Saccharification of lignocellulose

Student: Mohamed Warsame, Malmö University

Supervisor: Bengt Danielsson
Saccharification of lignocellulose

Warsame M. Sacchrification of lignocellulose. *Degree Project, Biomedical Technology 30 Credit points.* Malmö University: Health and Society, Department of Biomedical Laboratory Science 2012.

The increasing energy demand and the anticipated decline in crude oil production has led to an immense search for new energy sources. Plant cell walls contain lignocellulose that conserve great amounts of energy. These polysaccharides are of high importance for the search of renewable energy sources.

Pretreatment of the cell wall is necessary in order to hydrolyse it to its component sugars. Once degraded to monomeric sugars it can be fermented to either ethanol or biogas through established fermentation technologies. The aim of this thesis was to compare and evaluate some of the methods used for sacchrification of lignocellulose. Three treatments where compared to determine which is highest yielding. These are enzymatic hydrolysis, microwave irradiation and steam explosion.

Wheat straw was used as substrate and hydrolysed by three commercial enzyme mixtures. Samples were pretreated before the enzymatic reaction with either microwave or steam explosion. Results showed that a treatment of either microwave irradiation or steam explosion combined with enzyme hydrolysis gives the highest yield in monomeric sugars. The conclusions that can be drawn are that mechanical pretreatment increases yield drastically but is insufficient in its self. Further enzymatic treatment of wheat straw is necessary to obtain high amounts of simple sugars.

*Keywords:* Cellulose, Hemicellulose, Lignocellulose, Pretreatment, Saccharification, Wheat straw
Degradering av lignocellulosa


Den ökande efterfrågan på energi och den förväntade nedgången i råoljeproduktion har lett till ett enormt sökande efter nya energikällor. Cellväggen i växter består till stor del av lignocellulosa som i sin tur innehåller cellulosa och hemicellulosa. Dessa polysackarider är av stor betydelse för sökandet efter förnyelsebar energi.

Cellväggen måste förbehandlas innan den kan brytas ner till enkla sockerarter. Efter nedbrytning kan monosackariderna användas till produktion av etanol eller biogas genom väl etablerade fermenteringstekniker.

Syftet med denna studie var att jämföra och utvärdera några metoder som används vid degradering av lignocellulosa. Tre behandlingar har jämfört för att se vilken som ger mest avkastning i form av monosackarider. Vetehalm användes som substrat och hydrolyseras med hjälp av tre kommersiella enzymblandningar. Proverna förbehandlades före den enzymatiska reaktionen med antingen mikrovågar eller ångexplosion.


Nyckelord: Cellulosa, Hemicellulosa, Lignocellulosa, Förbehandling, Försockring, Vetehalm
Contents

Abstract .................................................................................................................. 2
Abstrakt .................................................................................................................. 3
Contents .................................................................................................................. 4
Introduction ............................................................................................................. 5
   Lignocellulose ................................................................................................. 5
   Lignocelluso as energy feedstock ................................................................. 7
   Lignocellulosic enzymes .............................................................................. 8
      Cellulases .................................................................................................... 8
      Hemicellulose ............................................................................................ 8
Pretreatments ....................................................................................................... 8
   Microwave ....................................................................................................... 9
   Steam explosion ............................................................................................ 10
Analysis ................................................................................................................. 10
   Enzyme thermistor ....................................................................................... 10
   HPLC ............................................................................................................... 10
Material and method ............................................................................................ 12
   Enzymatic treatment of filter paper ............................................................ 14
   Enzymatic treatment of wheat straw ........................................................... 14
   Microwave preatretment .............................................................................. 14
   Steam explosion ............................................................................................ 15
   Enzyme thermistor ....................................................................................... 15
Material ................................................................................................................ 12
   Equipment ..................................................................................................... 12
   Enzymes ....................................................................................................... 13
   Buffers .......................................................................................................... 13
Result ..................................................................................................................... 16
   Effect of cellulose being conserved in lignocellulose .................................. 16
   Microwave ..................................................................................................... 17
      Time ........................................................................................................... 17
      Power ........................................................................................................ 18
   Steam explosion ............................................................................................ 18
   Summary ......................................................................................................... 20
Discussion ............................................................................................................. 19
   Effect of cellulose being conserved in lignocellulose .................................. 19
   Microwave ..................................................................................................... 19
   Steam explosion ............................................................................................ 19
   Confounding factors ..................................................................................... 20
   Conclusion ..................................................................................................... 20
Acknowledgement ............................................................................................... 21
Reference: .......................................................................................................... 22
Introduction

Lignocellulose is both more abundant and cheaper than any other energy-bearing polymer on earth. Degradation of lignocellulosic material gives monosaccharides that can be used to create ethanol and biogas through fermentation. Lignocellulose is also the most recalcitrant biopolymer to degrade. The cellulose in lignocellulose is mostly in crystalline formation meaning that the glucose units in cellulose are in perfect alignment. [1]

Lignocellulose

Lignocellulose is an organic and renewable material found in plant cell walls. Lignocellulose is the most abundant organic resource on soil.[2] Agricultural industries such as forestry, paper and food produce numerous tons of lignocellulose residue. Lignocellulose is treated as waste in many countries despite its high potential value as energy production feedstock. [1] Even in countries where lignocellulose residue is reused it’s only a small amount of the total waste production being used as renewable energy. [2]

Table 1. Table of lignocellulosic residue world-wide from different agricultural industries[2]

<table>
<thead>
<tr>
<th>Lignocellulosic residues</th>
<th>10^6 ton/year</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton fiber</td>
<td>17-20</td>
<td>SICA (2005).</td>
</tr>
<tr>
<td>Bean straw</td>
<td>4.9-5.9</td>
<td>COFEMERMIR (2006).</td>
</tr>
<tr>
<td>Coffee straw</td>
<td>1.6-1.9</td>
<td>FIMARC (2005).</td>
</tr>
<tr>
<td>Almond straw</td>
<td>0.4-0.49</td>
<td>Agroalternativo-Argentina (2005).</td>
</tr>
<tr>
<td>Hazelnut husk</td>
<td>0.2-0.24</td>
<td>FIA (2005).</td>
</tr>
<tr>
<td>Sisal a henequen straw</td>
<td>0.077-0.093</td>
<td>FAO-esc (2008).</td>
</tr>
</tbody>
</table>

The quantity of lignocellulosic residue has increased immensely due to the expansion of the agricultural industries.[2] The summary in table 1 shows us that sugar cane bagasse, maize straw, rice shell and wheat straw are particularly abundant and together comprising a whopping 787 million tons a year of waste that could be reused as energy feedstock. [2]
Structure and composition

Plant cell wall consists of one layer primary cell wall and three layers secondary cell wall. Contrary to the primary cell wall the secondary layers contains lignin, hence the name lignocellulose. Lignin gives the plant a tough structure allowing high plants to stand. Lignin also prevents the cells from bursting because of the high pressure of water being transported upwards. Other than lignin, lignocellulose also consists of two other polymers. These are cellulose and hemicellulose. Cellulose and hemicellulose are two polysaccharides that are connected through hydrogen and covalent bonds. These polymers, together with proteins and waxes that can be found in plant cell wall, make up the secondary cell wall. [3]

Cellulose

Cellulose is made up of two long D-glucose chains linked together by a $\beta$ 1, 4 glucoside linkage. Cellulose polymers are bonded to hydrogen or van der Waal forces. [1] Most of the cellulose is in crystalline formation even though some cellulose fibrils can be in amorphous formation. [3]

Hemicellulose

Hemicelluloses are complex heterogeneous polymers consisting of polysaccharides. The polysaccharide comprises of several pentoses and hexoses. These are D-xylose, D-mannose, D-galactose, D-glucose, L-arabinose, 4-O-methyl-glucuronic acid, D-galacturonic acid and D-glucuronic acid and they’re joined together by $\beta$-1, 4 -, or $\beta$-1, 3 - glycosidic bonds[3, 4]. The amount of hemicellulose in plant cell wall vary between different species, also the hemicellulose composition can vary between plant species where some plant have higher portion of pentose sugars such as Xylose. In general plants of the grass family (Poaceae) e.g. wheat straw and switch grass have high hemicellulose content while softwood generally contain less[3].

Lignin and other components

Apart from the polysaccharides mentioned above and glycoproteins, plant cell wall in vascular plants contains lignin, suberin, cutin and cutan [4]. Lignin is a hydrophobic and aromatic polymer of inconsistent structure[3] Lignin consists of three fundamental monolignols. These are coniferyl alcohol, sinapyl and p-coumaryl. These monolignols are amorphous alcohols and are connected through different ether and carbon-carbon bonds[3]. Lignin protects cellulose and hemicellulose from microbial and chemical degradation by embedding and bonding to the polysaccharides.
Lignocellulose and its use as energy feedstock

Lignocellulose can be used as energy feedstock. In the process the cellulose and hemicellulose must be degraded to its component sugars. As earlier stated cellulose consist of D-glucose polymers and hemicellulose of different pentoses and hexoses. When cellulose and hemicellulose are hydrolysed into their individual monosaccharides it can be converted into ethanol or biogas through established fermentation technologies. The conversion of lignocellulose to individual sugars is hindered by the structural complexity of the biopolymer [3]. Lignin covers the polysaccharides and hinders enzyme accessibility.

Figure 1. Figure above illustrates a 3D model of how the different polymers of lignocellulose are connected. It also shows the formation and the different bonds.[4]

Figure 2 Atomic force microscope image of wheat straw (Parenchyma) cell wall. Cellulose and hemicellulose covered in lignin. Circular objects covering the fibres are lignin (white arrows).[3]
**Lignocellulosic enzymes**

Fungi and bacteria naturally degrade lignocellulose. These organisms produce extracellular enzymes specific for degrading lignocellulosic compounds such as cellulose, hemicellulose and lignin. Cellulose and hemicellulose degrading enzymes are known as cellulases and hemicellulases[6].

**Cellulases**

Enzymes that hydrolyse cellulose, cellulases, consist of three major groups of enzyme. Endoglucanase (EG, endo-1,4-D-glucanohydrolase) attacks the amorphous parts of the cellulose fiber. The internal β-1,4-Dglucosidic bonds are cut creating free chains. Exoglucanase degrades the polymer further by removing cellobiose units from the cellulose chain. The free cellobiose units, which consist of two connected glucose molecules are further degraded by beta-glycosidase into individual D-glucose molecules[6]. These enzyme have a high specificity in degrading cellulose to glucose.

**Hemicellulas**

The hemicellulosic system in plant cell wall is more complex and consists of several branched five and six carbon sugars [1,3,6]. Since hemicellulose is a polymer consisting of various sugar molecules, see hemicellulose, a vast number of different enzyme classes are needed to fully hydrolyse the whole polymer [4]. Xylose and arabinose comprise the significant fraction of hemicellulose and enzymes degrading these monosaccharides are used in biomass fermentation[4]. These enzymes are commonly known as xylanases and arabinases.

**Ligninases**

Fungi such as the white rot fungi degrade lignin naturally. Phenol oxidases such as lignin peroxidase (LiP), manganese peroxidase (MnP) and laccases break down lignin in oxidative processes. Lip and MnP are described as true ligninases because of their high efficiency of lignin degradation. Lip degrades all phenolic parts of the phenolic, which make up to 90 % of the polymer.

Lignin degrading enzymes could be considered demanding since efficient lignin degradation requires xylanase activity. This indicates that the ligninolytic system is part of secondary metabolism.

**Pretreatments**

Before the hemicellulose and cellulose can be hydrolysed to fermentable monomeric sugars a form of pretreatment is necessary. The intention of pretreating the biomass is to change the chemical and physical characteristics in order to make the substrate more accessible to hydrolysing enzymes. Several pretreatment technologies have been established and studied but none has yet proven to be ideal and ready for industrial use [5].
In order to consider a pretreatment successful the monomeric sugar yield from cellulose and hemicellulose should be high. Also the water and energy consumption of the method should be low.

![Diagram of pretreatment effect](image)

**Figure 3.** Simplified representation of pretreatment effect. The macroscopic structure is broken and the different components of lignocellulose are accessible after a successful pretreatment [6].

The pretreatments used can be divided into physical, thermal, chemical and biological. Pretreatments are usually combined for best result. For example the substrate is first milled, then subjected to a chemical treatment using either acid or alkaline [5].

**Microwave**

Microwave irradiation is different from conventional heating where the heat is delivered to the surface of the mass. While in irradiation the microwave energy heats the material throughout the volume. This results in a more rapid and uniform heating [6]. The main aim effect of microwave treatment is that hemicelluloses and to some degree even lignin is separated from the biomass. This increases enzyme accessibility and hydrolysis [6].

![Diagram of microwave irradiation](image)

**Figure 4.** Concept of microwave irradiation of lignocellulose.[5]

The irradiation in itself can auto hydrolyse substrate when water is used as heating medium but for best result the treatment is combined with an acid/alkaline catalyst.
Steam explosion

The steam explosion is an attractive choice when pre-treating biomass for fermentation. Steam treatment in its basic form without explosion is based on saturating the substrate with steam at high temperature and pressure. Temperatures usually range between 140 and 210°C[7].

Explosion phase is attained when the temperature and pressure is suddenly dropped, by opening the reaction chamber. The sample undergoes an explosive decompression thus leading the lignocellulose structure to rupture and the component to be more accessible. The process also leads to an aqueous phase (hydrolyzate) rich in hemicelluloses, mostly xylose, secreted from the fibres. The solid phase cellulose is also easier accessible [7].

Analysis

The saccharification of lignocellulose can be analysed using tools that quantify the concentration of sugar monomers. Precise quantitative analysis of sugar concentrations is an important part of the process. In this project enzyme thermistor and HPLC was used for analysis.

Enzyme thermistor

The enzyme thermistor is an analytical calorimetric device that measures energy changes in the solution. The energy changes are founded by enzymatic reactions between the immobilized enzymes in the controlled pore glass and the sample injected [8].

Phosphate buffer is introduced to the inlet at a flow rate of 0.5-2 ml/min Samples are introduced manually with a syringe. The sample passes through a heat exchanger and the controlled pore glass, CPG, with the fixed enzymes. The temperatures from both the columns are measured by a thermistor, which are connected to a Wheatstone bridge. The difference in temperature is measured and fed to readout device [8].

To determine the concentration of glucose the CPG is loaded with enzymes degrading glucose. In our case the CPG contains glucose oxidase and catalase. A calibration curve is then made of samples with known concentrations of glucose. Later the concentration of the unknown sample can be determined using the equation from the calibration curve. The method is accurate with a temperature resolution as low as 10^{-5} Celsius [8].

HPLC

HPLC is an abbreviation of High Performance Liquid Chromatography. It’s a separation and quantification technique that’s been used for nearly 35 years. A small volume of liquid sample is forced through a stationary phase containing porous material with different chemical properties. The molecules in the sample are then separated by chemical and physical interactions with the packing material in the column. Attributes such as volatility and polarity differentiate the molecules [9].

The separated components are identified at exit by external detection device. This could be a refractometer or spectrophotometer. A wide range of polymers and chemical compounds can be separated and detected using HPLC. The heart of this method is the column or stationary phase that determines which components are separated. When separating sugars a carbohydrate column is used [9].
Prior to quantification a calibration is needed. Known sugar concentrations are used to calculate the concentration in the sample. An advantage with HPLC is that all monomeric sugars are analysed simultaneously, contrary to the enzyme thermistor.

**Aim**

The aim of this study is to examine the saccharification process of lignocellulose and compare three pretreatment methods to determine which method provides the highest yield monomeric sugars. The effects of lignin will also be determined.
Material and method

Material

Equipment

The steam explosion machine was an in-house construction made by DTU.

![Schematic illustration of the steam explosion machine and its components.](image)

The steam generator is a 10 L tank with two heat coils and a thermometer. The heat coils are connected to the power regulator. This can be used to set the preferred temperature but for best result the power was controlled manually. The thermometer is connected to a data-logger which both displays and stores the information.

The reaction chamber is a 2.7 liter tank with a removable cover. At the bottom there's a pipe for liquid exit. The reaction chamber also has a steam emission pipe for quick emptying of steam.

Microwave
A conventional microwave was used for the irradiation test. Elextrolux EMS 2487W was provided by IES. The microwave had a maximum power of 450 W.

Wheat straw
Pre-milled wheat straw from Laga bioenergy AB was provided by IES. The straw length varied between 1-2 cm.

Grinder
A conventional coffee grinder was used to mill wheat straw. Wahl James Martin ZX595 Coffee Grinder was provided by IES.
Incubation
P-Selecta orbital incubator by Buch & Holm A/S

**Enzymes**

Accelerase 1500 *Cellulase enzyme complex for Lignocellulosic Biomass Hydrolysis* from Genencore is an enzyme complex specifically intended for lignocellulose hydrolysis. Accelerase 1500 contains Endoglucanase and Beta-glucosidase.

The enzymes are produced from genetically modified strains of *Trichoderma reesei*.

Recommended pH range: 4.6-5.0

Accelerase XY *Accessory Xylanase enzyme complex for biomass hydrolysis* XY contains xylanase, a hemicellulase, that degrades Xylan.

The enzymes are produced from genetically modified strains of *Trichoderma reesei*.

Recommended pH range: 4.5-7.0

Accelerase XC *Accessory Xylanase/Cellulase enzyme complex for biomass hydrolysis*

XC is an complementary enzyme complex that works synergistically with other Accelerase products such as 1500 and XY.

The enzymes are produced from genetically modified strains of *Penicillium funiculosum*.

Recommended pH range: 3.7-4.2

**Buffers**

- Tris buffer pH 4.5 0.1 M
- Phosphate buffer pH 7.0 0.1 M
- Citrate buffer pH 4.5 0.1 M

**HPLC**

- Column: Aminex HPX-87H 300 mm * 7.8 mm (Catalog #125-0140)
- BIO-RAD USA
- Eluant: Degassed 0.004 M H₂SO₄
- Mobile phase: 0.004 M Sulphuric acid (H₂SO₄)
- Detektor: UV absorbance at 215 nm
**Method**

*Enzymatic treatment of filter paper*

Four samples of 100 mg milled filter paper were prepared. The filter paper was milled using a conventional coffee grinder and then transferred to 15 ml test tubes. The four samples were divided into two test samples and two control samples. In the control samples, no enzymes were added.

Control:
- 9 ml distilled water
- 1 ml Tris buffer, 1M, pH 4.5
- 100 mg milled filter paper

Test:
- 8.74 ml distilled water
- 150 µl Accelerase 1500
- 75 µl Accelerase XC
- 35 µl Accelerase XY
- 1 ml Tris buffer, 1M, pH 4.5
- 100 mg milled filter paper

The samples were incubated for 2 hours in an orbital incubator at temperature 50°C. After the incubation time, the tests were centrifuged for 2 minutes at 13000 rpm and the supernatant was collected. A second centrifugation was then made using spin columns with filter for 5 minutes at 13000 rpm. The eluate was then frozen down ready for HPLC analysis.

*Enzymatic treatment of wheat straw*

Same material and method as paper was used in the treatment of wheat straw.

*Microwave pre-treatment*

In order to perceive the optimal conditions for pre-treatment, three parameters were investigated. These were time, power, and the water/substrate ratio. The volume was used as ratio. 200 mg of wheat straw had the approximate volume of 2 ml. Either 4.8 or 10 ml of water was then added. The max power of the microwave was 450 W.

Control:
- 9 ml distilled water
- 1 ml Tris buffer, 1M, pH 4.5
- 100 mg milled filter paper

Test:
- 8.74 ml distilled water
- 150 µl Accelerase 1500
- 75 µl Accelerase XC
- 35 µl Accelerase XY
- 1 ml Tris buffer, 1M, pH 4.5
- 100 mg milled filter paper
Totally 27 samples were prepared and treated identically with the exception of water levels. 1 gram of dried and milled wheat straw was put in a 50 ml baker. Thereafter 4-10 ml of distilled water was added and the samples then subjected to microwave treatment using various time and power. After treatment the fibres were washed and weighed. 100 mg was prepared for further enzymatic treatment while 100 mg was used as a control sample.

Control:
- 9 ml distilled water
- 1 ml Tris buffer, 1M, pH 4.5
- 100 mg microwave treated wheat straw

Test
- 8.74 ml distilled water
- 150 µl Accelerase 1500
- 75 µl Accelerase XC
- 35 µl Accelerase XY
- 1 ml Tris buffer, 1M, pH 4.5
- 100 mg microwave treated wheat straw

Steam explosion
The steam explosion test was done at DTU, Danmarks Tekniske Universitet, with supervision of their technician. 20 grams of dried wheat straw was loaded into the reaction chamber. Steam generator was filled with water and the reaction was started when the preferred temperature was reached in the steam generator. Thereafter the pipe that leads the steam into the reaction chamber was opened and the sample is subjected to heat and pressure. After the preferred treatment time, in this case 20 minutes, the steam emission pipe is opened. This leads to quick drop of pressure and temperature in the reaction chamber. After the sample was collected it underwent the same enzymatic treatment as the microwave treated fibres.

Enzyme thermistor
100 µl of the treated samples were used for enzyme thermistor. The sample was diluted with distilled water 1:10. 0.1 M phosphate buffer pH 7.0 was prepared for the enzyme thermistor flow through. The samples were injected manually at a flow rate of 2ml/min and the peak height measured. The CPG were pre-loaded with glucose oxidase.

HPLC
100 µl samples was filter with a syringe filter with pore size 0.45 µm and heated to 30°C. 20 µl of the samples was introduced to Column Aminex HPX-87H with a mobile phase consisting of 4 mM sulphuric acid.
Results

The tests were done at IES and DTU. The project can be divided into three major experiments.

- Effect of cellulose being conserved in lignocellulose
- Microwave pre-treatment
- Steam explosion pre-treatment

Degradation of cellulose conserved in lignocellulose was examined by comparing degradation of wheat straw and filter paper. In wheat straw the cellulose is conserved in biomass whereas in filter paper the cellulose has previously been extracted from the biomass and available for enzymatic degradation. Both substrates underwent the same treatment and the glucose yields were compared. After enzymatic treatment, glucose content in all samples were analysed using an enzyme thermistor.

Totally 27 microwave pre-treatments were conducted to examine the ideal conditions. The effects of time and power were examined and compared. Yield of monomeric sugar after microwave pre-treatment and further enzymatic treatment where analysed and compared. The samples were analysed using HPLC.

Only one successful steam explosion test was conducted due to time-limitation. The fibres after treatment was analysed with HPLC.

Degradation of cellulose conserved in biomass (lignocellulose)

In order to analyse the effect of lignin and determine how much lignin hinders the degradation of cellulose in wheat straw, where the cellulose is conserved in biomass, and in filter paper where the cellulose has been extracted from the biomass, were analysed and compared. Both substrates where milled and incubated with enzymes and figure 6 shows the amount of glucose obtained.

![Figure 6. Amount of glucose per gram of substrate hydrolysed in filter paper and wheat straw. The difference in cellulose proportion was taken into account.](image)

Degradation of cellulose conserved in lignocellulose was low during short treatment times and was increased upon prolonged incubation time. After 24
hours the difference were far greater as the amount of glucose after degradation of filter paper was almost four times the amount obtained from wheat straw.

**Microwave**

The amount of monomeric sugars hydrolysed after microwave pre-treatment was compared with the monomeric sugar yield after microwave pre-treatment and further enzymatic treatment. Effect of the parameters time and power during microwave pre-treatment was compared (Fig. 8 and 9).

![Figure 7. Amount sugars hydrolysed after microwave pre-treatment compared to substrate further treated with degrading enzymes.](image)

The amount hydrolysed monomeric sugars after microwave pre-treatment was generally very low and compared to further enzyme treated samples, the difference in glucose and xylose yield was high.

**Time**

Treatment times of 5, 15 and 20 minutes were compared to determine the effect of microwave pre-treatment time. Treatment power varied between 200W to 400W.

![Figure 8 Enzymatic hydrolysis of microwave pre-treated wheat straw. The substrates were pre-treated for 5, 15 and 20 minutes before enzymatic incubation.](image)
The effect of pre-treatment time was determined by comparing the average yield from three independent experiments. The yield after longer pre-treatment times was higher compared to shorter pre-treatment. The difference was most significant regarding xylose degradation where 20 minutes pre-treatment resulted in more than twice as much xylose compared to the 5 minute pre-treatment (Fig. 8).

**Power**

The influence of microwave power during pre-treatment was determined by comparing three different power settings. The average yield from pre-treatments using effects of 200 W, 300W and 400W, respectively, was compared.

![Power effect microwave](image)

*Figure 9 Enzymatic hydrolysis of microwave pre-treated wheat straw. Pre-treatment power was 200W, 300W and 400W.*

Figure 9 shows a clear difference in yield after varying the effect during microwave pre-treatment. The major difference occurred in xylose degradation where the 400 W pre-treatment yield was more than twice the 200 W yield.

**Steam explosion**

The amount of monomeric sugars hydrolysed after steam treatment was compared to the monomeric sugar yield after steam and further enzyme treatment of the substrate. Only one test was conducted.

![Steam explosion](image)

*Figure 10. Enzymatic hydrolysis of steam explosion pre-treated wheat straw. Pre-treatment was conducted during 20 minutes at a temperature of 180°C.*
The results after steam explosion were similar to results obtained after microwave pre-treatment. The mechanical treatment obtained after steam explosion alone gave a low yield of glucose and xylose. However, when the substrate was further treated with enzymes the yield increased considerably (Fig. 10).

Discussion

Degradation of cellulose conserved in lignocellulose
The first experiment comparing wheat straw and paper was conducted to determine the degradation of cellulose conserved in lignocellulose. The result presented in figure 6 showed, as expected, clear difference in the amount hydrolysed glucose from these two substrates. Filter paper was hydrolysed to a much higher degree than wheat straw due to the cellulose fibres not to be conserved in complex structures of lignocellulose. Lignin and hemicellulose directly hinders the enzyme accessibility to the substrate [5]. The test also showed that longer treatment times gave a higher yield. Filter paper consists merely of cellulose fibres whereas wheat straw consists of roughly 30 % cellulose, the results shown in figure 6 were compensated for this difference.

Microwave
To determine the optimal conditions for microwave pre-treatment, three aspects had to be considered and revised. These were microwave pre-treatment power and time together with further enzyme incubation of pre-treated substrates.

As earlier stated the microwave treatment can hydrolyse the substrate and result in monomeric sugars without enzymatic treatment. Microwave pre-treatment alone gave a very low yield of monomeric sugars (Fig. 7). The effects of enzymatic degradation after microwave pre-treatment resulted in a higher portion of hemicellulose to be degraded into xylose.

The pre-treatment time of microwave irradiation was also of importance. Longer treatment times give higher yield in simple sugars. The same applied to treatment power. Higher effects such as 400W gave a higher yield compared to 200W and 300W.

Steam explosion
Pre-treatment using steam explosion resulted in hydrolysis of higher amounts of hemicellulose compared to cellulose (Fig. 10). The reason for this discrepancy was due to a higher portion of hemicellulose recovered in the water eluate compared to cellulose after steam explosion pre-treatment [7]. Steam explosion combined with further enzyme treatment gave the highest yielding process. A total of 228 mg of glucose and xylose per gram wheat straw was extracted which is more than twice the amount obtained after microwave pre-treatment. However, we should also take in consideration the power consumption of the pre-treatments.
Summary
The average monomeric sugar yield from non-treated fibres, microwave treated fibres and steam pre-treated fibres were compared to determine the difference and also to conclude which pre-treatment that was most beneficial.

As seen in figure 11 mechanical treatment of the fibres increase the yields drastically. In comparison to microwave pre-treatment, steam explosion gave more than twice as much simple sugar.

Wheat straw was milled using a coffee grinder. Since this method gives an inconsistent straw length it could have had an impact on the result. Surface area is an important factor that either increases or decreases substrate accessibility for the degrading enzymes [3].

Conclusion
From the result obtained in this study it can be concluded that un-lignified biomass is easier to saccharify and gives higher yield in monomeric sugars. On the other hand lignocellulose is the cheapest and most abundant biomass. The cost of using un-lignified biomass or residue as fermentation feedstock should be revised and compared to the one using lignocellulose. The cost should be compared to that of pre-treated lignocellulose.

Both treatment time and power using microwave pre-treatment had an impact on the outcome of reducing sugars. Increased pre-treatment time and effect both increased the yield of monomeric sugars and this may indicate a longer pre-treatment time to be more important than higher input power. However, no distinct conclusion can be drawn due to the low amount of tests conducted.

Of the pre-treatments made, steam explosion gave the highest yield in reducing sugars, figure 11. However, considering time and power cost, microwave treatment could be preferable. It took up to 20 minutes using two heat coils á 1200W to acquire the preferred temperature in the steam generator. The water and energy consumptions using steam explosion is far greater than microwave treatment.
Since the steam explosion machine used was not designed for industrial use it is not yet valid to draw a conclusion on which method that is most energy-efficient. Also for better result, a microwave designed for biomass treatment should be used. These machines have a higher output power and contain a vessel where temperature and pressure are monitored. The result could also be further enhanced using a chemical catalyst during the mechanical pre-treatments such as an alkaline or a base. Steam treatment with sulphuric acid may enhance the yield with more than 60 % [10].

**Acknowledgement**

I would like to thank IES and Bengt Danielsson for making this project possible. I would also like to thank Laura Mendoza and Cheng Fang for their technical support and good advice.
Reference:


June 2007, Pages 932–939


http://polymer.ustc.edu.cn/xwxx_20/xw/201109/P020110906263097048536.pdf 2012-11-10