The effect of dilute-acid pretreatment on cellulose crystallinity and digestibility

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AGROTECHNOLOGY AND
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1 Abstract

Sulphuric acid (SA) is at the moment the most commonly used acid for dilute acid pretreatment of cellulose and lignocellulosic biomass. Although the pretreatment is effective it has some major drawbacks such as the formation of degradation products and corrosion of the reactors. It was however found that maleic acid (MA), a di-carboxylic acid, is (nearly) as effective in terms of pretreatment efficiency as sulphuric acid with lower amounts of degradation and corrosion. It is however not yet known why maleic acid performs so well. The aim of this research was thus to determine why maleic acid performs better in terms of pretreatment efficiency than can be expected from its acidity. It had been suggested by previous research that this is due to the specific orientation of the acid groups which mimic cellulase enzymes. Another theory is that when maleic acid is combined with a high concentration of NaCl it works similar to an ionic liquid breaking the crystallinity by interrupting the hydrogen bonding network.

This was investigated using several combinations of temperature and time with different acids including but not limited to; maleic, sulphuric and oxalic acid. Some variations on maleic acid were also tested. The pretreatment itself was only varied on temperature, duration and used acid, all the other parameters like volume, acid concentration, enzymatic hydrolysis and solid loading level were kept the same as much as possible. This pretreatment was performed on Avicel PH-101 microcrystalline cellulose and milled wheat straw. After the pretreatment, an enzymatic hydrolysis was performed; this hydrolysis was kept the same for every sample. The efficiency of the pretreatment was determined on the glucose yield directly after the pretreatment and the glucose yield after the enzymatic hydrolysis. The amount of degradation products and crystallinity after the pretreatment were also determined using respectively HPLC and XRD.

Sulphuric acid performed better than maleic acid at lower temperatures while maleic acid was clearly superior at 190°C, the yield of sulphuric acid was then severely inhibited by the formation of degradation products. An increase in degradation products is almost directly related to a decrease in glucose yield after the enzymatic hydrolysis. The maleic acid sodium salts at two different pH's showed that the pretreatment efficiency increases at lower pH but also the formation of degradation products increases in a more acidic environment. The ionic liquid effect only had a positive influence before the hydrolysis at a low temperature for a long duration. This could be interesting for a pure acid hydrolysis without enzymes. The high salt concentration however, inhibits the enzymes.

The biomimetic effect was not proven as oxalic performed better under some circumstances than maleic acid. Oxalic acid did give more degradation products even though the acidity is nearly the same. Even more remarkable was the seeming inhibition of maleic acid on the formation of levulinic acid as even butyric acid produced more levulinic acid. The crystallinity of each pretreated sample was measured using XRD but no clear results were found

The pretreatment on straw gave good insight in pretreatment efficiencies, nearly independent on the amount of degradation products formed. At low temperatures not much change between the acids was visible but at 190°C maleic acid proved that it had a more efficient pretreatment. Water had a significantly lower yield than the tested acids; this means that pretreatment is indeed acid catalysed.

The reason for the good pretreatment efficiency of maleic acid was not clearly found, the biomimetic effect was not conclusively proven although it did perform better on straw than any of the other acids. The ionic liquid theory performed only at a low temperature after a long duration which could be interesting for further research. No changes in crystallinity were found so this was also not clearly affected by maleic acid.

2 Introduction

The growing concern for the environment and declining oil reserves have spiked interest into more eco-friendly sources of fuels and chemicals. One of the most investigated sources is lignocellulosic biomass (Tilman, 2009). The annual production of agricultural commodities in Europe is about 80 x 10^6 tons of which almost half is cellulose, most of this cellulose is "packed" in lignocellulose (Röper, 2002). Lignocellulose or lignocellulosic biomass is often considered an agricultural by-product and does not compete with food supply. The main constituents of lignocellulosic biomass are cellulose, lignin and hemi-cellulose.

Cellulose is notoriously difficult to hydrolyse using only enzymes(Arioli, 1998), it is a β 1-4 glucan with an internal hydrogen bonding network which helps in the formation of crystalline fibrils. Cellulose is even more difficult to hydrolyse in lignocellulosic biomass, where the cellulose is packed in hemicellulose and lignin. Hemicellulose is a branched polysaccharide that connects with cellulose through hydrogen bonding. Lignin is an amorphous, aromatic polymer crosslinked to both cellulose and hemicellulose through a combination of several bonds and linkages (Rackemann, 2011). Cellulose will be used as a model for lignocellulosic biomass because it is the most common component with 32% to 44% (Ballesteros, 2004), it also makes the experiments better reproducible since lignocellulose can differ in composition. Lignocellulosic biomass, in the form of milled straw will also be used for a smaller set of experiments.

Lignocellulosic biomass and cellulose thus need to be pretreated before an efficient enzymatic hydrolysis can be performed. A pretreatment partly hydrolyses the cellulose and makes it more accessible to the enzymes, making the enzymatic hydrolysis more efficient. This pretreatment can be done in several different ways, but they usually are performed under harsh conditions i.e. high temperatures, acids or bases. These methods are fairly effective but have several major drawbacks. They catalyse the degradation of sugars to products as 5-hydroxymethylfurfural (HMF) and levulinic acid. Especially 5-HMF is problematic since it can inhibit the fermentation to ethanol. Furthermore, these harsh conditions accelerate the

corrosion of the reactors, raising the investment costs. The final drawback is the pH of the pretreated material after the pretreatment which is either very basic (pH >11) or very acidic (pH < 2,5), the following fermentation cannot be performed at these extreme conditions. Thus the pretreated material needs to be neutralized with acid or base resulting in difficult to dispose of salts (Mosier, 2005).

Because of these drawbacks, research into alternative pretreatment methods has become increasingly interesting. Some promising results have been reported, one of these is the use of diluted maleic acid. This acid was shown to perform (almost) equal in terms of glucose production at the same concentrations to dilute sulphuric acid, the most used acid for pretreatment (Kootstra, 2009). Due to the lower acidity of maleic acid, the production of degradation products, which is an acid catalysed process, was significantly lower than with sulphuric acid. Another benefit of the lower acidity is the lower level of corrosion and the smaller amount of base needed to neutralize the pH and as a result a smaller amount of salt is formed.

Due to the lower acidity the high sugar yield cannot yet be explained as it was thought that pretreatment was also acid catalysed. There might be some sugar-acid interactions or changes in crystallinity than can explain the efficiency of maleic acid pretreatment. It has been proposed that due to the specific orientation of the acid groups, maleic acid works similar to some cellulase enzymes, called a biomimetic effect(Lu, 2007). Stein et al (2010) reported that the addition of 30% (w/v) of NaCl to maleic acid could dramatically increase the efficiency of the pretreatment, they suggest that it works similar to an ionic liquid. Reducing the crystallinity by interrupting the hydrogen bonding network, making the cellulose more accessible to cellulase enzymes. High concentrations of salt can inhibit fermentation however they can be removed relatively easy by electrodialysis (Ragg, 1987).

2.1 Aim of this research

The aim of this research is to elucidate the effect of dilute acid pretreatment on cellulose crystallinity and digestibility. This research will focus on cellulose pretreatment using maleic acid at 50mM at different temperatures and durations. This concentration was chosen because it is within the range or dilute acid pretreatment and is used in a similar study by Kootstra et al. (2009) so some comparison is possible. Several variations on maleic acid will be tested at the same conditions; addition of 30% (w/v) NaCl to 50mM, maleic acid sodium salt acidified to maleic acid pH and maleic acid sodium salt with 50mM sulphuric acid. These scenarios will be compared to sulphuric acid as a reference. In addition oxalic acid will be used as di-acid reference and butyric acid as mono-acid reference. Finally Water will also be tested as a control experiment. These acids will be compared on sugar yield before and after enzymatic hydrolysis, crystallinity after pretreatment, amount of cellulose dissolved after pretreatment and the amount of degradation products formed. Milled straw will be pretreated with maleic acid, sulphuric acid, oxalic acid and water, and will be compared on the same parameters except crystallinity. With these experiments I will try to answer the following question: Why does maleic acid perform better than can be expected from its acidity.

3 Materials and method

All the reaction runs were performed in triplicate using a set of 3 identical reactors. The set of reactors was replaced due to leakage of the original set by similar reactors halfway the experiments, this did not affect the results. These reactors were ¾ inch Swagelok union reactors with an internal volume of approx. 9 ml, the second set of reactors can be seen in picture 1. Before the experiment, wheat straw was milled using a regular food blender, the straw was blended for about 10 minutes and then sieved using a retsch 425 µm sieve.

Picture 1 second set of reactors

Firstly, 0,500 grams of cellulose (Avicel PH-101) or milled straw was weighed into each of the 3 reactors. Then 5 ml of acid solution (or water as a control experiment) and a stirring bean were added, then the reactors were tightly closed. The reactors were individually placed in a preheated silicone oil bath. The oil bath is a 15 cm aluminium pan filled with silicone oil, on top of a IKA RCT basic magnetic stirrer/heating plate, with a ETS-D5 temperature controller.

The oil bath was first preheated to 6° C above the desired reaction temperature. When the reactors were inserted into the oil bath the temperature was immediately set at 1° C above the reaction temperature for the duration of the reaction. The first 6 minutes were counted as heat-up time and are not counted as reaction time. Within these 6 minutes the reaction mixture was heated to 5° C below the desired reaction temperature, this temperature difference was deemed small enough to start the reaction time. The magnetic stirrer was set at 600 rpm for the duration of the heat-up time and reaction time. Sulphuric and maleic acid were tested at 130°C, 160°C and 190°C for 10, 30 and 50 minutes. The other acids were tested at 130°C for 50 minutes, 160°C for 30 minutes and 190°C for 10 minutes.

When the reaction time passed, the reactors were taken out of the oil bath and cooled under running cold tap water, to stop the pretreatment quickly. When the reactors were cooled to around room temperature, they were opened. The content was taken out, rinsed and diluted with a total of 20ml distilled water for a more optimal cellulose concentration for the enzymatic hydrolysis. The diluted content of the first two reactors was adjusted to around pH 5 using 1 M and 0,1 M NaOH. The pH was measured using an Oakton Acorn pH5 meter. HPLC samples were then taken to check the amount of sugars and degradation products formed during the pretreatment. 0,629ml of multifect gc extra cellulase enzyme was added to the samples. These were then sealed with Parafilm and incubated for about 72 hours in a shacking incubator(NB Innova 44 or Kuhner ISF1-X) at 50° C shacking at 90 rpm. Cellulose and straw without pretreatment were also hydrolysed for 72 hours as a control experiment. After the hydrolysis, the enzyme in the samples was inactivated in a water bath of around 90° C for approximately 10 minutes. HPLC samples were taken again to check the hydrolysis efficiency, the rest of the samples were stored in a sample vial. As a hydrolysis test, cellulose pretreated with sulphuric acid at 160°C

for 30 minutes was hydrolysed for 24 and 48 hours with the regular amount of enzyme and half the amount of enzyme.

The diluted content of the third reactor was filtered using a Büchner funnel, on a pre-weighed filter paper, and dried in an oven for several days. The dried filter papers were then weighed to determine the amount of cellulose that has reacted to dissolvable substances. The filtrate was collected and stored in sample vials, an HPLC sample of the filtrate was taken. After drying in an oven at 115°C for several days, the filter paper was weighed and the solids were carefully scraped off. The solids were then ground in a mortar and pestle for XRD analysis. The XRD samples were weighed before analysis to ensure that the amount of sample analysed does not affect the outcome. In addition to the pretreated samples, untreated cellulose was also analysed using XRD as a control experiment.

The XRD had the following settings: scan type; continuous, step size [°2θ]; 0.050, start angle [°2θ]; 7.070, end angle [°2θ]; 40.020, time per step [s]; 5.00, scan speed [°2θ/s]; 0.010. The XRD is a Philips PW 1830, set at 40kV and 40 mA.

Acid solutions were prepared according to the following table

Solution	Volume	Addition	Measured pH
50 mM H_2SO_4	2 litre	5,44 ml 98% H_2SO_4	1,30
50 mM maleic acid	500 ml	2,931 g 99% maleic acid	1,56
50 mM oxalic acid	50 ml	0,318 g 99% oxalic acid	1,58
50 mM maleic acid salt (mal acid pH)	50 ml	0,349 g 99% maleic acid sodium salt + H_2SO_4	1,53
50 mM maleic acid salt with 50 mM H_2SO_4	50 ml	0,349 g 99% maleic acid Na salt + 0,136 ml 98% H_2SO_4	1,30
50 mM maleic acid with 30% (w/v) NaCl	50 ml	0,293 g 99% maleic acid + 15 g NaCl	0,71
50 mM butyric acid	50 ml	0,231 ml 99% butyric acid	3,01

Table 1 Preparation of used acid solutions

Every acid solution was tested without cellulose or straw at 190°C for 50 minutes to check the degradation of the acid in the most severe conditions. The pH of the solutions was measured before and after these reactions.

4 Results and discussion

4.1 Sulphuric acid and maleic acid compared for all conditions

4.1.1 Introduction sulphuric acid and maleic acid comparison

These two acids and water are the main subject of this research and are therefore investigated in more detail. Sulphuric acid is at the moment the benchmark for dilute acid pretreatment while maleic acid is the subject of this research making this the main comparison. The full data set for all acids can be found in the appendix.

4.1.2 Comparison sulphuric and maleic acid all conditions

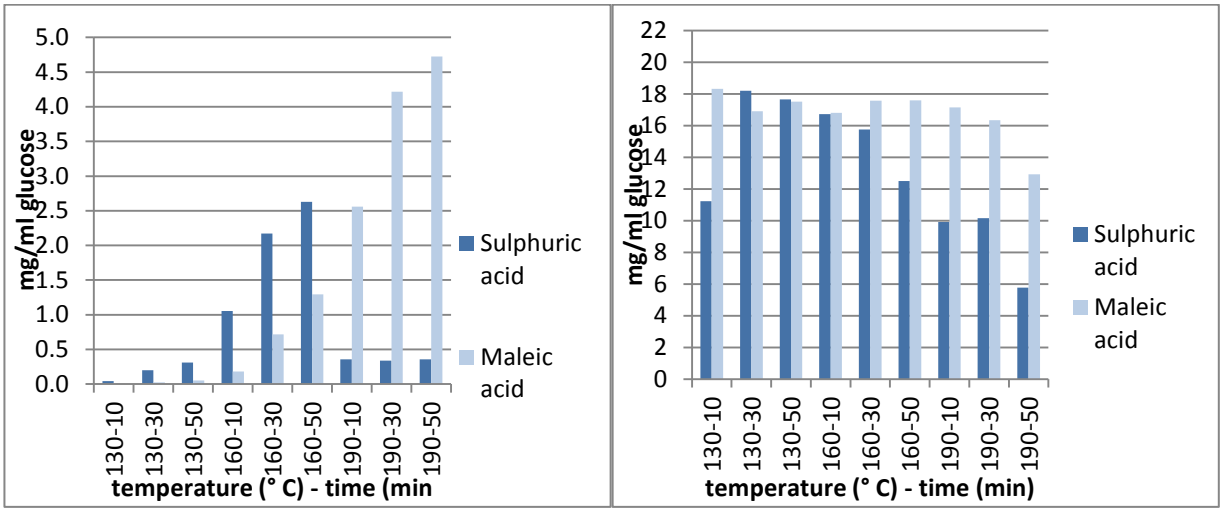

Figure 1 full comparison sulphuric and maleic acid at 50mM for glucose produced before pretreatment

Figure 2 full comparison sulphuric and maleic acid at 50mM for glucose produced after pretreatment

Figure 1 and figure 2 depict the glucose produced at all the tested conditions for maleic and sulphuric acid, the top of the graph in figure 2 (22mg/ml) depicts a 100% hydrolysis. Before the enzymatic hydrolysis (figure 1) sulphuric acid gives more glucose than maleic acid at 130 °C and 160 °C. The yield also increases with increasing temperature and duration, but at 190 °C the yield declines drastically. This is most likely due to the formation of degradation products. Maleic acid on the other hand shows a steady increase in glucose yield before the enzymatic hydrolysis with increasing pretreatment severity all the way to the most severe pretreatment at 190°C for 50 minutes.

After the hydrolysis, sulphuric acid shows a clear declining trend with increasing temperature. Except the 130°C for 10 minutes sample breaks the trend, this can be due to the pretreatment being too short at a too low temperature. The decline in sugar yield with increasing temperature can most likely be attributed to the formation of degradation products. The results of maleic acid are very similar for most pretreatment conditions. Only the most severe pretreatments (190°C - 30 and

190°C - 50)show a decline in sugar yield, attributable to the formation of degradation products.

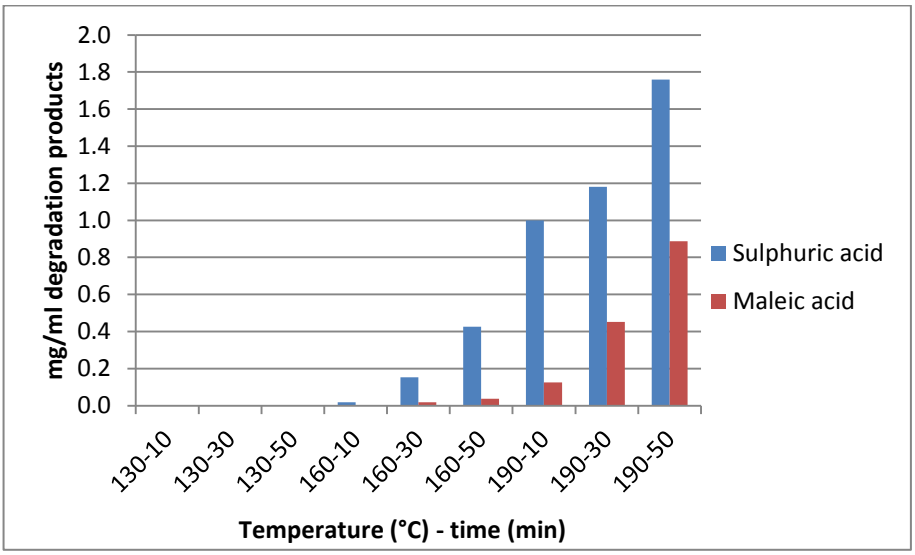

Figure 3 Total of HMF and levulinic acid formed after sulphuric and maleic acid pretreatment at 50mM

Figure 3 depicts the total of measured degradation products formed, this is an addition of levulinic acid and HMF. It can be clearly seen that sulphuric acid forms significantly more degradation products than maleic acid. The formation of degradation products starts at 160°C after 30 minutes for sulphuric acid, no significant amounts were formed with less severe conditions. The amount of degradation products formed increases with increasing severity (temperature and time) as can be expected. Maleic acid shows the same trend but the formation starts at more severe conditions, most likely due to the lower acid strength. Starting at 190°C significant amounts of degradation products are formed even with maleic acid.

4.1.3 Conclusion sulphuric acid and maleic acid comparison

The results for sulphuric acid are as expected until 160°C the steep decrease in sugar yield at 190°C shows how significant the effect of degradation product formation is. Not even taking into account the inhibitory effect on fermentation. Maleic acid performs as can be expected, steadily increasing with temperature and with a lower amount of degradation products formed. After the hydrolysis sugar yield with sulphuric acid pretreatment decreases and maleic acid stays the same except for the most severe conditions, again attributable to the formation of degradation products.

4.2 Sulphuric acid, maleic acid and water compared

4.2.1 Introduction to comparison of Sulphuric acid, maleic acid and water

As in the previous part, sulphuric acid and maleic acid are compared but this time for the short conditions set. Water is also in this chapter to show the effect of acid pretreatment compared to non-acidic pretreatment at the same reaction conditions. Water was tested at only three reaction conditions. These reaction conditions used for water were also used for the other acids coming after this chapter. These reaction

conditions were 130 °C for 50 minutes, 160 °C for 30 minutes and 190 °C for 10 minutes. These reaction conditions were chosen because an increase in temperature decreases the reaction time. this was seen in the extensive data set of maleic and sulphuric acid in chapter 3.1. 10 minutes was too short for a pretreatment at 130 °C with a low sugar yield as a result, while 50 minutes was too long at 190 °C resulting in the formation of degradation products for both maleic and sulphuric acid.

4.2.2 Results of Sulphuric acid, maleic acid and water

First the glucose yield after the pretreatment will be discussed. Figure 4 shows the amount of glucose produced after the pretreatment with water, sulphuric acid or maleic acid. The reactions conditions are shown in the table. it can be seen that sulphuric acid performs best at 160 °C and 30 minutes while maleic acid performs best at 190 °C at 10 minutes. Since the amount of cellulose dissolved with sulphuric acid pretreatment at 190 °C is roughly the same as with 160 °C, it can be suggested that the dissolved cellulose has degraded to by-products such as 5-HMF and levulinic acid. The glucose production with maleic acid is as expected, increasing with a higher temperature (Kootstra, 2009). Water pretreatment shows only a significant amount of glucose at 190°C. Showing that acidic reaction conditions most likely do improve the pretreatment efficiency.

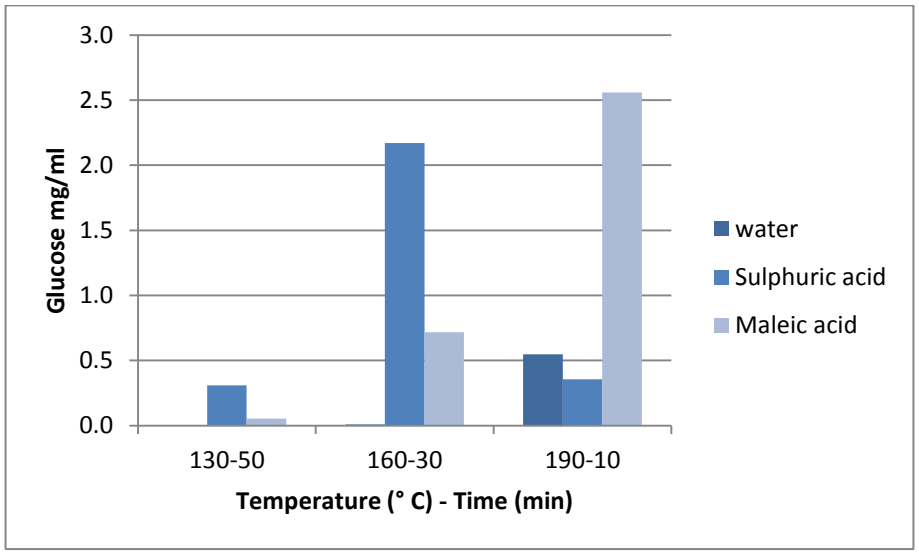

Figure 4 Glucose produced after the pretreatment

The amount of cellulose dissolved can give an idea on the formation of soluble products formed from the cellulose. Figure 5 shows the percentage of cellulose that has reacted to dissolvable products during the pretreatment with water, sulphuric acid or maleic acid at the specified reaction conditions (temperature and time). This figure shows different trends for the two acids and water. Water performs nearly identical at 130°C and 160°C but performs better at 190°C. This same trend can also be seen in the amount of glucose produced after the pretreatment. The negligible amounts of glucose produced while some cellulose has still reacted suggests that the cellulose was hydrolysed to soluble oligomers but net yet fully to glucose. Sulphuric acid dissolves the highest percentage of cellulose at 160°C but this does not increase

at 190°C. So the lower glucose yield can most likely be attributed to degradation. Maleic acid on the other hand shows a steady increase from 130°C to 190°C which also shows in the glucose yield.

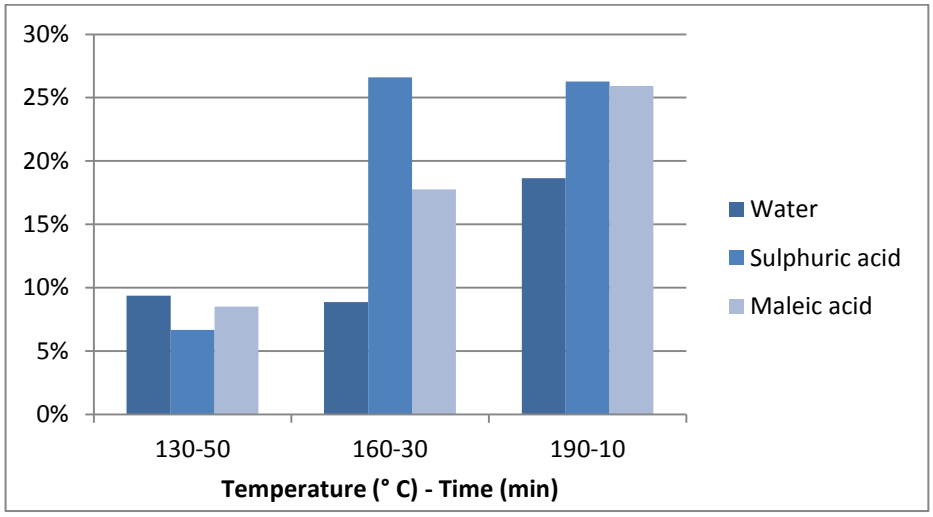

Figure 5 percentage of cellulose reacted to dissolvable products after the pretreatment

The formation of degradation products have a double negative effect on the pretreatment efficiency, they reduce the sugar yield and at high concentrations can inhibit fermentation later on. Figure 6 shows the amount of HMF (blue) and levulinic acid (red) produced after pretreatment with water, sulphuric acid and maleic acid at 190°C for 10 minutes. Maleic acid shows some promising results, with significantly less HMF and levulinic acid formed compared to sulphuric acid as expected. But the amount of degradation products formed is even slightly lower with maleic acid than with water. Suggesting that the formation of degradation products is not purely acid catalysed or maleic acid has some inhibiting effect on these reactions. Mosier et al. (2002) investigated acid catalysed reactions of glucose with maleic acid and several other acids; they found that the degradation only starts at a certain H^+ concentration. Below that concentration, the reaction follows the same kinetics as with water. This H^+ concentration was found to be around 25 mM or pH 1,60. 50mM maleic acid had a measured pH of 1,56, this is barely acidic enough for the acid catalysed reaction to start. So it is possible to conclude that after some acid degradation (which occurs at these temperatures) the reaction follows the water catalysed kinetics, resulting in low degradation product formation.

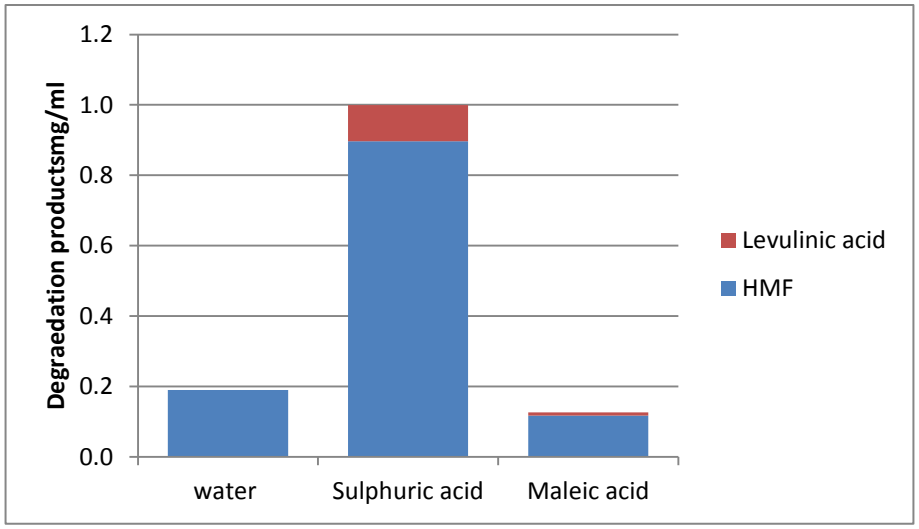

Figure 6 Amount of HMF and levulinic acid produced

It has to be noted that the hydrolysis was probably too effective for this research. This was concluded from the fact that the hydrolysis rate was very high. A hydrolysis test was performed (results in appendix) with 24 and 48 hours hydrolysis and half the amount of enzyme on a specified pretreatment; these yielded nearly the same results. Another control hydrolysis was performed without pretreatment, which yielded a glucose yield of 18 mg/ml.

Figure 7 shows the amount of glucose produced of the water and acid pretreated samples after the enzymatic hydrolysis. The top of the graph (22mg/ml) represents the theoretical 100% hydrolysis of cellulose to glucose. This figure shows a decline in glucose produced with increasing temperature for maleic acid, sulphuric acid and water. Maleic acid and water decline only slightly while sulphuric acid declines about 40%. This can at least partially be attributed to degradation product formation. Although other degradation products than levulinic acid and HMF could have been formed, these were not quantitatively analysed but could account for the large difference in glucose yield. The difference between maleic acid and water can be considered insignificant.

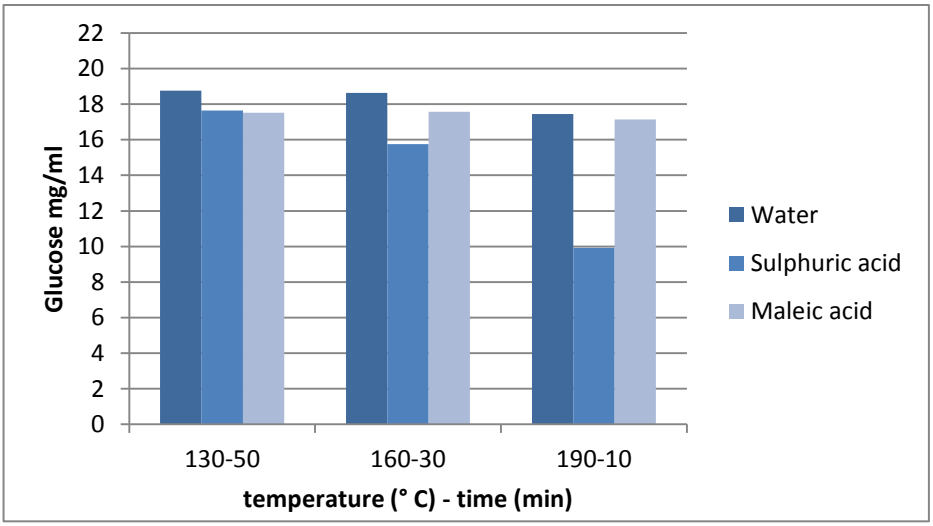

Figure 7 Amount of glucose formed after the enzymatic hydrolysis

4.2.3 Conclusions from sulphuric acid, maleic acid and water

The correlation between the amounts of degradation products formed and the glucose yield is very apparent when comparing maleic acid with sulphuric acid. While sulphuric acid performs better in terms of cellulose reacted to soluble molecules, this does not show in the glucose yield after the pretreatment at high temperatures. Water gave a very high glucose yield after the hydrolysis; this is due to the enzymatic hydrolysis being too strong.

4.3 Different types of maleic acid compared

4.3.1 Introduction to the different types of maleic acid

Some variations on maleic acid were tested; this was done to test if the structure of the acid explained to good pretreatment efficiency. The maleic acid sodium salt was tested at the same concentration as the other acids, namely 50 mM. But they were acidified using sulphuric acid to the pH of regular maleic acid or to the pH of sulphuric acid. This was done to test the effect of acidity on pretreatment efficiency, independent on the amount of maleic acid present. Also the addition of 30% of NaCl was tested to test the ionic liquid theory of Stein et al. (2010).

4.3.2 Results of variations on maleic acid

Figure 8 depicts the amount of glucose formed after the pretreatment with the different types of maleic acid solutions, i.e. maleic acid, maleic acid sodium salt (maleic acid pH), maleic acid sodium salt (H_2SO_4 pH) and maleic acid + 30% NaCl. The reaction conditions, temperature and time are noted in the figure. In comparing the four different maleic acid solutions some interesting results can be found in this figure. Regular maleic acid performs best at 190°C while the more acidic maleic acid sodium salt solution performs best at 160°C but only slightly better than regular maleic acid. The maleic acid sodium salt at the pH of maleic acid performed at each temperature about 20% worse than regular maleic acid.

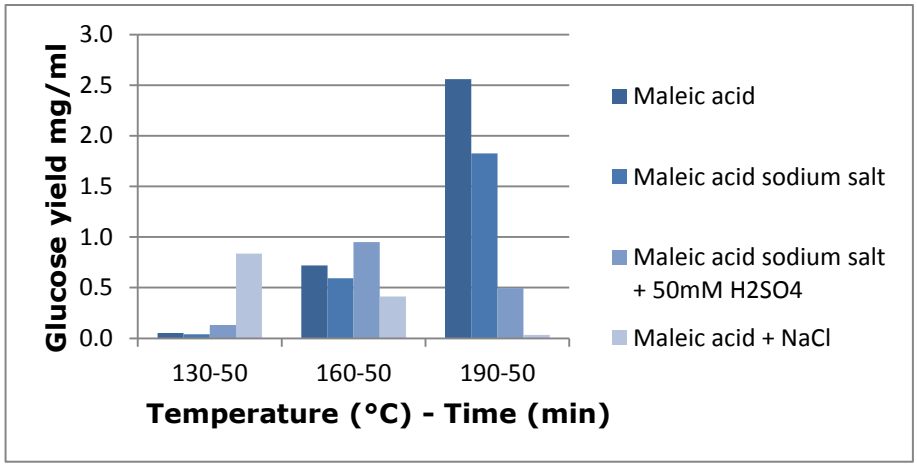

Figure 8 Glucose productions after the pretreatment with variations on maleic acid

But looking at the degradation product formation there is a big difference. Figure 9 shows the amount of HMF and levulinic acid formed after pretreatment with the different types of maleic acid at 190°C for 10 minutes. Maleic acid sodium salt at roughly the same pH as regular maleic acid gives the same amount of HMF and levulinic acid. But when the pH is decreased to 1,30 with H_2SO_4, the production of HMF increases, but not as high as sulphuric acid alone at the same concentration. More remarkable is the high production of levulinic acid, higher than any other experiment. It seems that the reaction rate of HMF to Levulinic acid is selectively increased under these conditions. This effect could not be found in literature. This could be interesting for the selective production of levulinic acid.

The difference in degradation products formed between maleic acid and maleic acid salt, and maleic acid salt with 50mM H2SO4 can likely be explained by the formation of degradation products below pH 1,60 as described by Mosier et al. (2005). As the first two are around that pH they most likely follow the same kinetics as water and the more acidic maleic acid sodium salt follows acid catalysed kinetics, most likely maleic acid + NaCl follows the same kinetics.

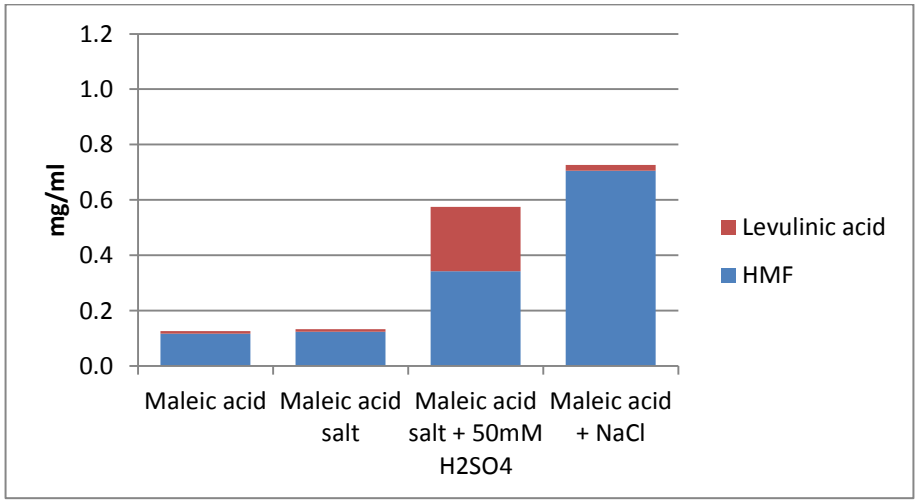

Figure 9 Productions of HMF and levulinic acid after the pretreatment

At 130°C maleic acid with NaCl has the highest glucose production but declines with increasing temperature and shorter reaction times. But the amount of cellulose dissolved after the pretreatment does increase with temperature. This is shown in figure 10 for the tested types of maleic acid. This increase in dissolution at higher temperatures could suggest a more time-dependent hydrolysis to glucose compared to a more temperature dependent hydrolysis. It has been shown that maleic acid with NaCl can effectively hydrolyse Avicel cellulose after several hours at 125°C (Stein, 2010). This could be a promising result, when pretreatment is performed at such a low temperature degradation products are barely formed and thus the reaction can be performed for a longer duration. If the reaction with added NaCl is performed for a longer duration such as described by Stein et al. (2010) an enzymatic hydrolysis might not be necessary.

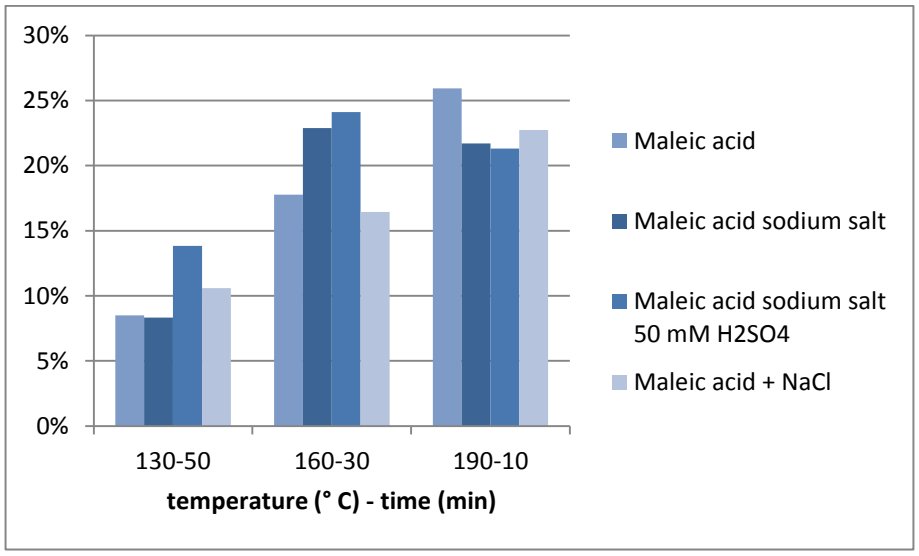

Figure 10 Percentage of cellulose reacted to dissolvable products after the pretreatment

Figure 11 shows the amount of glucose produced of the pretreated samples after the hydrolysis. The top of the graph (22 mg/ml) represents a 100% hydrolysis. Maleic acid with 30% NaCl produced consistently the lowest amount of glucose after the hydrolysis, this can be partly due to the formation of more degradation products. But it could more likely be attributed to the fact that enzymes don't work optimally under high salt concentrations. Datta (2010) found that certain types of cellulase enzymes retained only 65% of their activity at a salt concentration of 2 M, the concentration in the performed hydrolysis was around 1 M. This could have a significant effect on the cellulase activity and could therefore explain the lower sugar yields. No clear trends in glucose production after hydrolysis with the other types of maleic acid can be seen, except all of them decreasing slightly with increasing temperature. This slight decrease can most likely be attributed to the formation of degradation products at higher temperatures.

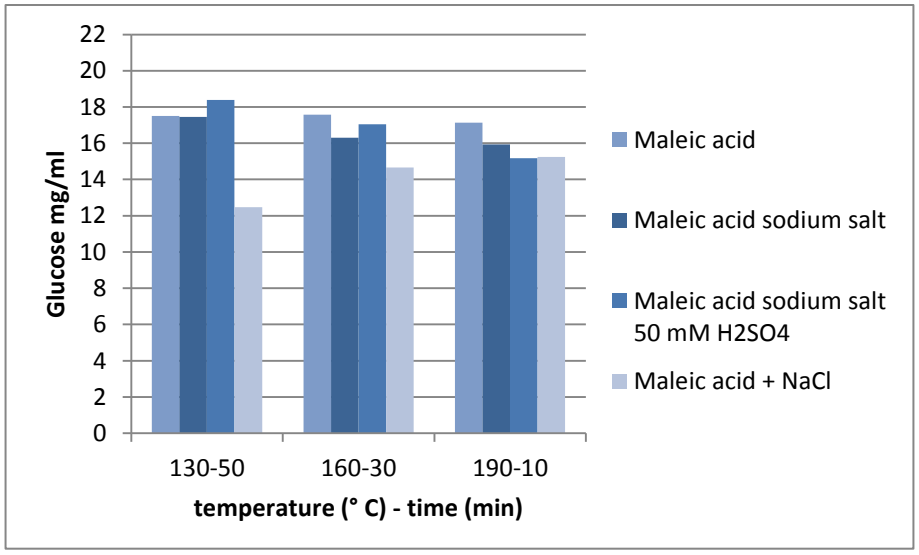

Figure 11 Amount of glucose produced after hydrolysis

4.3.3 Conclusions from variations on maleic acid

There was not much difference between maleic acid and the maleic acid sodium salts at lower temperatures but at 190°C there is a clear decline in glucose yield with the most acidic maleic acid sodium salt. This can be attributed to the formation of degradation products, although not as severely as with sulphuric acid. The differences in glucose yield after hydrolysis are not remarkably big. The addition of NaCl however did have a clear effect; at 130°C it gave the highest glucose yield after the pretreatment; however this declined at higher temperatures. But this high yield could be interesting for a purely acidic hydrolysis without enzymes. It was clearly visible that the high amount of salt did have a negative effect on the enzymes. The amount of degradation products formed is mostly as expected, except for the high production of levulinic acid with the more acidic MA sodium salt.

4.4 Di-acids and mono-acids compared

4.4.1 Introduction to the comparison of di-acids with mono-acids

With this comparison it could be possible to prove or disprove the biomimetic effect; maleic acid and oxalic acid have a similar acidity and are both di-acids. So the only effect on the pretreatment should come from the conformation of the acid groups. If maleic acid performs significantly better than oxalic acid could this be proof of some sort of biomimetic effect. Butyric acid was taken up into this comparison because it is a mono-carboxylic acid with the same chain length, if this acid performs almost equal to maleic acid, than the effect is carboxylic acid specific and not maleic acid specific.

4.4.2 Results from comparison of di-acid and mono-acids

Figure 12 shows the amount of glucose produced after the pretreatment with maleic acid, oxalic acid or butyric acid. The temperature and time of the reactions are shown. This figure shows that the glucose yield after the pretreatment is higher for

oxalic acid at 130°C and 160°C than for maleic acid, at 190°C the yield of oxalic acid dramatically decreases. This decrease can be attributed to the degradation of oxalic acid, degrading almost completely at 190°C. The glucose yield of maleic acid on the other hand increases from 160°C to 190°C. Butyric acid has very low glucose production; this could be expected since this mono-acid is much weaker than either maleic acid or oxalic acid.

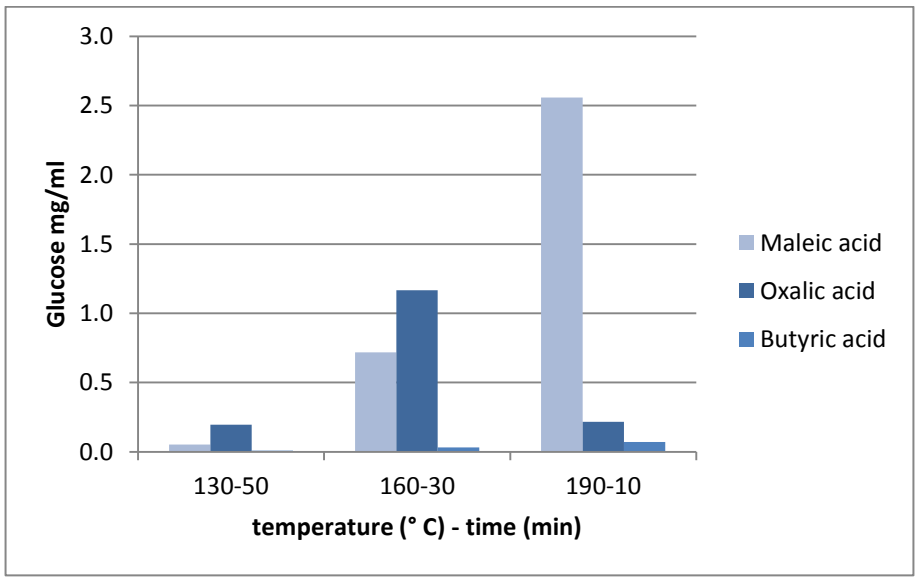

Figure 12 Glucose yield after pretreatment

Figure 13 shows the percentage of cellulose that was dissolved during the pretreatment with maleic acid, oxalic acid or butyric acid at the specified reaction conditions (temperature and time). The amount of cellulose dissolved after the pretreatment increases with a higher temperature for the di-acids. Butyric acid performs almost equally at 160°C and 190°C. As can be expected, the weaker butyric acid has dissolved the lowest amount of cellulose at every reaction condition. Oxalic acid dissolves the most at 130°C but is outperformed at 160°C and 190°C by maleic acid. The lower percentage at 190°C of oxalic acid can probably be attributed to the degradation of the acid. But as the glucose yield and the dissolution percentage do not follow the same trend for oxalic acid, it can be proposed that the hydrolysis to soluble oligomers happens very quickly to a certain point. The full hydrolysis to glucose however, takes more time and at that point, the oxalic acid has probably degraded to CO_2.

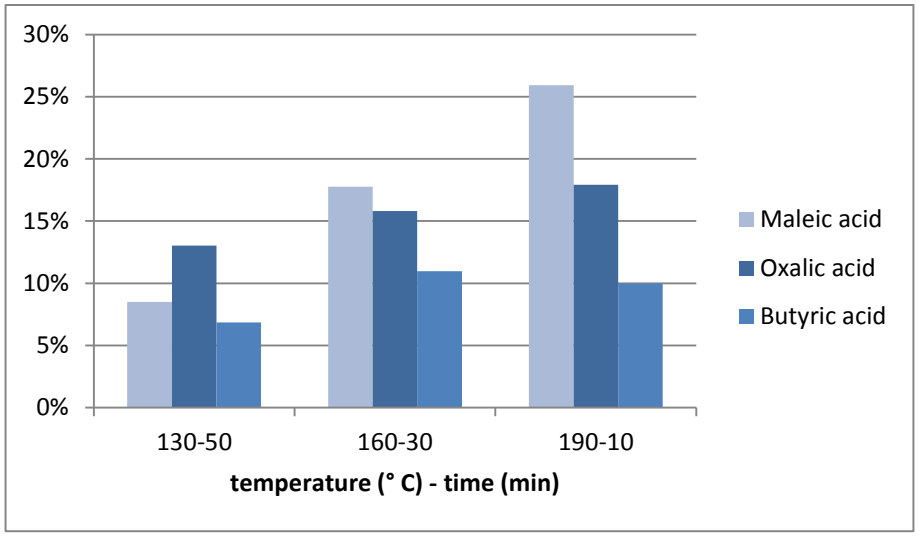

Figure 13 Percentage of cellulose dissolved during the pretreatment

Figure 14 shows the amount of glucose produced of the pretreated samples after the hydrolysis. The top of the graph (22 mg/ml) represents a 100% hydrolysis. This figure shows the same general trend as seen in figures 8 and 4, the glucose yield decreases with a higher temperature. As before, this most likely can be attributed the production of degradation products. Oxalic acid has the lowest glucose yield after the hydrolysis at the higher temperatures. This is somewhat unexpected since the degradation products form from glucose but the glucose production after the pretreatment of oxalic acid is very low at 190°C. This suggests that oxalic acid catalyses the degradation of glucose significantly more than maleic acid, this is contrary to what Lee et al. (2011) found. Also less cellulose has reacted to dissolvable products with oxalic acid compared to maleic acid.

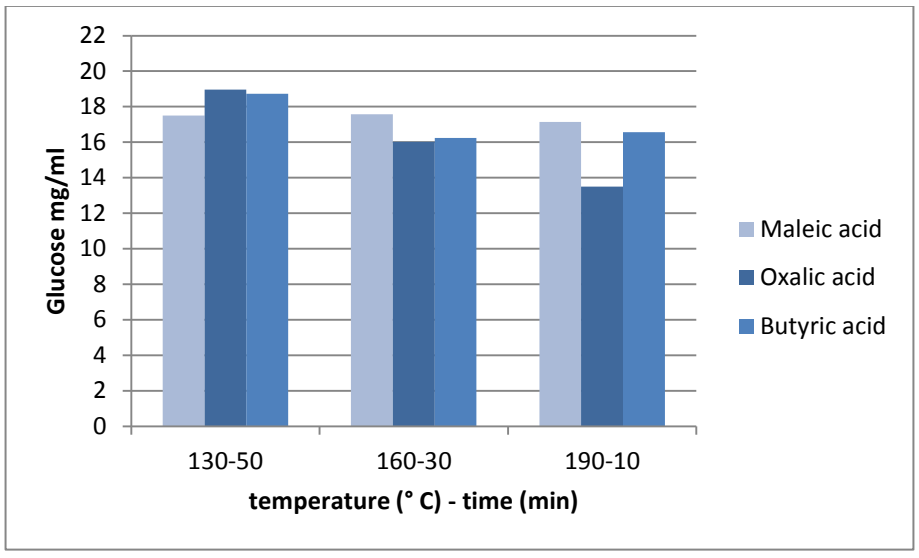

Figure 14 Amount of glucose formed after the hydrolysis

Figure 15 shows the amount of HMF and levulinic acid formed after pretreatment with maleic, oxalic and butyric acid at 190°C for 10 minutes. The formation of HMF is as can be expected, oxalic acid and maleic acid produce roughly the same amounts

of HMF. Butyric produces significantly less, this can be attributed to this reaction being (at least partly) acid catalysed and butyric acid is a much weaker acid with a pH of around 3 compared to the 1,6 of maleic and oxalic acid. Remarkable however is the formation of levulinic acid, both oxalic acid and butyric acid produce significantly more levulinic acid than maleic acid. This suggest some kind of inhibiting effect of maleic acid, since oxalic acid has roughly the same acidity, butyric acid has a much lower acidity so this does not explain the levulinic acid production. No explanation for this could be found in literature.

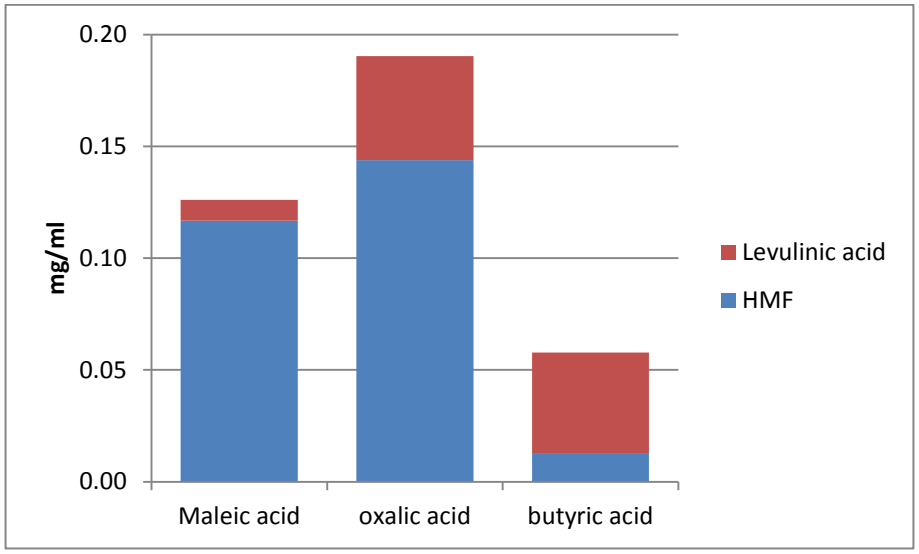

Figure 15 amount of HMF and levulinic acid formed

4.4.3 Conclusions from the comparison of di-acids and mono-acids

This comparison could have given insight into the biomimetic effect of maleic acid but it didn't; maleic acid didn't perform significantly better than oxalic acid. Butyric acid did perform worse than either maleic acid or oxalic acid, this is most likely due to the lower acidity and not due to the biomimetic effect. Another result is the seemingly inhibiting effect of maleic acid on levulinic acid production as both oxalic acid an butyric acid produce both about 5 times as much levulinic acid as maleic acid. While oxalic acid has roughly the same acidity, butyric acid on the other hand is significantly less acidic and should thus produce less, which it doesn't.

4.5 Straw pretreatment analysed

4.5.1 Introduction to straw pretreatment

This chapter can give insight into the more practical application of this pretreatment. Cellulose is a useful model for real biomass but the different components in lignocellulose can have different effects on the pretreatment efficiency and the degradation product formation. Milled wheat straw was tested for only a smaller set of experiments due to time constraints.

4.5.2 Results from wheat straw pretreatment.

The glucose production from straw after the pretreatment shows the clearest trend so far. This can be seen in figure 16, this figure shows the amount of glucose

produced after the pretreatment for the tested acids and water. Reaction conditions are shown in the graph. For every tested acid, the yield increases with an increasing temperature. The yield on straw, for all the acids and water, is lower than the yield on pure cellulose, this suggests that straw is much more difficult to hydrolyse. Because of this difficulty and the lower glucose yield, degradation product formation is significantly lower than for pure cellulose. Water performs worst at all the tested temperatures as can be expected. Maleic acid however is the worst performing acid at every reaction condition. Oxalic acid has a higher glucose yield than maleic acid at every temperature, although the difference becomes smaller at 190°C. This can be attributed to the degradation of oxalic acid at this temperature. Remarkable is the high yield of sulphuric acid, this suggests that the pretreatment of straw is indeed acid catalysed. The difference between pretreatment efficiency of cellulose and straw can likely be attributed to the other components present in straw. The hydrolysis of these components is likely acid catalysed and can therefore account for the higher glucose yield with the strongest acid. Because the lower glucose yield, degradation product formation does not have such a significant effect on glucose yield as with pure cellulose.

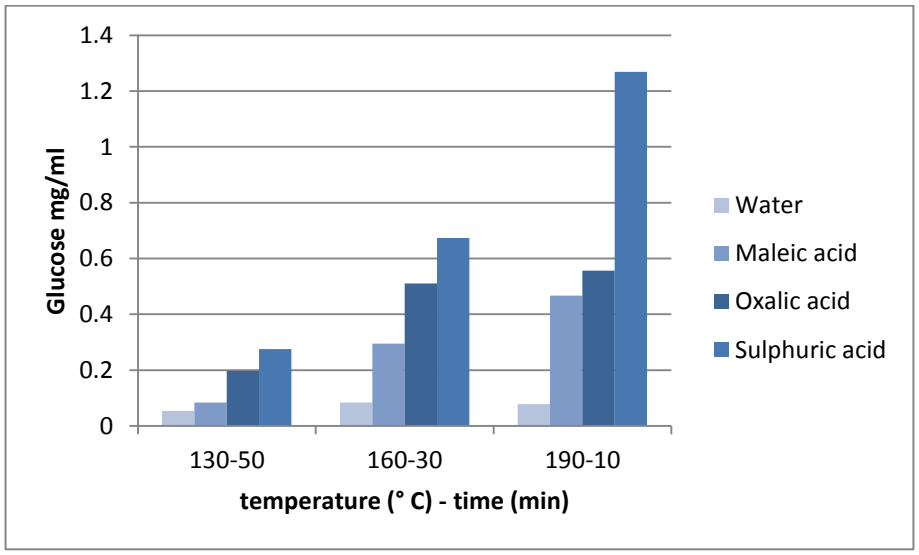

Figure 16 Amount of glucose formed from straw after pretreatment

Figure 17 shows the amount of xylose formed after the pretreatment with water and maleic, oxalic and sulphuric acid. The top of the graph (4 mg/ml) represents a 100 hydrolysis. Figure 14 shows that negligible amounts of xylose are formed at any temperature after the pretreatment with water. Xylose formation after pretreatment with acid shows an entirely different trend than glucose formation. At 130°C, maleic acid shows the lowest yield, than oxalic acid and sulphuric acid gives the highest yield. Sulphuric acid has its highest xylose yield at 130°C and it declines very slightly at 160°C but decreases significantly at 190°C. Saha et al. (2005) found very similar results for glucose and xylose yield with sulphuric acid. They also found that at 180°C after 15 minutes, some furfural (not analysed in these experiments because the main focus was on glucose which doesn't form furfural) was formed from xylose, this could account for the lower xylose yield at 190°C in these experiments. At 160°C, the

three acids perform very similar to each other, maleic and oxalic acid have the highest yields at these conditions. The order has changed at 190°C, here oxalic acid has the lowest yield, followed by sulphuric acid. Maleic acid has the highest xylose production at these reaction conditions. Again, the low yield of oxalic acid can be attributed to the degradation of the acid at this temperature.

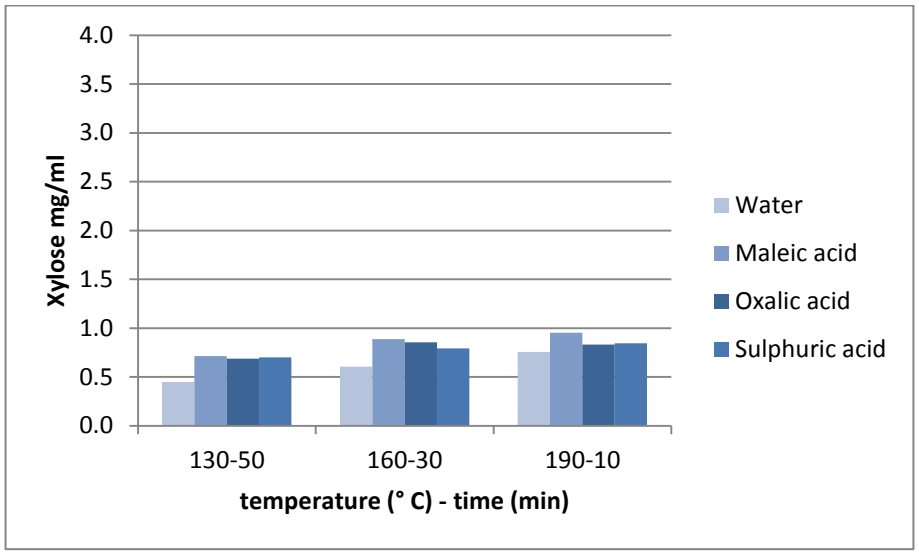

Figure 17 Amount of xylose formed after pretreatment

Figure 18 shows the amount of glucose produced from the water and acid pretreated and non-pretreated samples after the hydrolysis. The top of the graph (8,2 mg/ml) represents a 100 hydrolysis of glucans to glucose. The glucose yield after the hydrolysis shows that a pretreatment is indeed necessary for straw, as opposed to pure cellulose. The no pretreatment control experiment shows a very small yield compared to the pretreated samples. Water pretreated samples show a 3 to 6 fold increase in yield over the no pretreatment sample. The yield increases with higher temperatures, as can be expected for water. At 130°C, the acids perform quite similar to each other but all perform significantly better than water. At 160°C maleic acid gives the highest glucose yield after the hydrolysis, this suggests that maleic acid does have another effect on the straw than the other acids. Oxalic acid has only a slightly lower glycose yield than maleic acid and sulphuric acid is only slightly below that. At 190°C the degradation of oxalic acid is visible again, resulting in the lowest yield of the acids. Sulphuric acid only has a slightly higher yield than oxalic acid, while this does not degrade. Maleic acid performs better than the others at this temperature again and hydrolyses nearly 100%.

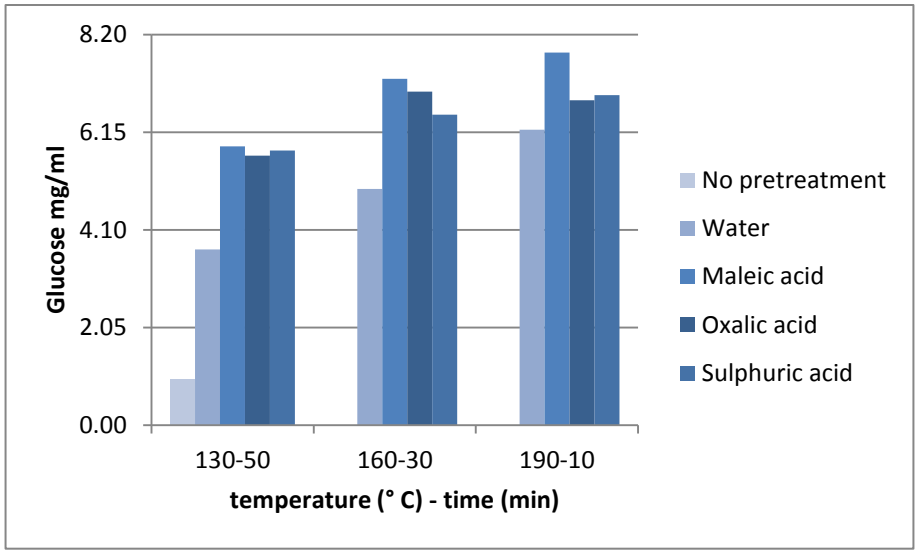

Figure 18 Glucose yield on straw after hydrolysis

Figure 19 depicts the amount of xylose measured after the pretreated and non-pretreated samples have been hydrolysed. The top of the graph (4 mg/ml) represents a 100% hydrolysis of all the xylan present. The straw without pretreatment does give some xylose after the hydrolysis, suggesting that the used cellulase enzyme mixture also has some xylanase activity. This can be further seen in xylose yield with water pretreated samples, while the pre-hydrolysed samples contain hardly any xylose, after the hydrolysis significant amounts have formed. This amount increases with pretreatment temperature, this suggests that xylan oligomers form during the water pretreatment but these are not yet fully hydrolysed to xylose. For almost all the reactions with acid, the xylose yield decreased after hydrolysis. While xylose does degrade to HMF (Qian, 2005), the amount of HMF formed (not shown) did not correlate with the observed decrease in xylose yield. No clear trend in xylose yield after the hydrolysis can be observed.

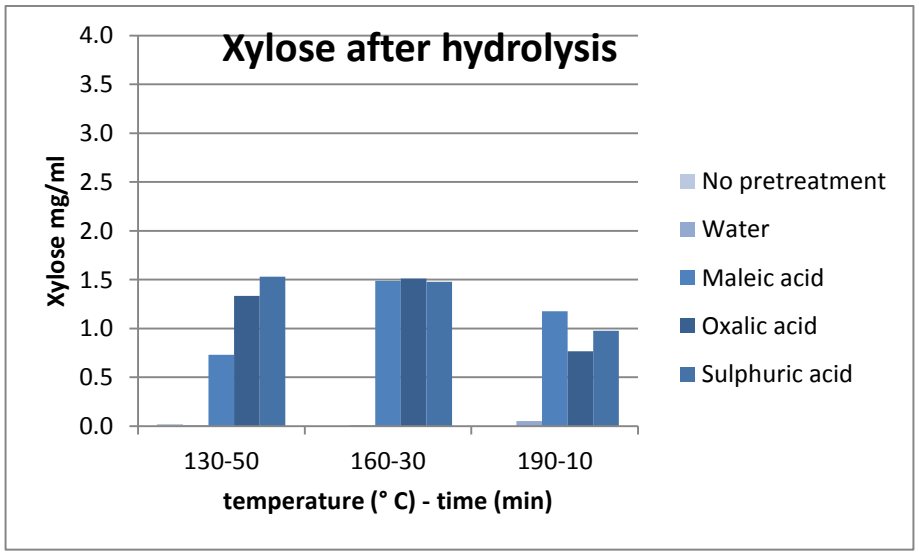

Figure 19 Amount of xylose formed after the hydrolysis

4.5.3 Conclusions from straw pretreatment

The pretreatment of straw gives more insight into pretreatment efficiency than cellulose as straw is more difficult to hydrolyse enzymatically. It can be concluded that maleic acid is indeed the best acid for pretreatment, the differences are not significant at low temperatures but at 190°C it is clearly visible. Another thing that can be noted is that xylose is more easily hydrolysed than glucose as the xylose yield is already quiet high at low temperatures, even with water. Very low amounts of HMF and levulinic acid were formed, this can be due to the lower concentration of glucose in the reaction mixture.

4.6 Acid degradation

Acid (50mM)	start pH	end pH
sulphuric acid	1.30	1.58
maleic acid	1.56	2.17
oxalic acid	1.58	2.99
maleic acid Na salt MA pH	1.53	1.78
maleic acid Na salt SA pH	1.30	1.96
maleic acid + 30% NaCl	0.71	2.65
butyric acid	3.01	3.36

Table 2 pH of tested acids at the start and at the end of an empty 50 minute pretreatment at 190°C

Table 2 shows the pH of all the tested acids before any reaction has occurred and after a pretreatment without cellulose or straw at 190°C for 50 minutes. Remarkable is that all the acids show some degradation on its own or as a results of reactions with the reactor wall. Sulphuric acid should normally not degrade but can react with the iron in the reactors explaining the increase in pH. Maleic acid also degrades a bit most likely due to reactions with the reactor wall. Oxalic acid is known to degrade on its own at high temperatures and that is clearly shown in the table with a significant decrease in acidity. The maleic acid sodium salts degrade both about the same, the amount of degradation was in between that of sulphuric acid and that of maleic acid. The very low pH of maleic acid with NaCl can be explained by the common ion effect an probably also by some effect of the salt on the pH meter. The measured pH difference, almost 100 times less acidic, of this acid mixture is very high but can at least partly be attributed to NaCl reacting. Butyric acid shows the least amount of degradation but this acid had the highest pH to start with.

4.7 Comparison of cellulose and straw

4.7.1 Introduction of cellulose and straw comparison

Comparing the glucose yield after hydrolysis for pure cellulose and straw gives us a good idea of pretreatment efficiency. While the cellulase enzymes work in optimal conditions with pure cellulose, resulting in nearly full hydrolysis, with straw they are not so efficient. Hydrolysed cellulose gives a better idea of the effect of degradation products on the overall sugar yield than the results from straw could give. But because the hydrolysis is so effective on pure cellulose, the pretreatment efficiency could not be truly investigated. The straw on the other hand hardly has any effect of

the degradation products but the pretreatment does have a bigger effect on the overall sugar yield. So a low yield on cellulose means more degradation products and a high yield on straw means good pretreatment efficiency.

4.7.2 Results for comparison of cellulose and straw at 130°C after 50 minutes

Figure 20 compares the achieved glucose yield, in percentage of the theoretical maximum, on water and acid pretreated and non-pretreated cellulose and straw after hydrolysis. The pretreatment was performed at 130°C for 50 minutes.

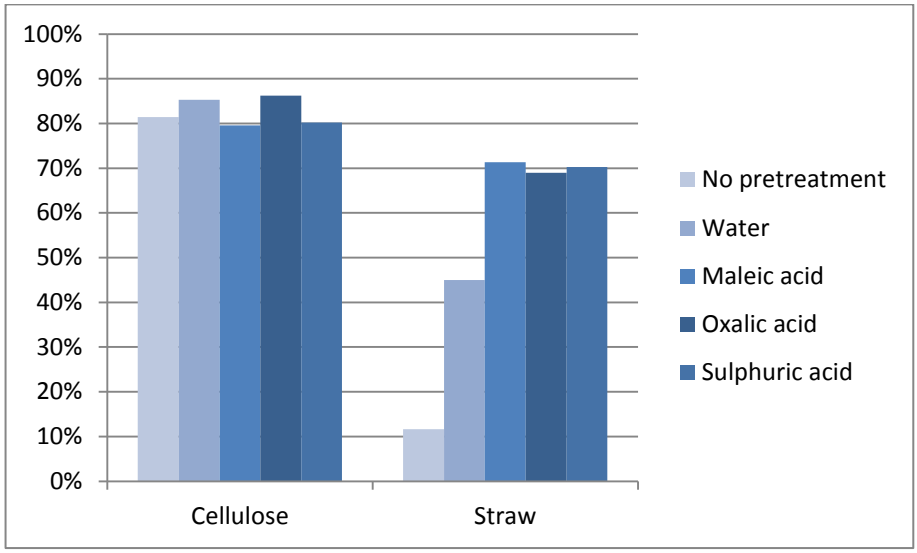

Figure 20 Glucose yield in percentage of theoretical maximum after pretreatment at 130°C for 50 minutes

Looking at the lowest temperature; 130°C for 50 minutes, we can see that there is hardly any formation of degradation products. The glucose yield for all the samples is very similar to the non-pretreated sample. Looking at the straw, we can see that that the glucose yield is not very high, especially for the water pretreated samples. The three tested acids perform very similar to each other at these conditions.

Figure 21 compares the achieved glucose yield, in percentage of the theoretical maximum, on water and acid pretreated and non-pretreated cellulose and straw after hydrolysis. The pretreatment was performed at 160°C for 30 minutes. At these conditions some differentiation can be observed. The stronger acids, e.g. sulphuric and oxalic acid, give lower glucose yields with cellulose. Maleic acid sits between water and oxalic acid in terms of yield and water performs best. The pretreated and hydrolysed straw tells a different story, here maleic acid shows its clear superiority compared to the other acids. The pretreatment is efficient in making the straw more easily hydrolysable while still keeping the amount of degradation products low. The other acids also perform well but slightly less than maleic acid. Sulphuric acid gives the lowest yield and oxalic acid is in between this and maleic acid. The water pretreatment gives a lower yield on straw than any of the acids.

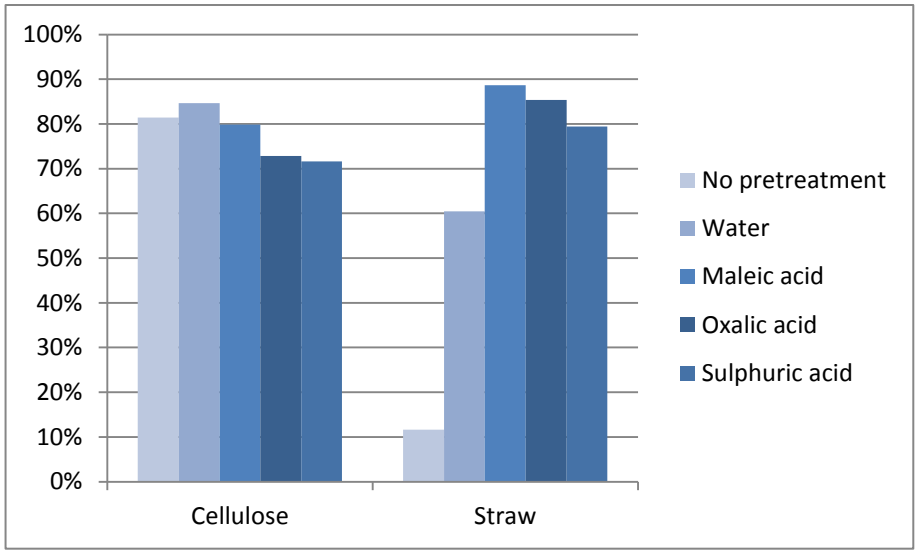

Figure 21 Glucose yield in percentage of theoretical maximum after pretreatment at 160°C for 30 minutes

Figure 22 compares the achieved glucose yield, in percentage of the theoretical maximum, on water and acid pretreated and non-pretreated cellulose and straw after hydrolysis. The pretreatment was performed at 190°C for 10 minutes. The differences visible at 160°C become more apparent at 190°C, water and maleic acid perform very similar in glucose yield on cellulose. From this we can conclude that the amount of degradation products formed for water and maleic acid are quiet similar. Oxalic acid has a 20% lower (80% vs 60%) glucose yield than maleic acid, this can also be seen in the higher amount of measured degradation products formed. Sulphuric acid has an even lower yield at only 45%. It has already been shown that sulphuric acid produced the highest amount of degradation products. This is as expected in advance. The glucose yield with straw shows that water has a relatively higher yield at this temperature than the acids, getting closer in terms of yield. Oxalic acid and sulphuric acid both form about the same amount of glucose from straw, this amount is slightly higher than that of water. Maleic acid is clearly the superior pretreatment acid, with a 95% glucose yield on straw. This cannot solely be attributed to its acidity since sulphuric acid is a much stronger acid. Also its not solely di-acid bound, otherwise oxalic acid would perform equally well. So there is a chance that the specific conformation of the two acid groups has a positive effect on the pretreatment efficiency.

4.7.3 Conclusions from the comparison of cellulose and straw

The first thing that should be noted is that the pretreatment on straw showed that acids do improve the efficiency; this could not be noted with pure cellulose. Maleic acid is clearly superior in terms of degradation products formed, as seen from the glucose yield on cellulose. But even more interesting is that maleic acid also performs better in terms of pretreatment efficiency on straw than the other acids. This combination of low degradation products and high pretreatment efficiency is very promising for the use of maleic acid in dilute acid-pretreatment.

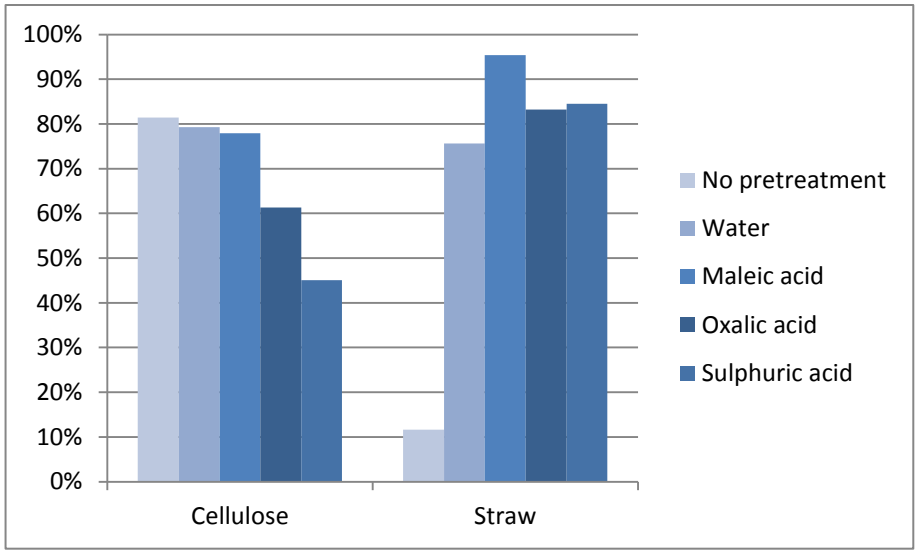

Figure 22 Glucose yield in percentage of theoretical maximum after pretreatment at 190°C for 10 minutes

4.8 XRD measurement results

XRD measurement results for pretreated cellulose can be seen in figure 23; every tested acid is depicted in the graph. The XRD analysis was performed for every cellulose sample but, as can be seen from the graph, no correlation between pretreatment conditions and crystallinity were observed. The only notable effect of the pretreatment can be seen with oxalic acid at 190°C, not with any other acid or any other temperature. The two small peaks visible at 18 and 30 2θ° only were observed at these conditions. These two small peaks are not yet reported in literature and cannot be logically explained. Surprising is the fact that the pretreatment with maleic acid and 30% NaCl did not have any noticeable effect on the crystallinity at any conditions (not all shown). Contrary to what Stein et al. (2010) reported. This could be due to the method used, differences in crystallinity might have been measureable with C NMR but this was not performed.

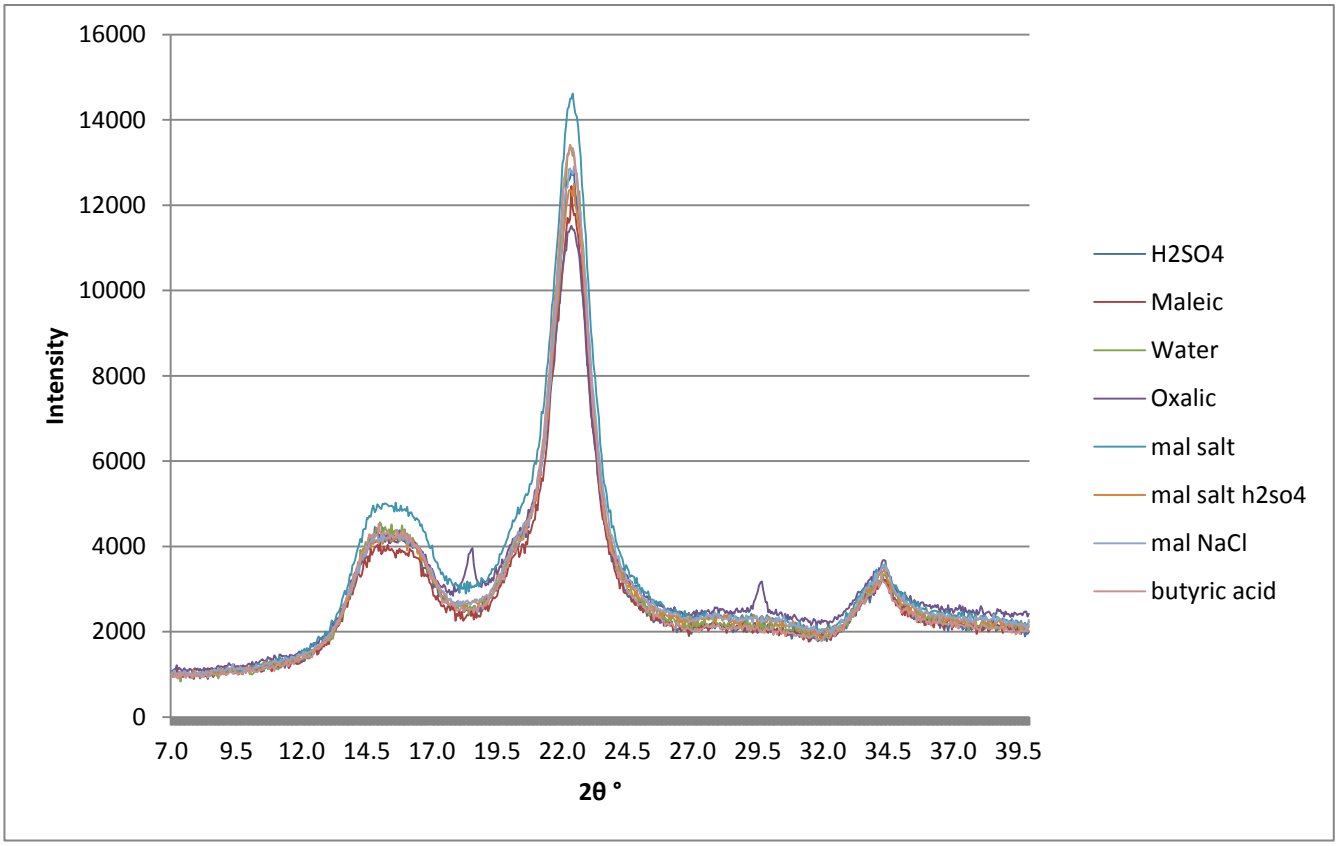

Figure 23 Raw XRD measurement data of all the tested acids at 190°C after 10 minutes.

5 Conclusion

In terms of glucose yield after the pretreatment, maleic acid performs better than sulphuric acid at 190°C while at lower temperatures sulphuric acid performs better. This difference can be attributed to the formation of degradation products which can also be seen in a lower glucose yield after hydrolysis for sulphuric acid at190°C.

From the variations on maleic acid, it can be concluded that regular maleic acid performs better in terms of glucose yield at high temperatures than the maleic acid sodium salts. A more acidic sodium salt gave a higher glucose yield after the pretreatment at low temperatures but declined at 190°C due to degradation products formed. So the acid itself affects the pretreatment efficiency and not the structure of the conjugate base.

The promising research by Stein et al. (2010) on salt assisted hydrolysis could only be partly reproduced here. Only at low temperatures for a long duration yielded this mixture a very high glucose yield after the pretreatment and at higher temperatures gave more degradation products and lower sugar yields than regular maleic acid. So the addition of 30% NaCl can be beneficial if a single acid hydrolysis is performed at low temperatures for a long time without enzymatic hydrolysis afterwards as the high salt concentrations inhibit the enzymes. This full acid hydrolysis could be interesting by reducing the costs of enzymes and reducing the number of processing steps. Stein et al.(2010) also suggested that the addition of salt would work in the same way as an ionic liquid by disturbing the hydrogen bonds. As a result of this, the crystallinity would change, but this change in crystallinity was not measured.

There was however a measureable difference in glucose yield on straw between the tested acids, here maleic acid performed better than the other acids. This difference was more apparent at higher temperatures, this does suggest some specific mechanism of maleic acid for pretreatment efficiency. This effect could not be found in cellulose because the enzymatic hydrolysis was too strong. But the effect cannot be explained, there were no measureable changes in crystallinity and the biomimetic effect was not proven although it also was not disproven.

The alleged biomimetic effect of maleic acid was not proven in these experiments, as maleic acid did not perform significantly better than oxalic acid. The theory was also not clearly disproven but no convincing evidence was found. Oxalic acid even performed better under some circumstances, only at 190°C where oxalic acid degraded, had maleic acid a significantly higher yield. The amount of degradation products formed however, was higher for oxalic acid than for maleic acid while they both have similar acid strength. Especially the formation of levulinic acid was significantly higher for oxalic acid but also for most other acids, this suggests some inhibiting action of maleic acid for formation of levulinic acid.

Even though the mechanism could not be proven it was still apparent that maleic acid does performs really well in terms of pretreatment efficiency on straw with low amounts of degradation products formed. This can open up new pretreatment methods for large scale applications because most of the benefits of maleic acid are

related to its lower acidity. The amount of corrosion to the reactors can be reduced which reduces costs. Also the amount of base needed to neutralize the acid is smaller for maleic acid than for sulphuric acid, this also reduces the amount of salt formed from this neutralization. So the overall conclusion is that maleic acid is a promising substitute for sulphuric acid in dilute-acid pretreatment in terms of glucose yield and degradation product formation although this research could not answer why this is the case.

6 Perspectives & Recommendations

Lignocellulosic biofuels are at the moment not yet profitable, this is mainly due to the difficulty to hydrolyse the raw materials to fermentable sugars. It is too expensive to only use enzymes and therefore a pretreatment is necessary. Pretreatment is regarded as one of the most expensive processing steps in the production lignocellulosic biofuels and therefore has a great potential to be improved (Mosier, 2005). The now most common pretreatment method is dilute sulphuric acid pretreatment but this method has several drawbacks raising the costs. Most of these drawbacks are related to the high acidity of sulphuric acid. If a weak acid with nearly the same effectivity as sulphuric acid could be used for the pretreatment many of these drawbacks could be reduced.

Maleic acid thus has the potential to reduce costs in the production of lignocellulosic bioethanol bringing us one step closer to the profitable production. If this could be achieved then a large portion of the used transportation fuels could become carbon neutral greatly reducing the amount of greenhouse gasses emitted (Stöcker, 2008).

This research focused mainly on the comparison between maleic acid and sulphuric acid. Although these acids give promising results, it could still be very interesting to test different acids such as oxalic acid, which also gave promising results, in more detail. Oxalic acid and others could not be investigated in great detail as this was not within the scope of this research. Expanding the reaction conditions for maleic and other acids to find the ideal pretreatment conditions for the specific acids should also be interesting for optimization of the process. Only a relatively small set of temperatures and durations were tested and only one acid concentration was tested. These were all tested at the same solid loading level which can also affect the relative yield (Kootstra, 2009).

For testing the biomimetic effect even further a mono-caboxylic acid should be tested that has the same acidity as maleic acid. If the efficiency can be attributed to a carboxylic acid in combination with a low pH instead of the conformation of the acid groups. If there really is a biomimetic effect than a significant difference should be visible in terms of glucose yield between this mono-acid and maleic acid.

The tested types of maleic acid had different effects on the measured parameters such as sugar yield and degradation product formation. This does show that maleic acid has some effect on the pretreatment efficiency in terms of glucose yield before and after hydrolysis not only related to its acidity. Although this research was not

able to show what effect this was exactly, it could be an interesting topic for further research. As HMF is a promising biobased platform chemical.

Another interesting effect of maleic acid is the seeming inhibiting effect on the formation of levulinic acid. Maleic acid produced consistently the lowest amounts of levulinic acid compared to the other tested acids even when similar amounts of HMF are produced. Only water had a lower production of levulinic acid but this can be explained by the reaction being acid catalysed. Especially remarkable is the difference between the two maleic acid sodium salts, an increase in acidity results in a significantly higher amount of levulinic acid. This effect could be interesting for further research, maybe for the production of HMF with small amounts of levulinic acid formed as by-product.

On the experimental side of this research some things should be considered for change for following researchers. It would firstly be very beneficial for the experiment to first do a screening of the hydrolysis efficiency of the cellulase enzymes. In these experiments, the concentration of enzyme was too high, as a result of this, the pretreated cellulose was almost completely hydrolysed independent of the used acid or conditions. Only the degradation product formation had a significant effect on the sugar yield after the hydrolysis. Even cellulose without pretreatment was nearly fully hydrolysed. However, the used enzyme concentration was just right for the milled straw, as this is more difficult to hydrolyse.

The crystallinity of the cellulose gave no measureable difference between the pretreatment methods or the non-pretreated samples. This could be due to two reasons; the used measurement technique or the crystallinity actually did not change. The used technique, XRD, needs very high quality spectra with a high signal to noise level to measure small changes in crystallinity. It could be that the used spectra were not of high enough quality to measure the small changes in crystallinity (Park, 2010) if they did occur at all. C-13 NMR can measure small changes in crystallinity but this method was not used in this research, although this could have been useful. It could have been useful to be able to measure in a more accurate way because crystallinity does affect the cellulase digestibility (Hall, 2010). It is possible that the crystallinity did not change at all but the addition of 30% of NaCl has already been shown to change the crystallinity (Stein, 2010). It should be noted that the overall crystallinity could change if cellulose reacted to soluble non-crystalline structures, but this would not be measureable because the crystalline fraction would still be measured.

Scanning electron microscope (SEM) imaging could have been useful since changes in structure that occurred during pretreatment could have been visualized (Sannigrahi, 2008). Other changes than crystallinity could be visualized using this technique and can give insight in the pretreatment mechanism. This technique could be useful in further research of this topic.

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8 Appendix

Sulphuric acid									
Temp.-time	start weight	weight after g	% dissolv.	glucose before	Glucose after	Yield %	HMF	Lev. Acid	Tot. degr.
130-10	0,500	0,476	5%	0,045	11,228	51%	0,0000	0,0000	0,0000
130-30	0,500	0,4497	10%	0,200	18,190	83%	0,0020	0,0000	0,0020
130-50	0,500	0,4667	7%	0,309	17,643	80%	0,0013	0,0000	0,0013
160-10	0,500	0,4215	16%	1,053	16,723	76%	0,0193	0,0000	0,0193
160-30	0,500	0,367	27%	2,170	15,758	72%	0,1257	0,0276	0,1533
160-50	0,500	0,3553	29%	2,627	12,503	57%	0,2399	0,1864	0,4263
190-10	0,500	0,3686	26%	0,356	9,921	45%	0,8966	0,1029	0,9995
190-30	0,500	0,2612	48%	0,339	10,150	46%	0,8188	0,3611	1,1799
190-50	0,500	0,2151	57%	0,357	5,773	26%	0,7999	0,9598	1,7596
Maleic acid									
Temp.	start weight	weight after g	% dissolv.	glucose before	Glucose after	Yield %	HMF	Lev. Acid	Tot. degr.
130-10	0,500	0,4617	8%	0,005	18,308	83%	0,0000	0,0000	0,0000
130-30	0,500	0,462	8%	0,025	16,901	77%	0,0001	0,0000	0,0001
130-50	0,500	0,4575	9%	0,053	17,510	80%	0,0002	0,0000	0,0002
160-10	0,500	0,4457	11%	0,180	16,805	76%	0,0012	0,0005	0,0017
160-30	0,500	0,4112	18%	0,718	17,570	80%	0,0186	0,0000	0,0186
160-50	0,500	0,4188	16%	1,292	17,597	80%	0,0329	0,0045	0,0374
190-10	0,500	0,3704	26%	2,558	17,142	78%	0,1168	0,0092	0,1260
190-30	0,500	0,2918	42%	4,216	16,333	74%	0,4359	0,0163	0,4522
190-50	0,500	0,2195	56%	4,725	12,925	59%	0,7980	0,0886	0,8866
oxalic acid	start weight	weight after g	% dissolv.	glucose before	Glucose after	Yield %	HMF	Lev. Acid	Tot. degr.
130-50	0,500	0,4348	13%	0,1950	18,96945	86%	0,000	0,000583333	0,00085
160-30	0,500	0,421	16%	1,1661	16,02865	73%	0,032	0,006466667	0,03821667
190-10	0,500	0,4104	18%	0,2175	13,4935	61%	0,144	0,04655	0,1904
Maleic acid salt	start weight	weight after g	% dissolv.	glucose before	Glucose after	Yield %	HMF	Lev. Acid	Tot. degr.
130-50	0,500	0,4584	8%	0,0406	17,4521	79%	8,3E-05	0,0004	0,00048333
160-30	0,500	0,3856	23%	0,594	16,3009	74%	0,0163	0,0059	0,02221667
190-10	0,500	0,3915	22%	1,8246	15,93	72%	0,125	0,009016667	0,13355
Maleic acid salt + 50mM	start weight	weight after g	% dissolv.	glucose before	Glucose after	Yield %	HMF	Lev. Acid	Tot. degr.
130-50	0,5	0,4308	14%	0,13	18,38	84%	0,001	0,028	0,029
160-30	0,5	0,3935	24%	0,949	17,05	78%	0,067	0,035	0,102
190-10	0,5	0,3794	21%	0,495	15,18	69%	0,343	0,2325	0,5755
Maleic acid + NaCl	start weight	weight after g	% dissolv.	glucose before	Glucose after	Yield %	HMF	Lev. Acid	Tot. degr.
130-50	0,500	0,447	11%	0,8368	12,4641	57%	0,001	0,003066667	0,00379167
160-30	0,500	0,4178	16%	0,4134	14,6668	67%	0,072	0,00766	0,07971
190-10	0,500	0,3863	23%	0,03275	15,2437	69%	0,705	0,021516667	0,7267
butyric acid	start weight	weight after g	% dissolv.	glucose before	Glucose after	Yield %	HMF	Lev. Acid	Tot. degr.
130-50	0,500	0,466	7%	0,009	18,7272	85%	0,0002	0	0,0002
160-30	0,500	0,445	11%	0,0321	16,244	74%	0,0013	0,015	0,0163
190-10	0,500	0,450	10%	0,07	16,567	75%	0,0128	0,045	0,0578
water	start weight	weight after g	% dissolv.	glucose before	Glucose after	Yield %	HMF	Lev. Acid	Tot. degr.
130-50	0,500	0,453	9%	0	18,7573	85%	0	0	0
160-50	0,500	0,456	9%	0,008366667	18,6235	85%	0,001	0,00235	0,00308333
190-50	0,500	0,407	19%	0,5479	17,43655	79%	0,190	0	0,18963333
Hydro test 160-30	start weight			glucose before	Glucose after	Yield %			
24 h	0,500			0,206	14,4875	66%			
48 h	0,500			0,1982	15,7429	72%			
24 h 1/2 enzyme	0,500			0,1867	14,3581	65%			
48 h 1/2 enzyme	0,500			0,2634	15,807	72%			
	start weight	weight after g	% dissolv.	glucose before	Glucose after	Glucose yield	xylose before	xylose after	Yield %
no pretreatment cell	0,500	0,5	0%	0	17,9185	81%	na	na	90%
no pretreatment straw	0,500	0,5	0	0,075	0,954	12%	0,015	0,2317	

Straw mal.	weight g	weight after g	% dissolv.	glucose before	Glucose after	Glucose yield	xylose before	xylose after	total before	total after
130-50	0,500	0,290	42%	0,084	5,850	71%	0,7311	1,221	0,8148	7,0711
160-30	0,500	0,258	48%	0,295	7,271	89%	1,4864	1,230	1,7809	8,5014
190-10	0,500	0,242	52%	0,466	7,823	95%	1,1761	0,873	1,6425	8,6961
Straw H2SO4	weight g	weight after g	% dissolv.	glucose before	Glucose after		xylose before	xylose after	total before	total after
130-50	0,500	0,285	43%	0,275	5,7610	70%	1,529	1,195	1,804	6,956
160-30	0,500	0,251	50%	0,673	6,514	79%	1,477	0,956	2,15	7,47
190-10	0,500	0,228	54%	1,269	6,929	85%	0,978	1,282	2,2467	8,2109
Straw oxalic	weight g	weight after g	% dissolv.	glucose before	Glucose after		xylose before	xylose after	total before	total after
130-50	0,500	0,312	38%	0,197	5,654	69%	1,332	1,191	1,529	6,845
160-30	0,500	0,281	44%	0,51	7,001	85%	1,509	1,087	2,019	8,088
190-10	0,500	0,267	47%	0,556	6,822	83%	0,767	1,231	1,323	8,0531
Straw water	weight g	weight after g	% dissolv.	glucose before	Glucose after		xylose before	xylose after	total before	total after
130-50	0,500	0,416	17%	0,054	3,687	45%	0,01	0,453	0,064	4,14
160-30	0,500	0,380	24%	0,084	4,956	60%	0,01	0,841	0,094	5,797
190-10	0,500	0,301	40%	0,078	6,203	76%	0,051	0,911	0,129	7,114