]. NaOH treatment in combination with ultrasound resulted in a very high reducing sugar yield of 89% but with a high energy input. A larger scale reactor with a much lower energy use has to be developed [264].

 

Table 10: Overview of alkaline pre-treatment of sugar cane bagasse

| Ref | Product | Pretreatment | Particle size | Chemicals | T (°C) | Time | Results (Yield/EH/fermentation) |
|---|---|---|---|---|---|---|---|
| [233] | EtOH | Alkaline | | NaOH, 5 wt% of dry SCB | 121 | 4h | Optimized enzyme formulation: Celluclast, Novozyme and Viscozyme L. Yield reducing sugars after alkaline–enzymatic hydrolysis for 4h was 11–20 wt% (based on dry SCB) (0.19 ml enzyme/g SCB),but with an optimized mixture 50 wt% was reached at sugar concentration of 70 g/l (0.57 ml enzyme/g SCB). |
| [246] | EtOH | Alkaline/peroxide | | Peroxide 0-5% at pH 8-13 | RoomT? | 8-48 h | 2% peroxide at pH 11.5 during 48h removed lignin best. Calculated ethanol yields very low: 0.004 g/g bagasse |
| [259] | EtOH | Alkaline/peroxide | 80% > 1.2 mm | $H_2O_2$ 0.25-1.84 g/g SCB<br>$Ca(OH)_2$ 0.25-0.65 g/g SCB | $H_2O_2$ 20-60<br>Lime 50-85 | $H_2O_2$ 1-24 h<br>Lime 8-54 h | Peroxide had higher glucose yields than lime with a max of 0.70 g/g glucose in pretreated SCB. Sugar concentrations < 2 g/l |
| [260] | Sugars | Alkaline | Milled | $Ca(OH)_2$ 0.4 g/g SCB | 70 | 36h | 6.5 times increase of hydrolysis rate with optimal enzymes combination compared to no pretreatment |
| [261] | Sugars | Alkaline | | $NH_4OH$ 0.114 M/g | 70 | 36h | 13 fold increase of hydrolysis rate with optimal recombinant enzymes treatment compared to untreated |
| [261] | Sugars | Alkaline | | NaOH 0.063 M/g | 55 | 24h | 9 fold increase of hydrolysis with optimal recombinant enzymes treatment compared to untreated |
| [262] | Sugars | Microwave-alkaline + acid | < 1mm | NaOH 10% w/w + $H_2SO_4$ 10% w/w | | | Enzymatic hydrolysis yields 0.67 reducing sugars/g chemically treated. Additional treatment with 10% $H_2SO_4$ gave enzymatic hydrolysis yield of 0.83 reducing sugars/g chemically treated. |
| [263] | EtOH | Alkaline/oxidative | >0.45 <0.9 mm | NaOH 1% + 0.3% $H_2O_2$ v/v (12.5 +3.75 w/w) | 30 | 20h | Cellulose conversion up to 78%. Two recycle stages possible but with more stages yield decreases. Savings due to recycling 26% on NaOH and 40% on water. In SSF 79% yield → 0.2 g ethanol/g bagasse |
| [265] | Sugars | Alkaline | 40 mesh | $Ca(OH)_2$ | 120 | 1 hour | Glucan conversion 60%, total sugar yield 68% |
| [266] | Sugars | Alkaline | <0.5 mm | $Ca(OH)_2$ | 90 | 90 h | Max glucose yield 0.23 g/g SCB (55% of cellulose) and total reducing sugar yield 0.41 g/g SCB at sugar conc of 1.5 g/l. |
| [267] | Sugars | Alkaline | As such and 0.25-1.4 mm | $Ca(OH)_2$ 0.25-0.65 g/g SCB | 55-85 | 10-60 h | Unscreened: TRS 0.39 g/g and glucose 0.20 g/g SCB. Glucan conversion 46% and polysaccharide conversion 58%.<br>Screened: TRS 0.37 g/g and glucose 0.22 g/g SCB. Glucan conversion 58% and polysaccharide conversion 61%. Sugar concentrations < 2 g/l |
| [268] | Sugars | Alkaline/proxide | As such and 0.25-1.4 mm | $H_2O_2$ 0.25-1.84 g/g SCB<br>$Ca(OH)_2$ 0.25-0.65 g/g SCB | $H_2O_2$ 20-60<br>Lime 60-70 | $H_2O_2$ 6-24 h<br>Lime 12-36 h | Lime results in higher glucose yields, 88% of converted cellulose in screened SCB. With 0.55 g/g SCB TRS was maximal in lime treatment of unscreened SCB. Sugar concentrations < 2 g/l |

| Ref | Product | Pretreatment | Particle size | Chemicals | T (°C) | Time | Results (Yield/EH/fermentation) |
|---|---|---|---|---|---|---|---|
| [269] | Sugars | Sono-assisted alkaline | <0.27 or <0.91mm | 0.25 or 2.5 | 30 or 50 | 5 or 50 | Highest experimental delignification was 82% and cellulose recovery of 98% at 0.27 mm, S/L=20, 2.9 %NaOH, 70 °C and 47 min. TRS yield after saccharification 89 %. |
| [270] | EtOH | Sono-assisted alkaline + acid | <0.26 mm | 2% NaOH w/v L/S=20<br>0.5-3% $H_2SO_4$<br>L/S =10 to 25 | 50 | Alkaline 20 min<br>Acid 15-75 min | Alkaline 99% cellulose- and 79% hemicellulose recovery, 75% lignin removal. 69% hexose and 81 % pentose yield at 2% $H_2SO_4$, L/S=20 and 45 min.<br>Fermentation 92% of theoretical ethanol yield. |
| [271] | Lignin removal | Alkaline | <20 mesh | $NH_4OH$ 0-0.3% v/v 2.4% w/w dry SCB | 30 | 10-40 days | 23 and 46% delignification after 40 days with respectively 0.03 and 0.3% $NH_4OH$. |
| [272] | EtOH | AFEX | <20 mm | $NH_4OH$ | 100-140 | 30 min | After AFEX treatment at 140 °C during 30 minutes followed by enzymatic hydrolysis at 6% glucan concentration. 90% glucan and 76% xylan conversion.<br>Results in 21.5 kg ethanol/100 kg dry SCB. |
| [273] | Sugars | Alkaline | | AFEX or $NH_4OH$ | AFEX 100<br>$NH_4OH$ 160 | AFEX 30 min<br>$NH_4OH$ 60 min | Better result with AFEX: 73% glucan conversion with addition of Biocat xylanase |

 

Long-time anaerobic storage of SCB at ambient temperature after mixing with $NH_4OH$ might be a cheap way to treat SCB. Lignocellulose pretreatment can then be done all year around and not only during the harvest period, resulting in smaller and therefore cheaper equipment. Kim found up to 45% of delignification after 40 days at 30 °C when SCB was treated in such a way [271].

The ammonium fibre expansion process AFEX shows very promising results. Krishnan found cellulose conversion of about 90% at a total sugar concentration of about 100 g/l. Prior achieved a lower glucan conversion of 73%, which probably can be explained by the lower temperature of 100 °C compared to 140 °C during the AFEX treatment [272].

### *4.6.2 Summary*

From these articles can be concluded that a pretreatment with only $Ca(OH)_2$ or NaOH results in moderate cellulose conversion and reducing sugar yields. To enhance these yields, additional chemicals like hydrogen peroxide or a combination with a physical treatment like ultrasound or microwave is needed. The AFEX system is very promising, it gives high conversion yields without additional chemicals or treatments.

## 4.7 Wet oxidation

Articles on the wet oxidation of SCB are limited; only the work of Martin and co-workers is mentioned here (see also Table 11). They found better results of wet oxidation with sodium carbonate compared to steam explosion. SSF yield was 83% of cellulose conversion (77% on cellulose in raw SCB) compared to 53% with steam explosion [257]. It was also found that treatment time had more effect than pH.
Due to the alkaline pH higher concentrations of phenolic degradation products were present compared to furfural. Enzymatic hydrolysis was more effective in washed pretreated SCB than in unwashed SCB. Enzymatic cellulose conversion was maximal 75% resulting in an overall cellulose conversion of 70% [274].

In a comparison with different oxygen pressures it was shown that 12 bar yielded lower glucan in the pretreated SCB than 3 bar but enzymatic hydrolysis yield was much higher. About 57% of the cellulose in the raw bagasse was converted into glucose [275].
In another comparison with steam explosion Martin again found that sodium carbonate assisted wet oxidation results in somewhat higher cellulose conversion than steam explosion [258]. Due to other raw material the cellulose conversion is in this case lower than in previous experiments (53%).

In these articles Martin showed that depending on the applied conditions 53 to 70% of the cellulose in the raw bagasse was converted in glucose by enzymatic hydrolysis. SSF could increase the cellulose conversion to 83%. It is also showed that the differences in raw material can result in large differences in conversion yields.

Table 11: Overview of pretreatment of sugar cane bagasse by wet oxidation*

| Ref | Product | Pretreatment | Chemicals | T (°C) | Time (min) | Results (Yield/EH/fermentation) |
|---|---|---|---|---|---|---|
| [257] | EtOH | Wet oxidation | 12 bar $O_2$ 3.3 % $Na_2CO_3$ or 4% $H_2SO_4$ w/w dry SCB | 195 | 15 | Max glucose yield 792 g/kg after enzymatic hydrolysis of washed residue of the $Na_2CO_3$-WO treatment at 11 g glucose/l. Max cellulose conversion 829 g/kg in SSF of the whole slurry originating from the $Na_2CO_3$-WO treatment (26 g cellulose/l) |
| [257] | EtOH | Steam explosion | None | 205 | 10 | 482 g/kg cellulose in SSF |
| [258] | Sugars | Wet oxidation | 12 bar $O_2$ 3.3 % $Na_2CO_3$ w/w dry SCB | 195 | 15 | 93 % cellulose recovery- 57% enzymatic cellulose conversion Overall cellulose conversion is 53% at 7 g glucose/l |
| [258] | Sugars | Steam explosion | None | 205 | 10 | 81% cellulose recovery- 45% enzymatic cellulose conversion Overall cellulose conversion is 36% at 5 g glucose/l |
| [274] | Sugars | Wet oxidation | 12 bar $O_2$ 3.3% $Na_2CO_3$ or 1% $H_2SO_4$ w/w dry SCB | 185-195 | 5-15 | pH 10, 15 minutes at 195 °C resulted in 92% cellulose recovery and 75% enzymatic convertibility. Overall 69% glucose yield of theoretical maximum at about 10 g glucose/l. Main hemicellulose degradation products are oligomers |
| [275] | Sugars | Wet oxidation | 3 and 12 bar $O_2$ 3.3% $Na_2CO_3$ w/w dry SCB | 195 | 10 | 56.5% of cellulose in raw SCB converted at 12 bar $O_2$ at about 5 g glucose/l |

**Particle size < 2 mm*

 

## 4.8 Organosolv

### *4.8.1 Overview*

Pretreatment of SCB with organic solvents are described in this section and summarized in Table 12. More detailed information can be found in Appendix 1.

Mesa tested organosolv with 50% ethanol/water mixture and showed higher glucose yields when $H_2SO_4$ was used as a catalyst compared to NaOH [276]. The tested cellulase and glucosidase loads showed no significant differences. A pretreatment at 175 °C with 1.25% $H_2SO_4$ as catalyst during 60 minutes followed by enzymatic hydrolysis with Tween 20 resulted in a glucose yield of 25 g/100g SCB and a glucose concentration of 29 g/l. This yield corresponds to a cellulose conversion of 55%. Fermentation yielded 93% ethanol of the theoretical maximum yield after 24 hours.
In the following process steps Mesa compared separate hydrolysis and fermentation (SHF) with simultaneous saccharification and fermentation (SSF) and pre-saccharification followed by SSF (PSSF). It was determined that the order in best performing was PSSF>SHF>SSF with 68, 66 and 59% of theoretical yield based on glucose content in the raw material. Depending on the chosen process parameters in the separate enzymatic hydrolysis, glucose concentrations varied between 33 and 72 g/l and sugar yields (glucose +xylose) between 23 and 35 g/100 g SCB [277]. An acid-stage followed by an organosolv-stage (ethanol) with NaOH as a catalyst showed somewhat higher yields. However, the costs of this extra process step will probably raise the costs per produced sugar unit [278].

Sindhu found that treatment with formic acid yielded higher amounts of reducing sugar than glycerol, acetone, methanol or acetic acid. After 24 h fermentation 19 g/l of ethanol was produced corresponding to an overall efficiency of 48% [279].
Kuo dissolved the cellulose component of SCB in NMMO and regenerated it for further enzymatic hydrolysis [280]. A cellulose conversion into glucose of 85% was reached. Nothing was mentioned on a feasible way of recovering the NMMO. Moreover, NMMO is a thermally unstable solvent that needs large investments in safety [281].

Zhao found better conversion with peracetic acid (PAA) than with $H_2SO_4$ and NaOH treatments under the same conditions, but optimal conditions for $H_2SO_4$ and NaOH were done at much higher temperatures [282]. Overall cellulose conversion was about 67%.

### *4.8.2 Summary*

The organsolv treatments on SCB described in above-mentioned articles yielded 48 to 68% conversion of cellulose; only Kuo found higher conversion yields probably at least 85%. Regarding these results the organsolv process does not seem to have an advantage over other processes.

Table 12: Overview of organosolv pretreatment of sugar cane bagasse

| Ref | Product | Particle size | Chemicals | T (°C) | Time (min) | Results (Yield/EH/fermentation) |
|---|---|---|---|---|---|---|
| [276] | EtOH | 2 mm | Ethanol (50%) with $H_2SO_4$ or NaOH as catalyst | 175 | 60-90 | 175 °C with 1.25% $H_2SO_4$ and 60 min resulted in 25 g glucose/100 g SCB. Tween 20 used in enzymatic hydrolysis |
| [278] | EtOH | | $H_2SO_4$ 1-4% w/w SCB Ethanol 30% v/v with 3% NaOH | Acid 120- 175 Organosolv 185 | Acid 40 Organosolv 60 | PSSF>SHF>SSF with 68, 66 and 59% of theoretical yield based on glucose content in raw material and this yields 198, 192 and 172 l ethanol per ton SCB. Enzymatic hydrolysis gave 33 to 72 g glucose/l and 23 to 35 g sugar/100g SCB |
| [278] | Sugars | < 1 cm | 10% $H_2SO_4$ w/w SCB Ethanol (10-30%) with 3% NaOH | Acid 120 Organosolv 175-195 | Acid 40 Organosolv 20-60 | Depithed SCB. Best yield 29.1 g glucose/100g SCB at 195 oC, 60 minutes and 30% ethanol. Corresponds to 58% cellulose conversion at 18 g glucose/l. |
| [279] | EtOH | <1 mm | Formic acid, glycerol, acetone, methanol and acetic acid | | | Formic acid at 60% concentration was best treatment, giving 19 g/l ethanol with an overall efficiency of 48% |
| [280] | EtOH | 30-45 mesh | N-methylmorpholine-N-oxide (NMMO) | 130 | 60 | 10% mass loss and 95 % cellulose hydrolysis cellulose conversion at least 85%. Reducing sugars about 6 g/l, 0.15 g EtOH/g dry SCB in SSF |
| [282] | Sugars | | Peracetic acid 20-60% C2H4O3 +3% $H_2SO_4$ | 65-80 | 60-120 | Optimum at 50%--L/S=6—80°C—2h gave cellulose content>70% and cellulose conversion of >80% after enzymatic hydrolysis at 20 FPU/g cellulose during 72h |

Table 13: Overview of miscellaneous pretreatments of sugar cane bagasse

| Ref | Product | Pretreatment | Particle size | Chemicals | T (°C) | Time | Results (Yield/EH/fermentation) |
|---|---|---|---|---|---|---|---|
| [283] | Sugars | Ionic liquids | 0.25-0.5 mm | [C4mim]Cl L/S ratio 10 | 110-160 | 30-180 min | 78% conversion of original glucan |
| [284] | Sugars | Ionic liquids | 0.5 mm | [BMIM]Cl [EMIM]oAc [EMIM]DEP L/S=25 | 100 | 0.5-8 h | [EMIM]oAc was best with 57% reducing sugar yield on theoretical maximum. |
| [285] | Sugars | CTMP | | NaOH or NaOH+$Na_2SO_3$ | 120 | 2 h | 14, 50 and 85% cellulose conversion for untreated , NaOH and NaOH+$Na_2SO_3$ respectively |
| [286] | EtOH | Milling | Start fraction <2 mm | none | | | Ball milling had greater effect on SCB than on straw For Wet disc milling it is the opposite. 84 and 77% glucose and xylose yield and with C5/C6 strain 80% ethanol yield on total sugars. An overall yield of 65% on total polysaccharides. |
| [287] | sugars | Alkaline | <1.5 mm | NaOH 4% w/v L/S ratio 25 | 20-40 | 18 h | 55.5 to 62.1 % dissolution of hemicellulose |

 

### 4.9 Miscellaneous

With ionic liquid pretreatments polysaccharide and glucan conversion yields vary between 70 to 80%. Energy demand is lower for ionic liquids but recovery of the liquid is an issue [283, 284].

With a combination of sodium hydroxide and sodium sulphite in a standard CTM-pulping process a cellulose conversion yield of 79% (based on cellulose in raw bagasse) could be reached in 96 hours of enzymatic treatment [285].

With ball milling a complete disruption of the native structure was reached without the use of chemicals resulting in a cellulose conversion of about 84%[286]. However, energy use is enormous.

### 4.10 Summary

Of the described pretreatments of SCB the majority are basically acid or alkaline treatments. Variants on those themes are mostly with extra chemicals or with a combined physical treatment like ultrasound or microwave. Sometimes a combination of acid and alkaline is described. The treatments that result in high cellulose conversion (>90%) are $SO_2$-catalysed steam or steam explosion processes, and the AFEX-system.

Cardona recently presented a review on the production of bioethanol from sugarcane bagasse [228]. In his remarks on pretreatment methods it is mentioned that dilute sulphuric acid has been successfully developed but that costs are high, but also the costs of alkaline pretreatment are so high that this is not competitive for large scale- plants. Wet oxidation and organosolv pretreatments are thought to be the most perspective technologies avoiding degradation products and detoxification stages. For more information, please refer to Klinke *et al* [288] and Pan *et al* [289].

<u>Dilute acid pretreatment</u>

In a substantial part of the literature on dilute acid treatment the authors aimed for high xylose yields in the hydrolysates of acid treated SCB. Their yield on sugars varied between 29 and 37% of dry SCB and maximum xylose yields varied between 74 and 86%. Sugar concentrations in the hydrolysates ranged from 17 to 47 g/l depending on the L/S ratio: low L/S ratios result in high xylose concentrations but also in high inhibitor concentrations like furfural and acetic acid. High L/S ratios will lead to lower concentration but higher yields. At higher severity of the acid treatment xylose yields will drop due to degradation of xylose into products that can inhibit subsequent fermentation. Detoxification of these products appeared best with ion exchange. For sulphuric acid-catalysed pretreatment the following order of effect was observed: temperature> acid concentration > residence time.

Literature on the enzymatic hydrolysis of the solids after dilute acid treatment show cellulose conversions of only 40-50%. A higher yield of 76% was reached when a mixture of $H_2SO_4$ and HAc was used in the pretreatment, but sugar concentration was very low (Rocha). After pH adjustment of total slurry (solids + acid hydrolysis liquid) Jeon reached a total sugar yield of 87%

at a concentration of 58 g/l. A number of process routes is possible, and the route to choose will be indicated by the constrains given by the final steps of fermentation and purification.

Steam and hot water pretreatments

Several authors used $SO_2$-catalysed steam pretreatment that gave cellulose conversions of over 90%. It appears that $SO_2$-catalysed steam explosion results in better yields than non-catalysed steam explosion. It also seems to give higher cellulose conversion yields than dilute acid treatment.

Steam treatment/explosion shares the same issues with dilute acid treatments. Higher yields or concentrations will result in more inhibitors making removal of inhibitors by a choice of removal methods or washing necessary. Applying severe conditions to get high yields can lead to uneconomic processes. Most of the sugar concentrations in the literature above were around 20 to 25 g/l, however an experiment of Geddes with phosphoric acid catalysed steam explosion resulted in a sugar concentration of about 50 g/l.

Alkaline

For pretreatment with lime there is a maximum effective lime concentration of 10%. Authors reported maximum cellulose conversions between 46 to 60%. Lime combined with hydrogen peroxide gave a cellulose conversion of 76%, however much of the expensive peroxide was used. Ammonium- and sodium hydroxide treatments are more effective than lime treatment. Sodium hydroxide with hydrogen peroxide gave the same yield as lime with peroxide, however peroxide load was much lower with sodium hydroxide. Long-time anaerobic storage of SCB at ambient temperature after mixing with $NH_4OH$ might be a cheap way to pretreat SCB. The ammonium fibre expansion process AFEX shows very promising results, it gives high conversion yields without additional chemicals or treatments. Krishnan found cellulose conversion of about 90% at a total sugar concentration of about 100 g/l. Treatment with only lime or NaOH results in moderate cellulose conversion and reducing sugar yields. To enhance these yields, additional chemicals like hydrogen peroxide or a combination with a physical treatment like ultrasound or microwave is needed.

Wet oxidation

In several articles by Martin it was shown that depending on the applied conditions 53 to 70% of the cellulose in the raw bagasse was converted to glucose by enzymatic hydrolysis. SSF could increase the cellulose conversion to 77%. It was also shown that differences in raw material can result in large differences in conversion yields. Better results were achieved with wet oxidation at pH 10 than at pH 6 or pH 3.

Organosolv

Organosolv treatments of SCB were carried out with ethanol, formic acid, glycerol, acetone, methanol, acetic acid and peracetic acid. These treatments yielded 48 to 68% conversion of cellulose. Only Kuo found higher conversion yields with NMMO, but it is not clear how much exactly, calculations show that it must have been at least 85%. Regarding the cellulose conversion the organsolv process does not seem to have an advantage over other processes.

 

Miscellaneous

Application of ionic liquids as cellulose dissolvent resulted in polysaccharide and glucan conversion yields of 70 to 80%. Recovery of the ionic liquid is still an issue. A combination of sodium hydroxide and sodium sulphite in a standard CMT (Chemo Mechanical Thermo) pulping process results in a cellulose conversion yield of 79%, based on cellulose in raw bagasse. Ball milling resulted in a complete disruption of the native structure. It was reached without the use of chemicals and resulting in a cellulose conversion of about 84%. However energy use is enormous.

### 4.11 Conclusions

This chapter describes the work done on pretreatment of sugarcane bagasse as described in literature. Pretreatment of sugarcane bagasse is a research topic studied by a number of researchers as demonstrated by the many references that are available on this topic. For this review the articles were categorized on pretreatment technique and conclusions on the most optimal conditions were drawn for each method.

In order to draw conclusions from these studies and to answer questions like "What is the best pretreatment method for the production of lactic acid from sugarcane bagasse" is very difficult for a number of reasons listed here below:

- Each author uses different definitions to describe their process in terms of yield, conversion and efficiency, which makes it impossible to compare the various methods described in literature.
- The choice of the best pretreatment does not only involve the sugar concentration and yield, but also formation of inhibitors. Different fermentation organisms are differently sensitive to inhibitors. So the end product of the total process and the chosen micro-organisms to produce that product influences the choice of pretreatment process or the need of a detoxification step. Most of the articles focus on the production of ethanol, some on the production of fermentable sugars. Often only a small part of the process is evaluated and not the whole chain (i.e. from biomass to (intermediate) product).
- The literature described in this chapter on pretreatment of sugar cane bagasse for fermentation purposes is strongly based on laboratory work. In most experiments the SCB is ground to small particles (mostly <1 mm) to enhance diffusion and homogeneity. In practice this would be a costly step resulting in poorer economy of the process. No articles were found that describe treatments at pilot or larger scale. Only a few articles describe experiments on bench scale or semi-technical scale: Geddes worked with 0.5 kg batches [290] and Rodrigues scales up to 350 litres [237]. The best results described in literature are reached on these laboratory scale-experiments under optimal conditions; yields on larger scale will probably be lower due to unfavourable, but economic more viable, conditions.
- Enzymatic treatments are often high in enzyme dose and treatment times are long, which is a logical step when determination of pretreatment-effect on maximum degradability of the polysaccharides is the main goal. Buffers are normally used to optimize enzymatic activity and sometimes antibiotics are applied to prevent consumption of released sugars by micro-

organisms; also sterilisation was applied. Those conditions can often not be applied under industrial conditions as fermentation is usually the following step.

- In a recent article Galbe writes "The various pretreatment methods need in the future to be reassessed at more industrial-like conditions, considering the whole integrated process, taking into consideration the influence on all process steps" [291].

 

# 5 Techno-economic studies

## 5.1 Introduction

In the previous chapter a more fundamental approach was followed for the pretreatment of biomass, and more specific sugarcane bagasse. This chapter provides techno-economic information on various pretreatment techniques.

## 5.2 Comparison of pretreatment technologies; process and techno-economic analysis

Comprehensive evaluation of six pretreatment processes to convert switchgrass to fermentable sugars and ultimately to cellulosic ethanol is described by Tao *et al.* [56]. The six pretreatment processes are ammonia fiber expansion (AFEX), dilute acid (DA), lime, liquid hot water (LHW), soaking in aqueous ammonia (SAA), and sulphur dioxide-impregnated steam explosion ($SO_2$). The process conditions of the pretreatments are listed in the table below. For a detailed description of the different pretreatments see Chapter 3.

Table 14: Pretreatment process conditions [56]

| | Chemicals | T (°C) | Time | P | Reactor | Solids* (%) | Chemicals for neutralization |
|---|---|---|---|---|---|---|---|
| AFEX | $NH_3$ (l) | 150 | 30 min | Elevated | Extruder-like reactor** | 55 | No |
| DA | $H_2SO_4$ (l) | 140 | 40 min | 40 psig | Horizontal screw-feed reactor | 30 | Yes (end pH 1) |
| Lime | $Ca(OH)_2$ | 120 | 4 h | Elevated | Reactor vessel | 20 | No |
| LHW | None | 200 | 10 min | Elevated | Tube reactor | 20 | No |
| SAA | $NH_3$ (l) | 160 | 60 min | 465 psig | Horizontal screw-feed reactor | 20 | No |
| $SO_2$ | $SO_2$ (g) | 180 | 10 min | Elevated | Horizontal screw-feed reactor | 30 | Yes (end pH 1) |

*Ttotal solids concentration during pretreatment stage
** In a more recent development, AFEX is carried out in packed beds that are operated in batch mode

The sugar yields after pretreatment are based on the feedstock composition of the milled switchgrass and the composition of switchgrass (i.e. 35 % cellulose, 22.5% xylan and 22.6% lignin dry weight) significantly influences the overall analysis. In Figure 6, the total monomer and oligomer sugar yields for each case are compared.

If only monomeric sugars are assumed as fermentable sugars to produce ethanol then AFEX, DA and $SO_2$ are the best options. However, the MESP (minimum ethanol selling price) for $SO_2$ is higher due to significant costs associated with handling and usage of $SO_2$ (onsite production will lower the costs). Significant amounts of total sugars are found as oligomers for lime, LHW and SAA pretreatment.

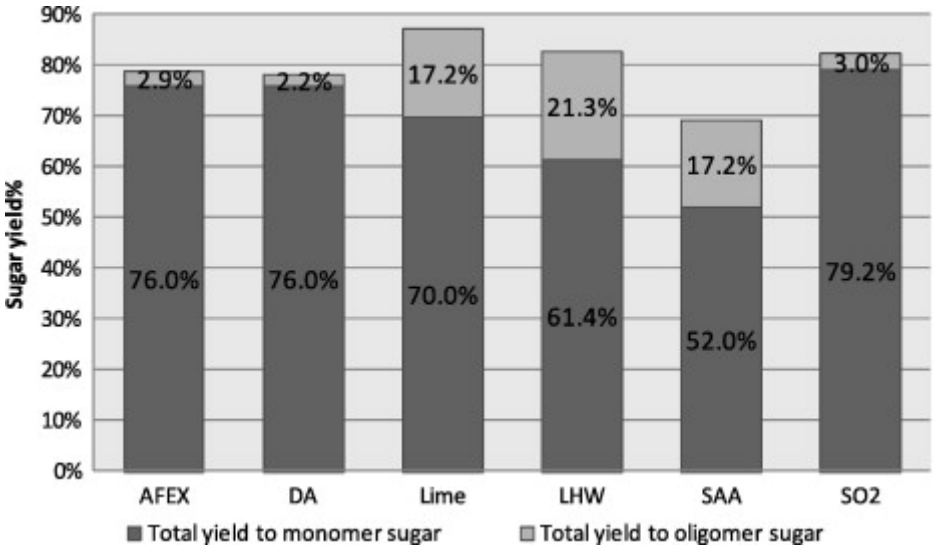


Figure 6: Total monomer and oligomer sugar yields [56]

If it is assumed that all soluble xylose and glucose sugars (both monomeric and oligomeric) can be fermented to ethanol (or oligomer sugar can be hydrolysed to monomers via use of an enzyme preparation with appropriate oligomer-hydrolysing activities), the MESP results for the six pretreatment options may be significantly different as shown in Figure 7. These findings indicate that if oligomeric sugars can be converted, much less differentiation exists between the pretreatments (except for SAA).

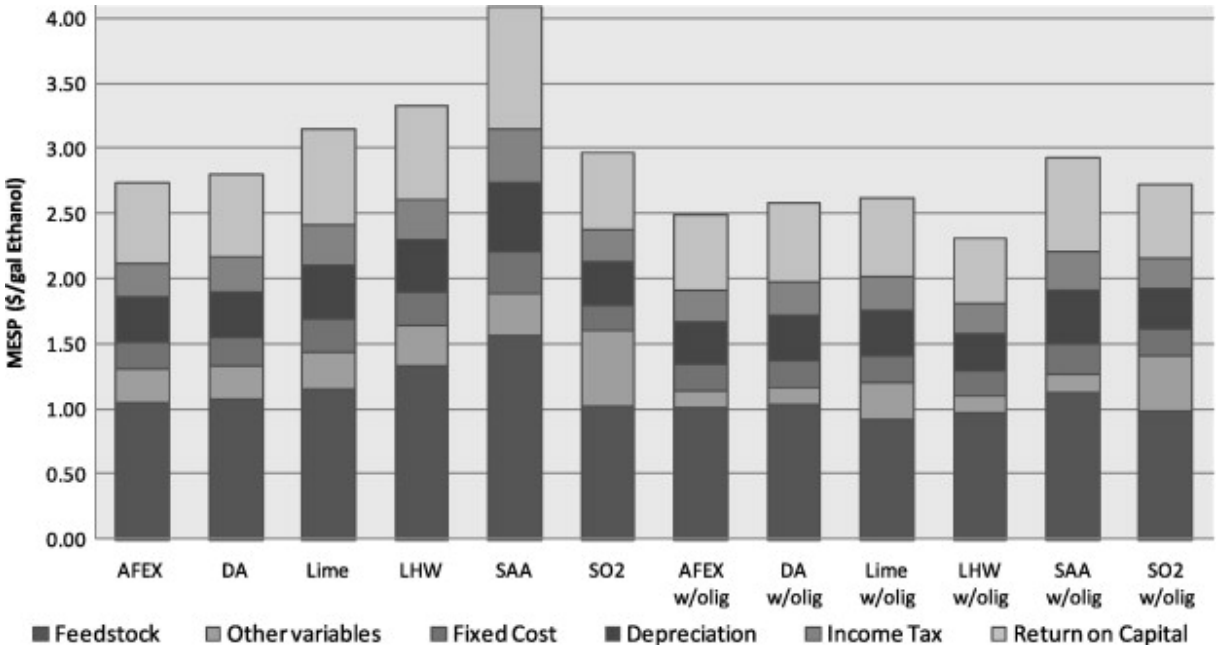


Figure 7: MESP (minimum ethanol selling price) with and without oligomer credits [56]

 

The six pretreatment technologies vary greatly in terms of their process design and projected total capital investment. Overall ethanol yield, which is largely based on the overall sugar yield achieved in pretreatment and subsequent enzymatic hydrolysis steps, is the single-most important factor in determining projected MESP. Capital costs associated to pretreatment are highest for lime, DA, and SAA pretreatments, and lowest for LHW, AFEX, and $SO_2$-explosion.
There are also significant differences in the fraction of pretreatment reactor costs in overall capital costs for pretreatment: for DA 76% of pretreatment capital is associated to the reactor, whereas for LHW, SAA, and lime, less than half of pretreatment capital costs are required for pretreatment reactors.
Based on the switchgrass composition the theoretical ethanol yield can be calculated, assuming that all the cellulose and hemicellulose is converted to ethanol. For the six pretreatments the % of theoretical ethanol yield varied between 40-60%, indicating that improvement can be obtained with microorganisms that can ferment oligomer sugars along with minor sugars such as arabinose, galactose and mannose.

### 5.3 NREL study: base case

The most extensive, publicly available techno-economic analysis of lignocellulosic biomass pretreatment is the analysis provided by the National Renewable Energy Laboratory [1]. This study analyses the cost of producing bio-ethanol from corn stover in the United States, and is regularly updated by incorporating new insights from on-going Research & Development. The latest update (May 2011) known to us, is used in this report. Besides production costs for bioethanol, the study provides good insights in producing fermentable sugars from lignocellulose, which are useful for other fermentation purposes.

In Table 15, data for the production costs of fermentable sugars from corn stover are presented. These data are directly taken from figure 19 of the NREL-study ('Economic summary for dilute sugar production'), except that US mass and volume units are converted to metric units, and 2007-$ are converted to € by using an average exchange rate in 2007 of 0.7353 $/€ [292].
An important quote from the study states that

*"It should be stressed that the sugar stream produced in this analysis is strictly "imaginary" . The purpose of this analysis is merely to separate the cost of producing sugars from the downstream costs of producing ethanol or other products."*

Table 15: Cost of producing fermentable sugars from lignocellulose (based on NREL 2011[1])

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sugar Production Process Engineering Analysis | | | | | | | | | |
| | | Corn Stover Design Report Case: DW1102A | | | | 0.7353 | Euro/$ | | | |
| | Dilute Acid Prehydrolysis with Enzymatic Saccharification | | | | | | | | | |
| | | All values in 2007 Euro/In metric units | | | | | | | | |
| | Minimum Sugar Selling Price | | | 0.1877 | EUR/kg, dilute sugars | | | | | |
| Sugar Production | | | (metric ton/Year) | 412,950 | | | | | | |
| Sugar yield | | | (kg/metric Ton Feedsto | 589.5 | | | | | | |
| Feedstock cost $/Dry metric Ton | | | (EUR/Dry metric Ton) | $47.42 | | | | | | |
| Internal Rate of Return (After Tax) | | | | 10% | | | | | | |
| Equity Percent of Total Investment | | | | 40% | | | | | | |
| | Capital Costs | | | | | Manufacturing cost (Eurocents/ kg sugar) | | | | |
| Pretreatment & Conditioning | | € | 24,264,900 | | Feedstock & Handling | | | | | 8.045 |
| Enzymatic hydrolysis | | € | 14,411,880 | | Sulfuric Acid | | | | | 0.266 |
| On-site Enzyme Production | | € | 13,455,990 | | Ammonia | | | | | 0.783 |
| Solids Recovery | | € | 5,367,690 | | Glucose (Enzyme production) | | | | | 2.101 |
| Wastewater treatment | | € | - | | Other Raw Materials | | | | | 0.173 |
| Storage | | € | 1,397,070 | | Waste Disposal | | | | | 0.360 |
| Boiler/Turbogenerator | | € | 48,529,800 | | Electricity | | | | | -1.096 |
| Utilities | | € | 5,073,570 | | Natural Gas (sugar concentration) | | | | | 0.000 |
| Total installed Equipment Cost | | € | 112,427,370 | | Fixed Costs | | | | | 1.587 |
| | | | | | Capital Depreciation | | | | | 1.585 |
| Added costs | | € | 93,383,100 | | Average Income Tax | | | | | 0.906 |
| (% of TPI) | | | 45% | | Average Return on Investment | | | | | 4.061 |
| Total Project Investment | | € | 205,810,470 | | Manufacturing cost ($/yr) | | | | | |
| | | | | | Feedstocks & Handling | | | | € | 33,235,560 |
| | | | | | Sulfuric Acid | | | | € | 1,102,950 |
| Installed Equipment Cost/Annual kg | | € | 0.28 | | Ammonia | | | | € | 3,235,320 |
| Total Project Investment/Annual kg | | € | 0.50 | | Glucose (Enzyme production) | | | | € | 8,676,540 |
| | | | | | Other Raw Materials | | | | € | 735,300 |
| Loan Rate | | | 8.0% | | Waste Disposal | | | | € | 1,470,600 |
| Term (years) | | | 10 | | Electricity | | | | € | 4,485,330- |
| Capital Charge factor | | | 0.131 | | Natural Gas (sugar concentration) | | | | € | - |
| | | | | | Fixed Costs | | | | € | 6,544,170 |
| Sugar concentration (g/L) | | | 127 | | Capital Depreciation | | | | € | 6,544,170 |
| Energy Efficiency (LHV Efficiency | | | 50.80% | | Average Income Tax | | | | € | 3,750,030 |
| | | | | | Average Return on Investment | | | | € | 16,764,840 |

 

Hence, the costing study is based on a set of assumptions, that each carry an intrinsic variance in their level of confidence. For instance, the plant life is estimated to be 30 years, which can be considered as very optimistic. Furthermore, in the cost analysis for fermentable sugars, the following assumptions and modifications to the ethanol cost study are made:

- Enzymatic hydrolysis is assumed to be carried out in a sterile way and nearly to completion so that a transferable sugar stream is produced
- A lignin press with counter-current washing is added after hydrolysis to separate lignin and unreacted insoluble solids from the dilute mixed sugar stream
- The combustion section for lignin is retained, and an electricity co-product credit is assumed for these solids
- A wastewater cost is kept to account for treatment of the pretreatment flash vapour; however, given that the beer column stillage (from the ethanol model) is not applicable in the sugar model, there is no on-site wastewater facility included. Instead, an operating cost of $ 0.09/kg COD is applied for disposal of the wastewater material to off-site treatment
- Fermentation, distillation, stillage treatment, and ethanol storage are completely removed from the sugar model. As in the ethanol process design, enzymatic hydrolysis is assumed to achieve 90% cellulose-to-glucose conversion

Data in Table 15 show a minimum selling price for fermentable sugars of 187 €/ton sugars produced from corn stover, in a facility that produces approximately 413 kton of sugars on an annual basis. If the feedstock is assumed to be corn stover, the associated yearly biomass feedstock requirement amounts to 700 kton of corn stover, on a dry matter basis. The data further show that feedstock costs (taken at 47.48 €/ton feedstock price including handling) amounts to 43% of total production costs, whereas capital depreciation and return on investment combined amount to 30% of total production costs. A full distribution of cost components is provided in Figure 8:

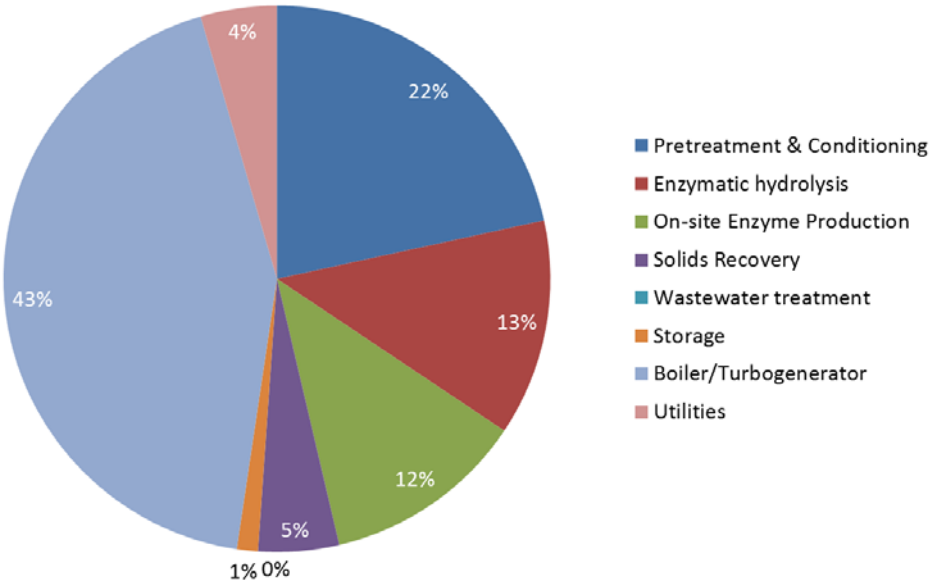


Figure 8: Distribution of cost components in lignocellulose biomass to sugars (for assumptions see Table 15)

It is very important to point out that the cost estimates in Table 15 is for sugars that are contained in diluted form (i.e. they cannot be directly compared to world sugar market price, or other market data), and that it contains a significant concentration of other organic and inorganic components. Table 16 presents the anticipated concentration of monomeric sugars (approximately 125 g/L) and other components in the dilute sugar stream (left column). The NREL study states that further upgrading the dilute sugar stream to a more concentrated sugar stream (486 g/L sugars; see Table 16, right column) would increase the price of fermentable sugars to 231 €/ton, an increase of 23%. This additional cost only covers the cost for concentration (*i.e.* removing water), it does not include costs for removing other components in the sugar stream that may inhibit fermentation.

Table 16: Composition of fermentable sugars stream in raw/diluted and concentrated [1]

| Component | Raw/Diluted stream (g/L) | Concentrated stream (g/L) |
|---|---|---|
| Glucose | 75.9 | 289.8 |
| Xylose | 42.1 | 160.8 |
| Arabinose | 5.1 | 19.6 |
| Mannose | 1.3 | 4.8 |
| Galactose | 3.0 | 11.5 |
| **Total Sugars** | **127.4** | **486.5** |
| Extractive organics | 31.2 | 119.1 |
| Solubilized lignin | 1.7 | 6.4 |
| HMF | 0.9 | 3.6 |
| Furfural | 0.9 | 0.1 |
| Ammonium Sulfate | 5.5 | 21.0 |
| Ammonium Acetate | 4.1 | 15.8 |
| Insoluble solids | 0.3 | 1.1 |

 

In order to assess the costs for producing fermentable sugars for a lactic acid facility and to compare this study with other pretreatment cost studies, the data from the NREL study are first downscaled to a lactic acid facility of 100 kt/y, which is described in the next section. Subsequently, a number of cost estimates from recent papers or projects are reviewed.

### 5.4 Adapting NREL data to lactic acid size facility (100 kt/y)

In order to come up with a cost estimate for producing fermentable sugars for a lactic acid production facility, the following assumptions are being made:

- In order to produce 100 kton of lactic acid per year, we assume that 133 kton of fermentable sugars are needed. By using the same conversion factor of lignocellulose into fermentable sugars as in the NREL-study, 225 kton of dry lignocellulose feedstock are needed per year to produce this amount of sugars. This is equivalent to 32% of the original size of the NREL study (where approximately 700 kton of feedstock is used).
- In order to estimate capital costs of the smaller facility, we assume a general scaling factor of 0.7 for scaling capital costs. This means that installed capital costs are 45% of the capital costs in the NREL study. The capital costs of this smaller facility are summarised in Table 17, and amount to a total project investment of 93 M €, which included indirect costs (*e.g.* field expenses, construction fees, contingencies etc.) according to NREL, are equivalent to 45% of total project investment.
- Cost for chemicals, enzymes, utilities and other operating costs in the smaller facility are similar to these costs in the larger facility, on a per unit basis. Feedstock costs per ton biomass are unchanged as well (i.e. for a smaller production facility, one could argue that transportation costs for biomass are lower, however this is not taken into accounting in the cost estimate).
- Finally, all costs related to capital, including fixed costs, capital depreciation, and income tax per year are estimated to be 45% of the costs of the larger facility (*i.e.* similar to the change in capital investment costs)

Table 17 shows the estimated annual manufacturing costs of fermentable sugars for the 100 kton lactic acid facility, which amounts to nearly 34 M€ per year. Given that 132 kton of fermentable sugars are produced per year, the manufacturing costs are equivalent to 256 €/ton of sugars produced, in the form of a diluted sugar stream (as discussed in the previous paragraphs). The data also show that downscaling the facility leads to an increase in cost of manufacturing cost of approximately 40%, compared to the earlier stated manufacturing costs of 187 €/ton of the original facility. Finally, the fraction of feedstock and other variable costs (e.g. enzymes, chemicals) naturally occupy a smaller fraction in total manufacturing costs in comparison to the larger facility. Interestingly, the manufacturing costs of the downscaled facility would be slightly less dependent on changes in feedstock cost (32% of manufacturing costs) as compared to the larger facility (43% of total manufacturing costs are composed of feedstock costs). In addition, it should be pointed out that additional costs for detoxification of the lignocellulosic hydrolysate are not taken into account.

Table 17: Capital costs and manufacturing costs for producing fermentable sugars for a 100 kton/y lactic acid facility, based on NREL design 2011 [1]

| | | | |
|---|---|---|---|
| Size Facility | 225,000 | dry tons feedstock/y | |
| | 132,525 | tons sugar/y | |
| Average scaling fact | 0.7 | | |
| | | | |
| Capital Costs | | | |
| Pretreatment & Conditioning | | € | 10,963,208 |
| Enzymatic hydrolysis | | € | 6,511,481 |
| On-site Enzyme Production | | € | 6,079,597 |
| Solids Recovery | | € | 2,425,194 |
| Wastewater treatment | | € | - |
| Storage | | € | 631,215 |
| Boiler/Turbogenerator | | € | 21,926,415 |
| Utilities | | € | 2,292,307 |
| Total installed Equipment Cost | | € | 50,829,417 |
| | | | |
| Indirect costs | | € | 42,191,739 |
| (% of TPI) | | | 45% |
| | | | |
| Total Project Investment (TPI) | | € | 93,021,156 |
| | | | |
| TPI per ton of feedstock | | € | 413.43 |
| TPI per ton of sugar | | € | 701.91 |
| | | | |
| | | | |
| Manufacturing Costs per y | | | |
| Feedstocks & Handling | | € | 10,682,859 |
| Sulfuric Acid | | € | 354,519.64 |
| Ammonia | | € | 1,039,924 |
| Glucose (Enzyme production) | | € | 2,788,888 |
| Other Raw Materials | | € | 236,346 |
| Waste Disposal | | € | 472,693 |
| Electricity | | € | -1,441,713 |
| Natural Gas (sugar concentration) | | € | - |
| Fixed Costs | | € | 2,944,877 |
| Capital Depreciation | | € | 2,944,877 |
| Average Income Tax | | € | 1,687,514 |
| Average Return on Investment | | € | 12,185,771 |
| | | | |
| Total operating cost | | € | 33,896,554 |
| | | | |
| Manufacturing Costs per ton of feedstock | | € | 150.65 |
| Manufacturing Costs per ton of sugar | | € | 255.77 |

 

## 5.5 Comparison of NREL (downscaled) base case with other pretreatment cost studies

In order to make a comparison of the NREL design, which is based on dilute acid pretreatment of corn stover, a number of recent studies are summarised below where a different pretreatment pathway is selected. Subsequently, key economic from these studies are taken to make comparisons with the NREL study. Three cases from the earlier described study by Tao [56] (which originate from the well- published CAFI study in 2005) are also taken for comparison purposes. The following studies were considered:

Lime pretreatment/EET [293, 294]

As part of a Dutch EET-funded study, a cost calculation was made to produce fermentable sugars for the production of lactic acid from wheat straw at a scale of 100 kton lactic acid per year. The total production costs for fermentable sugars are estimated at 190 €/ton sugar, with a capital investment required of 127 M€ for feedstock handling, pretreatment, enzymatic hydrolysis and simultaneous fermentation, and processing of non-fermentable residues. Costs for down-stream processing of lactic acid are not included. The main pretreatment procedure consists of heating biomass under addition of lime ($Ca(OH)_2$; 7,5% on dry weight biomass) under atmospheric conditions, followed by SSF. Part of the lime is used in the fermentation to control pH during fermentation.

Mechanical-Alkaline fractionation/Biosynergy (Harmsen *et al.*, unpublished )

As part of the European Biosynergy project, a combination of mechanical pretreatment (accomplished by extrusion) and alkaline pretreatment (accomplished by adding NaOH and heat) is used to pretreat lignocellulose. The scale of this process was 125 kton of biomass per year, with wheat straw as model feedstock.

Total investment costs for the facility are estimated at 31 M € , which include pretreatment section (both mechanical and chemical treatment), enzymatic hydrolysis, and recovery of the side product lignin. In this process, a large fraction of the lignin is recovered from the soluble (pretreatment liquor) by precipitation. Cost for thermal conversion of the remainder of the lignin, as well as waste water treatment, are not included.

AFEX [295]

In a US-based study that is focused on the AFEX pretreatment process, Bals estimated costs of producing ethanol from lignocellulose. The scale of the process is set at 300 kton of lignocellulosic biomass per year with corn stover chosen as model feedstock.

The total equipment cost required in this study is 75 M $. The process includes, among others feedstock handling, pretreatment, chemicals recovery, biological conversion, waste water treatment, and residue processing areas. It should be noted that in a later development, AFEX is carried out in batch-wise operated packed beds, rather than a continuous pretreatment reactor. The capital costs associated to the packed beds are not known.

To further compare with other studies, three pretreatment techniques that were described in an earlier report by Tao [56] were included as well: AFEX, lime and dilute acid. According to Tao, estimated total installed capital costs amount to 191 M€ (AFEX), 212 M€ (lime), and 192 M €

(dilute acid) respectively. The size of the facility is similar to the original NREL study, approximately 700 kton of biomass per year (on dry matter basis).

Before the comparisons of the different cost studies are discussed, it is important to point out that there are considerable differences in assumptions in the various studies, which make one-to-one comparison with the NREL study quite difficult, as well as estimating total manufacturing costs of sugars. Among others, these differences are:

- The scale of operation is variable: from 100 to 700 kton per year
- Installed capital cost estimates vary widely: some studies use the widely reported method by Peters and Timmerhaus [296] to estimate cost for installment, others use different methods to determine these costs
- Plant life varies from 15 years to 30 years
- Feedstock types vary as well: corn stover is generally considered to require less severe pretreatment conditions compared to wheat straw or switchgrass
- The number of process units that are included in the cost studies vary: some include costs for chemical recovery (AFEX), byproduct extraction, conversion of byproducts, waste water treatment, whereas others do not include these processes in the cost estimate
- Some studies incorporate on-site enzyme production (e.g. NREL) whereas in others studies enzymes are bought from commercial suppliers (*e.g.* lime, mechanical/alkaline, AFEX)
- Credits for co-products, such as electricity delivered to the grid, are different. In addition, there are differences in costs for chemicals, utilities, and labour, and cost estimates are often based on different years (2006 to later years).

An overview of some of the key assumptions made in these studies is provided in Table 18, and indicates the great differences in key assumptions.
Table 19 includes the installed capital cost of the various studies, which are converted in € by using the 2007 average exchange rate (as described earlier in this chapter). Capital investment required is both displayed for the original scale used in the study, as well as for a downscaled version of 225 kton lignocellulose per year, which is estimated by using a scaling factor 0.7 .

The comparison of the original scale facilities (see top section of the table) shows that total installed capital costs vary widely, from 161 € per ton of biomass processed per year (NREL) to 292 € per ton of biomass processed per year (lime pretreatment-NL study). Interestingly, there are significant differences between the NREL-dilute acid cost study and the dilute acid pretreatment study by Tao, as well as the two studies for AFEX pretreatment, even though all studies were done at same production scale. It is possible that in the study of Tao, installed capital from processes not directly related to pretreatment or by-product conversion are included (the study by Tao does not distinguish capital investments to a more detailed level, as was done in the NREL and Bals study).

 

The table also shows that if all the facilities are scaled down or up to the 100 kton/y lactic acid facility, total installed capital costs vary from 208 (mechanical/alkaline fractionation) to 292 (lime pretreatment) € per ton of feedstock processed per year. In other words, installed capital costs for the pretreatment facility would be in the range of 47 M€ to 66 M€. If for all systems a yearly production of 133 ktons fermentable sugars is assumed, the capital cost would amount to 53 to 79 € per ton fermentable sugars per year (at 15% capital cost per year).

Table 18: Main assumptions made in different cost studies, in stated units

| | **Dilute acid NREL** | **Lime NL** | **Mechanical /alkaline EU** | **AFEX US** | | **AFEX Tao** | **Lime Tao** | **Dilute acid Tao** |
|---|---|---|---|---|---|---|---|---|
| Feedstock type | Corn stover | Wheat straw | Wheat Straw | Corn stover | | Switchgrass | | |
| Feedstock price | 58.5 $/t | 38.5 €/t | 50 €/t | 50 $/t | | 69.5 $/t | | |
| Enzyme price | 0.34 $/gal (ethanol) | 0.12 €/kg | 1.4 €/l | n.a. | | 0.25 $/gal (ethanol) | | |
| Lignin conversion method | CHP | CHP | precipitation | CHP | | combustion | | |
| Energy credit | 0.12 kWh/lb | 0.078 €/kWh | | 0.047 $/kWh | | 0.04 $/kWh | | |
| Total monomeric sugar yield (%)* | 90 | 90 | 90 | n.a. | | 76 | 70 | 76 |
| | | | | | | | | |
| Return on investment (%) | 10 | n.a | n.a | 12 | | 10 | | |
| Plant life (y) | 30 | 15 | n.a | 25 | | 20 | | |
| *Total monomeric sugar yield = conversion yield of cellulose to glucose and hemicellulose to monomeric C5 sugars | | | | | | | | |

Table 19: Estimated installed capital costs and main variable costs of different pretreatment processes (where indicated, costs are displayed per ton of dry matter lignocellulose processed)

| | **Dilute acid NREL** | **Lime NL** | **Mechanical /alkaline EU** | **AFEX US** | | **AFEX Tao** | **Lime Tao** | **Dilute acid Tao** |
|---|---|---|---|---|---|---|---|---|
| Scale (original version) [kton feedstock/y] | 700 | 227 | 125 | 300 | | 700 | 700 | 700 |
| Installed equipment costs [M€] | 112 | 66 | 31 | 77 | | 140 | 156 | 141 |
| Installed equipment cost [€/ton feedstock.y] | 161 | 291 | 248 | 257 | | 200 | 222 | 201 |
| Scale (downscaled version) [kton feedstock/y] | 225 | 225 | 225 | 225 | | 225 | 225 | 225 |
| Installed equipment costs [M€] | 51 | 66 | 47 | 63 | | 63 | 70 | 64 |
| Installed equipment cost [€/ton feedstock.y] | 226 | 292 | 208 | 280 | | 282 | 313 | 283 |
| Biomass cost [€/ton feedstock] | 47 | 40 | 56 | 51 | | 51 | 51 | 51 |
| Enzyme cost [€/ton feedstock] | 12 | 32 | 28 | 16 | | 16 | 16 | 16 |
| Total variable costs (excl. feedstock) [€/ton feedstock] | 22 | 54 | 77 | 45 | | n.a. | n.a. | n.a. |

The relatively low cost installation of mechanical/alkaline fractionation is probably due to the fact that costs for a CHP installation for lignin combustion are not included (in this study, costs for precipitating lignin from the pretreatment liquor are included in stead, but capital costs associated with lignin precipitation are not as high as a CHP installation). As noted earlier, the larger differences in costs estimated may also be due to the different methods used for estimating costs for installing equipment.

Biomass costs are shown to vary from 40 to 56 €/ton lignocellulose (see lower section of the table). Again, scale factors for supplying biomass at a smaller facility are not included in these estimates.
Table 19 also shows that there are large differences between enzyme costs in the studies. For the Bals and Tao study, the enzyme costs are estimated at a standard 0.25 $/gallon ethanol produced, which amounts to 16 €/ton biomass processed. For the NREL studies, enzyme cost (12 €/ton biomass) displayed are only estimated costs for using glucose that is used for the on-site production of enzymes, and therefore these costs do not cover all costs associated to enzymes. For the other two studies, generally enzyme costs are higher primarily due to higher price estimates for buying cellulase enzyme from commercial suppliers. To what extent there are additional costs associated to the use of hemicellulases (in particular for alkaline pretreatment, where part of the hemicellulose remains in polymeric form), is not known. Most available enzyme cocktails express both glucanase and hemicellulase activities.

Other variable costs are shown to be highest for the mechanical/alkaline pretreatment. This is primarily related to the use of sodium hydroxide, which is not recycled in this version of the process. Variable costs for lime and AFEX are shown to be rather similar, whereas lowest variable costs are presented for the NREL-dilute acid design.
The variability in data and methods that are brought about in comparing these data, show that it is important to set up cost models that use similar costing methods for capital costs and variable costs such as enzymes, chemicals, and biomass feedstock.

 

## 5.6 Summary

The following conclusions can be drawn from the literature review of the techno-economic studies:

- An analysis of 6 pretreatment technologies on a comparable basis indicates that the expected yield of monomeric sugars from cellulose and hemicellulose ranges widely, from 52 to 79%. However, if oligomeric sugars produced in the pretreatment are accounted for as well, differences in sugar yield are much smaller
- Based on ethanol fermentation, manufacturing costs of the endproduct ethanol are lowest for AFEX and dilute acid, which follows in part from the higher monomeric sugar yields of these pretreatments. However, if oligomeric sugars are also fermented, differences in manufacturing costs among pretreatments are not very large
- Results of the widely published NREL design study (dilute acid pretreatment of corn stover) shows that manufacturing costs of fermentable sugars are estimated at 187 €/ton of diluted sugar stream produced (concentration sugars: 127 g/L). If this facility is downscaled to 100 kton, the manufacturing costs of the fermentable sugars is estimated at 256 €/ton sugar.
- Analysis of more recent costing studies that apply different pretreatment routes show that there are considerable differences in installed capital costs for pretreatment of lignocellulose. However, part of these differences are due to different costing methods applied in the studies, and a different set of pretreatment process steps included in the studies. It is therefore difficult to make a good assessment of pretreatment costs by comparing the available studies.
- It is recommended to set up a model that investigates different pretreatment technologies on a similar design basis and by using similar costing methods. In this model, the efficiency of converting cellulose and hemicellulose should be taken up, as well as costs for detoxification of the sugar streams produced.
- Life cycle analysis (LCA) should be used to compare different pretreatment methods, in particular with regard to factors that impact on the environmental footprint of the pretreatment technology, including water use, salts re-generation, and energy use.

# 6 Conclusions

Pretreatment is a crucial process step in the biochemical conversion of lignocellulosic biomass to fermentable sugars and finally to products like lactic acid or other fermentation products. Pretreatment is required to alter the structure of lignocellulosic biomass to make cellulose, and often, hemicellulose, more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars. Pretreatment has been recognised as one of the most expensive processing steps in cellulosic biomass-to-fermentable sugars conversion and several review articles provide a general overview of the field.
In summary, main pretreatment technologies that are developed for producing fermentable sugars from lignocellulose can be roughly divided in methods that rely on full or partial depolymerisation of carbohydrates (*sugar-removing* methods), and methods that rely on delignification (*lignin-removing* methods). In certain pretreatment methods, both sugar degradation and lignin removal takes place simultaneously. Although most pretreatment methods depend on the use of cellulose and hemicellulose degrading enzymes to produce fermentable monomeric sugars, certain pretreatments directly lead to monomeric sugar formation-without the use of enzymes (e.g. concentrated acid hydrolysis, Plantrose process).

Main *sugar-removing* pretreatment methods include dilute-acid, liquid hot water, supercritical $CO_2$ and acid-catalysed steam explosion. In general, these pretreatments are carried out at elevated pressures and temperatures, and as a result sugar degradation products that may inhibit fermentation are formed. Furthermore, recondensation of solubilised lignin fractions may occur and lead to inhibition of enzymes or microorganisms. To some extent, the tendency for acid-catalysed pretreatment methods to form fermentation inhibitors can be described or predicted by calculating the severity factor. However, the relation between severity factor and inhibitor formation may differ from feedstock to feedstock. To overcome fermentability issues and reduce inhibitor formation, many pretreatment methods are conducted in two stages (with hemicellulose-derived sugars extracted after the first stage, and further pretreatment of cellulose in the second stage). Furthermore, many pretreatment methods include other measures to improve fermentability of the resulting hydrolysate, such as detoxification.

Main *lignin-removing* pretreatment methods include pretreatment with calcium- or sodiumhydroxide, ammonia pretreatments, modified sulfite pretreatments, pretreatment with oxidising agents such as ozone or hydrogen peroxide, as well as organic solvents. In general, lignin-removing pretreatment methods are conducted at lower temperatures and pressures compared to the *sugar-removing* pretreatment methods. Therefore, fermentation inhibitors found in sugar streams from these pretreatments are more likely associated to lignin degradation products rather than sugar degradation products.

Pretreatment technologies for lignocellulose that have been implemented at the commercial/industrial scale for decades are related to the pulp and paper production and include kraft pulping and the sulphite process. In general, these processes were designed for woody biomass (hardwoods, softwoods) rather than herbaceous biomass (e.g. straw, bagasse). These processes can be adapted to lignocellulose to produce fermentable sugars, and it is believed that

 

process conditions could be less intensive compared to paper making. However, the processes are capital expensive due to various chemical recovery steps.
A worldwide review of industrial activitities on lignocellulosic biomass pretreatment for biofuels and biochemical shows that steam explosion is by far the most applied pretreatment technology by industrial companies today. It should be noted however that there is no "standard" technology for steam explosion: there are various technology providers that offer reactors in different configurations, and steam explosion is often combined with a variety of chemicals-both acids and bases. In cases where companies intend to market other products besides fermentation products (e.g. lignin) companies have selected other pretreatment methods than steam explosion. The industrial activities on using lignocellulosic biomass are primarily focused on upscaling pretreatment to the pilot or demonstration scale. It is very difficult to ascertain from the outside how successful these activities are, in particular in terms of being able to operate continuously, using different feedstocks, and achieving the targeted performance in terms of product yield or costs.

As part of this review, a large number of studies that investigated lactic acid fermentation by lactic acid bacteria from lignocellulosic biomass were reviewed. Main bottlenecks encountered in the efficient conversion of lignocellulosic biomass-derived sugars to lactic acid are the resistant nature of biomass towards hydrolysis of cellulose, high costs of enzymes and inhibition by hydrolysed sugars, formation of by-products due to fermentation of pentose sugars (co-production of acetic acid), and carbon catabolite repression caused by the heterogeneity of hydrolysate-sugar composition (i.e. sequential utilization of mixed sugars in fermentation).

A key question in the assessment and comparison of different pretreatments is the resulting sugar concentration in the hydrolysate, and the requirement for concentration of those sugars prior to fermentation. This study did not find evidence of specific pretreatment technologies that may lead to high sugar concentrations, as most pretreatment evaluations found in literature are based on enzymatic hydrolysis under laboratory conditions, carried out at low solids concentrations, and hence low sugar concentrations. Therefore, which pretreatment may lead to highest sugar concentrations is a remaining research questions, and should be subject to further experimental research. Another key question is whether the fermentation will accept a mixture of C6 and C5 sugars, or if (primarily) C6 sugars are preferred. In the latter case, alternative routes to convert C5 sugars could be considered.

Literature on pretreatment of sugar cane bagasse, *i.e.* the fibrous residue obtained after extracting juice from sugarcane, for fermentation purposes is strongly based on laboratory work. Of the described pretreatments of bagasse, the majority are acid or alkaline treatments. No articles were found that describe treatments at pilot or larger scale. Enzymatic treatments are often high in enzyme dose which serves to determine the maximum degradability of the polysaccharides, rather than fermentable sugar yield under economically viable conditions. Besides sugar conversion and sugar concentration, the choice for the optimum pretreatment for bagasse should also consider the formation of fermentation inhibitors. The best results described in the literature are reached

on laboratory scale under optimal conditions; yields on larger scale will probably be lower due to unfavourable, but economic more viable, conditions.

If a well-known economic costing study for lignocellulose pretreatment from 2011 [1] is adapted to a scale suitable for a 100 kton lactic acid facility, the estimated manufacturing costs for fermentable sugars are equivalent to 252 €/ton of sugars produced, in the form of a diluted sugar stream. The manufacturing costs of this facility are made up of feedstock costs (31%), other operating costs (14% including enzymes and chemicals), and capital costs (46%). Analysis of other costing studies that apply different pretreatment routes show that there is a considerable difference in installed capital costs for pretreatment of lignocellulose. However, part of these differences are due to different costing methods applied in the studies, and a different set of pretreatment process steps included in the studies. It is therefore difficult to make a good assessment of pretreatment costs by comparing the available literature.

What is the optimal pretreatment for producing fermentable sugars from lignocellulose for lactic acid production?

Within the framework of the BE-Basic program, a pretreatment technology could be defined as optimal if a fermentable substrate can be obtained at lower than 200 €/ton dry substance (i.e. sugar) and at concentrations exceeding that what is currently achieved in experimental research, taking into account substrate choice, generation and elimination of inhibitors and impurities, possibilities for valorization of by-products, and sustainability aspects. In the review of economic studies in this report (Chapter 5), it is shown that costs calculation in general are higher (187 € to 253 €/ton fermentable sugar) compared to the 200 € benchmark. Moreover, it should be noted that there is considerable uncertainty regarding the fermentability of the produced substrate from lignocellulose, as actual fermentation tests with lactic acid-producing microorganisms have not been reported on.

The main cost factors contributing to the higher production costs are installed capital costs, feedstock costs, enzyme costs, and other operating costs such as chemicals and waste disposal. If we take these major costs factors into account, and include the uncertainty regarding substrate fermentability to lactic acid, the following would be compelling research directions to drive down the costs of producing fermentable sugars for lactic acid fermentation:

**Reducing capital costs**: reducing installed capital costs can be approached by reducing the capital costs for thermal conversion costs (estimated at 43% of capital investments, see Figure 8), pretreatment reactor (22%), as well enzymatic hydrolysis and enzyme production costs (13% and 12%, respectively).

- *Thermal conversion costs* could be reduced by co-locating the pretreatment and lactic acid fermentation plant near an existing facility for electric power generation. Alternatively, costs for installing a boiler could be reduced by selecting a pretreatment that produces a more benign residu. In general, pretreatment methods that do not involve sulfates, chlorides or alkali metals (Na, K) in the process, could lead to such improvements.

 

- Options for reducing costs for *pretreatment reactors* could be approached in several ways: by selecting continuous pretreatment reactor with a shortest residence possible, or by choosing milder pretreatment conditions that would have a lower tendency for corrosive process conditions in the pretreatment process. In general, alkaline-based pretreatment methods would serve that purpose, although there will be a trade-off between lower capital costs and higher operating costs. Finally, pretreatment reactors that can handle high solids concentration could lead to reduced costs for pretreatment reactors.
- Reducing installed capital costs for *enzymatic hydrolysis* (i.e. reactors) could be approached by reducing the total hydrolysis time needed to convert cellulose and hemicellulose to monomeric sugars, or by combining enzymatic hydrolysis and fermentation through SSF (simultaneous saccharification and fermentation) .
- Options for reducing (on-site**)** *enzyme production costs* could be realised by reducing total enzyme load (g protein/g lignocellulosic biomass), which would likely involve developing specific enzymes for a certain feedstock-pretreatment combination. In addition, employing SSF could lead to reduced enzyme loadings as well, although this has not been reported so far for lactic acid fermentation.

**Feedstock costs** could be reduced by focusing on lowest cost feedstocks, and by increasing the amount of sugars produced per ton of feedstock. In order to do this, one of the primary performance indicators in the pretreatment review is the (potential) sugar yield (cellulose and hemicellulose conversion to sugars) through pretreatment and enzymatic hydrolysis. Another strategy is to focus on lowest cost lignocellulosic biomass, such as lignocellulosic biomass residues that have negative or zero value, or secondary biomass streams that bear no logistic costs.

**Fermentability issues** could be overcome by choosing milder pretreatment conditions, in terms of temperatures and pH. This could be accomplished by limiting the severity factor of pretreatment. Another strategy is to develop innovative detoxification methods. Finally, lactic acid-producing bacteria could be developed that are more tolerant to common fermentation inhibitors, as was already successfully done in the case of ethanol-producing yeasts. *Waste disposal and chemical use costs* could be realised through focusing on pretreatments with low chemical use (or high chemical recycling rates), and milder conditions during pretreatments. Reduction of water streams could be realised by adopting high solids concentrations throughout the process, including pretreatment and enzymatic hydrolysis.

Finally, the overall costs for producing fermentable sugars from lignocellulose could be reduced by integrating the fermentable sugar production in a *biorefinery setting*. In this case, co-products such as lignin are partially, or fully used to produce other higher-valued products, instead of electricity and heat. Production of lignin-derived chemicals and materials is currently a growing, and in the longer term this might lead to economically viable options.

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# Appendix 1: Pretreatment of sugar cane bagasse

**Dilute acid**

*Aim for high sugar concentration in acid hydrolysate*

A number of the authors were only interested in the sugar yields in the acid treatment liquid and did not hydrolyse or ferment the residue any further. Using HCl as a catalyst, Hernandez found reducing sugar yields in the acid hydrolysate of 36% of the dry bagasse without significant differences of the solid/liquid ratio that ranged from 5 to 15 [233]. From the HCl concentrations based on dry matter of SCB it can be calculated that the bagasse has a dry matter content of 50%, and this results in L/S ratio's on dry bagasse of 10 to 30, xylose concentrations about 35 g/l and glucose about 10 g/l.

Chandel also used HCl and studied the release of sugars in the acid hydrolysate and found a maximum sugar yield of 30% on dry SCB with mainly C5 sugars and high inhibitor yields [232]. Different detoxification methods were therefore applied and the order of best performance was ion exchange> activated charcoal> laccase> overliming> neutralization with respectively the following ethanol yields 0.48>0.42>0.37>0.30>0.220 (g/g sugar) in a fermentation with *C. shehatae*.

Um and Sarrouh were also focussed on the xylose sugars in the acid hydrolysate. Um studied the optimal conditions for xylose production in dilute acid hydrolysate of SCB [235]. Maximal experimental xylose yield was 79% at 170 °C and 0.24% H2SO4 during 15 minutes and 76% at 200 °C, 0.22% acid during 6 minutes. No sugar concentration levels were presented. The results were fitted in a model and the predicted maximum yields obtained with that model 80 and 78% for the above stated conditions.

Sarrouh studied the production of xylitol from SCB. Xylose concentration was enhanced by post-hydrolysis of the hydrolysate of dilute acid treatment SCB [234]. Post hydrolysis lead to an increase of 18 to 24 g/l xylose. Furfural, HMF, acetic acid and phenolics showed concentrations of respectively 0.08, 0.07, 1.5 and 3.8 g/l. Evaporation to equal xylose concentration leads to lower inhibitor content for the post hydrolysate. The xylose-xylitol conversion in the xylitol fermentation increased from 71 to 76% due to post hydrolysis.

Vargas Betancur carried out an interesting set of dilute acid treatments according to a central composite design in which L/S ratio, time and acid concentration were varied at a constant temperature of 121 °C [230]. The statistical evaluation resulted in a model that was validated by an experiment. High xylose concentrations request low L/S ratios however for high hydrolysis yields the acid concentration is the most important factor and high L/S ratios are needed to get high yields. Highest xylose concentration was 82 g/l at a yield of 60%. The highest xylose

hydrolysis yield was 74% (17.1% on dry matter) at a concentration 68 g/l. The concentrations of xylose, furfural, HMF and hydrolysis yield were related to the severity factor.

Rodrigues [237] used a severity factor based on the Arrhenius equation (H-factor) to be able to scale-up the process from laboratory 25 ml, to semi-pilot 25 l, to pilot scale 350 l in different type of reactors. The equation formula of this factor is similar to the alkaline H-factor used in the pulp and paper industry, however with another frequency factor and activation energy that was determined by Aguilar [236]

$$H = \int_0^{tf} x^{\left(35.127 - \frac{13107.64}{T}\right)}$$

Rodrigues showed that with the same H-factor of 5.45 at different temperatures and different reactors similar xylose (approximately 74%), furfural and HMF concentrations were obtained. The highest xylose yield of 83% was reached in a 25 l semi-pilot reactor but furfural concentration of 2 g/l was high. At an H-factor of 5.45 xylose yield was about 74% for the different reactors. Xylose concentration was 19 g/l and total sugars 23 g/l. The concentrations of furfural and HMF were low, respectively 0.08 and 0.007 g/l. Total lignin degradation products (phenolic compounds) was about 0.3 g/l.

Augilar tested a series of conditions ranging from 2 to 6 % $H_2SO_4$ (w/v) and 100 to 128 °C. The results were used to find the kinetic parameters that were later used by Rodrigues [236] and found 2% $H_2SO_4$ and 122 °C during 24 minutes as optimum by which about 90% of the hemicellulose was hydrolysed. This condition resulted in the following concentrations xylose 21.6 g/l, glucose 3 g/l and furfural 0.5 g/l.

Pattra treated SCB with different $H_2SO_4$ concentrations at 121 °C and a solid liquid ratio of 1:15 [238]. The optimum concentration was 0.5% with a total sugar concentration of 24.5 g/l . This is about 37% of the dry matter. With 11 g/l the glucose content is remarkable high and equals or exceeds the xylose levels. Pattra assumes that this is caused by removal of the rind. About 1% of the produced xyloses was degraded, resulting in 0.12 g/l furfural. The lignin content of this material is very low however also cellulose and hemicellulose are low, but the share of not determined fraction is very high. Pattra uses this hydrolysate to produce hydrogen. The best hydrogen yield was 1.7 mol $H_2$/mol of total sugar. Besides the higher yields at low acid concentrations Pattra cites that for economic reasons it is also wise to work at lower acid concentrations to minimize corrosion problems.

Nigam used a $H_2SO_4$ concentration of 7% acid at 125 °C and a L/S ratio of 5 [239]. That gave much higher xylose and arabinose and concentrations than Pattra found, however also a much higher acetic acid concentration of 11 g/l was found. A total sugar concentration of 58 g/l was

 

reached, this corresponds to 29% of dry matter. The hydrolysate was used as substrate to grow *Candida langeronii* to produce single cell protein.

Cheng recycled acid hydrolysate to increase the sugar content[231]. In two recycles (3 stages) the reducing sugar concentration is increased from 28 to 63.5 g/l. Further recycling only increase the sugar content by a few percent. Furfural and acetic acid levels were 2 and 8.4 g/l. After detoxification by boiling and electrodialysis 90% of the acetic acid and 45% of the furfural was removed. 88% of the sulphuric acid in the hydrolysate was recovered. Sulphuric acid savings would be 55%

*Aim for high sugar concentration in liquid and enzymatic saccharification of solid*
Other authors had combined interest of hemicellulose yield in acidic pre-treatment liquid and the enzymatic saccharification of the remaining solids.

Canilha describes dilute acid treatments with sulphuric acid and found within the tested variable window the following order of effect: temperature> acid concentration > residence time [240]. Statistic significant quadratic models can describe the influence of these parameters. Based on these models highest xylose recovery is around 70%, however the xylose balance in this acid treatment showed deficiency of xylose. Canilha supposes degradation reaction leading to other compounds than furfural and condensation reactions among hemicelluloses and lignin derivatives creating pseudo-lignin insoluble compounds. Highest cellulose saccharification with the chosen enzyme preparation +Tween 20 was 45%. Canilha could reproduce these results later in a 100-litre reactor.

Jeon investigated the acid pre-treatment of different lignocelluloses at fixed conditions of 2% $H_2SO_4$ (v/v) at 134 °C during 60 minutes [241]. The acid treatment was followed by an enzymatic hydrolysis, pH correction was done with solid $Ca(OH)_2$. SCB and wheat straw gave the highest sugar concentrations and recovery yields after acid and enzymatic pre-treatments. 72% of the available sugars were recovered in the SCB hydrolysate with a concentration of 48 g/l. After liquid/solid separation fermentability of the supernatant was tested with *Zymomonas mobilis* ZM4. Sugarcane bagasse showed the highest ethanol yield equivalent to 84% of the theoretical maximum yield. Reducing the temperature of the enzymatic treatment from 60 to 50 °C resulted in a higher sugar recovery yield of 87% at a total sugar concentration of 58 g/l. In general the herbaceous raw materials yield much better than the woody biomass.

Martin investigated the dilute acid treatment of SCB, rice hulls, peanut shells and cassava stalks at 122 °C using 2% $H_2SO_4$ (w/w slurry) and a L/S ratio of 10 [242]. SCB showed the best yields of all raw materials and at a treatment time of 60 minutes enzymatic convertibility is 66% resulting in an overall cellulose conversion of 40% of the cellulose in the raw bagasse. Biomass consistency (dry matter content) in enzymatic treatment was 2%, so glucose concentration must have been

lower than 10 g/l. This is low compared to his experiments with wet oxidation and steam explosion. It is clear that this dilute acid treatment has to be optimized.

Santos studied the effect solid load, enzyme load and application of Tween 20 on the cellulose yield in enzymatic hydrolysis of $H_2SO_4$ pre-treated SCB [243]. Pre-treatment took place at 150 °C at 17.5% solid concentration during 30 minutes. Two different commercial enzyme preparations were used. As expected sugar yields increased with higher enzyme load, however for one of the preparations there was clearly a maximum in yield after which increasing the load had no positive effect. Addition of Tween 20 increased the yield with increasing load within the chosen concentration range. The highest measured cellulose conversion was 65%. In an additional experiment increasing solid load had no significant effect on yield but gave significant higher concentrations at higher loads, maximum concentration of fermentable sugars was 27 g/l.

Rocha [244] pre-treated SCB with a mixed acid of 1% $H_2SO_4$ and 1% HAc at 190°C during 10 minutes at different liquid to solid ratios (L/S). At 6.7 L/S, hemicellulose dissolution was somewhat higher than at L/S 10, but both had levels of over 90% dissolution. For glucose it is the opposite with 17.1 and 14.6% respectively. Concentration of total sugars was about 14 g/l of total sugars but based on the amount in the residue the concentration should be more as twice as high. The enzymatic conversion yielded equally for both L/S ratios with 76% cellulose conversion after 72 hours. The enzymatic conversion of L/S ratio 10 seems to be a bit faster. Glucose concentrations are not presented but as enzymatic hydrolysis was performed at 2% consistency glucose concentrations are below 8 g/l.

Chen [245] used microwave assisted dilute treatments of 5 and 10 minutes at 130-190 °C. Analysis of residues treated for 5 minutes at 190 °C showed strong degradation of hemi-cellulose, strong degradation in structure and crystallinity and decrease in particle size. No significant difference was measured in reaction time, however total energy input and time needed to reach the set point are not mentioned.

Binod treated SCB with 10% H2SO4 (w/w) in a microwave. With only 0.25g reducing sugars/g treated SCB the results was much poorer than of microwave-alkaline treated SCB [262]. Only microwave power and no temperatures are presented.

**Steam (explosion) and hot water**

*Steam and Hot Water*

Jacobsen investigated the effect of different solid concentrations on xylose yields in hydrolysates of non-catalysed (auto)hydrolysis of SCB at 200 °C. Very thin reactors were used resulting in very fast heat transfer. The consequence is that SCB must be grind to small particles like in most cases found in literature [247]. Traditional kinetic models predict that monomer and oligomers yields

 

are independent of solid concentrations however Jacobsen finds that higher yields can be reached at lower solid concentrations and monomer. Also the monomeric fraction related to the available xylose (as xylan) or as fraction of the recovered xylose is higher at lower solid concentrations. At almost every solid concentration the maximum xylose yield of 75 to 80% is reached in ten minutes for solid concentrations from 1-10%. At 0.5% solids a maximum of 86% is reached. At this maximum 70-75% of the xylose is present as oligomers. Oligomer yields seem to increase at lower solids concentrations and fall more rapidly after reaching maxima.

Silva studied the fermentation of cellulosic hydrolysates obtained by enzymatic hydrolysis of hydrothermal pre-treated SCB [248]. A part of the treated material was also delignified in an alkaline step. The hydrothermal treatments show a cellulose conversion of about 25% and a hemicellulose conversion of 68 to 89%. These sugars are lost in the hydrolysate. Lignin degradation was about 38%. After alkaline treatment the total conversion losses were 34 to 45% for cellulose, 84 to 96% for hemicellulose and 65 to 81% for lignin. The extra alkaline treatment gave higher glucose yields in the subsequent enzymatic hydrolysis than the alternatives that were only hydro thermal treated. The hydrothermal treatment at 195 °C during 10 minutes followed by alkaline treatment gave the highest glucose yield of 89% at a concentration of 86 g/l. However with the presented data it can be calculated that either this enzymatic glucose yield must be 10% higher or the concentration must be 10% lower. Overall glucose yield is 49% of the theoretical maximum. So these two pre-treatment stages gave excellent conversion of cellulose in the residue, however much of the available cellulose has been dissolved and lost in these stages. Highest ethanol production of 20.5 g/l was reached with almost complete glucose conversion and 37% of xylose conversion.

Pereira studied the rheological behaviour of the biomass hydrolysate and the enzymatic conversion of steam pre-treated SCB at 10% dry weight [249]. The energy needed with a flat blade impeller was ten times higher than for a pitched blade impeller in the beginning of the reaction. The hydrolysis result was 45% cellulose conversion in 36 hours reaching a glucose concentration of 22 g/l.

Laser compared liquid hot water treatment (LHW) with steam treatment and found better results for the LHW treatment [250]. Glucan recovery after steam and LHW under different conditions was 93% or higher. The average of the four best LHW runs resulted in SSF yields of about 83% at 20 g/l cellulose. Xylan recovery in the solids was 84%. Steam treatments could not reach these levels. Strong rate inhibition was found at furfural levels of 0.15% or higher.

Washed SCB from the sugar mill Carrasco were pre-treated with a $SO_2$ catalysed steam pre-treatment [252]. Impregnation with 6% $SO_2$ on dry matter was applied at room temperature during 30 minutes, after which the material was subjected to a steam treatment at temperatures of 180 °C to 205 °C during 5 to 10 minutes. Hydrolysis was carried out with washed pre-treated

material at 2% water insoluble solids (WIS). At higher WIS also unwashed samples were hydrolysed. A commercial mixture of celluclast 1.5L and Novozym 188 was used. Maximum polysaccharides conversion was reached at 190 °C during 5 minutes. Glucan was for 92% converted and xylan for 82 %, the latter mainly in the acid hydrolysate. The maximum overall yield was 87% at a concentration of about 12 g/l. At 8% WIS and 72 hours enzymatic the glucose yield was about 40% lower than at 2% WIS. After 24 hours of enzymatic hydrolysis this difference was even more than 40%. Due the end-product inhibition the highest yields were reached at the lowest WIS and unwashed materials had a somewhat lower glucose yield and lower initial production rate.

Soares studied the sugar yields after enzymatic hydrolysis of steam treated SCB as delivered by the sugar mill [251]. Enzyme loading and alkaline washing both had a positive effect on the glucose yield. Milling of the SCB did not significantly influence the production of glucose by enzymatic hydrolysis. Washing with 1% NaOH resulted in removal of the majority of hemicellulose and lignin and a pre-treated SCB composition of 79.5% cellulose, 8% hemicellulose and 11.5% lignin. It also led to the largest amount of about 37% glucose on dry SCB in the subsequent enzymatic hydrolysis.

*Steam explosion*

Dias carried out a simulation of integration of first and second-generation bioethanol production [256]. The second-generation part included different pre-treatment methods of surplus bagasse and 50% recovery of trash from the field. An economic risk analysis showed the best results for steam explosion treatment, high solids loading for hydrolysis and 24-48 hours hydrolysis.

Ewanick showed high cellulose and ethanol yields after $SO_2$ impregnation and steam explosion [253] with two SCB samples. Water soaked SCB gave a better result than dry SCB probably due to a better penetration of $SO_2$. After this pre-treatment 84 and 88% of glucan was recovered in the residue and respectively 33 and 29% of the xylan. Enzymatic hydrolysis of the residue showed over 90% of cellulose conversion at a sugar concentration of about 23 g/l (22 g/l glucose). SSF showed over 80% of the theoretical ethanol yield.

Martin used different impregnating agents in steam explosion of SCB and tested the fermentability after enzymatic hydrolysis [254]. Both $SO_2$ (SD) and $H_2SO_4$ (SA) were applied at 1% w/dry SCB also non-impregnated (NI) SCB was treated. Treatment temperature and time were 205 °C and 10 minutes. $H_2SO_4$ showed high glucose and low xylose yield and such a high inhibitor release that fermentation was not possible. Sugar and acetate yields in the steam explosion hydrolysates are higher for $SO_2$ impregnated SCB than for non-impregnated SCB. With 1% on biomass the glucose production in SD en NI steam treatment was similar low. After enzymatic hydrolysis glucose yield in hydrolysates were respectively 35.2 and 33 g/100 g SCB differences of maximal 8% with a maximum cellulose conversion of over 80%. Xylose yields

 

were 5.9 to 16.2 g/100g SCB. Total sugar concentration was about 25 g/l and the maximum sugar yield of 52.9 g/100g dry SCB is lower than other authors find with optimized $H_2SO_4$ impregnations. Fermentation of NI en SD showed the same characteristics a similar lag phase, total fermentation of glucose and lower production rate than the reference fermentation with pure sugars. The lower yield of NI compared to SD is probably more than compensated by the cost reduction of not using SO2 and the additional safety measurements.
Martin also compared steam explosion without the addition of chemicals [257] with wet oxidation (WO) at pH 10. The yield on cellulose was 58% for steam explosion, that of wet oxidation was 54%. A subsequent SSF gave a cellulose conversion yield of 482 g/kg while wet oxidation resulted in 829 g/kg. Fermentation inhibition was tested with the prehydrolysates and steam explosion hydrolysate was found to inhibit most.
In a second comparison of steam explosion with wet oxidation Martin found again better results for WO with 3.3% w/w of $Na_2CO_3$ than for steam explosion without addition of chemicals [258]. Overall cellulose conversion was 40% for STEX and 53% for WO at concentrations of 4.4 and 8 g/l respectively .

Geddes pre-treated SCB with 1% Phosphoric acid at high temperatures during 10 minutes [255]. The reactor was equipped with large valves to minimize time needed to heat up and discharge. A total cycle lasted 10 minutes from which 9.5 minutes were at the right temperature. The yield in released sugars was equal for 180 and 190 $^oC$ but as can be expected the amount of inhibitors is much higher at 190 $^oC$. Total sugars recovered by phosphoric acid pre-treatment at 180 and 190 $^oC$ equals 70% of the bagasse dry weight and about 98% of total sugars in the initial untreated bagasse. After an initial liquefaction step SSF was applied with a hydrolysate resistant *Eschrichia coli* strain. After the initial liquefaction step, sugar concentration was about 50 g/l. Up to 207 kg ethanol/ton bagasse was produced without a solid liquid separation, which equals 57% of the maximum theoretical yield based on composition of initial bagasse.

Rocha investigated the composition of 20 most diverse varieties and origins of natural sugarcane bagasse [229]. Also the yields and composition of these varieties after a standard steam explosion treatment were determined. Moreover also the yields and composition of these steam exploded SCB samples after a subsequent alkaline delignification were determined. Steam explosion was carried out in a 200 litre reactor at 190 °C during 15 minutes and the subsequent alkaline delignification was carried out at 100 °C during 1 hour in a 1% NaOH solution w/v at a L/S ratio of 1:10. The mass yields in the consecutive treatments were 66.1 and 51.5 % leading to an overall cellulose recovery of 68.5% in these pulps. Standard deviations were low and cellulose contents in SCB and treated SCB were 43.1±1.4 and 86.8±2.7 showing excellent reproducibility of the processes. Average hemicellulose and lignin content was respectively 4 and 6%.

**Alkaline**

Chang studied the effect of lime concentrations, water loading, temperature and particle size in lime pre-treatment of SCB [265]. The pre-treated material was enzymatically hydrolysed with a mixture of cellulase and cellobiase during 72 h. Above 10% lime concentration yield only increased slightly. Water loads of 6 to 14 g/g dry biomass gave comparable results but the lower loads gave thick pastes that might create handling problems. Particles that passed a 40-mesh screen did not give higher yields than particles between 40-mesh and 1x1 mm. After a pre-treatment with 10% lime (on d.m.) during 1 hour at 120 °C enzymatic hydrolyses showed 60% conversion of glucan and 80% conversion of xylan, resulting in a total sugar yield of about 68%. The large effect on xylose is thought to be caused by removal of the acetate groups, which improve the accessibility for hydrolytic enzymes. A lime recovery study with ten washing steps and carbonating the wash water with $CO_2$ showed 86% recovery of the calcium. Precipitation and burning will give lime again. Losses of glucan and xylan in lime treatment were negligible

Fuentes investigated the kinetics of SCB lime treatment [266] and found a quadratic model for glucose yield with time, temperature and lime concentration. Optimization performed using this model shows a maximum glucose yield of 0.23 g/g SCB and total reducing sugars of 0.41 g/g SCB at a lime loading of 0.4 g/g SCB during 90 hours at 90 °C. The enzymatic hydrolysis was carried out at an extreme low dry matter content of 0.3% resulting in sugar concentrations of about 1.5 g/l. The work of Fuentes shows that at lower temperature, but at higher lime loadings and longer treatment times than Chang used, a substantial amount xylan dissolves, which is lost for further hydrolysis and fermentation.

Fu finds that SCB treated with 10% $Ca(OH)_2$/g dry SCB at 100 °C during 2 hours results in a better anaerobic fermentation into carboxylate salts than 30% aqueous ammonia treated SCB at 55 °C for 24 hours with a loading of 10 ml/g biomass [297].

Rabelo studied the effect of very high loads of lime ranging from 0.15 to 0.65 g/g dry SCB on screened (0.15-1.4 mm) and unscreened SCB [267]. Best conditions for non-screened SCB were 54 hours at 80 °C and a lime loading of 0.25 g/g SCB resulting in total reducing sugars (TRS) yield of 0.39 g/g and glucose yield of 0.20 g/g SCB. This corresponds to a glucan conversion of 46% and total polysaccharide conversion of 58%. Best conditions for screened SCB were 65.6 hours at 70 °C and a lime load of 0.4 g/g SCB resulting in a TRS yield of 0.367 g/g SCB and a glucose yield of 0.218 g/g. This corresponds to a glucan conversion 58% and total polysaccharide conversion of 61%. Time and temperature are in that order the most important factors. Highest yields were reached at high pre-treatment time combined with low temperature and at low pre-treatment time and high temperature. After lime treatment the SCB was washed before enzymatic hydrolysis at a very low consistency of <2 g/l took place.

 

Both glucose and TRS yields correspond well with those found by Fuentes but are much lower than those found by Chang. It seems that the higher temperatures and short pre-treatment time used by Chang has an advantage, however Chang used higher enzyme loads in his experiments. Rabelo also studied the release of sugars in lime versus alkaline peroxide pre-treatment of screened (0.25 -1.4 mm) and unscreened SCB [268]. Lime treatment resulted in higher glucan conversions and TRS yields than alkaline peroxide treatment. Effects of screened and unscreened can be different at each individual process condition. Highest glucose yield is found in the lime treatment of screened SCB. Highest Total reducing sugars were found highest in the lime treatment of non-screened SCB.
Remarkable is that TRS and glucose yields of the lime treatments are much higher than in Rabelo's experiments of 2009 where the same experimental procedures are used. In the most recent article Rabelo describes the ethanol production of SCB treated with alkaline peroxide (pH 11.5) and with lime [259]. In this article the alkaline peroxide data from the 2008 article [268] is compared with the lime data of the 2009 article [267] and now declares the alkaline peroxide treatment as the best treatment with higher glucose amounts after enzymatic hydrolysis. This creates doubts on the accuracy of the sugar measurements or calculations especia lly for the high yields in the 2008 article. Also the chemical analysis of the raw material differs so mewhat with the previous described. Highest yields are reached with high peroxide concentrations. Time and temperature do not have a significant influence at these high peroxide levels. Therefore treatment at 25 °C for 1 hour yielded a glucose amount corresponding to 76% conversion of the cellulose. This high yield was reached at low enzymes dose however the peroxide dose of 1.8 g/g dry mass was extremely high and no information is given on the fraction that is consumed. Also very high were the Liquid/Solid ratios in both chemical pre-treatments as well in enzymatic hydrolysis. Hydrolysates had therefore to be evaporated to a glucose concentration of 6 g/l before ethanol fermentation. Compared to fermentation of pure glucose this concentrated hydrolysate did not show any inhibition. The very high levels of $H_2O_2$ will make the process very costly and not economic viable lower $H_2O_2$ levels resulted in low sugar yields.

Beukes pre-treated sugarcane bagasse with 0.4 g lime/ g SCB and subsequently washed before a hydrolysis with recombinant enzymes [260]. The optimal enzyme combination has a hydrolysis rate with treated SCB that is 6.5 times as fast as the optimal enzyme combination with untreated SCB. The total conversion is 4 times as high. No yield figures were given.
Beukes also studied the effect of $NH_4OH$ and NaOH treatments and found 13 and 9 fold increase in hydrolysis rate with optimal recombinant enzyme mixtures [261] with total sugar yields of 71 and 60% on dry SCB. SCB was washed after alkaline treatment.

Hernandez also studied alkaline pretreatment under the conditions of 4 hours at 121 °C with 10% NaOH on dry bagasse [233]. This treatment was followed by pH adjustment and enzymatic treatments during 4 hours at 55 °C with 0.19 ml/g bagasse of different commercial Novozymes

 

enzymes. Reducing sugar yields of 11-20% on dry bagasse were found. An optimal mixture of Celluclast, Novozymes and Viscozyme L. at a total enzyme load of 0.57 ml/g bagasse yielded more than 50% reducing sugars at a concentration of about 70 g/l.

Binod used alkali in combination with microwave (also with acid ) [262]. After enzymatic hydrolysis, reducing sugar yield on pre-treated SCB was 0.67g/g pre-treated bagasse. If the alkaline treatment is followed by an acid microwave treatment, enzymatic yield increases to 0.83 g reducing sugars/g pre-treated bagasse. It is not clear what the energy input on dry matter SCB is. Thoroughly washing was applied after the chemical-microwave treatment.

Cheng describes five stages of recycling of the alkaline liquid in alkaline/oxidative pre-treatments [263]. In spite of somewhat higher cellulose losses in the alkaline liquid a 1% NaOH+ 0.3% $H_2O_2$ treatment resulted in a much higher cellulose conversion of 70% in the subsequent enzymatic treatment than a 1% NaOH without hydrogen peroxide that showed 55% conversion. If 0.6% $H_2O_2$ was added the conversion increased to 78.1%. The consumption of NaOH is around 60% and stays on this level during the four following recycles. The consumption of $H_2O_2$ it is about 92% and increases to over 99% in the fourth recycle. In the recycling procedure NaOH and $H_2O_2$ concentrations were brought back to the desired level. With two recycles the composition of the SCB residues was the same however in the following stages the lignin and hemicellulose removal dropped somewhat resulting in lower cellulose conversion. In a subsequent SSF 79% of the glucose was converted to ethanol giving a yield of 0.2 g ethanol/g bagasse.

Dawson compared alkaline peroxide treatment with dilute acid treatment with $H_2SO_4$ both probably at room temperature [246]. Optimal acid treatment was found at 0.8M $H_2SO_4$ during 24 hours. It resulted in 2.8 times as much ethanol than the optimal 2% alkaline peroxide treatment during 48 hours. However both yields are very low with respectively 0.013 and 0.04 g ethanol/g bagasse.

Velmurugan investigates the process variables in sono-assisted alkaline treatment of SCB [264]. These variables are particle size, S:L ratio, NaOH concentration (w/v), temperature and treatment time. Within the chosen ranges all changes in process parameters had a significant effect on delignification with sonication time as the most significant and particle size as the least significant variable. For reducing sugars yield particle size is a more important factor and after sonication time the most significant. Highest experimental delignification was 78% with a cellulose recovery of 98% at 0.43 mm, SLR 1:20, 2.5% NaOH, 60 $^oC$ and 20 minutes. The reducing sugar yield after saccharification was 89 %. Respons Surface Methodology gave a maximum respons of 96% reducing sugar yield after enzymatic hydrolysis at a pre-treatment of 0.27 mm particle size, SLR 1:25, 2.9% NaOH, 70 $^oC$ and 47 minutes. This treatment combines high lignin delignification with high cellulose recovery and performs better than 2% NaOH at 121 $^oC$ during 1 hour [298]. This type of treatment shows high efficiency at low temperature

 

however energy input is extreme at this scale and design of a suitable reactor and energy optimization need to be done. The sono-alkaline treated SCB was washed before enzymatic hydrolysis.

Velmurugan  had previously studied the sono-assisted acid hydrolysis of SCB that has been treated with sono-assisted alkali. Both treatments were carried out at 50 °C [270]. Alkaline treatment was done with a L/S ratio of 20 and 2% NaOH solution resulting in 75% lignin removal and 21 % hemicellulose removal. Sono- acid treatments were carried out with this material at different L/S ratios, time and $H_2SO_4$ concentration. The optimum conditions are L:S ratio of 20:1 at 2% $H_2SO_4$ (w/v) during 45 minutes resulting in 69 and 81% of theoretical yield of hexose and pentose respectively. In the following fermentation ethanol yield was 92% of the theoretical yield. The saccharification yields were lower than reported in the article of 2012 [264] where more severe conditions were applied.

Kim pre-treated SCB for a long period with ammonium hydroxide at 30 °C without agitation in closed bottles and studied the delignification, so this was a kind of alkaline ensiling [271]. The tested concentrations of $NH_4OH$ were 0, 0.03 and 0.3%. Due to unfavourable pH conditions microorganisms were unable to reproduce after 10-days of storage. With 0.03% $NH_4OH$ delignification became visible after 20 days and with 0.3% $NH_4OH$ after 10 days. Up to 45% delignification was reached after 40 days at 0.3% $NH_4OH$. No enzymatic hydrolysis or fermentations were applied.

Krishnan pre-treated SCB and cane leafs with the ammonia fiber expansion (AFEX) process [272]. Small-scale experiments (22 ml) show increasing glucan and xylan conversion (after enzymatic hydrolysis) with increasing temperature, with maximum values at the maximum applied temperature of 140 °C at a 1:1 ammonia dose on biomass. At 2:1 ammonia supply the maximum glucan and xylan conversion of about 86% was already reached at 120 °C. The use of extra xylanases improved the conversion of xylan and it also had a small positive effect on the glucan conversion. Larger scale pre-treatment (2 L) followed by high solid loading enzymatic hydrolysis and fermentation showed glucan to glucose conversion of about 70%, but the total glucan conversion including the gluco-oligomers was about 90%. Total xylan conversion was about 76%. The total sugar concentration was about 100 g/l. Fermentation of this hydrolysate with a recombinant yeast yielded 21.5 kg ethanol per 100 kg dry SCB at a concentration of 33.7g/l with an ethanol yield of 92%.

Prior investigated the enzyme activities (effect) of four commercial xylanase preparations in combination with Spezyme CP 10FPU/g glucan and Novozym 188 (20 CBU/g glucan on ammonium treated and AFEX treated SCB [273]. Multifact and Biocat boosted the hydrolysis not only in xylan conversion but also substantial in glucan conversion. Greater amounts of both glucose and xylose were released from AFEX treated bagasse than from $NH_4OH$ treated bagasse.

 

Glucan conversion was 73% with Biocat at 50% protein level of the total protein level of spezym CP/Novozyme 188 mixture. Conversions were lower than reported by Krishnan, which probably can be explained by the difference in temperature during the AFEX treatment, 100 and 140 °C respectively.

**Wet oxidation**

Martin studied the cellulose convertibility and ethanol fermentation of SCB pre-treated with 15 minutes wet oxidation (WO) at 195 °C and 12 bar $O_2$ or 10 minutes steam explosion (STEX) at 205 °C [257]. WO was carried out with and without addition of $Na_2CO_3$ or $H_2SO_4$. STEX was carried out without any addition of chemicals. Cellulose recovery of STEX was lower than for the three WO treatments with highest recovery of 92.8% in the treatment with $Na_2CO_3$. The enzymatic convertibility was increasing with increasing pH and went up to 79.2 % of the available cellulose in washed pre-treated solids, resulting in a total conversion of 73.7% of the cellulose in raw bagasse at about 11 g glucose/l. Enzymatic conversion of non-washed slurries resulted in 4-18% inhibition. Formic acid is likely the main inhibitor as the inhibition is strongly correlated to the concentration of formic acid. With cellulose conversion of 829 g/kg in SSF of whole pre-treated and washed slurry (about 26 g/l cellulose) again the best results were gained with the $Na_2CO_3$ added WO with faster and more ethanol production than neutral WO and steam explosion (482 g/kg). Overall cellulose conversion was 57 % for WO and 24% for STEX. These conversion yields were higher than in the separated hydrolysis and even higher than with the washed material. However, the maximum yield in this work was lower than the more than the 900 g/kg cellulose that Laser [250] found in his work on liquid hot water treatment. However the SSF of Laser lasted 14 days and the medium was enriched with yeast extract and peptone. Martins SSF lasted 5 days only.

Martin studies the effect of different pH, temperature and time conditions with wet oxidation of SCB [274]. Due to removal of lignin and hemicelluloses the highest cellulose enrichment was found with $Na_2CO_3$ at 195 °C during 15 minutes and pH 10 with a concentration of almost 70% of the treated SCB. Cellulose recovery was 92% and hemicellulose dropped to 7% recovery under these conditions and almost half of the lignin was removed. Degraded hemicelluloses are mainly present as oligomers. At 185 °C increasing pre-treatment time had more effect than pH adjustment. The mildest condition resulted in the lowest amount of non-cell wall material (NCWM) in the solid fraction. Carboxylic acids are the main degradation product with formic acid as the main acid in most treatments. Furfural is low in most treatments and longer time and low pH gave higher concentrations. Phenolic degradation products were found in the range of 1.3-to 4.0 g/100 gram of dry SCB. With 75% the enzymatic convertibility was highest for the washed and most enriched sample (pH 10 at 195 °C during 15 minutes). Not washing of the slurry resulted in lower convertibility. Due to the high amount of oligomers, the xylan conversion was higher for the unwashed samples than for the washed. The overall cellulose conversion was 69% at a concentration of about 10 g/l.

 

Martin compares wet oxidation treatment at different oxygen pressures on SCB, rice hulls, peanut shells and cassava stalks [275]. 3 bar oxygen pressure results in general in higher glucan and xylan recoveries and less degradation products than 12 bar of oxygen pressure. SCB gave the highest yields and especially in the following enzymatic treatment the convertibility was much higher than of the other materials. Enzymatic conversion was with all raw materials higher for 12 bar than for 3 bar oxygen. For SCB this was 44% more, resulting in the highest total conversion of 56.5% of cellulose in the raw bagasse resulting in a concentration about 5 g glucose/l. This is lower than in previous described experiments were the maximum conversion yield was 73.7%. But it must be noted that in these previous experiments the SCB was from another harvest and the pre-treatment time was 5 minutes longer and that SSF fermentation resulted in even more conversion. Oxygen pressures were applied before heating up the reactor.

In a separate study Martin studies the release or forming of phenolic compounds in wet oxidation and steam explosion [299]. Under the chosen conditions WO produces higher concentrations of total phenolic compounds and different composition of the phenols than steam explosion does.

Martin compared wet oxidation of SCB with $Na_2CO_3$ supply with steam explosion [258]. Again WO gave higher cellulose recovery (93%) than STEX (81%) and a better cellulose conversion in the following enzymatic hydrolysis (57%). Overall glucose yield was 53% on cellulose in raw bagasse. The glucose yields of both treatments however are much lower than in previous experiments. The author attributes this to the difference in raw material, which was harvest in another year and on another place. During WO aliphatic acids were produced in high amounts and the production of furan aldehydes was very low, whereas during STEX the production aliphatic acids was low and furan aldehydes were produced in considerable amounts.

**Organosolv**

Mesa did experimental work on the optimization of enzymatic hydrolysis of SCB treated with a 50% ethanol/water mixture with as catalyst $H_2SO_4$ or NaOH on a 50-liter scale [276]. $H_2SO_4$ gave higher glucose yields than NaOH. It was found that cellulase loadings of 15 and 25 FPU did not differ significantly. The same was found for the 10 and 15 ratios of cellulase/β-glucosidase. Substrate concentration had an effect on the glucose concentration but not on the glucose yield. The addition of the surfactant Tween 20 increased glucose concentration and yield. A pre-treatment at 175 $^{o}$C with 1.25% $H_2SO_4$ as catalyst during 60 minutes with Tween 20 in the enzymatic hydrolysis resulted in a glucose yield of 25.1 g/100g material at a concentration of 29 g/l. This yield corresponds to a cellulose conversion of 55%. Fermentation yielded 92.8% ethanol of the theoretical maximum yield after 24 hours.

Mesa also studied a two-stage process in which the first stage was an acid pre-treatment and the second stage an organosolv ethanol process with NaOH. The first stage solubilizes the hemi-

cellulose fraction and creates the possibility to use this fraction as a substrate for other products. Moreover this stage can improve the organsolv stage and it contributes to a decrease in the use of chemicals. Mesa used a standard acid first stage and studied the effect of changing the temperature, the ethanol concentration and the treatment time of the organsolv pretreatment on enzymatic hydrolysis of depithed SCB [278]. In the acid pre-treatment about 73% of the xylan, less than 1% of the lignin and more than 80% of the unidentified components was removed resulting in glucose and lignin enriched SCB. The organosolv further enriches the glucose and lignin concentrations about 10% due to further dissolving of xylan. Cellulose, xylan and lignin recovery after these two pre-treatments was 87, 13 and 82%. Of the tested conditions, the condition with the highest temperature, time and ethanol concentration (195 °C, 60 minutes and 30% ethanol) gave the highest glucose concentration of 67.3% glucose in the residue. This was easily hydrolysed with a total yield 29.1 glucose/100g SCB at a glucose concentration of 18 g/l. Overall conversion of the cellulose in SCB was 58%. Mesa always uses the names of the monomers instead of polymer saccharides, this is confusing and leads to wrong conclusions of the readers. The yield is higher than in the previous described organsolv treatment however an extra process stage is involved.

Mesa studied different fermentation processes on SCB treated with acid at 175 and 120 °C before ethanol organosolv treatment [277]. These fermentations were separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) and pre-saccharification followed by SSF (PSSF). The order in best performing is PSSF>SHF>SSF with 68.4, 66.1 and 59.4% of theoretical yield based on glucose content in raw material and these yields are 198, 192 and 172L ethanol per ton SCB.
Depending on the chosen enzymatic hydrolysis process parameters, glucose concentrations varied between 33 and 72 g/l and sugar yields (glucose +xylose) between 23 and 35 g/100 g SCB

Sindhu investigated the effect of several organic solvents and organic acids on SCB [279]. Treatment with formic acid yielded higher amounts of reducing sugar than glycerol, acetone, methanol and acetic acid. Optimal conditions were 60% formic acid concentration, 0.6% $H_2SO_4$ as catalyst, 15% solid loading, particle size >0.6 mm, 121 °C and 90 minutes. After 24 h fermentation 18.5 g/l ethanol was produced corresponding to an overall efficiency of 48%.

Kuo pre-treats SCB with N-methylmorpholine-N-oxide (NMMO), a cellulose dissolution solvent used in the Lyocell process for cellulose fibre preparation [280]. 10% weight was lost of SCB dissolved in NMMO and regenerated afterwards. The cellulose part of that loss is not mentioned neither was the original chemical composition. The chemical composition after regeneration is close to that of the mean in Table 4. A 95% cellulose hydrolysis after NMMO and enzymatic hydrolysis is claimed. From these figures it can be calculated cellulose conversion yield into glucose was at least 85%. The concentration of reducing sugars was about 6 g/l. In SSF the glucose to ethanol conversion yield was approximately 86%. The total ethanol yield was 0.15 g/g

 

dry bagasse. In spite of the high conversion yields the ethanol yield based on dry bagasse is low, suggesting a low glucan content in the original bagasse or substantial cellulose loss in the NMMO treatment

Zhao studied the effect of L/S ratio, temperature, time and peracetic acid concentration (PAA) in pre-treatment of SCB in peracetic acid with sulphuric acid as catalyst on enzymatic hydrolysis [282]. He found better conversion with PAA than with $H_2SO_4$ and NaOH treatments under the same conditions, but optimal conditions for $H_2SO_4$ and NaOH are at much higher temperatures. Optimum conditions were found at 50% $C_2H_4O_3$ at 80 °C and a L/S ratio of 6 during 2 hours. Delignification was about 90%. Resulting in over 80% conversion of original cellulose (in raw bagasse) in glucose by enzymatic hydrolysis at 20 FPU cellulase during 72 hours. Overall cellulose conversion was about 67%.

**Miscellaneous**
Kimon pre-treats SCB with ionic liquid ([C4mim]Cl) in the temperature range of 130 to 160 °C. At 160 °C or at long treatment times at 150 °C, the unrecoverable fraction becomes too high. At 150 °C during 90 minutes 85% of the solids are recovered from the ionic liquid. Already after 3 hours of enzymatic hydrolysis 100 % saccharification of glucan is reached (78% of initial glucan). This result is far better than the 31% with a dilute acid treatment (7.5% w $H_2SO_4$ /w dry SCB during 10 minutes at 160 °C). After 121 hours enzymatic treatment of the acid pre-treated SCB 72% saccharification was reached [283].

Yoon compared ionic liquid treatment with alkali and with acid treatments [284]. The ionic liquid treatments [EMIM]oAc resulted in much higher reducing sugar yields after enzymatic hydrolysis than [BMIM]Cl and [EMIM]DEP. Yoon concludes highest reducing sugar yields after enzymatic hydrolysis for alkali followed by ionic liquid and acid. Reducing sugar yield was 73% [EMIM]oAc and 71% (57 and 56% on dry bagasse) for the alkaline treatment. However the calculated energy requirement for the alkali treatment is more than twice than that of the ionic liquid treatment. Acid yield seems to be very low due to high losses in the acid treatment.

Mendes pre-treats the SCB with a chemi thermo mechanically (CTM) process [285]. SCB impregnated with NaOH or NaOH combined with Sulphite were treated during 2 hours at 120 °C. NaOH concentrations were 5% and sulphite concentration was 10% on dry bagasse. After washing the treated bagasse was mechanically disrupted in disk refiner as commonly used in pulp and paper processes. Refining of untreated SCB did not result in better cellulose conversions however it provide a homogeneous material for subsequent enzymatic hydrolysis. From the experiments it was obvious that cellulose conversion increases with increasing lignin and hemicellulose removal. The combined NaOH and Sulphite treatment resulted therefore in the highest cellulose conversion of 85 % (on cellulose in pretreated samples) after 96 hours after

enzymatic hydrolysis. Cellulose conversion into glucose was 79% of the original present cellulose in SCB.
Da Silva studied the improvement of sugarcane bagasse and sugarcane straw by ball milling (BM) and wet disc milling (WDM) without applying any chemical pre-treatment. Bagasse was more susceptible to BM than straw, while straw was more susceptible to WDM than bagasse. Enzymatic hydrolysis yield of bagasse after BM was 84 and 77% for glucose and xylose respectively. With straw these maxima were 82 and 62%. The enzymatic glucose yields after WDM were 49 and 82% for bagasse and straw respectively. BM decreased the crystallinity strongly however it took more time and thus energy for the straw sample. BM completely disrupted the native structure of bagasse within 30 minutes. WDM showed defibrillation and reduction of fibre length. With S.cerevisiae IR-2 strain about 90% of the theoretical ethanol yield (based on glucose) was reached in 24 hours, which was even a little bit higher than the reference. The ethanol yield on total sugars was 56%. By using the C5/C6 strain MA-RA4 of the same yeast about 80% yield on total sugars was reached with of course a substantial increase in ethanol concentration. The WDM energy consumption with straw was 40 MJ/kg biomass, which corresponds to 11000 kWh/ton. An enormous amount of energy however no chemical pre-treatment is required.

Xu characterizes the alkali and acidic organic solvent-soluble hemicellulose polysaccharides in SCB [287]. Typical laboratory procedures are used as dewaxing the SCB with toluene-ethanol and lignine removal of the alkali resistant hemicellulose with acidic dioxane. The main result is that the post treatment with dioxane resulted in substantial degradation of the alkali insoluble hemicellulose. The alkaline dissolved hemicelluloses were more linear and acidic than those of the subsequent acidic dioxane.

 