BIOMASS REFINERY AND PROCESS DYNAMICS GROUP

THESIS BIOMASS REFINERY AND PROCESS DYNAMICS

MODELLING THE PRE-TREATMENT OF LIGNOCELLULOSE

Rick van Rijn

09-07-2013

Supervisors:
Ellen Slegers
Richard Gosselink

Examiner:
Ton van Boxtel

WAGENINGEN UNIVERSITY
AGROTECHNOLOGY AND
FOOD SCIENCES

WAGENINGEN UR

Modelling the pre-treatment of lignocellulose

Name course	:	Thesis Biomass Refinery and Process Dynamics
Number	:	YBT-80324
Study load	:	24 ects
Date	:	09 July 2013

Student	:	R. van Rijn
Registration number	:	92-06-04-721-010
Study programme	:	BBT (Biotechnology)

Supervisor(s)	:	Ellen Slegers; Richard Gosselink
Examiner	:	Ton van Boxtel
Group	:	Biomass Refinery and Process Dynamics
Address	:	Bornse Weilanden 9 6708 WG Wageningen The Netherlands Tel: +31 (317) 48 21 24 Fax: +31 (317) 48 49 57

WAGENINGEN UNIVERSITY
AGROTECHNOLOGY AND
FOOD SCIENCES

ABSTRACT

The pre-treatment of lignocellulose is an important step in the biorefinery chain. A lot of research has been done on the different pre-treatment processes available. However, there are no quantitative ways to compare the different processes. To solve this problem, in this work a model based approach has been used to evaluate some of the available pre-treatment methods, i.e. acid and base catalysed, organosolv and steam explosion. Several data sets on processing of straw were analysed and the severity of the pre-treatment process conditions were correlated to the sugar yield. This resulted in a model for each pre-treatment. The limited data size and different severity ranges makes it challenging to compare the models. Therefore, recommendations were made for further experimental and theoretical work.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

The development of sustainable energy sources is essential to replace the depleting fossil resources. Fossil energy sources will run out eventually and sustainable alternatives must be developed. Sustainability is the key to solving the energy crisis. Alternative fuels are becoming increasingly more important in today's world and have many benefits in comparison with fossil fuel. Biofuel for example is considered carbon neutral, emits less pollution such as the greenhouse gas carbon dioxide and can be made from renewable resources[1].

The use of biomass to create biofuel is an important alternative and is undergoing extensive research. 1st Generation biofuel is made from readily fermentable sugars, derived from crops such as corn or sugarcane. Therefore, it competes with food consumption and there is evidence that usage of these feedstocks increases greenhouse gas emissions[2]. 2nd generation biofuel, which is made from biomass that does not threaten food supplies or biodiversity, is considered an important solution for the energy problem. Lignocellulosic biomass is one of the most abundant resources in the world and is mostly found in agricultural residues and paper industry and municipal waste streams. Lignocellulose consists of cellulose, hemicellulose and lignin that are very tightly bound together. The composition varies between the type of feedstock that is being used. Lignocellulosic biomass can be used to produce biofuel, biobased chemicals or materials[3].

The usage of lignocellulosic biomass has undergone extensive research and much is known about the biorefinery process that produces biofuel[4]. The lignocellulose has to be treated to break down the lignin structure and disrupt or open the crystalline structure of cellulose. This increases the bioavailability of the sugar polymers and makes them accessible for enzymes that hydrolyse the sugar polymers into monomers, such as glucose. The sugars can then be fermented to biofuels, such as ethanol or butanol.
It is difficult to compare the different methods that are available to treat lignocellulose. The energy and chemical requirement differ between the various methods that are available. The pre-treatment method chosen also has an effect later on in the process, for example fermentation inhibitors can be formed. There are comparison tables available, such as the one shown in Table 1, which is derived from Harmsen *et al.*, 2010[5].

TABLE 1: THE TABLE FROM HARMSEN ET AL, 2010[4], SHOWING THE ADVANTAGES AND DISADVANTAGES OF THE DIFFERENT PRE-TREATMENT METHODS

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

+ = positive characteristic: E.g. high yield of fermentable sugars, no or low fermentation inhibitors, no residue formation, no or low need for recycling of chemicals, low investment costs, high applicability to different biomass types, proven at pilot scale, low operational costs
- = negative characteristic: E.g. low yield of fermentable sugars, high amount of fermentation inhibitors, high residue formation, need for recycling of chemicals, high in vestment costs, low applicability to different biomass types, not (yet) proven at pilot scale, high operational costs

Not a lot of quantitative research has been done on the pre-treatment step of lignocellulose. As the table above shows, only qualitative information is well known[4, 5]. In this work we aim at a model based approach for a quantitative process design analysis. The advantage of such a model based approach is that the effect of the pre-treatment can be predicted according to the process conditions. For example, the yield of a certain sugar such as glucose can be estimated when a certain pre-treatment is used under set conditions. Also, changed feedstock composition or physical properties can be related to the outcome of the pre-treatment process.

As Table 1 shows there are a lot of pre-treatment options available. Acid, alkaline, organosolv and steam explosion treatments were chosen to be modelled, since much is known about the processes themself and there was useful data available for those pre-treatments.

The choice was made to focus on the release of available sugars as most data sets showed little variation in fractionation, making it difficult or irrelevant to predict. The release of available sugars directly relates to the amount of ethanol that can be produced.

2. LITERATURE REVIEW

2.1. LIGNOCELLULOSE

Lignocellulosic biomass refers to higher plants, softwood, hardwood and agricultural plants. Lignocellulose consists mainly of cellulose, hemicellulose and lignin, but also water, small amounts of protein and minerals. The interaction between these three components is illustrated in Figure 1.

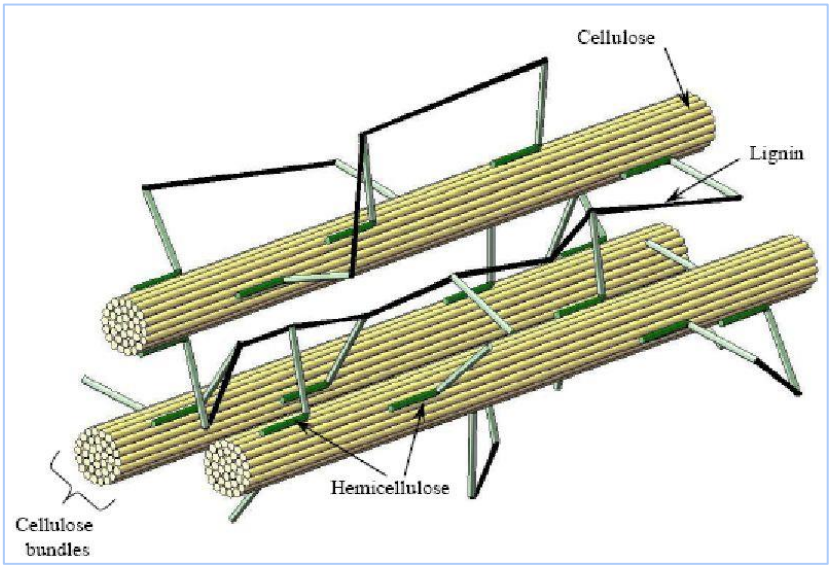

FIGURE 1: GENERAL STRUCTURE OF LIGNOCELLULOSE. THE CELLULOSE, HEMICELLULOSE AND LIGNIN BIND TOGETHER TO FORM A COMPLEX STRUCTURE[6].

Cellulose is the structural component of the primary cell wall in plants and is the most common organic polymer found on earth. It consists of multiple units of glucose, covalently linked to each other. The degree of polymerization varies with the type of raw material, but ranges from 300 to 1700 in wood to 800 to 10.000 in cotton and other plant fibres[7]. This means that the polymer is made up from 300 to 1700 individual glucose units, linked together as a chain. The cellulose chains form microfibrils by grouping together, and these microfibrils are bundled together to form the cellulose fibres[8]. Under normal atmospheric conditions (20°C, 60% relative humidity) cellulose is insoluble in water. Cellulose could be soluble in acid solutions, since the cellulose is hydrolysed under acid conditions[5].

Hemicellulose is a copolymer, consisting of C5 and C6 sugars, such as xylose and arabinose, and provides structural integrity to the cell. It consists of shorter chains than cellulose and unlike cellulose it is a branched polymer. It therefore has a more amorphous structure with less strength than cellulose and thus can be hydrolysed more easily[6]. Hemicellulose is almost

insoluble in water at lower temperatures and becomes soluble at higher temperatures as its hydrolysis starts. Acid and alkali also improve the solubility of hemicellulose in water[5].

Lignin is a polymer of aromatic compounds with phenyl propane as the predominant monomer. It lacks a defined primary structure and forms a protective layer in the cell walls of the plant. Hemicellulose and lignin are covalently linked together, giving the cell wall and whole plant structure mechanical strength. It plays an important role in the cells development, as it influences the transport of nutrients, metabolites and water in the cell. Lignin also connects individual cells, creating a composite material with high durability[5].

The composition of lignocellulose depends on the type of biomass that is considered. Table 2 gives a good overview of the different types of biomass and its composition:

TABLE 2: LIGNOCELLULOSE COMPOSITION OF DIFFERENT BIOMASS SOURCES[9].

Lignocellulosic materials	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwoods stems	40–55	24–40	18–25
Softwood stems	45–50	25–35	25–35
Nut shells	25–30	25–30	30–40
Corn cobs	45	35	15
Grasses	25–40	35–50	10–30
Paper	85–99	0	0–15
Wheat straw	30	50	15
Sorted refuse	60	20	20
Leaves	15–20	80–85	0
Cotton seed hairs	80–95	5–20	0
Newspaper	40–55	25–40	18–30
Waste papers from chemical pulps	60–70	10–20	5–10
Primary wastewater solids	8–15	NA	24–29
Swine waste	6	28	NA
Solid cattle manure	1.6–4.7	1.4–3.3	2.7–5.7
Coastal Bermuda grass	25	35.7	6.4
Switch grass	45	31.4	12

As can be seen in Table 2, there are large differences in composition between different biomasses. Different biomasses therefore might require a different pre-treatment step. Biomass with a high lignin content benefits more from a pre-treatment that removes lignin, such as the

organosolv pre-treatment. Biomass that contains a relative high amount of proteins might need a two-step pre-treatment process, to extract the relative high value proteins first.

The lignocellulose complex is made up of four main types of bonds: Ether type of bonds, carbon-to-carbon bonds, ester bonds and hydrogen bonds[10]. These bonds connect the different components to form the complex structure and an overview is given in Table 3:

TABLE 3: OVERVIEW OF THE LINKAGES BETWEEN THE MONOMER UNITS THAT FORM THE POLYMERS AND BETWEEN THE POLYMERS TO FORM LIGNOCELLULOSE[5]

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

To simplify the pre-treatments reactions, the interpolymer linkages are broken in the pre-treatment step and the intrapolymer linkages of hemicellulose and cellulose are broken in the enzymatic hydrolysis.

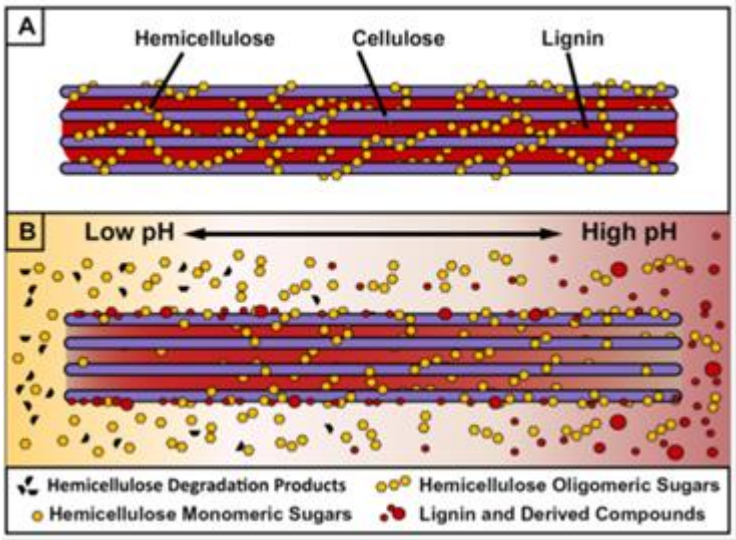

FIGURE 2: EFFECT OF THE PH ON THE STRUCTURE OF LIGNOCELLULOSE[11]

As illustrated in Figure 2 above, there is a large difference of the effect of low pH or high pH. When the process is performed at low pH, the hemicellulose is hydrolysed to monomeric sugars,

which can be partly degraded to furfural for example. Also, part of the lignin is removed from the complex, but could be precipitated at the fibre surface. The degradation of monomeric sugars does not occur at high pH. At high pH, the lignin is removed almost completely, and the hemicellulose is partly hydrolysed. At higher pH no formation of furfural could occur, though organic acids such as lactic acid may be formed.

2.2. BIOREFINERY CHAIN OF LIGNOCELLULOSE

An overall process of the biorefinery of lignocellulose for the production of cellulosic ethanol is illustrated in Figure 3. The biorefinery of lignocellulose starts with the pre-treatment. The pre-treatment step is necessary to make the lignocellulose more available for further processing. The type and conditions of the pre-treatment depend on the type of biomass used and the desired products in biorefinery. For example, to produce a high quality lignin product stream it is more beneficial to extract the lignin using the organosolv or alkaline pre-treatment. The different pre-treatment processes will be explained in the next chapter. After the pre-treatment, the lignocellulose is more susceptible for enzymatic hydrolysis. The severity of the pre-treatment determines the enzymatic susceptibility. Increasing the severity increases the bioavailability; however it also increases the amount of unwanted side products formed. These side-products can inhibit the microorganisms present in the fermentation step. The tendency to form these inhibitors differs between pre-treatment process.

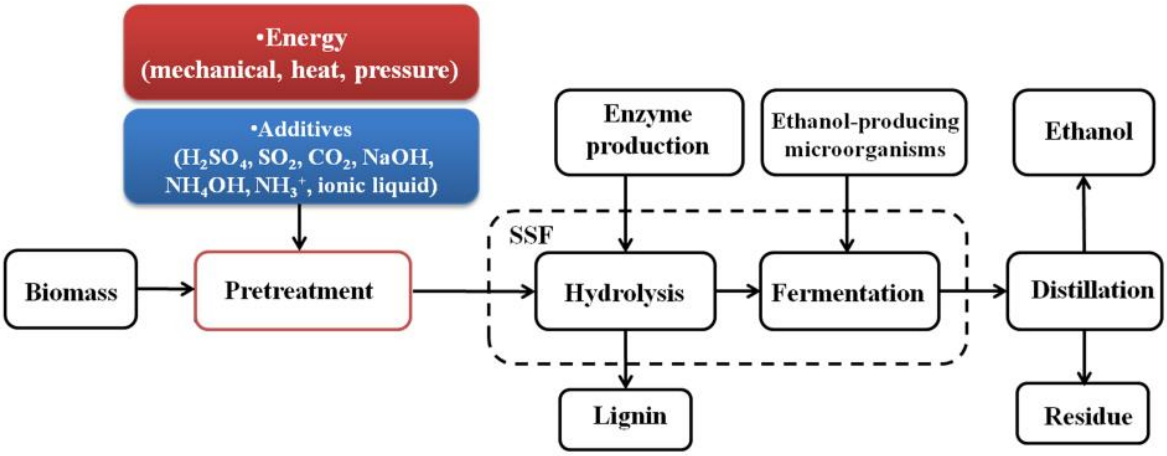

FIGURE 3: OVERALL PROCESS FLOW DIAGRAM FOR THE PRODUCTION OF ETHANOL FROM LIGNOCELLULOSE[12]

After the pre-treatment the enzymatic or acid hydrolysis follows. Enzymatic hydrolysis is preferred nowadays, as it is considered a cheaper alternative and produces very little degradation products[1]. The hydrolysis of cellulose and hemicellulose yields monomeric sugars, ready to be fermented to alcohol.

This fermentation is done by microorganisms. Different options are available, such as fungi or bacteria. Fungi, such as yeast, are promising, as they are relatively insensitive to the fermentation inhibitors, and are capable of producing the necessary hydrolysing enzymes themselves. This is named consolidated bioprocess, and looks very promising for the future. After the fermentation, the mixture is distilled to yield the final product, the biofuel.

2.3. PRE-TREATMENTS

In this section the available pre-treatment methods for lignocellulose will be described. The common goal of the pre-treatment step is to make the cellulose and hemicellulose more available for (enzymatic) hydrolysis and to create a fractionation. This thesis is focussed on four different pre-treatments: Steam explosion, organosolv, acid and alkaline pre-treatment. These pre-treatments are well studied at laboratory, pilot and demonstration scale and were the pre-treatments on which the most data was available.

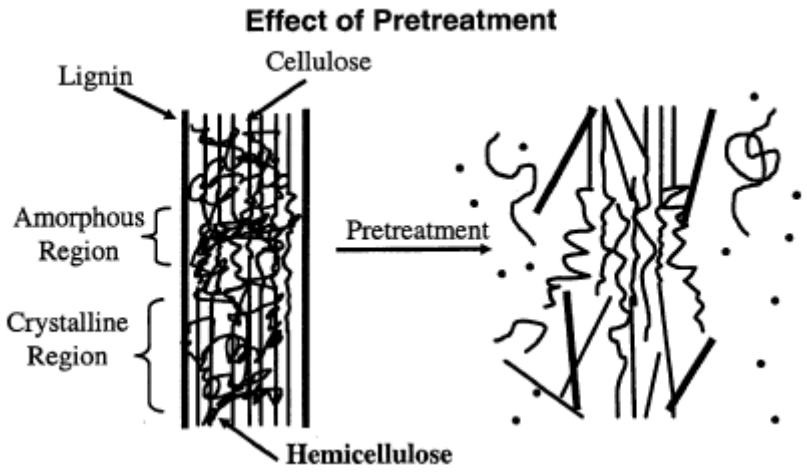

FIGURE 4: ABSTRACT VIEW OF THE EFFECT OF THE PRE-TREATMENT STEP[13]

2.3.1. ALKALINE

Alkali-based pre-treatment delignifies lignocellulose by breaking the ester bonds that link lignin and hemicellulose. This dissolves the lignin, which is the basis for separation in this process and improves the bioavailability of the hemicellulose and cellulose. The most used alkalis are lime, ammonia and sodium hydroxide, each with different reaction times and temperature. The disadvantage of this treatment is that the retention time is much longer than the other pre-treatment methods[13]. The key parameters are: Alkaline strength, temperature and residence time[14].

2.3.2. DILUTE ACID

The dilute acid pre-treatment involves adding an acid, such as sulphuric acid or maleic acid. The acid hydrolyses the hemicellulose and therefore makes the cellulose more bio-available. The disadvantage of this method is that it is not suitable for lignocellulosic biomass that contains a high amount of lignin, such as softwood. Also, if the conditions are too severe, fermentation inhibitors will be formed. The process can be run continuously, for a solids loading of 5-10% and temperatures above 160°C, or as a batch, for a solids loading of 10-40% and temperatures below 160°C[5]. The key parameters in this process are: Acid concentration, temperature and time.

2.3.3. ORGANOSOLV

The organosolv method uses an organic solvent to solubilize the hemicellulose and lignin. The organic solvent, most commonly ethanol, is added at high temperature (100-250°C) and pressure, with or without a catalyst. This will break alpha aryl-ether linkages of the lignin, hereby solubilizing the lignin and creating a separation. The process yields three fractions: Dry lignin, hemicellulose stream and a cellulose fraction[15].
Lignin solubility increases with the ethanol concentration, and reaches an optimum around 70% ethanol[16]. The key parameters in this process are: Catalyst loading, temperature and time.
Other organosolv pre-treatments use organic acids such as formic acid and acetic acid, but these pre-treatments were not considered in this thesis.

2.3.4. STEAM EXPLOSION

Steam explosion involves adding water to the lignocellulose, after which the mixture is heated to 160-220°C under pressure. When that pressure is suddenly released, the water will instantly vaporize, causing a high amount of shear forces to be applied to the lignocellulose. This will break the bonds between hemicellulose, cellulose and lignin. This creates a pulp which can be more easily degraded in the hydrolysis step. The key parameters in this process are: Temperature and residence time.

2.4. FACTORS LIMITING ENZYMATIC HYDROLYSIS

Enzymatic hydrolysis is important, as it releases the monomeric sugars from the polymers after the pre-treatment. The monomeric sugars are needed in the fermentation step. The enzymatic hydrolysis can take place either during the fermentation (Consolidated Bioprocess, CBP) or in a separate reactor. The accessibility of the cellulose fraction to the enzyme determines the reaction rate and total % conversion that can be reached[17]. To increase the accessibility for enzymatic attack a pre-treatment step was done. This bioavailability is changed by many factors. The structural modifications depend highly on the type of biomass and the type of pre-treatment used. The enzymatic hydrolysis is greatly affected by these factors[18].

ENZYME RELATED FACTORS

It is important to use the right mix of different enzymes. Three types of enzymes are need to hydrolyse cellulose: Endoglucanase, exoglucanase and β-glucosidase[19].

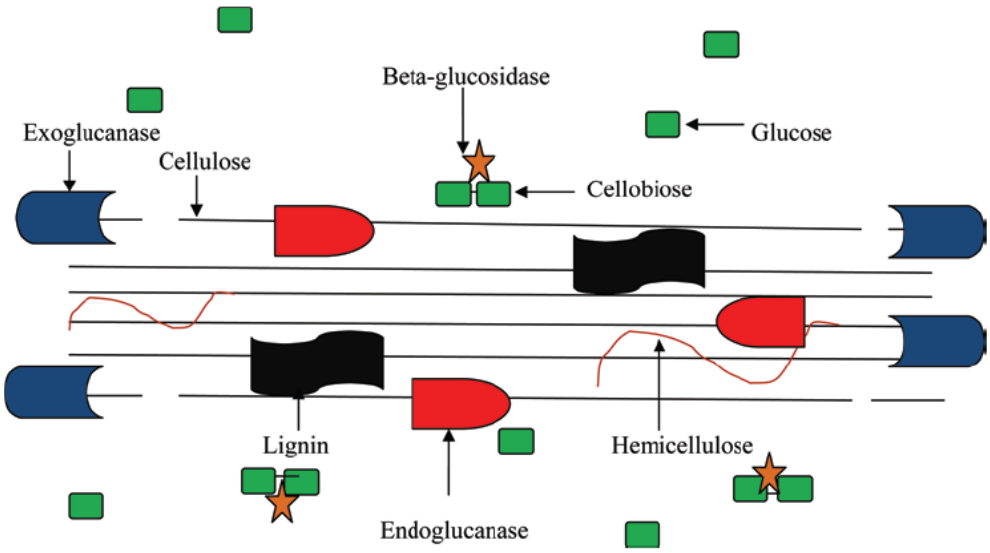

FIGURE 5: THE MECHANISTIC ACTION OF THE THREE DIFFERENT CELLULASE ENZYMES[20].

Exo-cellulase hydrolyses the individual cellulose fibres to break it into smaller sugars, endo-cellulase breaks the non-covalent interactions in the crystalline structure and B-glucosidase hydrolyses the disaccharides into glucose. Adding to much of one enzyme can lead to enzymes inhibiting themself, by binding together. It has been show that the addition of Xylanase increases the accessibility of cellulose and thus increases the fermentable sugar yield[21]. For hydrolysis of the hemicelluloses additional hemicellulases are used[22].

CELLULOSE CRYSTALLINITY

The crystallinity of the cellulose greatly affects the speed of the reaction. A higher crystallinity index (CrI) decreases the effectiveness of the enzymes. The crystallinity index describes the relative amount of crystalline structure in cellulose. More crystalline means the cellulose is more ordered and therefore more difficult to hydrolyse[23]. Cellulose with a high CrI will still hydrolyse, but will take days instead of hours[24]. Some pre-treatments could increase the CrI due to the fact that more amorphous material such as hemicellulose or lignin is removed.

LIGNIN CONTENT

The presence of lignin is a physical barrier for the enzymes, preventing the enzymes from reaching the cellulose, thus reducing the efficiency of the hydrolysis. Lignin has also been shown to non-productively bind the cellulase, inhibiting its function[18]. Removing the lignin therefore has a positive effect on the reaction rate and total % glucose yield[24].

HEMICELLULOSE CONTENT

By removing the hemicellulose the mean pore size of the substrate is increased which increases the accessibility of the cellulose[25]. But, since hemicellulose is made up of fermentable C5 and C6 sugars, recovery of those sugars can increase the total fermentable sugar production.

FERMENTATION INHIBITORS

During the pre-treatment of lignocellulose certain compounds may be formed that can inhibit the fermentation step. These compounds are degradation products of the sugars. The three major inhibitors are acetic acid, furfural and hydroxymethylfurfural (HMF). The formation of these components depends on the severity and type of pre-treatment that is used[26]. Increasing the temperature and the residence time will increase the amount of unwanted by-products formed. Examples are Furfural and HMF, which can be possible fermentation inhibitors[27]. Therefore, a balance must be made between making more sugar available and keeping the concentration of fermentation inhibitors to a minimum.

3. METHODS

To analyse the data and create a model, the release of sugars was related to the severity of the pre-treatment. The severity R_0 can be mathematically described by the following formula[28]:

$$R_0 = \int_0^t Exp(\frac{T(C°) - 100°}{14.75}) \cdot dt$$

The function for R_0 combines the two important parameters, temperature and residence time into a single reaction ordinate[29]. The effect of alkaline or acid conditions can be included according to the following formula[30]:

$$Log(R_0") = Log(R_0) + |pH - 7|$$

$$R_0" = R_0 + 10^{|pH-7|}$$

The effect of alkaline and acidity is equal by the use of the absolute value. This severity factor should be able to predict the amount of sugars released. A higher severity factor should increase the amount of sugar available.

For the organosolv pre-treatment, the ethanol concentration is not taken into consideration within the severity factor, as it has been shown that it is not an important parameter[31, 32].

3.1. DATA SETS

The six available data sets are given in Table 4. The biomass and process conditions are given for each pre-treatment.

TABLE 4: THE SIX AVAILABLE DATA SETS AND THEIR CHARACTERISTICS

Pre-treatment type	Biomass	Temp. (°C)	Time (min)	Acid %	Alkaline %	Ethanol %	Ref.
Alkaline	Wheat straw	70	240	-	0-12 NaOH, $Ca(OH)_2$	-	[33]
Alkaline	Rice straw	55/95	60-180	-	0-10 $Ca(OH)_2$ 0-4 NaOH	-	[34]
Dilute-acid	Barley straw	160-190	30-90	0-2 H_2SO_4	-	-	[35]
Dilute-acid	Wheat straw	130-170	10-50	11-89 mM Maleic acid	-	-	[36]
Organosolv	Wheat straw	160-210	60-120	0-30 mM H_2SO_4	-	50-80	[37]
Steam explosion	Wheat straw	190-210	2-10	0.2 H_2SO_4	-	-	[38]

- = Not applicable

3.2. SUGAR RELEASE EQUATIONS

All data sets were analysed and the R_0" was calculated for all the process conditions. The equations used to fit the data are:

- Michaelis-Menten equation
- Adjusted Michaelis-Menten equation
- Haldane equation
- Adjusted Haldane equation (1&2)
- Linear equation

3.2.1. MICHAELIS-MENTEN EQUATION

The Michaelis-Menten equation describes the rate of enzymatic reactions, by linking the reaction rate v to the substrate concentration [S], and is defined as follows:

$$v = \frac{V_{max}[S]}{K_m + [S]}$$

Here, V_{max} stands for the maximum reaction rate possible, at the highest possible substrate conditions. K_m is the Michaelis constant, which is the substrate condition at which the reaction rate is half of V_{max}.

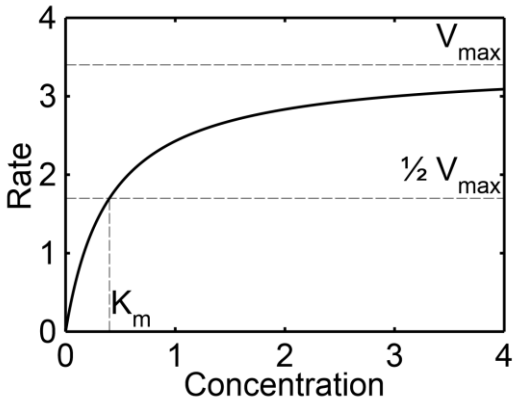

FIGURE 6: MICHAELIS-MENTEN EQUATION

To make the equation more suitable for the lignocellulose pre-treatment process, the parameters were changed:

$$Sugar\ \% = Sugar_{max} \times \frac{R_0"}{K_m + R_0"}$$

Here, *Sugar* represents the yield of either glucose, xylose or arabinose in %, $Sugar_{max}$ stands for the theoretical maximum yield possible, $R_0"$ is the severity factor, combining temperature, time and catalyst, and K_m is the Michaelis-Menten constant.

3.3.2. ADJUSTED MICHAELIS-MENTEN EQUATION

The adjusted Michaelis-Menten (MM) equation is derived from the normal equation by adding a degradation factor. Some data sets showed a decline in sugar yields at higher $R_0"$. The lower sugar yields are caused by the formation of degradation products, such as furfural and HMF[26]. This degradation factor should be able to model the decline after a certain severity. The adjusted Michaelis-Menten equation looks as follows:

$$Sugar\ \% = Sugar_{max} \times \frac{R_0"}{K_m + R_0"} - K_d \times R_0" + K$$

Here, *Sugar* represents the yield of either glucose, xylose or arabinose in %, $Sugar_{max}$ stands for the theoretical maximum yield possible, $R_0"$ is the severity factor, combining temperature, time

and catalyst, K_m is the saturation constant, K_d is the degradation factor and K is an extra parameter to compensate for the degradation factor at low R_0".

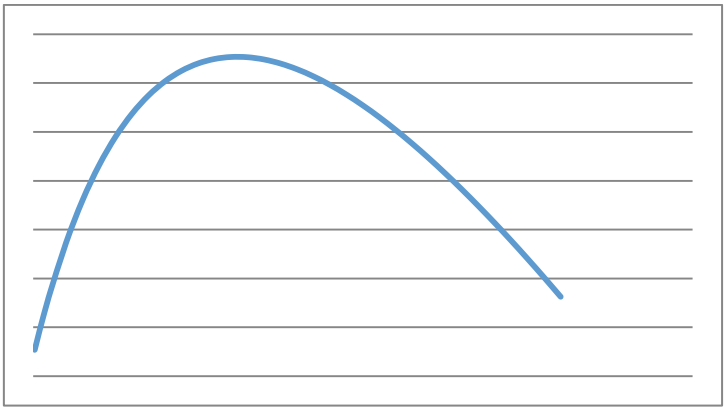

FIGURE 7: EXAMPLE OF THE ADJUSTED MICHAELIS-MENTEN EQUATION

3.4.3. HALDANE EQUATION

The Haldane equation is used to describe the enzyme-kinetics that show substrate inhibition[39]. It is similar to the Michaelis-Menten equation and is defined as follows:

$$Sugar\ \% = \frac{Sugar_{max} \times R_0"}{K_m + R_0" + \frac{R_0"}{K_d}}$$

Where, *Sugar* represents the yield in %, $Sugar_{max}$ stands for the theoretical maximum yield possible, $R_0"$ is the severity factor, K_m is the saturation constant and K_d is the degradation factor.

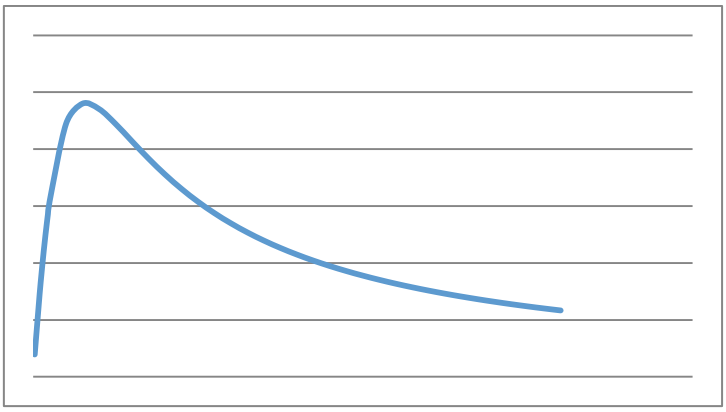

FIGURE 8: COMMON FORM OF THE HALDANE EQUATION

3.5.4. ADJUSTED HALDANE EQUATIONS

To make the Haldane equation more suitable for describing the sugar yield, two variations were derived, where a parameter was added and the notation was changed:

$$Sugar\ \% = Sugar_{max} \times \frac{R_0" - K_R}{K_m + R_0" + \frac{R_0"^2}{K_d}}$$

Where *Sugar* is the yield of sugar as a percentage of the theoretical yield, S_m is the limiting factor, $R_0"$ is the severity factor, K_R is the van Rijn constant and K_m and K_d are the Michaelis constant and the degradation constant, respectively.

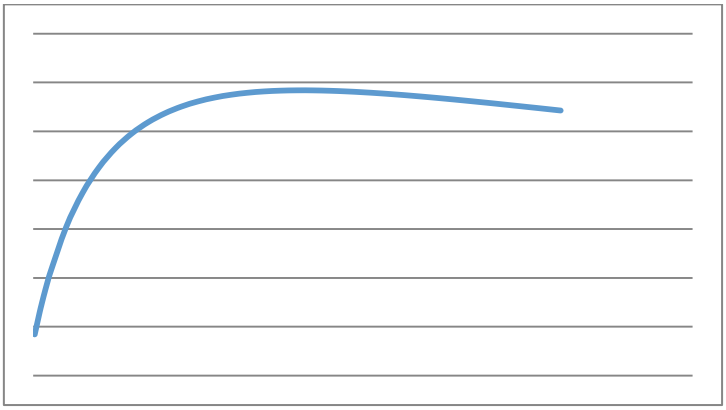

FIGURE 9: EXAMPLE OF THE ADJUSTED HALDANE EQUATION 1

The other variation of the Haldane equation was made, for a better fit in some data sets:

$$Sugar\ \% = S_m \times \frac{R_0" - K_R}{K_m + R_0"^2 + R_0" \times K_d}$$

Where sugar % is the yield of sugar as a percentage of the theoretical yield, S_m is the limiting factor, $R_0"$ is the severity factor, K_R is the van Rijn constant and K_m and K_d are the Michaelis constant and the degradation constant, respectively. The difference between the adjusted Haldane equation 1 and 2 is that the degradation factor is used differently.

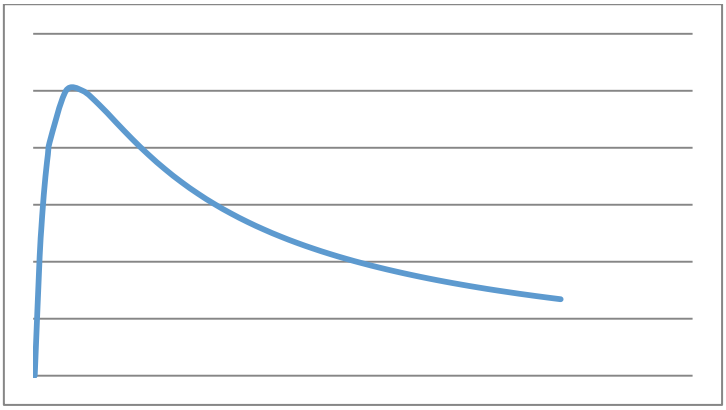

FIGURE 10: EXAMPLE OF THE ADJUSTED HALDANE EQUATION 2

3.6.5. LINEAR EQUATION

The linear equation is the most simple equation. It has two variables, one that defines the slope and one that defines the intercept. It has the form of:

$$Y = Ax + B$$

Where A defines the slope and B defines the intercept. The equation looks like this for the pre-treatment step model:

$$Sugar\ \% = K_R \times R_0" + K$$

The linear equation is only used when no other equation could be fitted. The pre-treatment process can only be linear if only a small range is considered.

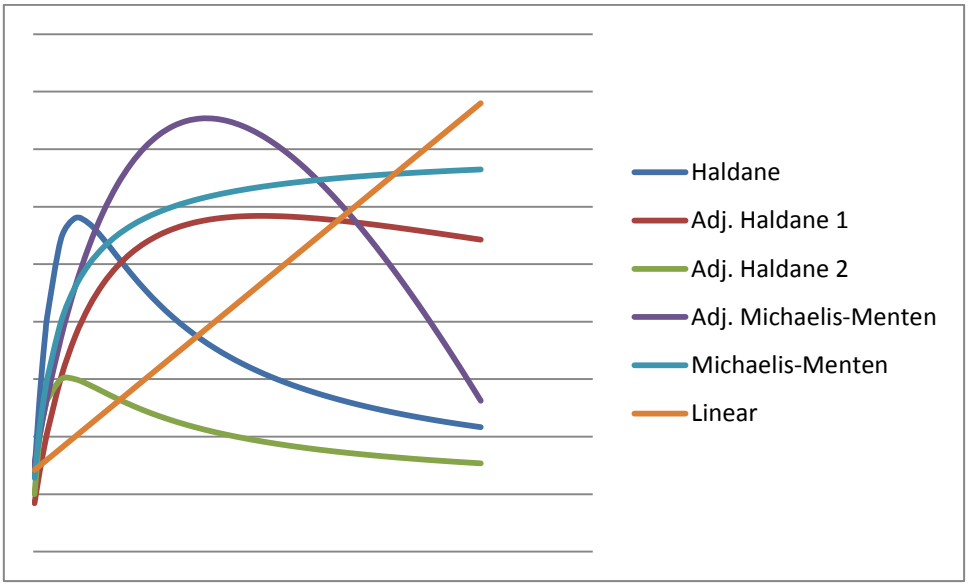

FIGURE 11: THE DIFFERENT EQUATIONS THAT ARE USED TO FIT THE DATA

Figure 11 shows the different equations explained before and gives a nice overview that clearly shows the differences between each equation.

3.3. FITTING THE EQUATIONS

The equations were fitted using Matlab, a numerical computer environment. In Matlab, the script nlinfit was used. This script returns the values of the parameters, based on an initial guess and nonlinear regression. The parameters are estimated using iterative least squares method. The

least squares method means that the end result minimizes the sum of the squares of the difference between the fit and the data points.

The best equation was selected on the basis of the R^2 and the standard error of fit. The R^2 or coefficient of determination indicates how good the values obtained from the model match the value the model should be able to predict. It is calculated as follows:

$$R^2 = 1 - SS_{resid}/SS_{total}$$

Where SS_{resid} is the sum of squared residuals and SS_{total} is the sum of the squared differences from the mean of the dependent variable. The value of R^2 should be between 0 and 1, where 0 indicates the worst possible fit and 1 indicates a perfect fit.

The standard error of fit, also known as the root mean square error, is an estimate of the standard deviation of the random component in the data, and is calculated as follows:

$$RMSE = \sqrt{MSE} = \sqrt{\frac{SSE}{v}}$$

Where *RMSE* stands for the root mean square error in %, *MSE* is the mean square error, *SSE* is the summed square of residuals and *v* stands for the residuals degrees of freedom, which is calculated as follows:

$$v = n - m$$

Where *n* is the number of response values and *m* stands for the number of fitted coefficients. The standard error of fit ranges from 0 to 100%, where 0 % indicates the best fit and 100 % the worst possible fit.

4. RESULTS

In this section, the results of the fitting in Matlab will be shown. The graphs containing the fit and data points will be shown, along with the reaction equation and it's parameters. The parameters are presented in a table, with further explanation about the units of the variables given in the methods section. The fit will be analysed and suggestions will be made on improvement of the model and experimental work.

4.1. PRE-TREATMENT OF WHEAT STRAW WITH NaOH

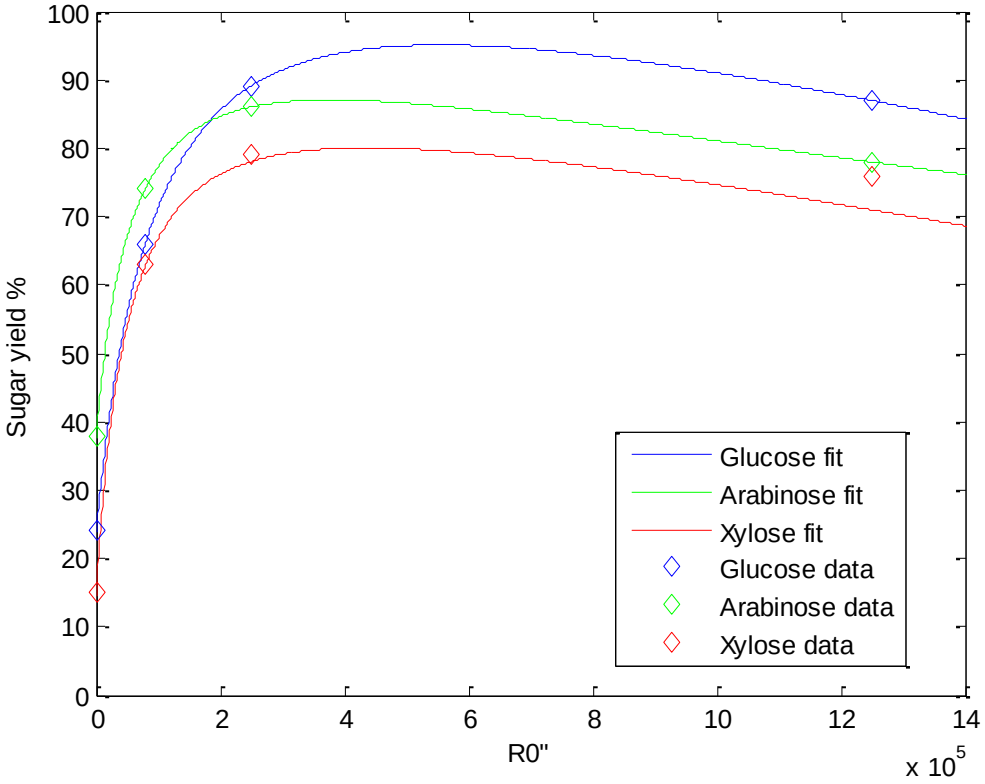

FIGURE 12: GLUCOSE, ARABINOSE AND XYLOSE YIELD DATA FROM THE WHEAT STRAW NaOH PRE-TREATMENT FITTED TO THE ADJUSTED MICHAELIS-MENTEN EQUATION, ADJUSTED HALDANE EQUATION AND ADJUSTED MICHAELIS-MENTEN EQUATION, RESPECTIVELY

In Figure 12 the fit of the glucose yield data from the wheat straw NaOH pre-treatment is shown. The data is fitted to the adjusted Michaelis-Menten equation. It is a perfect fit, with a R^2 of 1 and the standard error of fit is $2*10^{-14}$ %. The reaction equation with the parameter values are as follows:

$$Glu = Glu_{max} \times \frac{R_0"}{K_m + R_0"} - K_d \times R_0" + K$$

Glu_{max}	98.7908
K_m	$9.7827*10^{4}$
K_d	$2.2650*10^{-5}$
K	23.6911

The arabinose yield data from the wheat straw NaOH pre-treatment is fitted to the adjusted Haldane equation 2. It is a perfect fit, with a R^2 of 1 and the standard error of fit is $8*10^{-15}$ %. The reaction equation with the parameter values are as follows:

$$Ara = Ara_{max} \times \frac{R_0" - K_R}{R_0"^2 + K_d \times R_0" + K_m}$$

Ara_{max}	$4.4611*10^{8}$
K_R	$-2.1491*10^{4}$
K_d	$4.3640*10^{6}$
K_m	$2.5461*10^{11}$

In Figure 12 also the fit to the xylose yield data of the wheat straw NaOH pre-treatment is shown. The data is fitted to the adjusted Michaelis-Menten equation. It is also a perfect fit, with a R^2 of 1 and the standard error of fit is $4*10^{-5}$. The reaction equation with the parameter values are as follows:

$$Xyl = Xyl_{max} \times \frac{R_0"}{K_m + R_0"} - K_d \times R_0" + K$$

Xyl_{max}	81.9563
K_m	$5.1505*10^{4}$
K_d	$1.777*10^{-5}$
K	14.5077

Since the data set only has 4 measured process conditions, the fit is only applicable for a small range of conditions and therefore not very reliable. Extra measuring points might improve the model and therefore should be better at predicting the maximum sugar yield and sugar degradation. In this model, the maximum is not 100 %, but around 95%. This might be due to the fact that degradation products, such as organic acids, are already formed before 100% yield is reached, or that the cellulose never becomes completely bioavailable.

The arabinose yield has a lower maximum than glucose yield and has the fastest degradation for this pre-treatment. This likely means that more degradation products are formed regardless of the severity or that the hemicellulose fraction is simply less bioavailable as the xylose yield also

has a lower maximum. The equation parameter Ara_{max} is very high. This is odd compared with other models, which all have values around 100, which would be expected. This high value is probably corrected or caused by the high value for K_m, so the model still describes the arabinose yield accurately.

For this data set, it would be beneficial to measure between the last two data points, since the optimum is in that range but not measured properly. This would result in conditions with an alkaline loading of 10 or 11 % at the same temperature and time, 70 degrees and 240 min respectively.

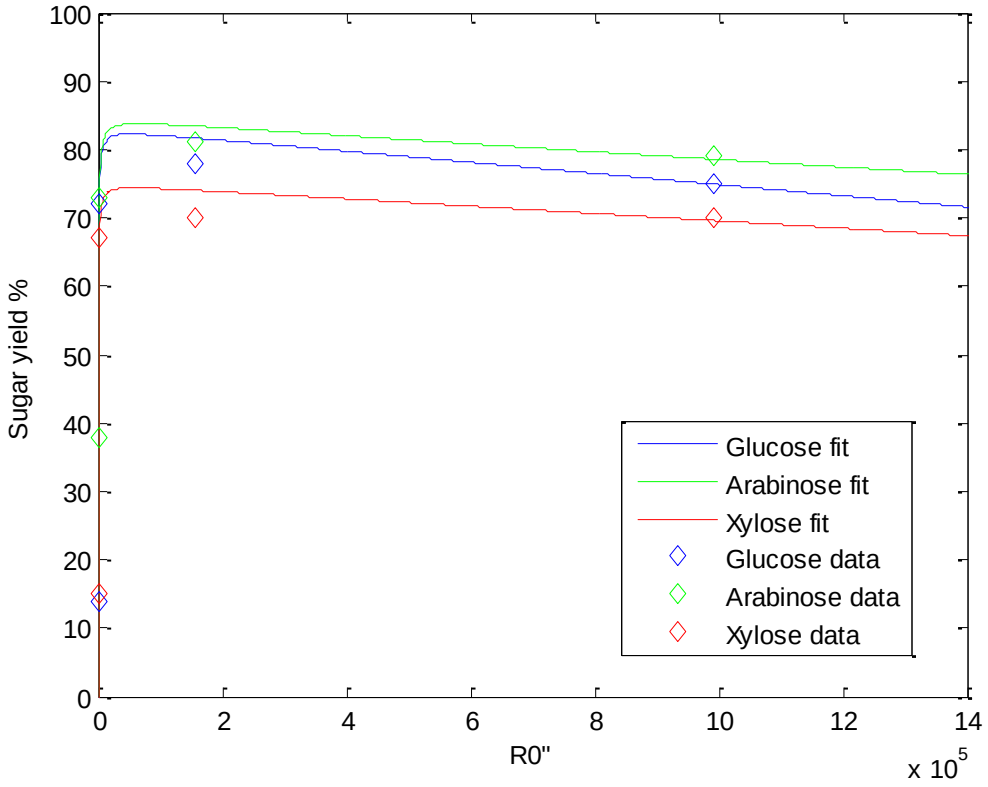

FIGURE 13: GLUCOSE, ARABINOSE AND XYLOSE YIELD DATA FROM THE WHEAT STRAW Ca(OH)2 PRE-TREATMENT FITTED TO THE ADJUSTED MICHAELIS-MENTEN EQUATION, HALDANE EQUATION AND ADJUSTED MICHAELIS-MENTEN EQUATION, RESPEECTIVELY

In Figure 13 the fit to the glucose data from the wheat straw Ca(OH)2 pre-treatment is shown. The R^2 is a satisfactory 0.9917 with a standard error of fit of 2.7862 %. The reaction equation with the parameter values are as follows:

$$Glu = Glu_{max} \times \frac{R_0"}{K_m + R_0"} - K_d \times R_0" + K$$

Glu_{max}	1346
K_m	$1.3456*10^3$
K_d	$8.2081*10^{-6}$
K	$-1.2629*10^3$

The arabinose data from the wheat straw Ca(OH)2 pre-treatment is fitted to the Haldane equation 1. The R^2 is 0.9799 with a standard error of fit of 2.8508 %. The reaction equation with the parameter values are as follows:

$$Ara = Ara_{max} \times \frac{R_0"}{R_0"^2/\mathrm{K_d} + \mathrm{R_0}" + K_m}$$

Ara_{max}	84.6211
K_d	$1.2954*10^7$
K_m	347.4153

The xylose yield data is fitted to the adjusted Michaelis-Menten equation. The fit has a R^2 of 0.9855 and the standard error of fit is 3.2601 %. The reaction equation with the parameter values are as follows:

$$Xyl = Xyl_{max} \times \frac{R_0"}{K_m + R_0"} - K_d \times R_0" + K$$

Xyl_{max}	11669
K_m	1.5949
K_d	$5.4176*10^{-6}$
K	$-1.1594*10^4$

Visually, the fit does not look exactly right, but this is what Matlab gave as the best solution. It has a very steep section at low R0", jumping to ~80% almost instantly, showing that a higher severity is not necessary for the maximum possible yield, with a side note that there is a big gap between the last two data points, making it possible that a higher yield could have been obtained. This pre-treatment with calcium hydroxide shows less degradation than in the previous alkali treatment with sodium hydroxide.

Again, the parameter values of the glucose and xylose models are a bit off. They are a factor 10 and 100, respectively, higher than what would be expected. However, the model should be accurate for the range measured in the data set.

There is a gap between the 3rd and 4th measurement. As this is where the highest sugar yield is expected. It would be beneficial to measure in that range, resulting in conditions with an alkaline loading of 10 or 11 % with the same temperature and time.

4.3. PRE-TREATMENT OF RICE STRAW WITH NAOH

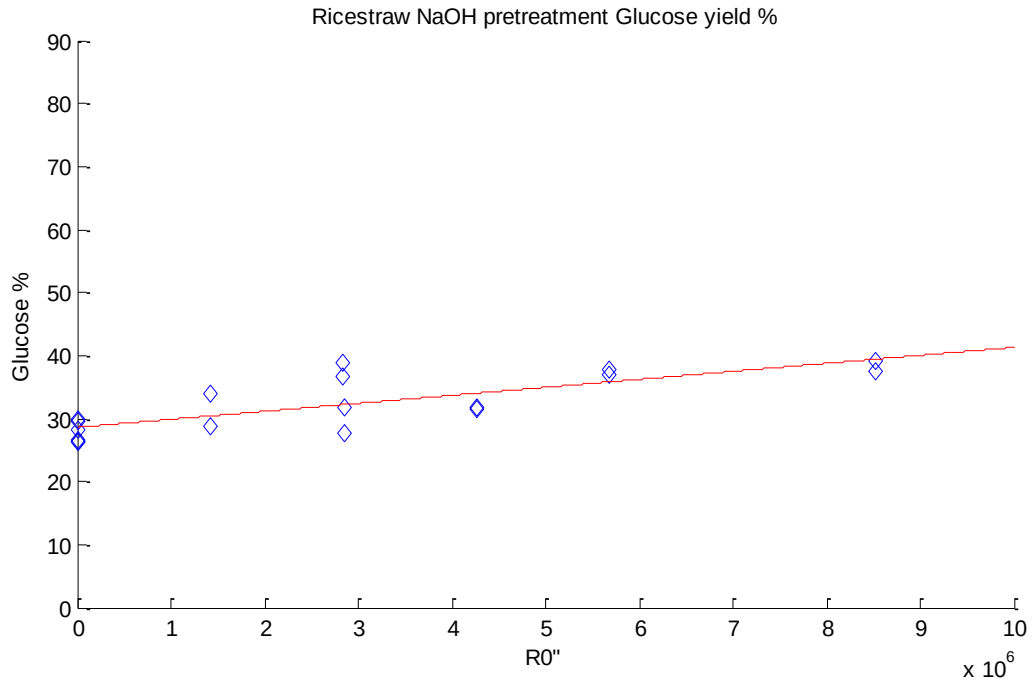

FIGURE 14: GLUCOSE YIELD DATA OF THE NaOH PRE-TREATMENT OF RICE STRAW, FITTED AS A LINEAR LINE

Figure 14 shows the glucose data from the NaOH pre-treatment of rice straw, fitted as a linear equation. The R^2 is 0.6132, and the standard error of fit is 2.8624%. The reaction equation with the parameter values are as follows:

$$Glu = K_R \times R_0'' + K$$

K_R	$1.2693*10^{-6}$
K	28.6044

The fit to this data set is not what one would expect. The data set also showed some irregularities, such as the four points at the severity of about $3*10^6$. There is a large difference in sugar yield at the same severity. This probably lies in the fact that the pH had to calculated based on the acid loading. The pH in the reactor was not specified in the data set, and there is a difference between the theoretical pH and the actual pH in the reactor. Lignocellulose has some buffer capacity, which affects the pH. Increasing the pH values of the highest alkaline loading conditions made the data set more similar to the other data sets. There was a more Michaelis-Menten equation like trend visible. The pre-treatment of rice straw with NaOH is not very effective in this experiment. Only a maximum sugar yield of 40% could be reached, which is very low compared to the yields reached with other pre-treatments. This is probably due to the low temperature, 55°C, that was being used. A higher temperature should improve the sugar yield.

4.4. PRE-TREATMENT OF RICE STRAW WITH CA(OH)2

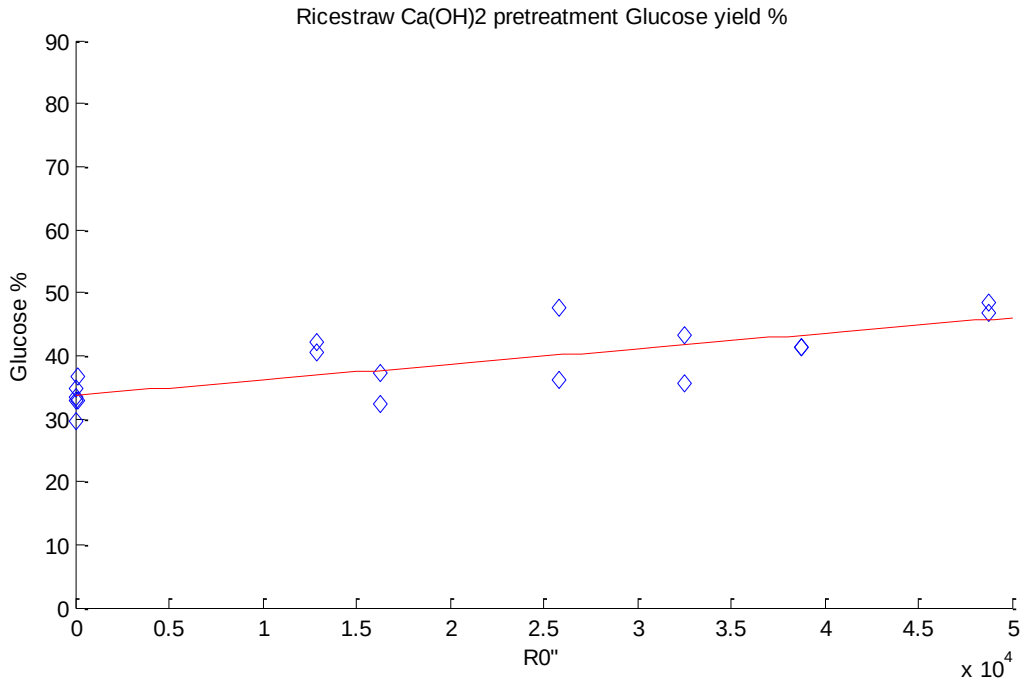

FIGURE 15: GLUCOSE YIELD DATA FROM THE RICE STRAW PRE-TREATMENT WITH CA(OH)2, FITTED TO A LINEAR LINE

In Figure 15 the glucose data form the Ca(OH)2 pre-treatment of rice straw is fitted to a linear line. The R^2 is 0.5895 and the standard error of fit is 3.635%. The reaction equation with the parameter values are as follows:

$$Glu = K_R \times R_0'' + K$$

K_R	$2.4812*10^{-4}$
K	33.6523

For this pre-treatment, the same is true as for the pre-treatment of rice straw with NaOH. The pH was not specified in the data set and had to be calculated based on the acid loading. This does not accurately reflect the real process conditions. Changing the pH gave the more expected form of the Michaelis-Menten equation. Again, the glucose yield is very low at almost 50 %. The temperature used in this pre-treatment was higher than the one used in the pre-treatment of rice straw with NaOH, which could explain the difference in maximum yield.

Using a higher temperature or longer time could increase the effectiveness of the pre-treatment of rice straw with NaOH and Ca(OH)2.

4.5. PRE-TREATMENT OF BARLEY STRAW WITH H_2SO_4 (DILUTE ACID)

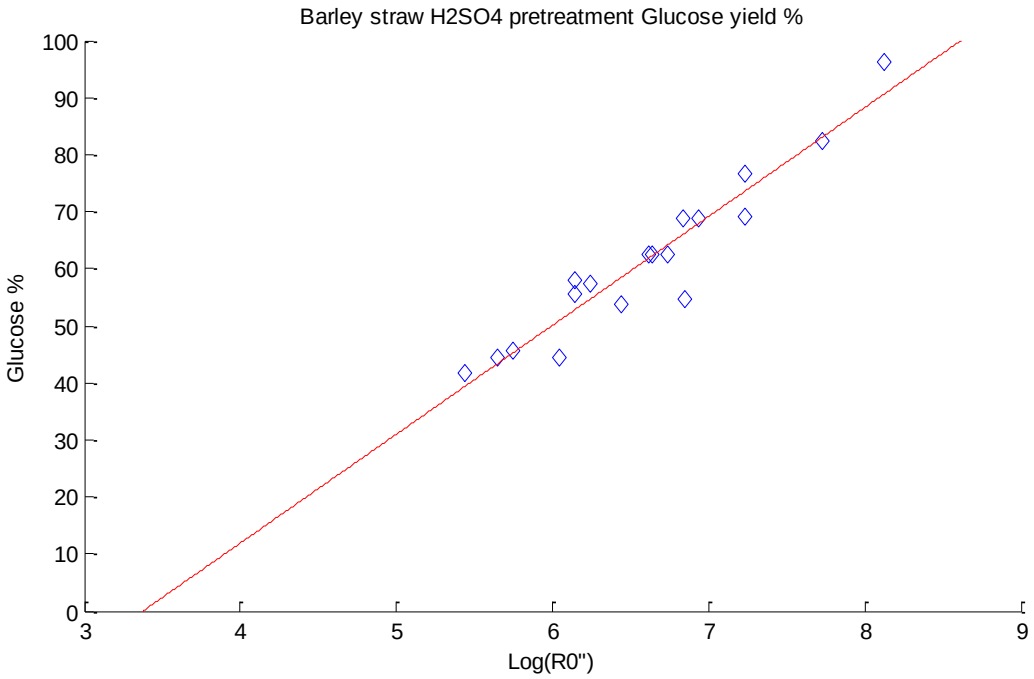

FIGURE 16: GLUCOSE YIELD DATA FROM THE PRE-TREATMENT OF BARLEY STRAW WITH H_2SO_4 FITTED TO A LINEAR LINE. NOTE THAT THE LOG OF R0" IS NOW PLOTTED AGAINST THE GLUCOSE YIELD.

Figure 16 shows the data from the barley straw pre-treatment with H2SO4, fitted to a linear equation. Instead of plotting the R0" against the glucose yield, for this data set plotting the log of the R0" gave a very good linear fit. The R^2 is 0.9073 with a standard error of 4.3247%. The reaction equation with the parameter values are as follows:

$$Glu = K_R \times Log(R_0") + K$$

K_R	19.0813
K	-64.5005

For this data set, an assumption had been made that the container contained 1.5 L. this assumption was needed to calculate the yield of the glucose in %. The data set only showed the output of glucose in g/L; therefore the volume of the reactor contents needed to be known but was not specified in the article, only the full volume of the reactor, which was 2 L. Common practice is to fill 75 % of the reactor. This decreases the reliability of the model.

For this data set, the choice was made to correlate the sugar yield to the logarithm of R_0". This created a much better fit than the relation with R0". The logarithm of the severity is used in many researches, to decrease the distance between the measuring points when plotting the data. Using the logarithm makes it difficult to compare to the other pre-treatments. What can be seen is that there is no degradation visible in this severity range. The severity range used in this pre-

treatment is similar to the other pre-treatments, which shows that glucose degradation is not an important issue with Maleic acid pre-treatment under the conditions used.

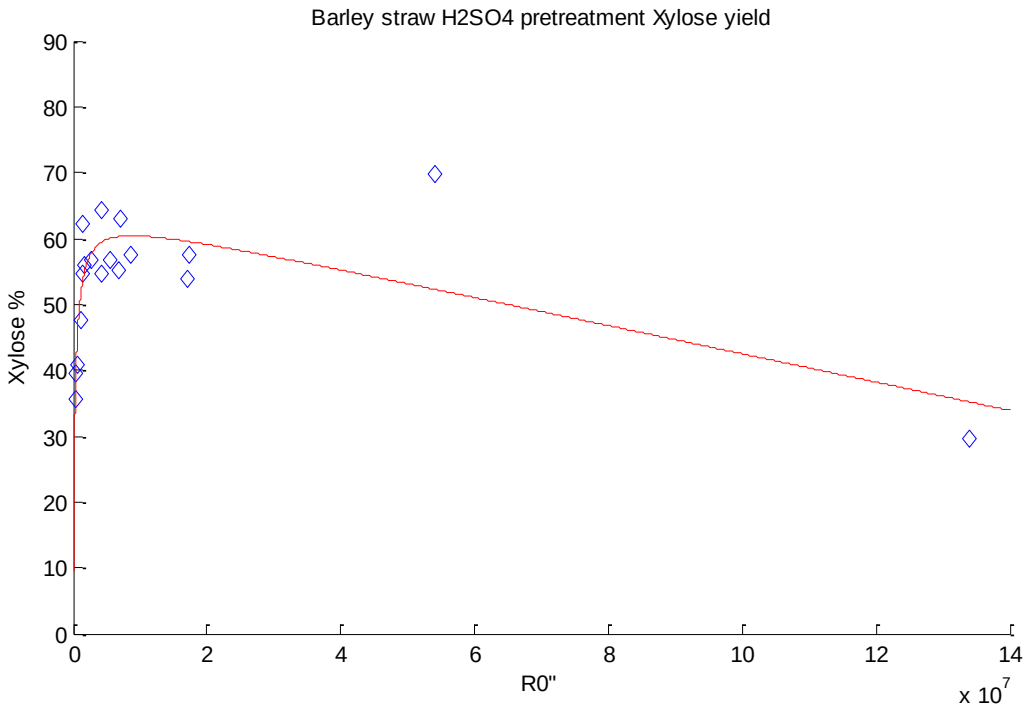

FIGURE 17: THE XYLOSE YIELD DATA FROM THE PRE-TREATMENT OF BARLEY STRAW WITH H2SO4 FITTED TO THE ADJUSTED MICHAELIS-MENTEN EQUATION

Figure 17 shows the data from the dilute acid pre-treatment of barley straw, fitted to the adjusted Michaelis-Menten equation. The R^2 is 0.6972 and the standard error of fit is 5.8054%. The reaction equation with the parameter values are as follows:

$$Xyl = Xyl_{max} \times \frac{R_0"}{K_m + R_0"} - K_d \times R_0" + K$$

Xyl_{max}	54.7196
K_m	$3.3678*10^5$
K_d	$2.1629*10^{-7}$
K	9.6096

As seen in the graph above, a lot of degradation occurs at high severity. Since there are not many data points at higher severity, the fit is not very good. The maximum of 70% xylose yield is not taken into account very well in the model. There are not enough measuring points between the severity of $4*10^7$ and $10*10^7$, which is where the maximum yield of xylose is expected. More measuring points should reveal the maximum xylose yield possible. The dilute acid pre-treatment does not look like a viable option if xylose recovery is also important. The conditions

needed for a high glucose yield will simultaneously result in a high amount of xylose degradation.

4.6. PRE-TREATMENT OF WHEAT STRAW WITH MALEIC ACID

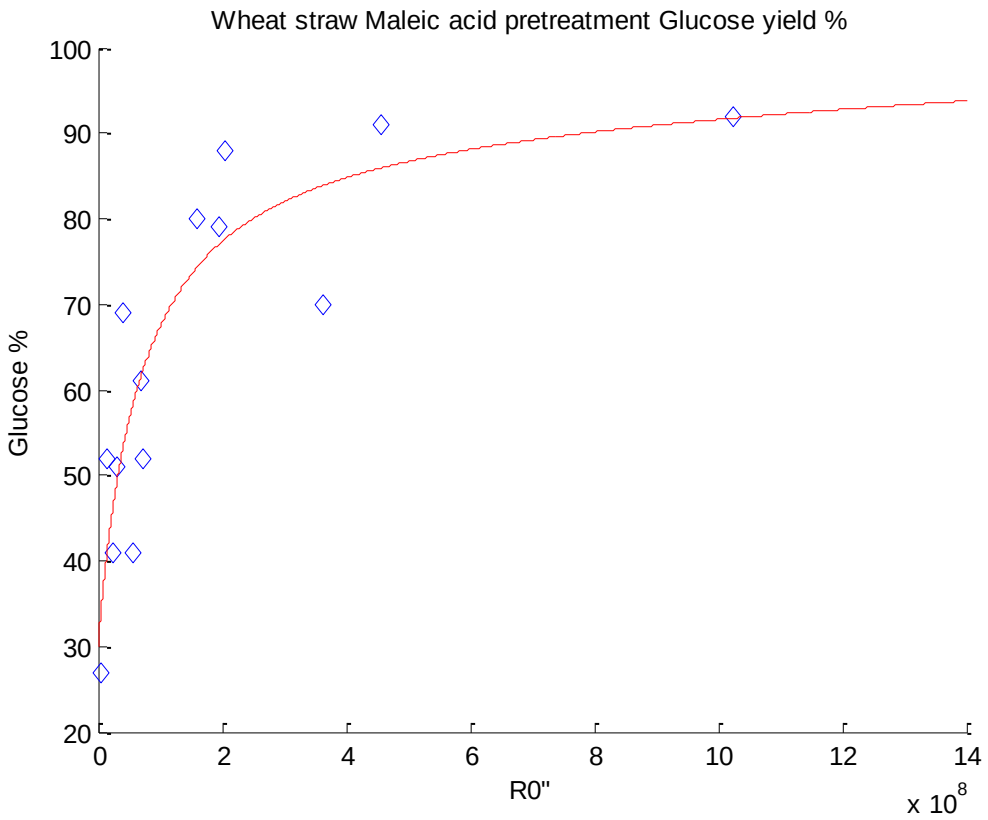

FIGURE 18: THE GLUCOSE YIELD DATA FROM THE PRE-TREATMENT OF WHEAT STRAW WITH MALEIC ACID, FITTED TO THE ADJUSTED MICHAELIS-MENTEN EQUATION

In Figure 18 the data from the wheat straw pre-treatment is shown, fitted to the adjusted Michaelis-Menten equation. The R^2 is 0.7733 and the standard error of fit is 9.8139%. The reaction equation with the parameter values are as follows:

$$Glu = Glu_{max} \times \frac{R_0"}{K_m + R_0"} - K_d \times R_0" + K$$

Glu_{max}	63.1662
K_m	$7.0143*10^{7}$
K_d	$-2.6125*10^{-9}$
K	29.9595

The fit to the data is negatively influenced by one outlier in the data set. The point at a severity around $4*10^{8}$ with a yield of 70% clearly does not comply with the rest of the data. The process conditions at this data point are characterized by a high temperature and relatively long time

with a low amount of acid added. The low amount of acid has a stronger effect than the higher temperature and longer time. This might lie in how the severity factor is calculated. Maybe there should be more emphasis on the acid loading. Going to a higher severity would probably not benefit the glucose yield, as the plateau already seems to be reached at a glucose of ~90%

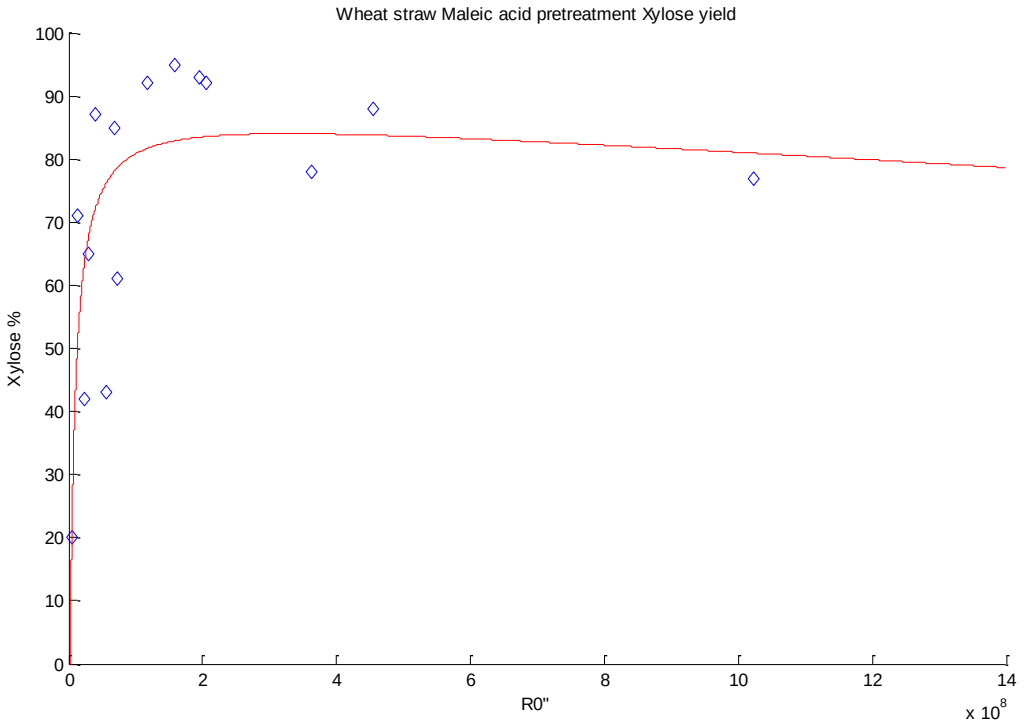

FIGURE 19: XYLOSE YIELD DATA FROM THE MALEIC ACID PRE-TREATMENT OF WHEAT STRAW, FITTED TO THE ADJUSTED MICHAELIS MENTEN EQUATION

Figure 19 shows the data from the pre-treatment of wheat straw with maleic acid, fitted to the adjusted Michaelis-Menten equation. The R^2 is 0.5679 and the standard error of fit is 14.7949%. The reaction equation with the parameter values are as follows:

$$Xyl = Xyl_{max} \times \frac{R_0"}{K_m + R_0"} - K_d \times R_0" + K$$

Xyl_{max}	119.6094
K_m	$6.1606*10^{6}$
K_d	$6.5775*10^{-9}$
K	-31.2176

This data set shows a high amount of variation, which explains why the fit is not very good. The maximum sugar yield reached at a severity of around $2*10^{8}$ is not properly taken into account by the model. This probably also has to do with the same outlier explained earlier.

4.7. PRE-TREATMENT OF WHEAT STRAW WITH ETHANOL ORGANOSOLV

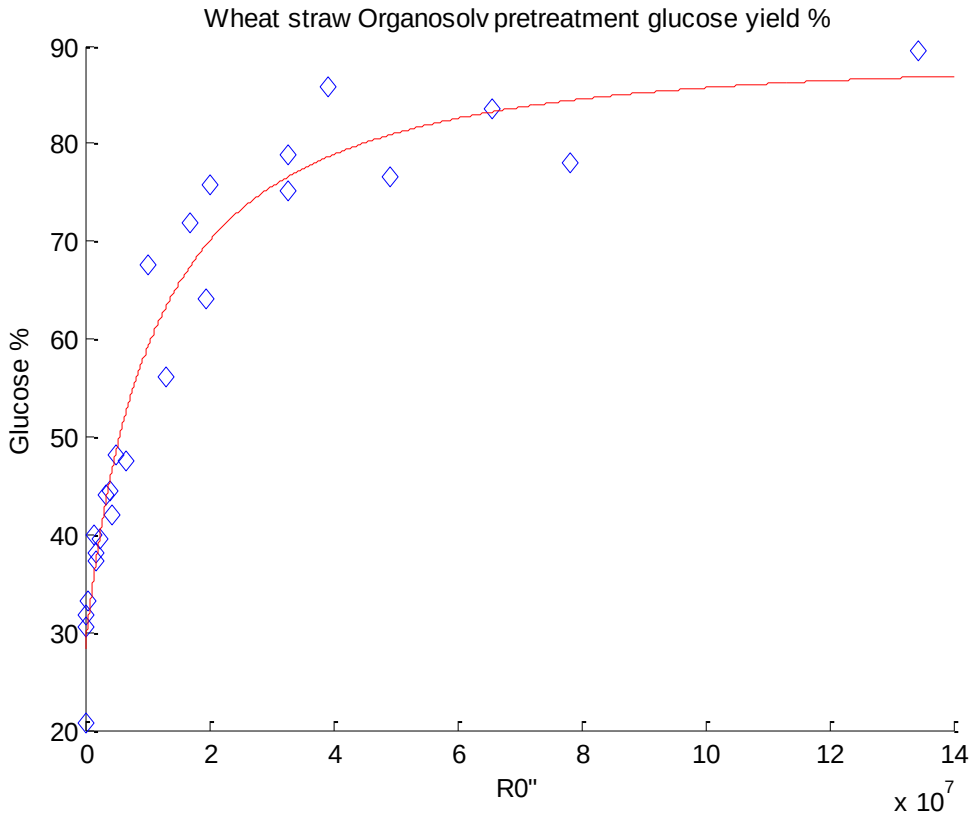

FIGURE 20: ENZYMATIC DIGESTIBILITY DATA FROM THE ORGANOSOLV PRE-TREATMENT DATA FITTED TO THE ADJUSTED MICHAELIS-MENTEN EQUATION.

In Figure 20 the fit to the enzymatic digestibility data from the wheat straw ethanol organosolv pre-treatment is shown. The R^2 is 0.9534 with a standard error of fit of 4.4528 %. The reaction equation with the parameter values are as follows:

$$Glu = Glu_{max} \times \frac{R_0"}{K_m + R_0"} - K_d \times R_0" + K$$

Glu_{max}	65.3839
K_m	$1.118*10^{7}$
K_d	$1.37*10^{-8}$
K	28.2569

As explained before the ethanol concentration was not taken into account and seems justified, given that the model fits very well to the data. There is little to no degradation observed in this data set, given that the highest severity gave the highest yield in sugars. However, degradation could occur when the temperature and acid concentration are high enough.

4.8. PRE-TREATMENT OF WHEAT STRAW BY STEAM EXPLOSION

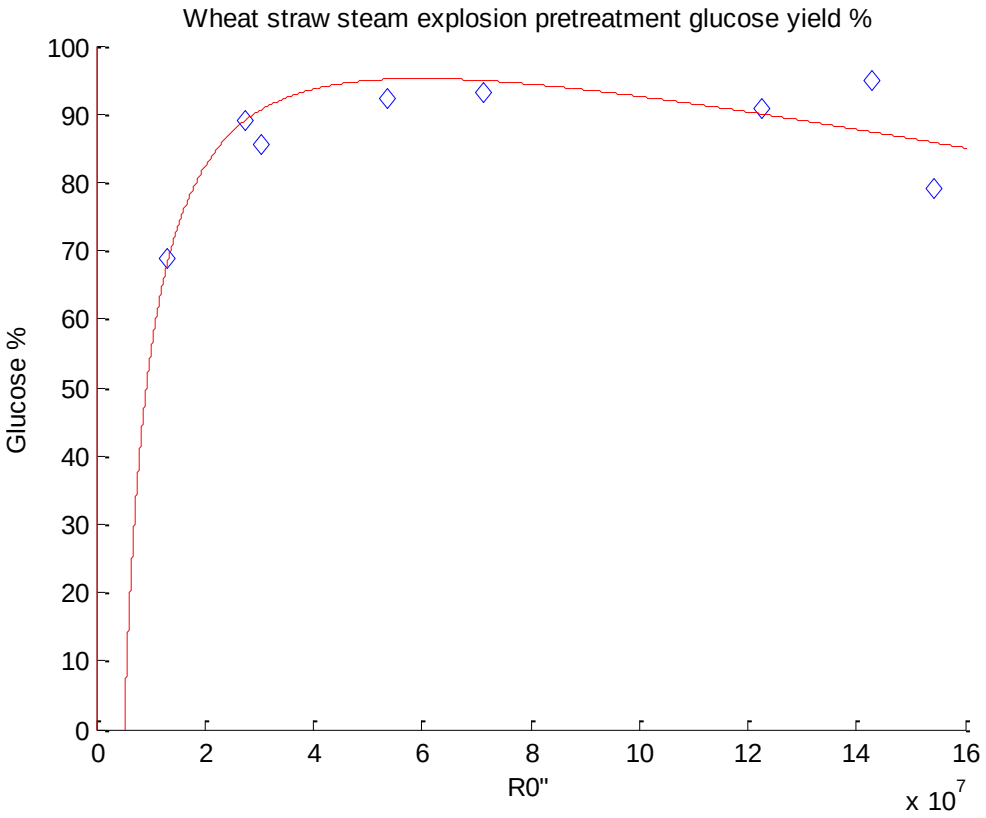

FIGURE 21: THE GLUCOSE YIELD DATA FROM THE STEAM EXPLOSION PRE-TREATMENT OF WHEAT STRAW, FITTED TO THE ADJUSTED MICHAELIS-MENTEN EQUATION

In Figure 21 the fit of the data from the wheat straw steam explosion pre-treatment is shown. The R^2 is 0.7291 and the standard error of fit is 4.9965 %. The reaction equation with the parameter values are as follows:

$$Glu = Glu_{max} \times \frac{R_0"}{K_m + R_0"} - K_d \times R_0" + K$$

Glu_{max}	116.8034
K_m	$1.7242*10^7$
K_d	$2.7888*10^{-7}$
K	22.6962

As seen above in the graph, the model does not accurately predict the glucose yield below a severity of $1*10^7$. This should not be a problem, since the steam explosion pre-treatment is not usually done at low severity. The problem might lie in the fact that the wheat straw was first impregnated with H_2SO_4, which lowered the pH significantly. When the pH is removed from the severity factor, thus only using the R_0, a better fit is obtained and shown in Figure 22.

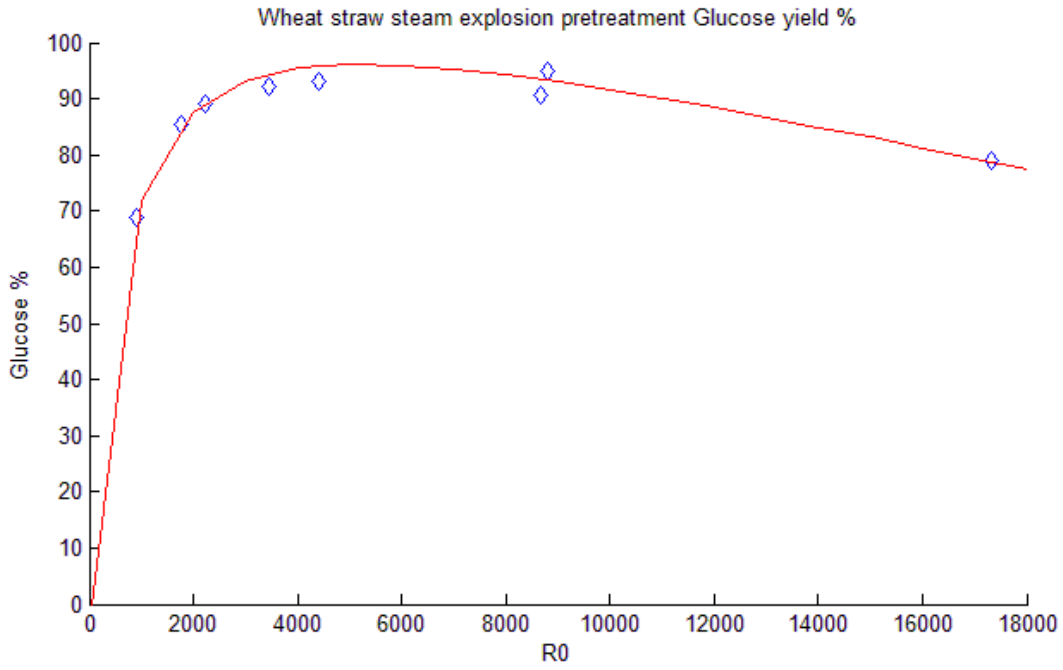

FIGURE 22: THE GLUCOSE YIELD DATA FROM THE STEAM EXPLOSION PRE-TREATMENT OF WHEAT STRAW, FITTED TO THE ADJUSTED MICHAELIS-MENTEN EQUATION. NOTE THAT R_0 IS USED, MEANING THAT THE pH IS NOT TAKEN INTO ACCOUNT

The fit has a R^2 of 0.92414 and the standard error of fit is 2.6442%. The reaction equation with the parameter values are as follows:

$$Glu = Glu_{max} \times \frac{R_0}{K_m + R_0} - K_d \times R_0 + K$$

Glu_{max}	122.7218
K_m	593.1379
K_d	0.0021
K	-2.9083

The time and effect of first soaking the wheat straw in an acid solution is not taken into account into the severity factor. Removing the pH from the severity improved the fit, but does not give a realistic view of the process since an acid catalyst is added that does affect the pre-treatment.

Degradation is observed in both graphs at higher severity. However, the degradation is much more visible in the graph with only R0, showing that the degradation is more likely due to the higher temperature and time than due to the decrease in pH.

4.9. COMPARING DIFFERENT PRE-TREATMENTS

To analyse the differences between the pre-treatments, several overlay graphs have been made. The comparison between the two alkaline pre-treatments, shown in Figure 23, shows that the NaOH pre-treatment is more effective as it gives rise to a higher glucose yield, but it does require a higher severity.

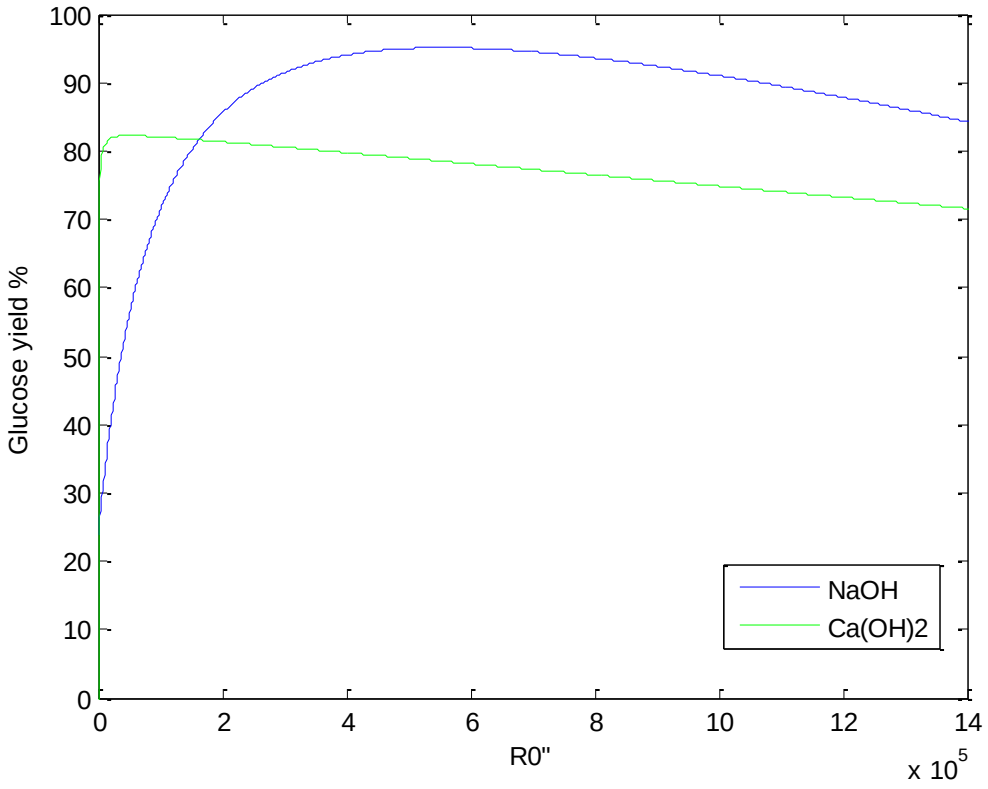

FIGURE 23: COMPARISON BETWEEN THE TWO ALKALINE PRE-TREATMENTS ON THE GLUCOSE YIELD

The Ca(OH)2 pre-treatment reaches its maximal sugar yield almost instantly compared to the NaOH pre-treatment, showing that a high severity is not necessary. Degradation of glucose starts very early for the Ca(OH)2, The NaOH pre-treatment also shows a higher amount of degradation. This trend is also seen in the xylose and arabinose yield pre-treatment comparison graphs(graphs not shown).

The comparison between all the different pre-treatments is shown in Figure 24.

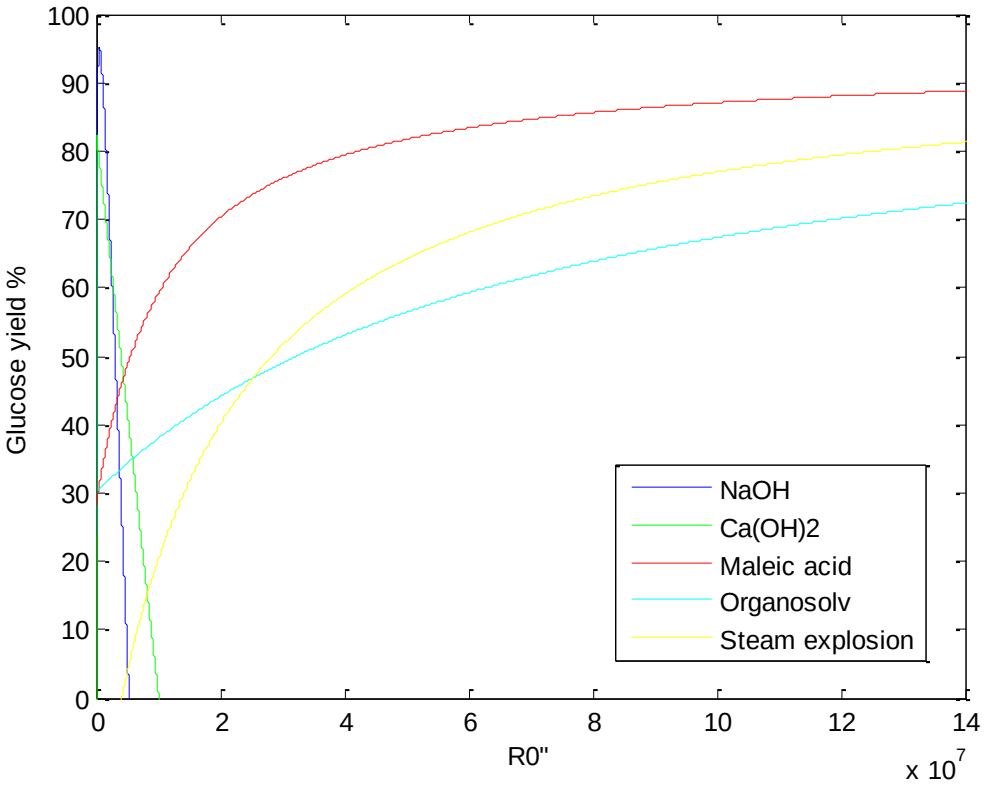

FIGURE 24: COMPARISON OF FIVE PRE-TREATMENTS ON THE EFFECT ON THE YIELD OF GLUCOSE

Again, this comparison graph clearly shows that the alkaline pre-treatments are very different from the other pre-treatments. The alkaline treatment is more effective at lower severities than the other pre-treatments. This difference probably lies in how the R_0" is calculated. The influence of time on the severity is relatively low and linearly scaled. The influence of the temperature and pH on the severity is exponential. Therefore, having a lower temperature and longer time leads to a relatively low calculated severity, but as seen in the alkaline pre-treatment still a high yield. The other pre-treatments are pretty similar. They all reach a maximum sugar yield of 90-100% and in the same severity range.

The comparison of the alkaline and acid treatments, shown in Figure 25, shows clearly that the acid pre-treatment requires a severity that is much higher than the severity needed with the alkaline pre-treatments.

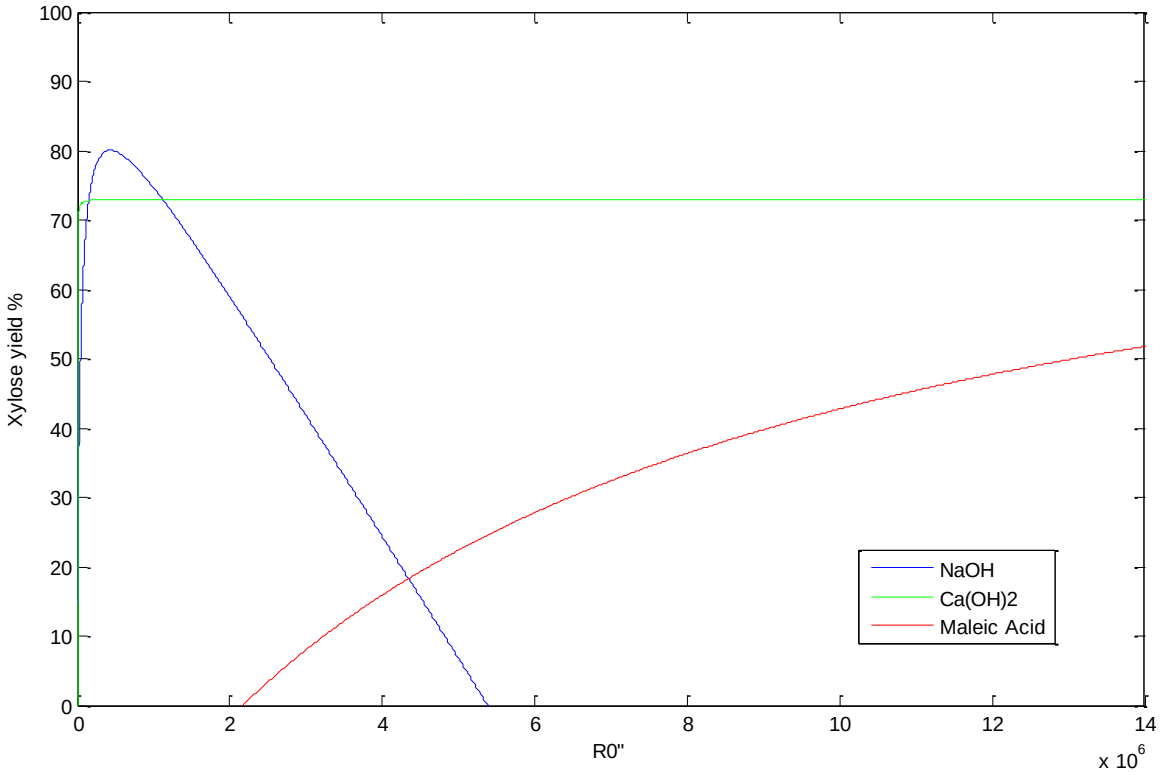

FIGURE 25: COMPARISON OF THREE DIFFERENT PRE-TREATMENTS ON THE EFFECT ON THE XYLOSE YIELD

The maximum sugar yield of the acid pre-treatment can reach cannot be shown in this graph as the alkaline pre-treatments would barely be visible. The maximum sugar yield possible does not differ much, however the maleic acid pre-treatment can reach a xylose yield of about 95%, which is significantly higher than the alkaline pre-treatments. This 95% xylose yield does require a higher severity than needed in the alkaline pre-treatments, giving rise to an economical issue.

4.10. RECOMMENDATIONS EXPERIMENTAL WORK

While working on this thesis it turned out that only limited data sets were available. The reasons for this were that many experimental data was confidential and could not be shared. In addition, a lot of literature on the pre-treatment of lignocellulose did not always specify enough details necessary. This limited the scope of this thesis. Therefore, certain recommendations can be made for future experimental work.

Many data sets miss data points at medium severity. This is where the optimum is expected to be. This optimum is important as this gives the highest possible amount of biofuel from the lignocellulose substrate. Cost wise, it may be important to determine the plateau of the sugar yield, so that the pre-treatment is not unnecessary severe, to reduce the costs. So, more experimental data is required for broad range of process conditions to optimise the modelling of the pre-treatment processes.

To create a satisfactory model, it might be beneficial to look more into depth on the degradation that occurs at higher severity. A lot of research stops at a certain severity where degradation occurs, which is logical if the goal is to find the maximum yield of sugars for the lowest costs for example. However, for modelling purposes it is be beneficial to measure also at very high severities, to accurately model the degradation.

5. DISCUSSION AND CONCLUSION

The pre-treatment of lignocellulose is a complex process. A lot of factors play a role in opening the lignocellulose, making it more bioavailable. Little is known about the kinetics of the process which complicates the modelling process.

Not much research has been done on the kinetics of the pre-treatment process. The precise effect of the pre-treatment on the opening of lignocellulose, release of sugars and accessibility of sugars remains largely unknown. Therefore, semi black box modelling was used in this work, meaning that the parameters of known equations were numerically estimated. This gives a more realistic view than fitting to a certain polynomial, as this gives a poor view on the physical properties of the process.

The trend of the models was expected. From literature it was known that degradation products are formed at higher severities, leading to a lower sugar yield. This decline in sugar yield is shown in almost all models. The plateau seen in most models was also predicted. In processes such as these, a 100% yield is never reached. A plateau of around 90% is to be expected. Though, for some models the plateau was a little lower, indicating that the pre-treatment might not be optimal.

When comparing the different pre-treatment technologies there is a large difference of effectiveness when looking only at the relationship between R_0"and sugar yield. The alkaline treatment is much more effective at lower severities than the other acidic pre-treatments. This difference probably lies in how the R_0" is calculated. The influence of time on the severity is relatively low and linearly scaled. The influence of the temperature and pH on the severity is exponential. Therefore, having a lower temperature and longer time leads to a relatively low calculated severity, but as seen in the alkaline pre-treatment still a high yield. The problem with the severity factor also comes forward in the steam explosion pre-treatment, where the lignocellulose was first soaked in an acid solution. This is not taken into account in the severity factor, as the steam explosion pre-treatment data show that the pH alone is not enough. This makes it difficult to compare pre-treatments on the severity factor alone. It is still useful when predicting what a certain pre-treatment will do to the availability of the lignocellulose and might be useful for looking at how different biomass type give different results with the same pre-treatment. The severity factor concept might need an update to increase the comparability between different pre-treatments. Changing the effect of the pH or the alkaline/acid loading might be a good way to start.

The effect of the pre-treatment on different types of biomass could not be modelled since the data available was not suitable for this purpose. The only data sets that could compare the effect of two types of biomass were the alkaline pre-treatment of wheat straw and rice straw. Due to the problems with the rice straw pre-treatment, that the pH was not specified in the article, the fit for this data set was not satisfactory. However, it is expected that since the composition of rice straw is similar to wheat straw, the effect of the pre-treatment should be similar. Biomass that is more different from each other, for example wheat straw and woody biomass, are expected to react differently to a pre-treatment process.

6. REFERENCES

1. Mussatto, S.I., et al., *Technological trends, global market, and challenges of bio-ethanol production.* Biotechnology Advances, 2010. **28**(6): p. 817-830.
2. Searchinger, T., et al., *Use of U.S. Croplands for Biofuels Increases Greenhouse Gases Through Emissions from Land-Use Change.* Science, 2008. **319**(5867): p. 1238-1240.
3. Kumar, P., et al., *Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production.* Industrial & Engineering Chemistry Research, 2009. **48**(8): p. 3713-3729.
4. Menon, V. and M. Rao, *Trends in bioconversion of lignocellulose: Biofuels, platform chemicals & biorefinery concept.* Progress in Energy and Combustion Science, 2012. **38**(4): p. 522-550.
5. Harmsen, P., W. Huijgen, L. Bermudez, and R. Bakker, *Literature Review of Physical and Chemical Pretreatment Processes for Lignocellulosic Biomass.* Food & Biobased Research, 2010.
6. Lope, T., A. Phani, and K. Mahdi, *Biomass Feedstock Pre-Processing – Part 1: Pre-Treatment*. Biofuel's Engineering Process Technology2011.
7. Klemm, D., et al., *Cellulose: Fascinating Biopolymer and Sustainable Raw Material.* Angewandte Chemie International Edition, 2005. **44**(22): p. 3358-3393.
8. Agbor, V.B., et al., *Biomass pretreatment: Fundamentals toward application.* Biotechnology Advances, 2011. **29**(6): p. 675-685.
9. Sun, Y. and J. Cheng, *Hydrolysis of lignocellulosic materials for ethanol production: a review.* Bioresource Technology, 2002. **83**(1): p. 1-11.
10. Faulon, J.-L., G.A. Carlson, and P.G. Hatcher, *A three-dimensional model for lignocellulose from gymnospermous wood.* Organic Geochemistry, 1994. **21**(12): p. 1169-1179.
11. Garlock, R.J., et al., *Comparative material balances around pretreatment technologies for the conversion of switchgrass to soluble sugars.* Bioresource Technology, 2011. **102**(24): p. 11063-11071.
12. Ayla Sant'Ana da Silva, R.S.S.T., Rondinele, et al., *Sustainable Degradation of Lignocellulosic Biomass - Techniques, Applications and Commercialization*, ed. D.A. Chandel2013: inTech. 275.
13. Mosier, N., et al., *Features of promising technologies for pretreatment of lignocellulosic biomass.* Bioresource Technology, 2005. **96**(6): p. 673-686.
14. Chen, Y., et al., *Understanding of alkaline pretreatment parameters for corn stover enzymatic saccharification.* Biotechnology for Biofuels, 2013. **6**(1): p. 8.
15. Zhao, X., K. Cheng, and D. Liu, *Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis.* 2009. **82**(5): p. 815-827.
16. Ni, Y. and Q. Hu, *Alcell® lignin solubility in ethanol–water mixtures.* Journal of Applied Polymer Science, 1995. **57**(12): p. 1441-1446.
17. Esteghlalian, A.R., et al., *Do Enzymatic Hydrolyzability and Simons' Stain Reflect the Changes in the Accessibility of Lignocellulosic Substrates to Cellulase Enzymes?* Biotechnology Progress, 2001. **17**(6): p. 1049-1054.
18. Alvira, P., et al., *Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review.* Bioresource Technology, 2010. **101**(13): p. 4851-4861.
19. Hasunuma, T., et al., *A review of enzymes and microbes for lignocellulosic biorefinery and the possibility of their application to consolidated bioprocessing technology.* Bioresour Technol, 2013. **135**: p. 513-22.
20. Chandel, A.K., et al., *The realm of cellulases in biorefinery development.* Crit Rev Biotechnol, 2012. **32**(3): p. 187-202.

21. García-Aparicio, M., et al., *Xylanase contribution to the efficiency of cellulose enzymatic hydrolysis of barley straw.* 2007. **137-140**(1-12): p. 353-365.
22. Pryor, S.W., B. Karki, and N. Nahar, *Effect of hemicellulase addition during enzymatic hydrolysis of switchgrass pretreated by soaking in aqueous ammonia.* Bioresource Technology, 2012. **123**(0): p. 620-626.
23. Park, S., et al., *Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance.* Biotechnology for Biofuels, 2010. **3**(1): p. 10.
24. Chang, V. and M. Holtzapple, *Fundamental factors affecting biomass enzymatic reactivity.* 2000. **84-86**(1-9): p. 5-37.
25. Chandra, R.P., et al., *Substrate pretreatment: the key to effective enzymatic hydrolysis of lignocellulosics?* Adv Biochem Eng Biotechnol, 2007. **108**: p. 67-93.
26. Bellido, C., et al., *Effect of inhibitors formed during wheat straw pretreatment on ethanol fermentation by Pichia stipitis.* Bioresource Technology, 2011. **102**(23): p. 10868-10874.
27. Zhang, Y., L. Wang, and H. Chen, *Formation Kinetics of Potential Fermentation Inhibitors in a Steam Explosion Process of Corn Straw.* 2013. **169**(2): p. 359-367.
28. Daniel Montané, J.S., Xavier Farriol, *Fractionation of Wheat Straw via Steam-Explosion Pretreatment. Characteristics of the Lignin Obtained by Alkali Delignification of the Steamed Straw.* Holzforschung - International Journal of the Biology, Chemistry, Physics and Technology of Wood, 1997. **51**(2): p. 135–141.
29. Overend, R.P., E. Chornet, and J.A. Gascoigne, *Fractionation of Lignocellulosics by Steam-Aqueous Pretreatments [and Discussion].* Philosophical Transactions of the Royal Society of London. Series A, Mathematical and Physical Sciences, 1987. **321**(1561): p. 523-536.
30. Pedersen, M. and A.S. Meyer, *Lignocellulose pretreatment severity – relating pH to biomatrix opening.* New Biotechnology, 2010. **27**(6): p. 739-750.
31. Huijgen, W.J.J., et al., *Catalytic organosolv fractionation of willow wood and wheat straw as pretreatment for enzymatic cellulose hydrolysis.* Journal of Chemical Technology & Biotechnology, 2011. **86**(11): p. 1428-1438.
32. Jiménez, L., et al., *Influence of process variables on the properties of pulp obtained by ethanol pulping of wheat straw.* Process Biochemistry, 1999. **35**(1–2): p. 143-148.
33. Harmsen, P., *WUR Data.*
34. Cheng, Y.-S., et al., *Evaluation of High Solids Alkaline Pretreatment of Rice Straw.* 2010. **162**(6): p. 1768-1784.
35. Panagiotopoulos, I.A., et al., *Effect of pretreatment severity on the conversion of barley straw to fermentable substrates and the release of inhibitory compounds.* Bioresource Technology, 2011. **102**(24): p. 11204-11211.
36. Kootstra, A.M.J., *Processing lignocellulosic by-product streams using organic acids*, 2010, Wageningen Universiteit (Wageningen University): Wageningen. p. 175 pp.
37. Wildschut, J., et al., *Ethanol-based organosolv fractionation of wheat straw for the production of lignin and enzymatically digestible cellulose.* Bioresource Technology, 2013. **135**(0): p. 58-66.
38. Linde, M., et al., *Steam pretreatment of dilute H2SO4-impregnated wheat straw and SSF with low yeast and enzyme loadings for bioethanol production.* Biomass and Bioenergy, 2008. **32**(4): p. 326-332.
39. Cornish-Bowden, A., *Fundamentals of enzyme kinetics*1995, London: Portland Press.