Biomass Refinery and Process Dynamics

Thesis Biomass Refinery and Process Dynamics

Understanding and predicting the alkaline pre-treatment of *Miscanthus* through modelling

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11-02-2014

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WAGENINGEN UNIVERSITY
AGROTECHNOLOGY AND
FOOD SCIENCES

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Name course	:	Thesis Biomass Refinery and Process Dynamics
Number	:	YBT-80324
Study load	:	24 ects
Date	:	11 February 2014
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Study programme	:	BBT (Biotechnology)
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Acknowledgements

I wish to thank various people for their help and contribution to this thesis; first of all, my supervisors, Ellen Slegers and Richard Gosselink who provided me with excellent advice during my work. Working with them was a real pleasure and I would do it again without hesitation. Special thanks should be given to my lab supervisors, Hans van der Kolk and Jacinta van der Putten on who I could always rely during my lab work. I would also like to especially thank my father for his long range support on which I could always count.

Introduction

With the increase of the world population, it is only logical that the global need for energy increases as well. Fossil energy sources will soon not be able to follow this demand anymore as they will eventually run out. Therefore, alternative and renewable energy sources are being studied. An intensively researched technology is the production of biofuels. Biofuels offer alternatives and advantages compared to fossil fuels, for instance, they emit less pollution, they are made from renewable resources and have the potential to be cheaper than gasoline[2].

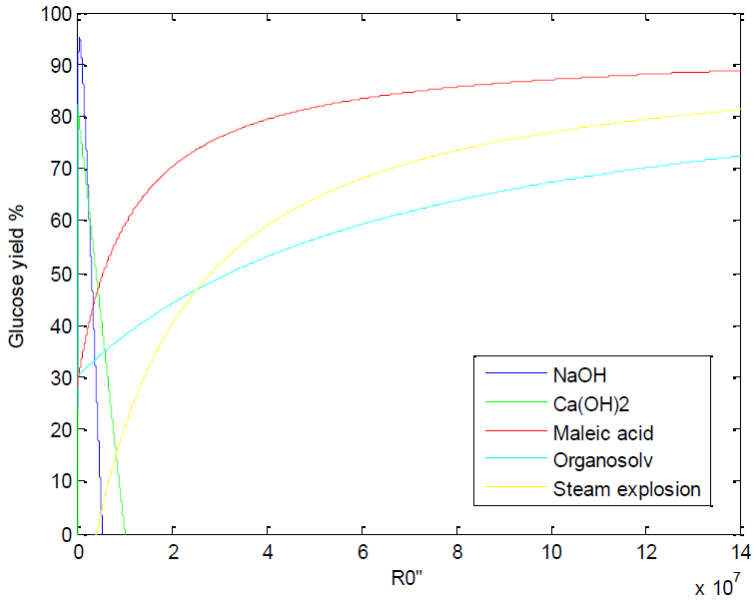


Figure 1: Comparison of five pre-treatments on the effect on the yield of glucose[1]

To be able to produce biofuels, biomass needs to be degraded and fermented. These biomasses can vary from paper to waste streams or wood. Four distinct categories of biofuels can be distinguished. The 1st generation biofuels are derived from sugar rich biomass such as corn or sugarcane, which competes with the worldwide food supplies, and thus raises ethical problems. 2nd generation biofuels are made out of biomass which do not interfere with food consumption. 3rd generation biofuels are fuels which are produced out of algae. And 4th generation biofuels combine engineered biomass with second generation biomass conversion techniques. This engineered biomass has a higher yield and an increased carbon storage. The results are carbon negative biofuels[3], this means that these fuels reduce the carbon footprint on earth. This last generation is new and is still in the initial stage of research. For now, the 2nd generation biofuels offer the highest potential in the production of sustainable renewable energy resources as they do not compete with the food consumption. In addition, most of the research about biofuels is done on this generation[4]. The most abundant 2nd generation biomass is lignocellulosic biomass. Lignocellulose is a raw material composed mostly out of cellulose, hemicellulose and lignin. The most common lignocellulosic biomasses are wood and straw. In the Netherlands, for the sole purpose to produce biofuels, the total yield is expected to be about 1 million ton of pure biomass [5].

Lignocellulose has a very strong and dense structure. Lignin is tightly bound to cellulose and hemicellulose, and cellulose is found in a crystalline structure. These properties make enzyme hydrolysis impossible if no pre-treatment is applied. Many different pathways for the pre-treatment of lignocellulose are known nowadays. Rick van Rijn compared the most important of them in his study[1], and correlated the sugar yield with the intensity of the applied pre-treatment RO" as shown in Figure 1.

As seen above in Figure 1, alkaline pre-treatments (with NaOH) have been observed as the treatments with the highest sugar yield and the severity of the treatment is also relatively low. However, most of the research focusses on the optimal conditions, and not on the whole process. As we can see in Figure 1, in the case of alkaline pre-treatments there is a loss in sugar yield at higher severity, and this still is not fully understood. In this work we aim at understanding the alkaline pre-treatment of *Miscanthus* with NaOH. We will be especially looking into the influence of the pH and temperature on the bioavailability of the sugars within *Miscanthus*. Based on a central composite design, experiments have been performed. The results of these experiments will be used to understand and model the kinetics of alkaline pre-treatments.

Literature review

Miscanthus

Miscanthus, more commonly known as 'elephant grass" is a tall herbaceous perennial grass, which can reach a height of 3-4 meters [6]. This plant originates from eastern Asia, but is nowadays also grown in America and Europe. It grows in dense clumps due to the underground rhizomes out of which this species grows.

Currently, *Miscanthus* is used a lot for ornamental purposes. But this plant has a lot of potential in the field of biomass conversion into marketable bio based products: it has a high rate of carbon fixation as it can yield up to 6,5 tons/hectare of biomass during 25-30 years without significant reduction in growth during this period [7]. Research on the production of biofuels out of *Miscanthus* revealed that one hectare of this species can save up to 7 tons of CO_2 per year [8].

Figure 2: *Miscanthus* crops at the University of Illinois, USA

Lignocellulose

The term lignocellulose refers to the dry matter of a plant, called the lignocellulosic biomass. It is the main component of *Miscanthus*, and it consists mainly of cellulose, hemicellulose and lignin (see Table 1 and Figure 3).

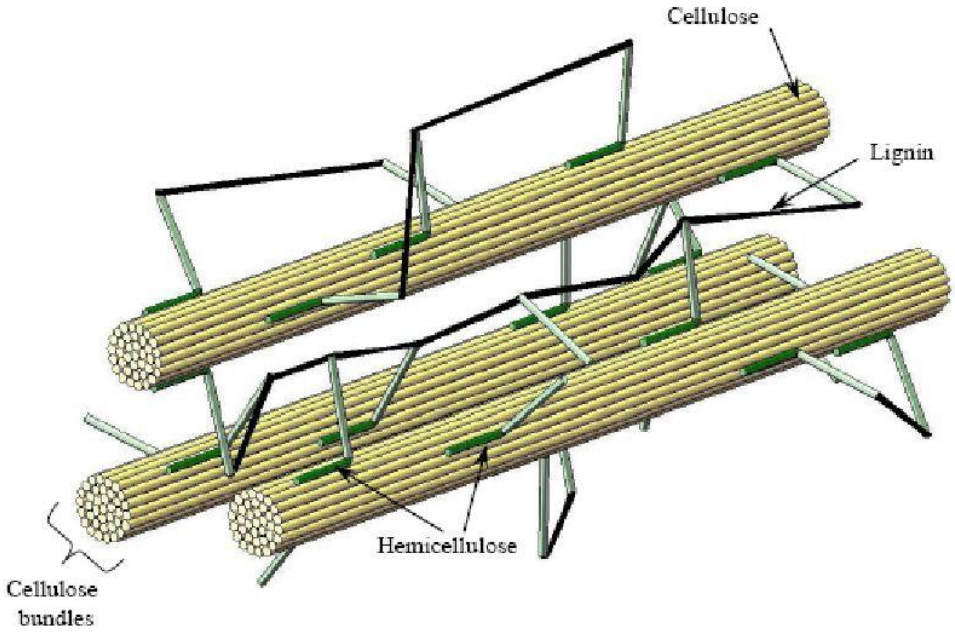


Figure 3: Structure of lignocellulose [9]

Cellulose is the main structure component of cell walls and is the most abundant organic compound in the world. Cellulose is a linear polymer of glucose units which are linked to each other through a β-1,4-glucosidic bond and form a crystalline structure. The amount of glucose units composing the cellulose fibre is defined by the degree of polymerization of glucose, this can vary from 300 to 17000 units. Commonly a degree of polymerization between 800 and 10000 units is encountered[10]. In lignocellulose, cellulose occurs in the form of bundles, which is composed out of several cellulose fibres. These bundles are glued together by lignin.

Hemicellulose is a polymer chain composed of C_5 sugars, such as xylose or arabinose, or C_6 sugar monomers. These chains are relatively short (150-200 monomers), and unlike cellulose, they are branched polymers. Because of the absence of a crystalline structure, hemicellulose is hydrolysed more easily compared to cellulose.

Lignin is the aromatic component giving the plants their structure and strength by binding cells, fibres and vessels together. After cellulose it is the most abundant carbon source on earth. It's structure is a complex polymer composed mostly of phenyl propane units as backbone, but all lignins show variation in their chemical composition.

Alkaline pre-treatment

In this section the chosen pre-treatment technique, alkaline pre-treatment, will be described. The goal of a pre-treatment is to increase the bioavailability of hemicellulose and cellulose, so that these sugar chains can be hydrolysed more easily by enzymes. This significantly increases the yields of fermentable sugar monomers such as glucose and xylose.

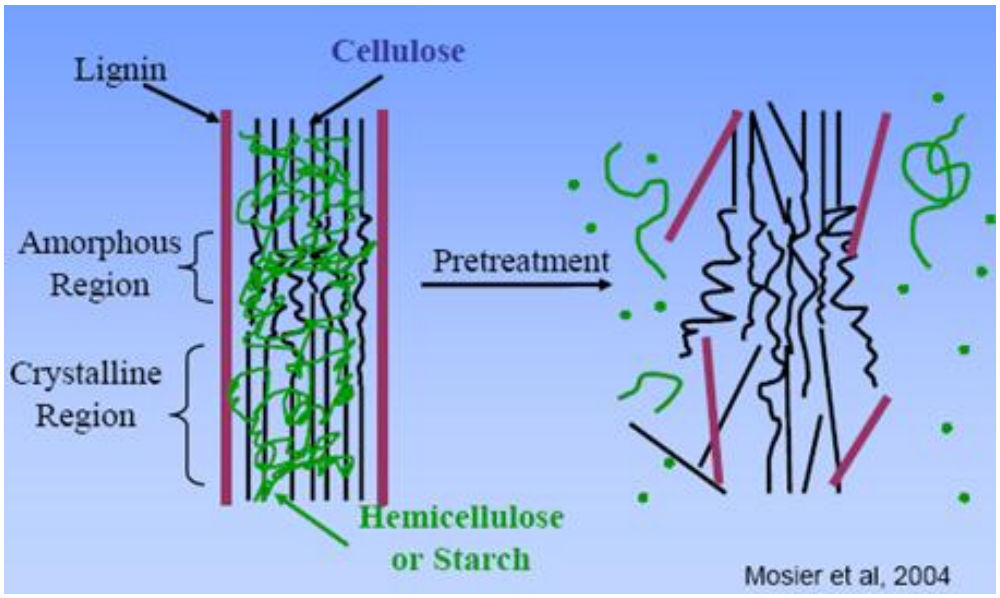
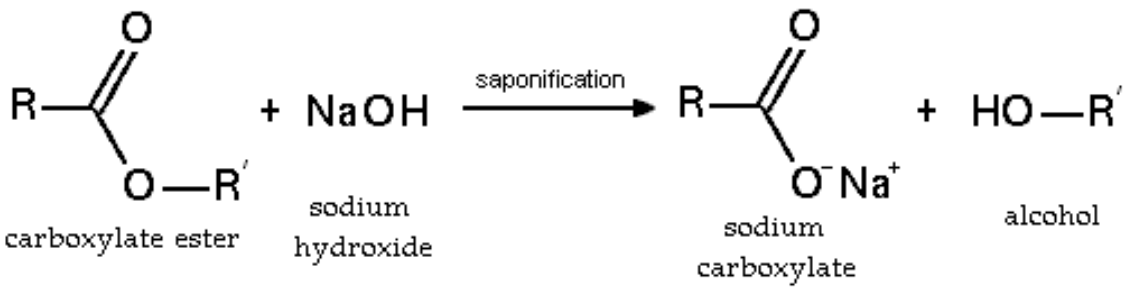


Figure 4: Schematic representation of the effect of pre-treatment on lignocellulosic biomass[11]

During an alkaline pre-treatment, the increase in availability of cellulose and hemicellulose is partly due to the delignification process (solubilisation of lignin). These alkaline conditions break the ester linkages between lignin and hemicellulose and in lignin through the saponification process, yielding alcohol and salts (Eq. 1). Other effects of the high pH is the disruption of the cellulose crystallinity and partial solubilisation of hemicellulose(Fig. 4). Alkaline treatment also increases the pore size of biomass, the so called swelling process [12]. All of these mechanisms contribute to the increase in bioavailability of (hemi)cellulose to obtain higher yields of fermentable sugars.

Equation 1: saponification process with sodium hydroxide



Furthermore, depending on the alkaline strength and the temperature, the ether bonds connecting cellulose and lignin are broken as well, causing a high removal of lignin through dissolution. The shielding function of lignin towards hemicellulose and cellulose is not possible anymore, resulting in the increase of availability of these two components for enzymatic hydrolysis[13]. It should be noted that at higher and more severe pre-treatment conditions, inhibitors such as organic acids or phenols may also be present. These compounds are degradation products from the monomeric sugars and lignin [14]. They inhibit the scarification process by competitive inhibition of the enzymes during the hydrolysis step.
Disadvantages of the alkaline pre-treatment is the relatively long treatment time compared to other pre-treatments [1]. However, in contrary to the other chemical pre-treatment (such as acidic pre-treatment) only relative low amounts of inhibitors are produced, which favours the high sugar yield. Additionally, the alkaline pre-treatment can be performed at milder temperature ranges compared to most acidic pre-treatments.

Materials and Methods

Preparation and analysis of Miscanthus

Miscanthus (harvested February 2005) was milled twice. First milled so the chips could pass through 8×8 mm sieves, and then through a mill transforming these chips in smaller chips with sizes varying between 0.5 mm and 4 mm. Milled *Miscanthus* was kept in a sealed plastic barrel at room temperature until use.

Chemical composition was analysed as described by TAPPI methods [15-19], with the following modifications: (1) Samples were extracted with ethanol:toluene 2:1, ethanol at 1500 psi and 100°C and hot water (1 hour) at boiling temperature. (2) The extracted samples were dried at 60 °C for 16 hours. (3) Monomeric sugar and lignin content of the extracted material was determined after a two-step hydrolysis with sulphuric acid (12 M for 1 hour at 30 °C; 1M for 3 hours at 100 °C). (4) Acid soluble lignin in the hydrolysate was determined by spectrophotometric determination at 205 nm.

A Dionex system with Carbopak PA1 column with pre-column was used at 30°C, with a gradient of sodium hydroxide in de-ionized water as mobile phase (1 mL/min) and fucose as internal standard. The Dionex HPLC method was also used for determination of monomeric sugars in the aqueous phase of both pre-treated and enzymatically hydrolyzed *Miscanthus.*

To determine the dry matter content of *Miscanthus,* oven drying at 105°C was used. A sample was weighed in an aluminium dish and left for drying overnight in an preheated oven at 105°C. The weight of the dried samples corresponds to the dry matter, so the ratio between the initial sample and the dried samples corresponds to the dry matter content:

$$\text{Dry matter content} = \frac{mass_{initial\ sample}}{mass_{dried\ sample}} \times 100\ \%$$

Dry matter content of the milled Miscanthus starting material was 93% (w/w) (24h at 105°C).

Table 1:Chemical composition of Miscanthus after grinding (FBR, Wageningen UR;22-06-11)

Extractives in original material			Average polysaccharide contents (%)						Uronic acids	Lignin		Total
ethanol / toluene (%)	ethanol (%)	water (%)	Arabinan	Xylan	Mannan	Galactan	Glucan	Rhamnan	(%)	AIL (%)	ASL (%)	(%)
3.50	1.08	4.07	1.46	15.87	0.02	0.34	38.96	0.00	1.34	20.82	1.04	88.52[1]

[1] The remaining 11.48% mostly consists out of proteins and salts

Experimental set up of Miscanthus pre-treatment

All chemicals were of research grade and used as received. Milled *Miscanthus* (7.5 g; 6.975 g dry biomass) was mixed with 75 mL of alkaline solution (1M) or with de-ionised water resulting in 10% (w/w) dry *Miscanthus* solids loading. This mixture was prepared in 100 mL stainless steel reactors fitted with thermocouples. Four reactors at a time were heated in a Haake B bath with a Haake N3 temperature controller (Thermo Fisher Scientific, Waltham, MA), filled with silicon oil (DC 200 fluid, 100 cSt, Dow Corning, Midland, MI)(Fig. 5). Sample core temperature was recorded (Picotech data collector and software; Picotech, UK). Pre-treatments were performed between 100°C and 160°C. Average heating up time was 15 minutes, starting from when the desired process temperature was reached. The temperature difference between the oil and the inside of the reactor did not exceed 15°C during heating, and not more than 5°C during the holding time. After the reaction time, the reactors were cooled by quenching in water at room temperature.

Figure 5: experimental set-up for the pre-treatment step

Enzymatic hydrolysis of pre-treated Miscanthus

After pre-treatment, the content of the reactors was transferred into glass flasks and 100 mL of water was added. The content of the flasks was then washed using a Buchner funnel connected to a side arm flask with a tube leading to a vacuum pump(Fig. 6). The biomass was washed with tap water until the pH was around 6-7.

Figure 6: Set-up of the washing step

After determination of the dry mass content of each sample, 2 grams of dry biomass was transferred into 100 mL erlenmeyers. 32 mL of water and 8 mL of sodium acetate buffer (pH 5.0) was added. By using 5M H_2SO_4 and 1M NaOH solutions, the pH was fine-tuned to pH 5, the optimum pH for the used enzymes. These enzymes are GC220 (supplier:Genencor) and Novozyme 188 (Supplier: Novozymes), they were added in a 10:1 ratio, and the amount of GC220 added relative to the amount of dry biomass was 0.25 gram enzyme/gram biomass. So 0.5 gram of GC 220 was added, and 0.05 gram of Novozyme 188. After enzyme addition, the Erlenmeyers were closed with aluminium foil and placed in an Innova 42 incubator shaker (50°C, 150 rpm, 2 in. stroke; NBSC, Edison, NJ). Two samples of 1 mL were taken at t=2, t=4, t=24 and t= 48 hours. The enzymes were inactivated in a boiling-water bath for 5 minutes, then the samples were stored at -20°C for further analysis.

The glucose yield from cellulose was calculated as follows:

$$Glucose\ yield\ (\%) = \left(\frac{GH}{GS} \times Y_G\right) \times 100(\%)$$

Where GH is the amount of glucose (g) present in the aqueous phase of the sample after enzymatic hydrolysis, measured using the HPLC, and GS is the amount of glucose present in the sample of dry *Miscanthus* (g glucose equivalents in cellulose). This yield equation is modified with the insertion of a yield factor. This is to take into account the effect of glucan conversion, where there is a 10% conversion into glucose, hence $Y_G = 0.9$.

Xylose yield from hemicellulose conversion was calculated similarly, using xylan/xylose content. Only the conversion factor of xylan to xylose Y_X is slightly different. In this case, we have a 12% conversion into xylose, resulting in $Y_X = 0.88$.

Central Composite Design (CCD)

A CCD is an experimental design which is often used in the Response Surface Methodology. This methodology is used to investigate the relationships between the explanatory variables, in this case temperature, pH and pre-treatment holding time, and the response variable, in this case the sugar yield. The CCD gives a sequence of designed experiments to perform in order to obtain the most accurate data possible, while minimizing the number of experiments. This design can be divided into three distinct sets experiments:

- A factorial design in the studied factors
- A set of centre points, whose values of each factor are the medians of the value used in the factorial design. This is a single point, but it is repeated multiple times, in order to improve the precision of the experiment.
- A set of axial (or star) points. These points are identical to the centre point, except for one factor. The varied factor will take on values both below and above the median of the factorial level, typically outside of their range, to study the extremes. This is done by applying a step α defined by the following formula;

  $$\alpha = 2^{\frac{k}{4}}$$

  With k the number of variables, in this case three (pH, temperature and time), so here α= 1.682.

Figure 7 gives a good image of the build of this experimental design:

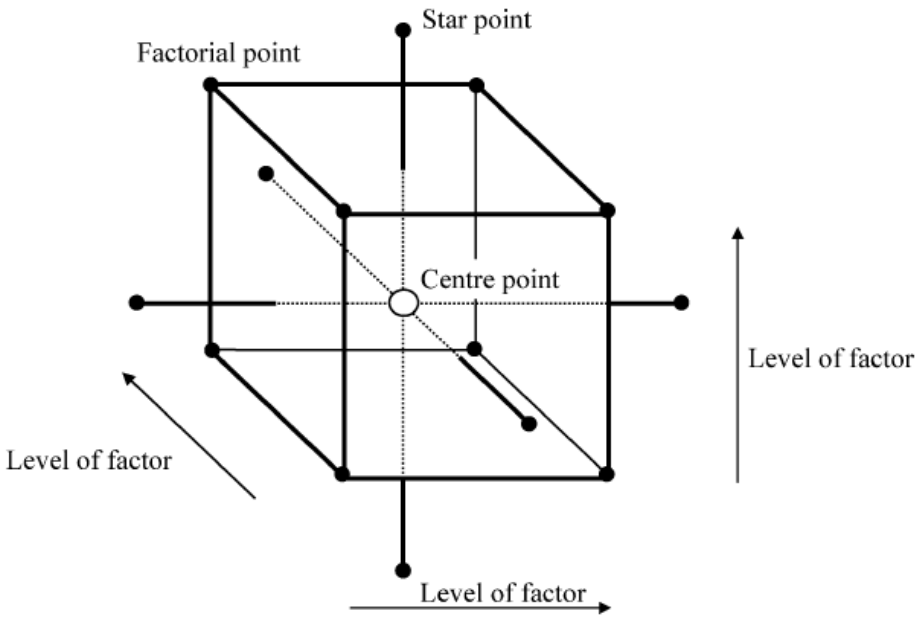


Figure 7: Central Composite Design (CCD) for three factors[20]

The CCD is used to build a second order (quadratic) model for the response variable without needing the full three-level factorial experiment. Because of this, the amount of experiments is considerably reduced, while still varying the conditions within the defined boundaries. This makes the results approximate, but it is easy to use and to estimate, even when little is known about the process itself. For my alkaline pre-treatment experiments the boundaries of the selected conditions are summarized in Table 2.

Table 2:Boundaries of main variables used for the Central Composite Design

	Minimum	Maximum	Scale
Time	1 hour	4 hours	linear
Temperature	100°C	160°C	linear
pH	7	13	Logarithmic: $pH=14+\log([OH^-])$

Modelling with MATLAB®

MATLAB is a numerical computer environment for numerical computation, visualization and programming. It enables to analyse data, develop algorithms and create models.

Out of the experiments described above, the sugar yields obtained after HPLC analysis are put together into a data matrix linking the sugar yields to the corresponding pre-treatment conditions (time, temperature and pH).

After resampling the data using the *interp1* script, the script *fminsearch* was used. This function is an unconstrained nonlinear optimization, it finds the minimum of a scalar function of several variables, starting at an initial estimate. The parameters are returned using the iterative least square method. This means that the net result minimizes the sum of the squares of the difference between the fit and the data points. In the current case, this script was coupled to the *ode45* solver function. This function solves numerically differential equations using a variable step Runge-Kutta Method. We used it to solve the differential equations describing the chosen model.

Results and Discussion

In this section, the results of the effect of temperature, time and pH during the pre-treatment of *Miscanthus* on the sugar yield as well as the modelling in MATLAB to derive the reaction kinetics are shown. Along with this, some recommendations for further experimental work are given based on the observations made during this work.

Lab work of the pre-treatment experiments

The performed experiments are shown in Table 3. The glucose and xylose yields as a function of the pre-treatment time at 130°C and pH=10 are given in Figure 8. In *Miscanthus* other sugars than glucose and xylose are also detected, but these are liberated in low amounts and therefore they were not used in the modelling.

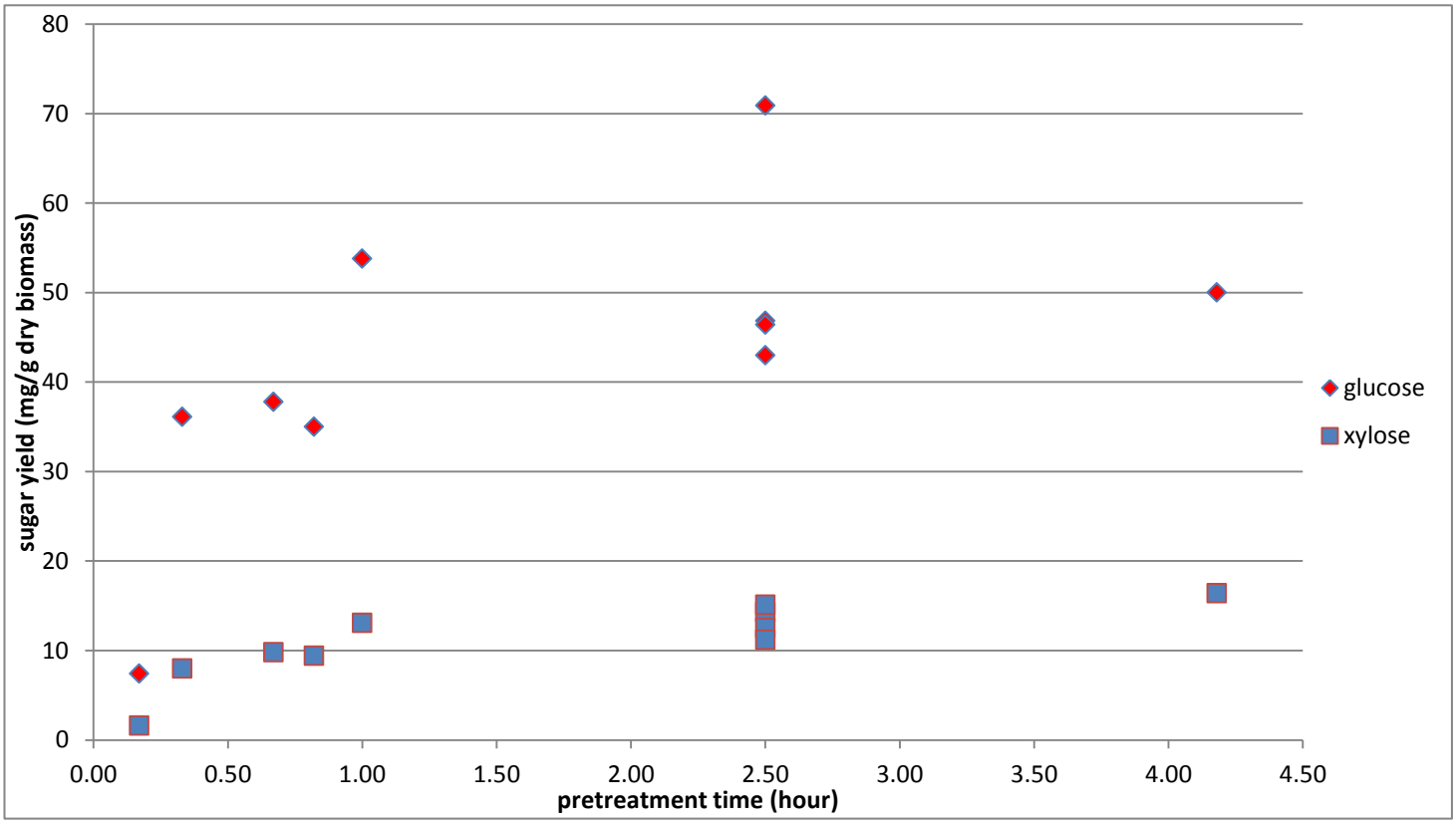


Figure 8:Sugar yields at 130 degrees Celsius and pH 10

Table 3: Central Composite Design of the experiments with results[2],[3]

	Central Composite Design					Coded values central composite design		
	experiment	*Time* (hour/min)	*Temperature* (°C)	*pH (theoretical)*	*pH (measured during the experiments)*	*Time*	*Temperature*	*pH*
Factorial design	17	1.50/90	110	8.00	8.75	-1	-1	-1
	19	1.50/90	110	12.00	13.00	-1	-1	1
	21	1.50/90	150	8.00	7.50	-1	1	-1
	23	1.50/90	150	12.00	12.83	-1	1	1
	18	3.50/210	110	8.00	8.20	1	-1	-1
	20	3.50/210	110	12.00	12.90	1	-1	1
	22	3.50/210	150	8.00	7.30	1	1	-1
	24	3.50/210	150	12.00	12.82	1	1	1
Centre Points	9	2.50/150	130	10.00	10.10	0	0	0
	10	2.50/150	130	10.00	9.90	0	0	0
	13	2.50/150	130	10.00	10.01	0	0	0
	14	2.50/150	130	10.00	10.30	0	0	0
Axial points	11	4.18/250	130	10.00	9.85	1.682	0	0
	12	0.82/50	130	10.00	9.85	-1.682	0	0
	7	2.50/150	163	10.00	13.00	0	1.682	0
	25	2.50/150	96	10.00	10.15	0	-1.682	0
	15	2.50/150	130	13.36	12.92	0	0	1.682
	16	2.50/150	130	6.64	6.60	0	0	-1.682
extra measuring points for short times	26	0.17/10	130	10.00	10.20			
	27	0.33/20	130	10.00	10.20			
	28	0.67/40	130	10.00	10.20			
	29	1.00/60	130	10.00	10.10			

[2] The experiments from which the sugar yields have been measured are highlighted in green, For the exact values of these sugar yields see Appendix A.

[3] Due to practical reasons, caused by a failing HPLC, the analysis of all the samples could not be done. Consequently it has been decided to measure only the samples linked to the centre points (T=130°C, pH=10), while varying the time of the pre-treatment, in order to build a basic kinetic model for one temperature – pH combination.

In the CCD, we only get values around pre-treatment times of 1.5 hours, 2.5 hours, 3.5 hours 4 hours and 50 minutes. To get more insight into the kinetics of the process, aside from the experiments performed for the CCD, additional experiments have been performed with shorter pre-treatment times (experiments 26 to 29).

In Figure 8, a clear trend is observed: indeed after 1/1.5 hours of pre-treatment, it seems that a maximum has been reached. After 4 hours of pre-treatment, no decrease in xylose yield is observed, so a simple model appears sufficient to describe the data.

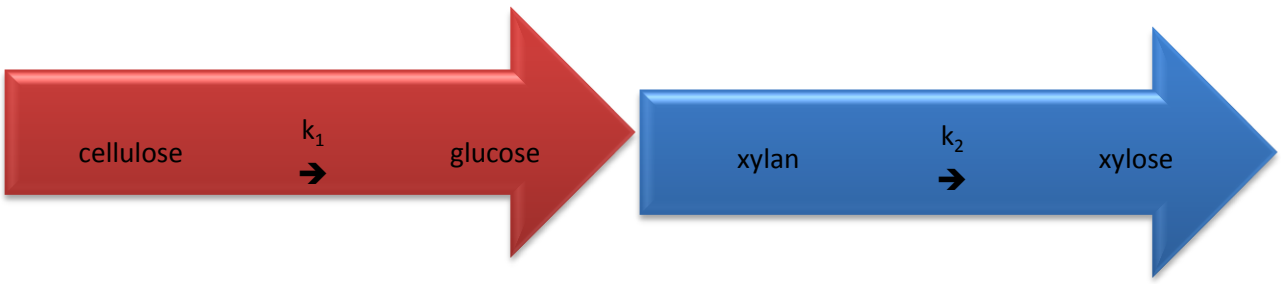


Figure 9:Adopted kinetic models for the sugar degradation

Mathematically, the model in Fig. 9 can be translated as follow:

$$\frac{d([Cellulose])}{dt} = -k_1 \times [Cellulose] \quad (1)$$

$$\frac{d([Glucose])}{dt} = \frac{k_1}{Y_1} \times [Cellulose] \quad (2)$$

$$\frac{d([Xylan])}{dt} = -k_2 * [Xylan] \quad (3)$$

$$\frac{d([Xylose])}{dt} = \frac{k_2}{Y_2} \times [Xylan] \quad (4)$$

As seen in table 3, 10 experiments were performed, where [Glucose] and [Xylose] have been measured to be able to determine the reaction rate constants. These reaction rate constants can be expressed through a modified Arrhenius equation taking into account the effect of pH as well as temperature:

$$k_i = A_{0_i} \times [OH^-]^{m_i} \times e^{(\frac{-E_{A_i}}{RT})} \quad (5)$$

with A_0 the pre exponential factor (min^{-1}), E_A the activation energy ($J.mol^{-1}$), m the reaction order of the hydroxide ion concentration, R the ideal gas constant and T the temperature (K). i=1,2 referring to cellulose and xylan respectively.

In this equation, m can be seen as a shape factor. This means that it determinates the importance of the [OH^-] in this equation. Along with this, A_0 can be interpreted as a balance factor for the whole reaction rate. Thus this frequency factor adapts itself to the values of m and E_A. This means that several combinations of A_0, m and E_A can lead to the same reaction rate, as well as the values of the reactions rates k_1 and k_2 should be in the same order of magnitude when calculated.

During the experimental work, a relatively high decrease in pH has been observed during the pre-treatment step (in some cases the pH dropped from 12.8 to 7.8). This is most likely caused by the organic acids formed during this step. As only the sugar yields were measured during this work, the exact yields of organic acids and their influence on the other reactions is not clearly known. Additional experiments to study the influence of organic sugars on the pre-treatment can be useful for better understanding of the pre-treatment.

Concerning the pH, during the experiments the importance of its influence became quite clear. Indeed, already after the pre-treatment step, a clear difference between the solution at milder and at higher pH values could be seen, as seen in figure 11.

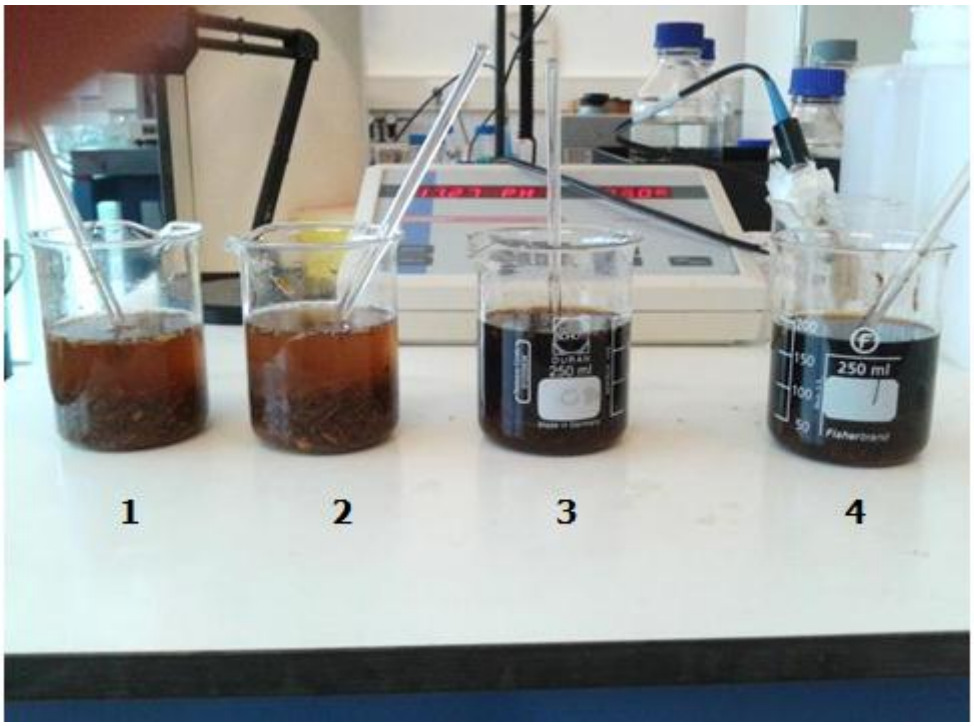


Figure 10: Black liquor after the pre-treatment step, at pH 7 (1 and 2) and pH 12.5 (3 and 4)

This liquid is called "black liquor" due to its colour. It is an aqueous solution of lignin fragments, hemicelluloses and the inorganic chemicals used during the pre-treatment. Here we can clearly see that the liquor is much darker for the pre-treatment at higher pH, thus containing more solubles. Furthermore, in the liquor at milder pH, we can still see some *Miscanthus* chips. Even after the enzymatic hydrolysis of these chips they remain solid, while the chips pre-treated at higher pH were completely dissolved in the hydrolysis solution.

The relatively high variation between the data points in figure 8, for example between the points around t=2.5 hours, is presumably due to the variation in pH between the theoretical value (pH=10) and the actual value measured during the experiments, as seen in table 2. This variation is due to the buffer capacity of the *Miscanthus* biomass itself. The buffer capacity is the ability to resist changes in pH, in this case by absorbing OH^- ions. As a consequence, it is very hard to predict the exact amount of alkali that is needed to reach a specific pH. To counter this inaccuracy, in further work, it would be more appropriate to work with NaOH loading or $[OH^-]$ instead of pH. As in this work sometimes different amounts of alkali were needed to reach the same pH, the results are less accurate.

For further work on this subject, one could also look into the intermediate steps of cellulose and hemicellulose degradation. In this process, these 2 fractions are not directly hydrolysed to monomers. There is an intermediate step taking place which is the formation of oligomer intermediates[21]. In the case of cellulose hydrolysis, cellobiose units may be present before the appearance of glucose. And in the case of hemicellulose, xylan, glucuronoxylan, arabinoxylan, glucomannan and xyloglucan may be possible intermediates[12].

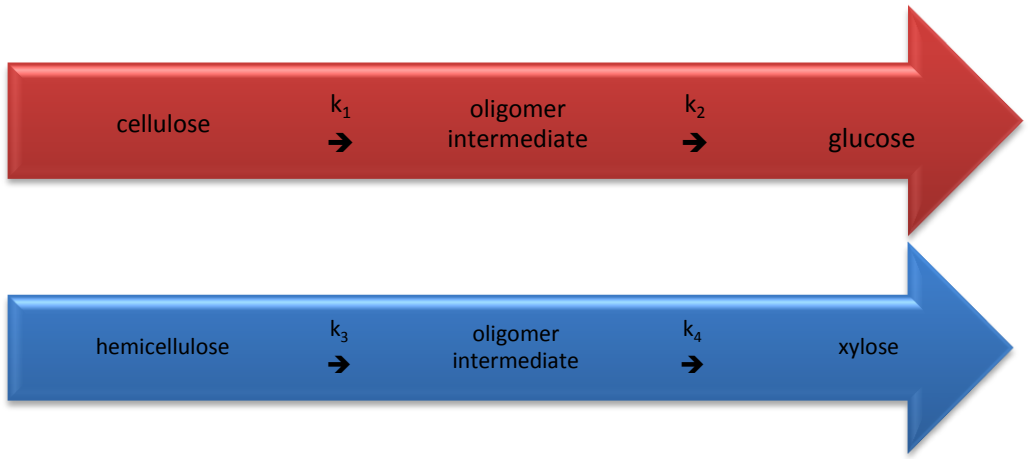


Figure 11:kinetic models with oligomer intermediates for degradation of (hemi)cellulose to its monomers

As one can see in figure 11, the inclusion of this intermediate step brings along a change in the adopted model because two extra reaction constants are needed. For practical reasons of time management, this model was not adopted, but might be an interesting approach for a follow-up thesis.

Modelling

A model relating the pre-treatment time with the sugar yield at a pH of 10 and 130°C was created. Equation (5) was combined with equations (1) - (4) to describe the degradation kinetics of cellulose and xylan:

$$\frac{d([Cellulose])}{dt} = -A_{0,1} \times [OH^-]^{m_1} \times e^{(\frac{-E_{A,1}}{RT})} \times [Cellulose] \quad (6)$$

$$\frac{d([Glucose])}{dt} = \frac{A_{0,1} \times [OH^-]^{m_1} \times e^{(\frac{-E_{A,1}}{RT})}}{Y_1} \times [Cellulose] \quad (7)$$

$$\frac{d([Xylan])}{dt} = -A_{0,2} \times [OH^-]^{m_2} \times e^{(\frac{-E_{A,2}}{RT})} * [Xylan] \quad (8)$$

$$\frac{d([Xylose])}{dt} = \frac{A_{0,2} \times [OH^-]^{m_2} \times e^{(\frac{-E_{A,2}}{RT})}}{Y_1} \times [Xylan] \quad (9)$$

The estimated parameters during this process were A_0, m, E_A and Y. As an initial guess for these parameters, values from Gurgel[22] and Dussan[23] were used for cellulose and xylan degradation respectively. As an additional input we implemented [OH^-] in the model. In each experiment this value was different as it is derived from the pH which as explained above also varied a lot. The obtained fit with the corresponding data is displayed in Figure 12.

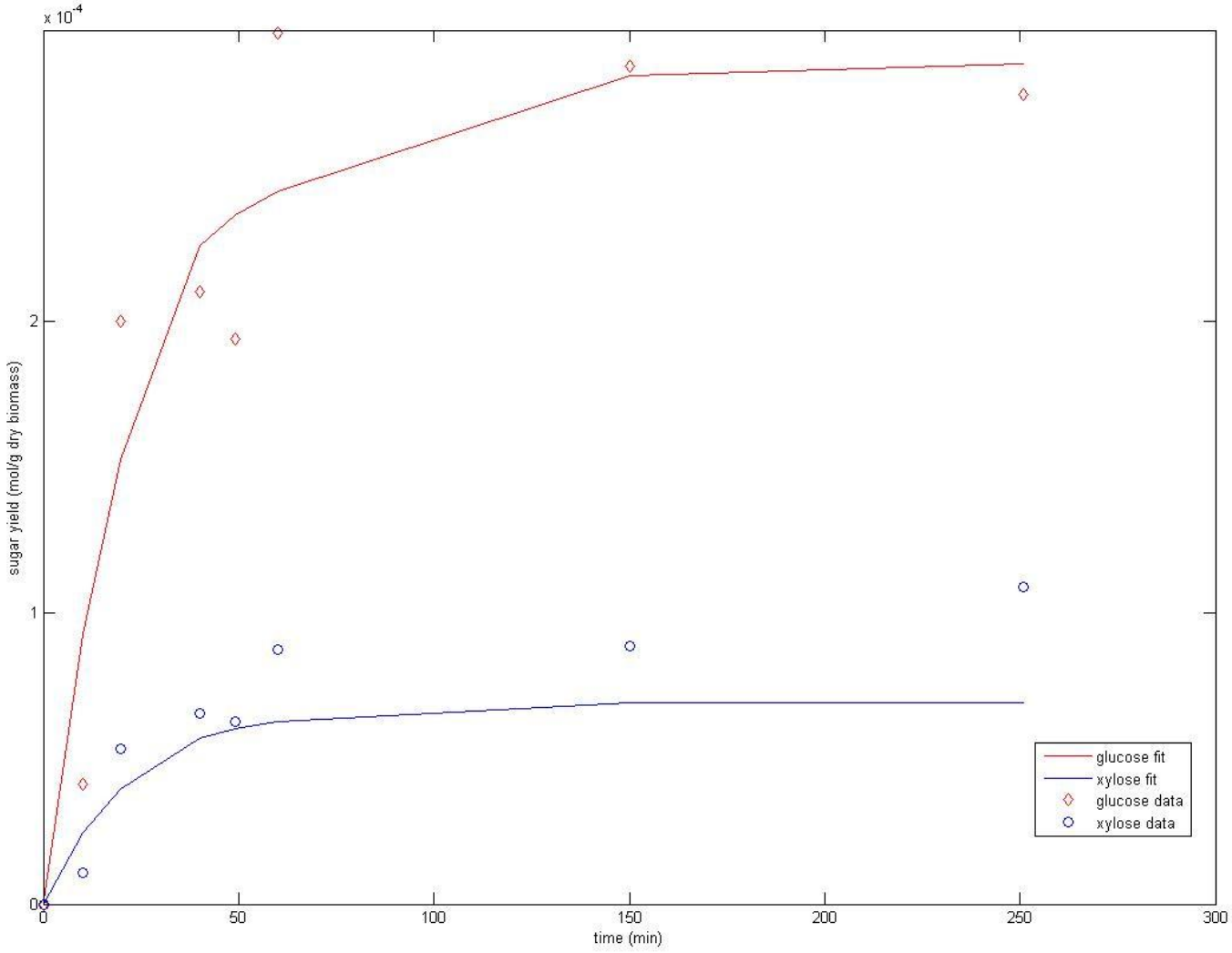


Figure 12:Glucose and xylose yield data fitted to the chosen model at pH=10 and T=130˚C

It should be noted that in Figure 12, the sugar yield is expressed in mol sugar/ g dry biomass, in contrary to mg sugar/ g dry biomass used in figure 1. This unit had to be changed so that it would match with the other units used in the reaction equation.

The trend obtained from the model was expected, at least concerning the first part of the curve. The build-up of the sugar yield is similar to the trends found in the literature[24]. After reaching a maximum, it was expected that there would be a decrease in sugar yield due to the formation of degradation products at higher severities. But even after 4 hours of pre-treatment under the conditions used in this work, the sugar yield is still at its maximum. Thus, with these new experiments, it can be said that the pre-treatment time does not highly influence the sugar yield after the maximum has been reached. It should be noted that only one data point was available for pre-treatment times higher than 4 hours, so the trend could be better verified if more data points were available.

The parameter estimates for the model fit are given in Table 4 and 5:

Table 4:Parameter values of cellulose degradation

parameters	value
A_0	6.71×10^{18} min^{-1}
m	2.03
E_A	96.6 kJ.mol^{-1}
Y_1	3.73

Table 5:Parameter values of xylan degradation

parameters	value
A_0	2.42×10^{11} min^{-1}
m	0.62
E_A	80.2 kJ.mol^{-1}
Y_2	30.65

If the reaction rates k_1 and k_2 are calculated from the values obtained above, we get the following result:

$$k_1 = 3.92 * 10^{-2}\ min^{-1}$$

$$k_2 = 4.32 * 10^{-2}\ min^{-1}$$

These values are consistent with each other, being in the same order of magnitude. This confirms that the estimation has been well performed. Following these values, we can also conclude that the conversion of xylan to xylose is slightly faster than the conversion of cellulose to glucose. Furthermore, the activation energy for the conversion of xylan is lower than for cellulose, indicating that xylan degradation proceeds more easily. This result is consistent with values from literature [25], in which it also mentioned that xylan degradation occurs more easily than cellulose degradation.

When looking at the data point with the highest yield (t=2.5 hours, glucose yield= $3*10^{-4}$ mol sugar.g^{-1} dry biomass, Figure 12), it looks like the degradation of glucose is occurring, with a maximum yield after 1 hour of pre-treatment. The real pH values measured during the experiments (varying between 9.85 and 10.3) highly influence the sugar yield, causing a different trend than the one obtained with the fit. This can be explained by the modified Arrhenius equation. In this equation, the [OH^-] is taken into account as well. In our model, we implemented the corresponding [OH^-] for each data point. Because of this, the reaction order m of the hydroxide ions concentration is relatively high, with m_1=2.03, indicating that the sugar yield is strongly linked to the [OH^-] and thus the pH. Indeed, when looking into the values in literature [26], we observe reaction order with values around 1.2-1.3, rarely above 1.6. So it is only logical that the sugar yield is in some way proportional with the pH value.

Because of the [OH^-] variation that occurred during the experiments, it becomes clear that the pH / [OH^-] has a high influence on the sugar yields. Because of the restraints due to the breaking down of the Dionex HPLC system, the influence of temperature on the sugar yields could not be investigated within this thesis period. Therefore we only worked with samples which were taken at 130°C.

As seen above, 8 parameters had to be estimated. It should be noted that this estimation is based on 10 experimental measurements. To get the most accurate possible parameter estimation, we need

$N >> P$

Where N is the number of experiments and p the number of parameters to be estimated. In this case, the ratio of 8 parameters to 10 experiments is quite high. It would have been more accurate to estimate less parameters, but in that case, the model would not reasonably fit the data. For this same reason, the degradation of the sugars can't be taken into account in our model, unless we get more data points. This because if we add the degradation of sugar in the model, we have to estimate at least 2 new reaction rates. This high ratio makes a new parameter estimation even more statistically unreliable. For this same reason, including the intermediate degradation steps with the oligomers as seen in Figure 11 was not an option. This because in that case, at least 6 extra parameters should be estimated because of the appearance of 2 extra reaction rate constants.

As described in the thesis of Rick van Rijn[1], after reaching a severity R0" of $6*10^5$, the sugar yield from the pre-treatment of biomass with NaOH reaches its maximum, at higher severities, the sugar yield decreases. Only R0" is a lumped factor of the time, pH and temperature, so it is unknown in which way each of these factors affects the sugar yield. If we calculate the R0" under the conditions of 4 hours of pre-treatment, we get a severity of $2.5*10^3$. So the severity of our experiments can be increased to get an even higher maximum yield. It can also explain why no sugar degradation is observed, because the conditions are not severe enough. This is also in accordance with the fact that the pH greatly influences the sugar yield. The pH can still be increased, and given its logarithmic scale with the $[OH^-]$, this means that the maximum sugar yield is far from being reached.

To get a complete picture of the alkaline pre-treatment effect on lignocellulose biomass, the remaining samples should be analysed and included in the model. Because in that case we work with different pH values and temperatures, the model will probably need some adjustments. And since the whole experiment was designed using a Central Composite Design, the obtained trends will give accurate prediction of the sugar yields within the boundaries of the system. To update this model and be able to add the degradation part if needed, the recommendation would be to measure data points at a pre-treatment time of around 1.5/2 hours and 3/3.5 hours. To verify if still no sugar degradation is occurring, it would be beneficial to perform experiments with pre-treatment times higher than 4 hours multiple times, to increase the reliability of the actual results.

Conclusion

During this work, a total of 37 individual pre-treatments and corresponding enzymatic hydrolysis were performed during 2 months of laboratory work. The aim of these runs was to get insights in the influence of pH, temperature and time on the sugar yields. High values for the reactions orders, especially in the case of glucose hydrolysis indicates a very high sensitivity on [OH^-] of the reaction yield. We found that at pH=10 and 130°C the reaction reached a maximum yield within about 2 hours without signs of degradation of the sugars within 4 hours. This data was fitted to a basic kinetic model to get primary insights on the kinetics of the degradation of lignocellulose in *Miscanthus*. The model indicated that there was no sugar degradation within the boundaries of our model, meaning the severity of the pre-treatment conditions can be increased. The model provided a good fit for the studied samples, because no degradation is observed. If sugar degradation occurs, the fitted model may have to be modified, to include a degradation step. And if enough data is available, intermediate steps can be added as well. The activation energies obtained through the parameter fit were 96.6 $kJ.mol^{-1}$ and 80.2 $kJ.mol^{-1}$ for cellulose and xylan degradation respectively. This indicates us that xylan degradation occurs more easily than cellulose degradation. Based on the observations made during the laboratory work and the obtained variation in the data points, it has been concluded that in further work, it would be more efficient to use the [OH^-] instead of the pH to determine the alkaline conditions of the pre-treatment. Analysis of all the samples in further work will allow a complete modelling of the degradation of lignocellulose. But from the current model, we get a good indication of the behaviour of the sugar yield in alkaline pre-treatments of *Miscanthus*, and the corresponding kinetics.

Appendix

A. Sugar yields from the analysed experiments

Table 6: sugar yields from the analysed experiments

Experiment	YIELD Glucose (mg/g dry biomass)	YIELD Xylose (mg/g dry biomass)
9	46.9	14.3
10	43	12.5
13	46.4	11.2
14	70.9	15.1
11	50	16.4
12	35	9.4
26	7.4	1.6
27	36.1	8
28	37.8	9.8
29	53.8	13.1

These sugar yields have been measured after 24 or 48 hours of enzymatic hydrolysis. As seen in figure 13, while performing the experiments it has been noted that the maximum sugar yield was achieved around this hydrolysis time. So to be able to compare all the samples with each other, the sugar yields from this time period have been selected for the end result.

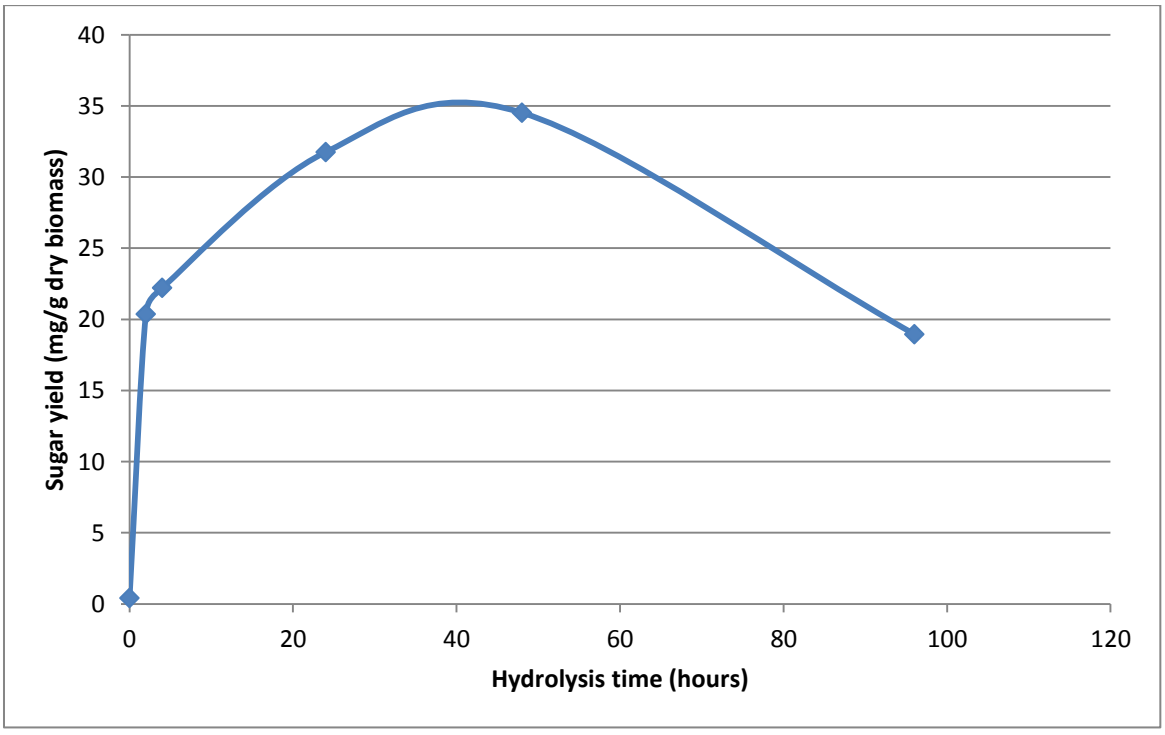


Figure 13:profile of the evolution of the sugar yield during hydrolysis

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