Potential of Steam Pretreated Jerusalem Artichoke Stem for Ethanol Production

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Picture on front page: Flower of Jerusalem Artichoke Photo by Larisa Koshkina.

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Abstract

To reach the goals set for the reduction of green house gas by the European union the use of fossil fuels has to be reduced. To do this alternative fuels such as bioethanol have to substitute fossil fuels. Second generation bioethanol is produced from lignocellulosic substrates, such as forest and agricultural residues, whereas first generation bioethanol is obtained from material as sugar cane and corn containing sugar and starch. A possible raw material for production of ethanol from lignocellulosic biomass could be the stem of jerusalem artichoke. Lignocellulosic materials are recalcitrant, and therefore they need to be pretreated in order to make them more degradable. Steam pretreatment is the way of pretreatment most commonly used at commercial scale production of ethanol from lignocellulosic material.

In this study it was investigated if the stem of jerusalem artichoke is a suitable material to produce ethanol using steam pretreatment as method for pretreatment. Jerusalem artichoke stem was pretreated at 7 different conditions, and the pretreated material were subjected to enzymatic hydrolysis. Two differently pretreated material (pretreated at 200°C for 5 minutes and 10 minutes) were selected for simultaneous saccharification and fermentation. The main aim of this study was to get an as high conversion from the sugars in the raw material to ethanol as possible.

The highest final ethanol yield that was received was 76% of the theoretical maximum for material treated at 200°C for 10 minutes. With this set of parameters the corresponding overall glucan to glucose conversion was 81%. These results were approximately in the same range as those of other studies using jerusalem artichoke as raw material. The highest concentration of ethanol that was received was 10,7g/l. This is low compared to 40g/l which is usually considered to be what must be achieved. However the parameters and procedure are not optimized and there is still room for improvements.

Contents

1 Preface

This master thesis was performed at the Department of Chemical Engineering, Faculty of Engineering, Lund University.

First and foremost I would like to thank Krisztina Kovacs for all the help she has provided throughout this thesis.

I would also like to thank everyone at the department for always giving me an extra hand when needed and the Swedish University of Agricultural Sciences, Alnarp for providing me with the raw material.

2 Populärvetenskaplig sammanfattning

Bioetanol producerat från blast av jordärtskocka.

I tider av ökad vetskap om fossila bränslens påverkan på vår miljö har efterfrågan på nya sätt att producera alternativa drivmedel bara blivit större och större. Många av dagens biobränslen behöver stora arialer av jordbruksmark för att tillgodose produktionen med tillräckligt råmaterial. Däribland bioethanol. Vissa anser att produktionen av råmaterial till bioetanol därför konkurrerar med produktionen av föda och det finns ett ständigt sökande efter nya råmaterial att använda för att minska den utnyttjade arialen. Ett material som har lovande egenskaper i detta anseende är jordärtskocka.

Jordärtskocka är en växt i solrosfamiljen som först odlades av indianer i Nordamerika. Den odlas främst för sina knölar som är den ätliga delen av växten. Dess stam kan bli 3m och utgör således en stor del av växtens biomassa. Den växer mycket snabbt och skapar därmed en stor mängd biomassa per areal och år. Jordärtskockan kan överleva i torra klimat, anpassar sig väl till många olika typer av jordmån, tål minusgrader och knölarna tål flera månader med tjäle. Detta gör att jordärtskockan kan växa på många ställen där andra grödor inte skulle överleva.

För att kunna producera etanol av blasten från jordärtskocka behöver den förbehandlas då jästen annars inte kan tillgodose sig kolhydraterna i materialet. En metod som redan används industriellt som förbehandling vid tillverkning av etanol från liknande material är så kallad ångexplosion. Det är en metod där man använder högtrycksånga för att luckra upp fibrerna i materialet. Med ångexplosion i kombination med enzymatisk hydrolys frigörs sockermolekyker som jästen kan äta och samtidigt producera etanol. Enzymatisk hydrolys är en metod för att dela upp kedjor av sockermolekyler till fritt socker.

För att undersöka hurvida blasten från jordärtskocka kan fungera som råmaterial för etanolproduktion analyserades olika behandlade material. Temperatur på ångan och tiden som materialet exponerades för ångan ändrades för att se vid vilka parametrar som materialet blev tillräckligt förbehandlat utan att bryta ner socker i materialet.

Då några av proverna som producerades inte förbehandlas som väntat var det inte möjligt att dra en slutgiltig slutsats angående vid vilka parametrar som materialet borde förbehandlas.

När de förbehandlade materialen fermenterades, uppnådes så mycket som 76% av det teoretiskt maximala utbytet. Detta kan anses vara ett acceptabelt utbyte för en process som inte optimerats.

För de parameteruppsättningar som materialen som fermenterades blev förbehandlade vid fick man ut 64 -76% av hur mycket etanol som maximalt hade varit möjlig med de förbehandlade materialen. Vilket kan anses vara ett acceptabla utbyte för en process som ännu inte är helt optimerad. Under förbehanlign av dessa prover gick 14-19% av glukosen förlorad. Därmed var den totala mängden glukos som konverterades till etanol under processen 55 och 62%

3 Introduction

The energy sector was in 2007 accountable for 80% of the greenhouse gas released in the European Union and at the same time it was calculated that the emission would increase 55% globally within the next 25 years. A new EU policy was issued to handle these problems, which included the goals to reduce the emission of greenhouse gas with 20% by 2020 and 40% before 2030 and that 20% of the total consummation of energy should come from renewable energy by 2020 and 27% by 2030 [1].

To be able to reach these goals alternative ways to provide energy are needed to reduce the usage of fossil based fuels, particularly in the transportation sector. One renewable alternative fuel that is suitable for transportation is bioethanol.

First generation bioethanol which includes most commercial produced ethanol worldwide is made from crops as corn, wheat, and sugar cane and beets. Due to concerns about food prices and the impact of land usage, the European Commission has suggested to limit production of biofuel from "food crops" to 7% of the energy usage for transportation [2]. Even if there are studies that question the validity of these concerns the demand for alternative ways to produce ethanol is increasing [2].

Second generation bioethanol, contrary to first generation ethanol, can be made from agricultural and forestry residue and will not, if using these types of raw material, increase the usage of land and instead use already existing material and there will be no food vs. fuel conflict.

One such raw material that is considered promising for ethanol production, due to its high biomass per area ratio its resistance to pesticides and its ability to grow in various climates is the stem from jerusalem artichoke. To be able to produce ethanol from this material it has to be treated before fermentation. One such treatment is called steam pretreatment which is the pretreatment most used when producing ethanol from lignocellulosic material on a commercial scale [3].

In this study the possibility to use steam pretreatment as pretreatment prior to fermentation on jerusalem artichoke stem has been investigated. The aim of this study was to find at which set of process parameters the pretreatment should be run by evaluating differently pretreated material. This was done by studying the samples composition, hydrolysability and by investigating the ethanol yields that could be reached for the different parameter sets.

4 Literature Review

4.1 Lignocellulosic biomass

Lignocellulosic biomass contains primarily polymeric sugars, such as cellulose and hemicellulose, lignin and minerals in smaller amounts (ash). In Figure 4.1 the structure of the biomass is shown.

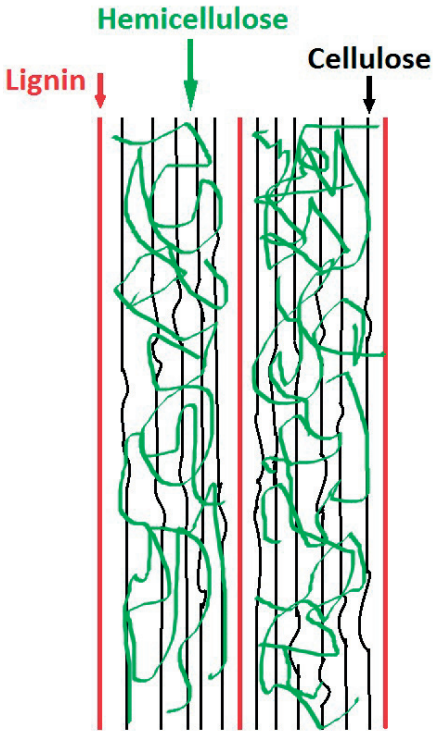

Figure 4.1: The arrangement of cellulose, hemicellulose and lignin in lignocellulosic biomass

- Cellulose
  Cellulose is a polysaccharid consisting of cellobiose as repeating unit which is a disaccharide with two glucose molecules (Figure 4.2). The glucose molecules are linked with a β-1,4-glycosidic bonds The cellulose content of lignocellulosic biomass is usually 35-50% (dry mass). Between 50 and 90% of the cellulose is laterally bound by hydrogen bonds and forms crystalline structures. The crystalline cellulose can be considered as a composite material built from nanometerscale microfibrils and poses a major problem for the hydrolysis due to its low surface area. Cellulose cannot be fermented if not divided into its monomeric sugars.

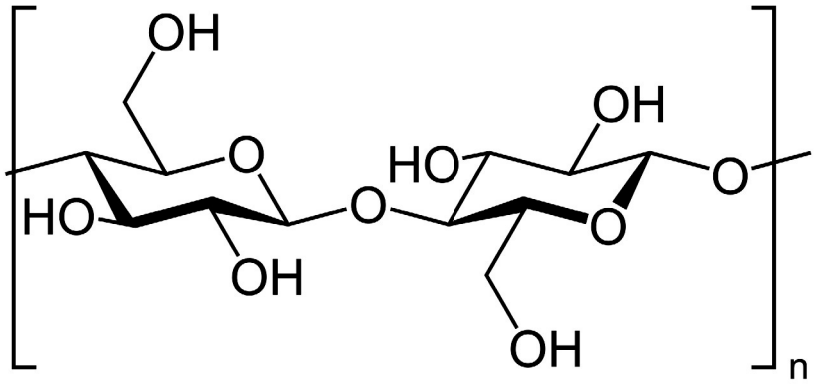

Figure 4.2: Structure of cellulose

- Hemicellulose
  The content of hemicellulose is up to 35% of the total lignocellulosic mass. Similarly to cellulose, hemicellulose consists of chains with sugars as mono-mers (Figure 4.3). Unlike cellulose the chains in hemicellulose are branched and contain different types of sugars such as xylose and arabinose, and aldohexoses, such as mannose, glucose, and galactose. Due to its branched chains and its irregularity hemicellulose is amorphous on contrary to cellulose. The composition of sugars differs depending on the material. In agricultural residues and hardwood the most abundant sugar is usually xylose and mannose in softwood. Parts of the hemicellulose (glucose and mannose) can be fermented with regular bakers yeast.

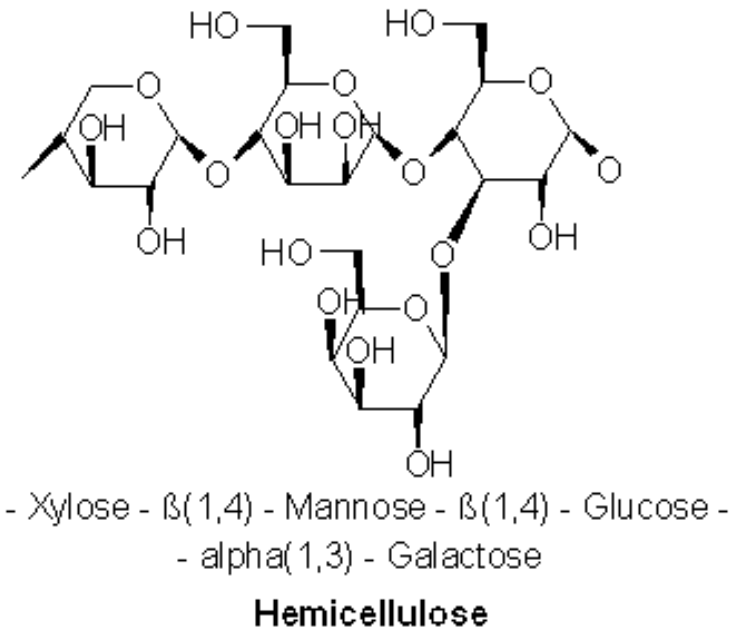

Figure 4.3: Structure of hemicellulose

- Lignin
  The lignin content is about 10-25%. Lignin is distinctly different from the other macromolecules in lignocellulosic biomass. Lignin is a branched polyaromat and consists primarily of three phenylpropane compounds, guaiacyl propanol, syringyl propanol and p-hydroxyphenyl (Figure 4.4). Lignin remains a solid after most hydrolysis methods and cannot be fermented to ethanol [4].

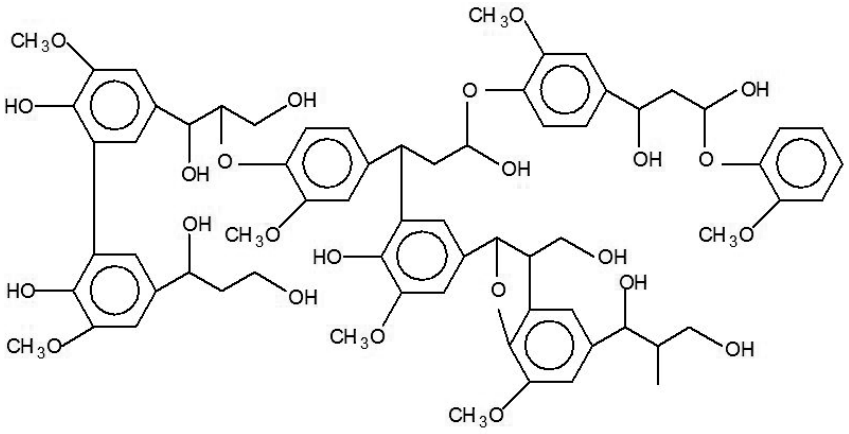

Figure 4.4: Structure of lignin

Besides cellulose, hemicellulose and lignin, lignocellulosic biomass consists of a number of other compounds in smaller amounts e.g. fats, pectins, proteins and inorganic compounds. Moreover the material also contains various extractives, mainly non-structural monomeric sugars [5]. The structure of lignocellulosic biomass has several features that makes it hard to deconstruct the macromolecules enzymatically. Most of the bio-polymers (cellulose, hemicellulose and lignin) are interconnected intimately and form lignin-carbohydrate complex which reduces the accessibility of cellulose to enzymes and this make it difficult to cleave the macromolecules to its monomers [6].

4.2 Jerusalem artichoke

Despite the name, Jerusalem artichoke (JA) (Helianthus tuberosus L.) is not a type of artichoke, neither has it any relations to Jerusalem. JA is a plant in the sunflower family that was first cultivated by Native Americans in North America [7]. It is mostly grown for its tubers which is the edible part of the plant. Its stem reaches about 1-3m and is a significantly big part of the plants biomass. JA can tolerate an annual precipitation of between 31 and 282 cm, an average temperature range of 6.3-26.6 °C, and pH of 4.5-8.2. It can adapt well to many types of soil, tolerate temperatures below zero and the tubers can withstand several months with ground frost [8].

JA grow aggressively and should therefore, when planted, be managed with caution. However due to the quick growth of the plant the need for pesticides is reduced and the annual amount of biomass per area is large. Due to its high biomass per area ratio, its good resistens against pests and its ability to grow in various climates JA is considered a promising plant for production of ethanol [9] [10].

Contrary to most lignocelullosic materials the stem of JA (JAS) contains a relatively high amount of inulin. Inulin is a polymeric sugar which consists of chains of fructose. Inulin works as a way to store carbohydrates for the plant and most of the inulin is located in the tubers of the plant. Depending on the time of the harvest different amount of inulin will be in the tubers and in the stem [11]. Besides the use of the tubers as food JA is also grown for the inulin alone. The inulin is then extracted from the tubers. The inulin is used as a sweetener in food and is claimed to have many health benefits [12] [13]. The inulin can also be used as a substrate to produce substitutes for petroleum-based polymers [14].

There has been many studies made on ethanol produced from the tubers of JA [15] [16] [17]. Researchs on the possibility of fermenting JAS is in contrast very limited. There are only three reports so far on ethanol production from JAS and in two of them the pretreated JAS was mixed with the tubers prior to fermentation [9] [10]. The third was made on JAS only and used acid pretreatment as pretreatment method [18].

4.3 Ethanol from lignocellulosic biomass

Producing ethanol from lignocellulosic material is not as easy as to ferment free sugars or starch based materials. The reason for this is that the yeast used for fermentation is not able to digest polymeric sugars as cellulose and hemicellulose, and therefore it is necessary to treat the material before the fermentation in order for the yeast to be able to produce ethanol [19].

4.3.1 Pretreatment

The goal for the pretreatment of the raw material is to reduce the crystallinety of the cellulose, remove lignin and/or hemicellulose, and increase the porosity of the material. The impact of the pretreatment is illustrated in Figure 4.5. There are four main requirements for the pretreatment that have to be considered [19]:

- Improve the sugar formation or possibility to form sugars by hydrolysis
- Avoid loss of carbohydrate
- Avoid formation of inhibitors to the hydrolysis or the fermentation
- Cost efficiency

There are several different types of pretreatment e.g. physical (grinding and milling), physico-chemical (steam pretreatment etc.), chemical (alkali, dilute acid etc.).

4.3.2 Steam Explosion

One of the most widely used method for pretreatment is steam explosion. Of the different pretreatment methods it is the one most used for commercial scale production of ethanol [3] and is the method that was used in this project. This method is using pressure to open up the structure of the raw material. The material is first treated with high pressure saturated steam, the

pressure is then rapidly decreased to atmospheric pressure, which results in that the structure undergoes an explosive decompression. The steam is typically at a pressure of about 0.70-4.85MPa with corresponding temperature of 160-260^{o}C. The rapid flashing of the material to atmospheric pressure causes the material to fragment and the surface area is increased [19].

To decrease the time and temperature of the treatment acid can be added to the raw material prior to the pretreatment. The acid improves the hydrolysis and can decrease the formation of inhibitors and helps the removal of hemicellulose [19].

The intensity of steam explosion has some limitations. Degradation products are formed that are inhibitory to microbial growth and the enzymatic hydrolysis. It can therefore be necessary to wash the pretreated biomass to get rid of these compounds if the material is treated harshly. However if the material is washed, soluble sugars will also be removed which affects the yield. Other limitations are destruction of some of the xylan fraction and incomplete disruption of the lignin-carbohydrate matrix. There are several factors that affect the pretreatment e.g. [19]:

- Duration of treatment
- Temperature
- Particle size
- Acid/catalyst concentration
- Moisture content

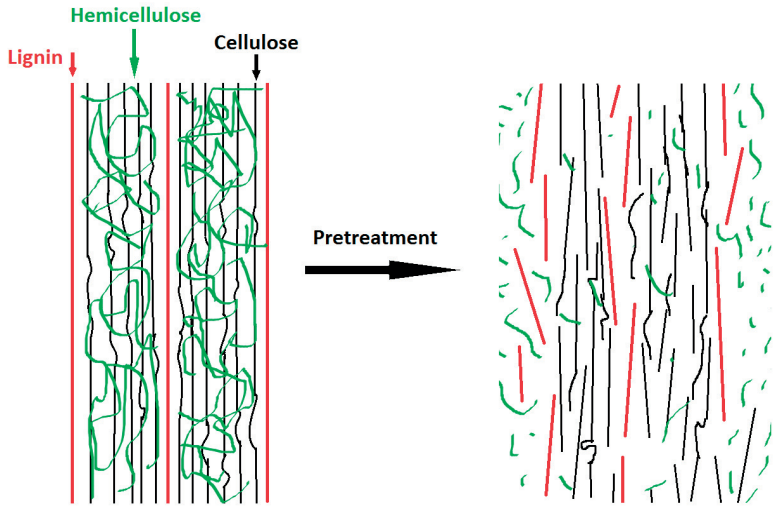

Figure 4.5: Schematic illustration of the impact of pretreatment on the raw material

After pretreatment the material will consist of a solid fraction of mainly lignin and cellulose, and a liquid fraction, containing hemicellulose-derived monomeric sugars, small amounts of other carbohydrates, sugar degradation products, dissolved lignin , and other compounds depending on the material and catalyst used. Some of these compounds can inhibit the hydrolysis and/or the fermentation [20].

4.3.3 Inhibitors

Two byproducts which reduce the ethanol productivity drastically are furfural and 5-hydroxymethylfurfural (HMF). They are derived from pentose and hexose sugars, respectively, under harsh conditions [21]. When degrading further furfural forms formic acid and HMF forms both formic acid and levulinic acid. When hemicellulose is hydrolysed, acetic acid is formed. These three acids can also inhibit the fermentation and deactivate the hydrolysis enzymes in high enough concentrations [21].

The formation of HMF, formic acid and levulinic acid can be seen in Figure 4.6

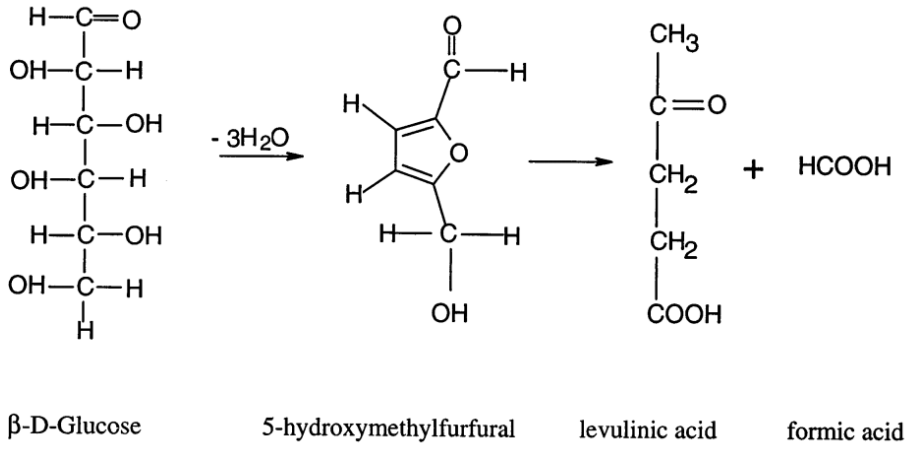

Figure 4.6: The formation of HMF, levulinic and formic acids from glucose

4.3.4 Enzymatic Hydrolysis (EH)

Hydrolysis is basically a reaction where a chemical bond is split with the addition of water. Though some hydrolysis already occurred in the steam pretreatment it is not nearly enough to get the amount of free sugars for a sufficient fermentation. The structure of the material has opened up but most of the polymers are still intact and still not digestible for the yeast. To get more free sugars a hydrolysis step is needed. Enzymatic hydrolysis (EH) is one way to hydrolyse polysaccharides.

EH is a heterogeneous reaction in multiple steps where initially insoluble cellulose is broken down with endoglucanases and exoglucanases into fragments containing 4-5 glucoses. This is followed by a liquid-phase hydrolysis with soluble polysaccharides which are cleaved catalytically with β-glucosidase to mono-saccharides into single glucose.

Due to the steam treatment the enzymes are allowed to access the polymers better and can therefore more easily break them down to sugars [22].

4.3.5 Fermentation

Due to the inhibitors that may occur in the pretreated material the environment in the material tends to be quite harsh. It might therefore be necessary to make some adjustments to the slurry depending on the amount of byproducts in it to make the fermentation possible. For example the pH could be adjusted or the solid fraction could be washed to get rid of most inhibitors. After pretreatment and enzymatic hydrolysis the samples mainly consist of lignin and dissolved sugars primarily xylose, glucose and in the case of JAS some fructose. Most yeasts cannot ferment 5 carbon sugars (such as xylose), thus when using such a yeast the maximal yield can be approximated as the glucose and fructose to ethanol yield.

$$C_6H_{12}O_6(180.16g/mol) \rightarrow 2CO_2(44.01g/mol) + 2C_2H_5OH(46.07g/mol) \tag{4.1}$$

The maximum glucose/fructose to ethanol yield can be calculated as follows.

$$yield_{ethanol} = \frac{2 * 46.07}{180.16} = 0.51 \frac{g_{ethanol}}{g_{glucose}} \tag{4.2}$$

Some factors that make a yeast good for fermentation are [23]:

- High yield and productivity from several types of sugar
- Tolerance for high ethanol and inhibitor concentrations
- Tolerance to low pH, because the risk of contamination decreases with low pH

Saccharomyces cerevisiae (S. cerevisiae) commonly referred to as bakers yeast is a yeast widely used to produce ethanol from starch and sugar and will likely be used in lignocellulosic biorefineries too. Though there are other microorganisms that could be used, in this study the focus has been on S. cerevisiae. S. cerevisiae is well established in large scale production and its use in industry is well documented. It got a high tolerance to ethanol and relatively high tolerance to many of the byproducts that can form after pretreatment of lignocellulosic material [24].

Fermentation with S. cerevisiae is generally very efficient for the 6 carbon sugars glucose and mannose. Galactose and 5 carbon sugars are not fermented by wild-type S. cerevisiae. However there are strains that are able to ferment galactose and efforts are made to engineer S. cerevisiae strains that can digest xylose [24].

4.3.6 Simultaneous saccharification and fermentation

EH and fermentation can be performed separately, in separate hydrolysis and fermentation (SHF) or at the same time, simultaneous saccharification and fermentation (SSF). SHF has the advantage that both steps can be performed at optimal condition whereas in SSF there have to be compromises. The optimal temperature for S. cerevisiae is around 30°C while the optimal temperature for the hydrolysis is around 50°C. This poses a problem when deciding which parameters to run the process at. The temperature has to be high enough for the enzyme to work properly without killing the yeast [25]. On the other hand the hydrolysis in SHF is inhibited

by the sugars released which is not the case in SSF due to the consumption of sugars by the yeast [26]. The losses of sugars are also minimized. Less material are lost in the process with fewer steps. Studies on steam pretreated material made previously have shown that SHF gives lower yields than SSF [27] [28]. Another advantage for SSF is the aspect of productivity. When hydrolysis and fermentation is run at the same time the productivity is doubled [26].

5 Materials and Methods

5.1 Raw material

Jerusalem artichoke stem and leaves were provided from the Swedish University of Agricultural Sciences in Alnarp.

5.2 Milling

To reduce the size of the material a knife mill was used. Different sizes of pieces and particles, 5 cm and down were received after the milling. The result of the milling can be seen in Figure 5.1. After milling the dry matter content was 90,5%

Figure 5.1: Picture of milled JAS. Matches for scale

5.3 Steam pretreatment

Prior to steam pretreatment the material was soaked in 1% (w/w) acetic acid for one hour. The ratio between JAS and acid was 1:20. The material was then pressed in a filter press to remove most of the water. Earlier studies were used to get an approximate time and temperature to pretreat at [29] [30]. In total 7 different parameter sets were performed. The different parameter sets are displayed in Table 5.1

Table 5.1: Duration and temperature of the pretreatment for different samples, all samples were soaked in 1% acetic acid for 1h prior to pretreatment

Sample	Time (min)	Temperature (°C)
1	5	180
2	5	190
3	5	200
4	5	210
5	10	190
6	10	200
7	10	210

All samples were run in a 4 lite steam explosion unit and after analysis two parameter sets were performed in a 10 liter unit. In the smaller batches about 200g dry matter was used and in the large batch 800g. Pictures of the units can be seen in Figure 5.2. Figure 5.3 shows the material after pretreatment.

Figure 5.2: Left: Large pretreatment unit. Right: Small pretreatment unit.

Figure 5.3: Material after steam pretreatment

5.4 Enzymatic hydrolysis (EH)

The material pretreated in the smaller pretreatment unit was hydrolysed to see how well the enzymes could split sugars for the differently pretreated materials. The material was hydrolysed with a water insoluble solid (WIS) content of 4% with Cellic Ctec2 provided by Novozymes AB (Bagsvaerk, Denmark), The WIS-content was regulated by adding appropriate amount of acetate buffer which also helped to keep the pH at a good level for the enzymes to work properly (pH 4,8). EH was performed in centrifuge tubes of 50ml (Figure 5.4) in an incubator at 45°C. Four samples for each pretreatment were run during 96h at two occasions. First with duplicate samples taken several times during the hydrolysis to see how the hydrolysis for the different pretreatments changed over time followed by one run with samples taken only at 72 and 96 hour to get a more exact glucose content at the end of the EH. During both runs the pH was checked and changed if needed to a pH of approximately 4,8. The amount of solved sugars were then measured.

Figure 5.4: Picture of centrifuge tubes used for EH

5.5 Simultaneous saccharification and fermentation (SSF)

As mentioned the SSF was run with two different pretreated materials (200°C, 5 min and 200°C, 10 min). Prior to the SSF the 10 liter pretreatment unit was used to get a larger amount of pretreated material for the fermentation. The fermenters used for the SSF were 2 L laboratory fermenters (Infors AG, Bottomingen, Switzerland), which can be seen in Figure 5.5

Before the start of the fermenters, glassware and pipettes that where to be used were sterilized in an autoclave at 121°C for 20 min. The following was added to the fermenters:

- Pretreated material (pH adjusted with 10% NaOH to pH 4.8) an water to get a total amount of 1000g and a WIS content of 5%.
- $(NH_4)_2HPO_4$ as nutrition to a concentration of 0.5 g/L.
- Enzyme, Cellic Ctec2 from Novozymes AB (Bagsvaerk, Denmark), enzyme loading of 10 FPU/g WIS.
- Yeast 5 g/l, S. cervisiae (Ethanol Red) from Fermentis AB, (France). Suspended in distilled water, ratio 5:1. Incubated for 30 minutes at 30°C before added to the fermentor

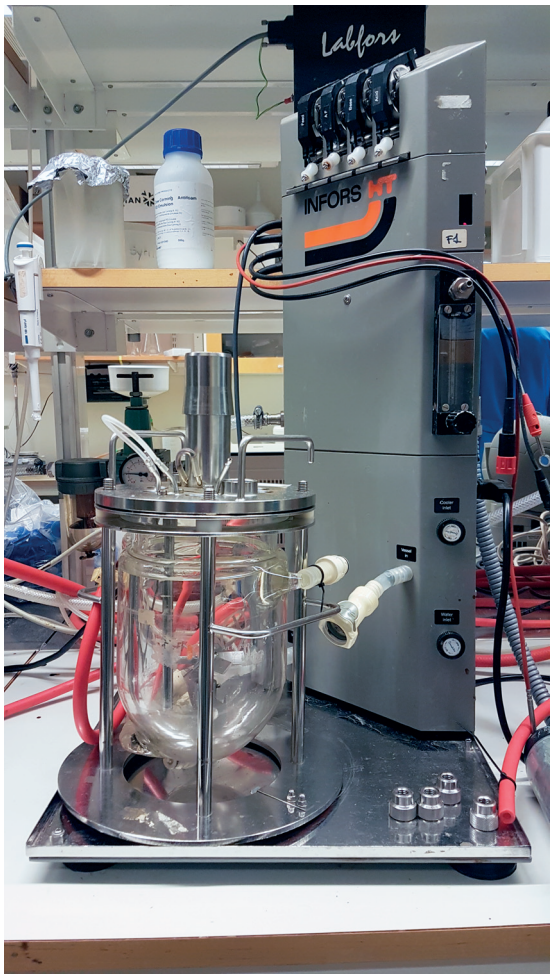

Figure 5.5: Picture of fermenters used for SSF

5.6 Analysis

5.6.1 Dry matter content

By drying the material until it had a constant weight using a Mettler Toledo HB43 Halogen Moisture Analyzer the dry matter content of the raw material was obtained.

5.6.2 Water insoluble solids (WIS)

The total solids (TS) and total dissolved solids (DS) were analysed using a standard method from The National Renewable Energy Labratory (NREL) [31]. The slurry of the pretreated material and separated liquid fraction were weighed before and after being dried at 105°C. The WIS content was calculated with the following equation.

$$\%WIS = \frac{\%TS - \%DS}{1 - \%DS} \tag{5.1}$$

%DS, dissolved solids in process sample %TS, total solids in process sample.

5.6.3 Extractives

The total amount of extractives (water and ethanol extractives) in the raw material was calculated using standard method from NREL [32].

5.6.4 HPLC-equipment

The analysis for ethanol, and byproducts in the liquid fraction of the pretreated material before, during and after the fermentation was performed in an HPLC with refractive index detector (Shimadzu,Hercules, CA), at 65°C. 5mM H_2SO_4 was used as eluent at the flow rate of 0.5mL/min. The sugar content of the raw material and the samples of the pretreated material was measured using an ICS-3000 chromatography system (Dionex, Sunnyvale, CA) which is a high-performance anion-exchanger chromatograph with pulsed amperometric detection. The columns that were used were a CarboPac PA1 gard column and a PA1 analytical column. Water was used as eluent at the flow of 1mL/min. Before the detector 200mM NaOH was added with a flow of 0.5mL/min. The column was cleaned between all samples with 170mM sodium acetate with dissolved 200 mM NaOH. The hydolysate was analysed for sugars using a chromatograph with a refractive index detector (RID). Glucose, xylose, mannose, galactose, arabinose and cellobiose were separated at 85°C in an ion-exchange column. As eluent distilled water at a flow rate of 0.5 mL/min was used. Prior to analysis all samples were filtered through a 0.2μm filter to get rid of particles.

5.6.5 Composition analysis

The composition of the raw material after extraction and the solid fraction of pretreated material were analyzed for structural carbohydrates, lignin, extractives and ash using standard methods from NREL [33]. The liquid fraction of the pretreatment samples was analysed for sugar, organic acid, HMF and furfural content using standard method from NREL [34].

5.6.6 Ethanol yield

The ethanol yield was calculated as follows.

$$Y_{ethanol} = \frac{m_{C_6} * WIS * 0.51}{C_{C_6} * \rho_s} \tag{5.2}$$

Where m_{C_6} is the percentage of C_6 sugars in the pretreated material. C_{C_6} the concentration of ethanol after fermentation ρ_s the density of the slurry after fermentation. 0.51 comes from eq 4.2

5.6.7 Sugar recovery

The sugar recovery was calculated using eq 5.3 for the liquid fraction and eq 5.4 for the solids

$$R_{lq} = \frac{C_{sugar} * \rho_w * m_{pre_w}}{m_{dry} * \%_{sugar}} \tag{5.3}$$

$$R_S = \frac{m_{pre_{sugar}} * m_{pre} * WIS}{m_{dry} * \%_{sugar}} \tag{5.4}$$

Where C_{sugar} is the concentration of sugar, m_{pre_w} the total weight of the water in the slurry after pretreatment, ρ_w the density of water, m_{dry} the total amount of raw material (dry mater) added to the pretreatment and $\%_{sugar}$ the amount of the sugar in the raw material.

6 Results and Discussions

The goal of this study was to find at which sets of parameter the pretreatment of JAS should be run and to investigate the possibility to use steam pretreated JAS to produce bioethanol.

To get as much sugars from the material as possible several pretreatments were performed to investigate the temperature and the duration of the steam pretreatment. Before steam pretreatment, the material was soaked in 1wt% acetic acid for 1h.

Due to shortage of material it was essential not to waste material and it was therefore decided to start with three different pretreatment samples to be able to draw a conclusion if the material should be more or less harshly pretreated. After analyzing the composition of the first samples the rest of the pretreated samples were prepared.

Due to the shortage of raw material it was decided not to do a fermentation on each batch of pretreated material. Instead a smaller steam pretreatment unit was used to pretreat the material at different parameters. An EH was performed on the differently pretreated material. From the results from the EH along with composition analyses two parameter sets were chosen to pretreat a larger amount which later was fermented.

The main factors that were analysed were:

- Composition of raw material
- The overall glucan yield through the pretreatment and the EH
- Ethanol yield from fermentation
- Amount of inhibitors in pretreated material
- Recovery of xylose in the pretreatment
- Sugars and oligomers in the liquid fraction of the pretreated material
- Loss of inulin during pretreatment

6.1 Composition of the raw material

The composition of the JAS used during the study was measured using standard methods [32] [33] [34]. The composition of the raw material is presented in Table 6.1.

Table 6.1: Composition of raw material including stem and leaves from JA

	Amount	Standard Deviation
Lignin	13,4%	0,1%
Arabinan	0,7%	0,3%
Galactan	3,0%	0,1%
Glucan	22,2%	1,0%
Xylan	11,4%	0,2%
Mannan	1,8%	0,1%
Glucose[a]	6,1%	0,7%
Inulin[ab]	8,8%	2,4%
Sucrose[a]	0,6%	0,6%
Other extractives	19,5%	0,9%
Total	87,4%	0,9%

[a] Part of the extractives

[b] Assuming all inulin is solved and fully hydrolysed during extraction [32] and all fructose in the extractives are derived from inulin

According to earlier studies made on JAS the composition seem reasonable for most components. However the inulin content is significantly higher in those studies (with an inulin content of about 20%). [9] [10] [18]. It is possible that the amount of inulin is underestimated due to assumptions made during the analysis. This is further discussed in section 6.6. The amount of glucan is lower than in many other lignocellulosic materials such as hardwood [35], softwood [35], corn stover [36], corn cobs [37] and bagasse [38]. The fraction not accounted for primarily contains non solvable fats, pectin and proteins. The amount of extractives is relatively high. This is due to the large amount of inulin in the material. About a third of the extractive from the JAS was inulin.

6.2 Steam pretreatment of JAS in the 4-l unit

The different parameter sets that were tested can be seen in Table 5.1 in the materials and methods section.

After pretreatment the composition of the solid fraction of the material was determined using a standard method [33]. The result from the composition analysis of the solid fraction of the pretreated material can be seen in Table 6.2.

Table 6.2: Composition analysis of the solid fraction of the pretreated material

	Lignin	Galactan	Glucan	Xylan	Mannan	Ash	Total
180°C 5min	24,5%	2,1%	28,8%	11,3%	2,2%	1,2%	70,1%
st div	0,3%	0,1%	1,1%	0,1%	0,1%	1,0%	1,7%
190°C 5min	28,2%	1,6%	34,7%	10,2%	2,2%	1,6%	78,4%
st div	0,6%	0,1%	1,0%	0,5%	0,0%	0,2%	0,9%
200°C 5min	28,2%	1,5%	36,7%	8,5%	2,0%	1,9%	78,8%
st div	0,4%	0,0%	0,7%	0,0%	0,0%	0,1%	1,2%
210°C 5min	26,0%	1,8%	33,7%	10,9%	2,1%	1,5%	75,9%
st div	1,0%	0,1%	1,2%	0,2%	0,0%	0,6%	2,1%
190°C 10min	28,3%	1,6%	34,2%	7,9%	2,0%	1,2%	75,2%
st div	0,1%	0,1%	0,1%	0,3%	0,1%	0,1%	0,7%
200°C 10min	26,2%	1,9%	31,0%	8,9%	1,9%	1,1%	71,1%
st div	1,4%	0,2%	3,6%	1,6%	0,2%	0,0%	4,2%
210°C 10min[a]	34,2%	1,2%	28,4%	3,8%	1,3%	1,7%	70,6%

[a] Only one measurement, no standard deviation calculated.

Glucan contents were 28,4-36,7%, lignin content 24,5-34,2%. The highest xylan content was found in the least severely treated material (180°C, 5min), while the most severely treated material (210°C, 10min) had the lowest xylan content. This is in accordance with the fact that hemicellulose removal increases with the severity of the pretreatment. Two of the pretreatment samples did not follow this trend (200°C, 10min and 210°C, 5min) They had a higher content of xylan compared to the other samples than would be expected which. One explanation for this could be that these samples were not pretreated as much as expected.

The total determined compounds were less than 80% for all samples which is lower than expected. This might be due to that some of the sugar concentrations are underestimated.

The amount of sugars and oligomers in the liquid fraction for each pretreatment was measured with standard method [34]. The results are displayed in Table 6.3 and Table 6.4.

The WIS content of all the pretreated materials was calculated. The result is displayed in Table 6.5 along with the total weight of the pretreated material, total WIS and the WIS recovery. The WIS for the different samples reached between 4,6 and 9,4% and the WIS recovery between 42 and 58%.

Table 6.3: Amount of free sugars in liquid fraction after pretreatment

	Sucrose (g/l)	Galactose (g/l)	Glucose (g/l)	Xylose (g/l)	Mannose (g/l)	Fructose (g/l)
180°C 5min	0,65	0,13	0,58	0,09	0,12	2,18
190°C 5min	1,29	0,26	0,56	0,20	0,16	1,53
200°C 5min	0,86	0,29	0,56	0,29	0,18	1,41
210°C 5min	0,41	0,13	0,44	0,15	0,09	1,20
190°C 10min	0,79	0,25	0,49	0,18	0,11	1,07
200°C 10min	0,30	0,11	0,30	0,10	0,06	0,97
210°C 10min	0,33	0,19	0,35	0,26	0,08	0,85

Table 6.4: Amount of sugar oligomers i liquid fraction of pretreated material

	Galactose (g/l)	Glucose (g/l)	Xylose (g/l)	Mannose (g/l)
180° 5min	2,19	0,65	1,59	0,36
190°C 5min	2,75	1,35	5,26	0,87
200°C 5min	1,89	0,94	5,42	0,82
210°C 5min	1,02	0,47	2,11	0,38
190°C 10min	1,22	0,66	3,14	0,52
200°C 10min	0,91	0,54	1,65	0,37
210°C 10min	0,84	0,56	1,95	0,45

The glucan recovery was calculated using eq 5.3 and eq 5.4. The glucan recovery is displayed in Table 6.6 and the xylan recovery in Table 6.7. The glucan recovery was low compared to other studies [39].

Table 6.5: Mass balance over the pretreatment. The dry weight of the raw JAS before impregnation was 200g in each case

	Tot out (g)	WIS%	WIS out (g)	WIS recovery
180°C 5min	1233,5	9,4%	116,2	58,1%
190°C 5min	1113,8	9,2%	102,1	51,1%
200°C 5min	1269,8	7,0%	89,1	44,6%
210°C 5min	2177,7	4,9%	106,3	53,1%
190°C 10min	1564,8	6,5%	101,5	50,7%
200°C 10min	2467,4	4,6%	113,9	56,9%
210°C 10min	1770,8	4,8%	84,3	42,1%

Table 6.6: Glucan recovery in the solid and the liquid fractions after pretreatment

	Solids	Liquid	Total
180°C 5min	59,3%	2,7%	61,9%
190°C 5min	62,8%	3,3%	66,1%
200°C 5min	57,9%	3,6%	61,5%
210°C 5min	63,5%	6,8%	70,3%
190°C 10min	61,4%	4,2%	65,6%
200°C 10min	62,6%	8,0%	70,6%
210°C 10min	42,3%	4,5%	46,8%

Table 6.7: Xylan recovery in the solid and the liquid fractions after pretreatment

	Solids	Liquid	Total
180°C 5min	57,5%	8,9%	66,5%
190°C 5min	45,7%	23,5%	69,1%
200°C 5min	33,0%	33,6%	66,6%
210°C 5min	50,6%	41,7%	92,4%
190°C 10min	35,2%	30,3%	65,5%
200°C 10min	44,2%	41,9%	86,1%
210°C 10min	14,1%	26,9%	41,0%

The WIS recovery was low even considering the high amount of extractives in the raw material (some of the extractives will be lost during the impregnation and the rest will be in the liquid fraction after pretreatment). This is probably due to a loss of material in the pretreatment, which also could be an explanation for the low sugar recovery in the samples. The WIS and sugar recoveries are dependent on the amount of material that is not retrieved from the pretreatment unit which makes the margin of error of these values big due to the fact that the emptying of the unit is made by hand. This uncertainty is bigger for a small batches because of the higher percentile loss of material. Another source of uncertainty is the difficulty to take a homogeneous sample from the pretreated material, especially form the less pretreated samples. It was not possible to calculate how much of the material was lost during the impregnation and how much that was lost during pretreatment with the data collected. For future studies the dry

matter content after filtration could be measured to see how much material that was lost in the impregnation alone.

Glucan recoveries of the samples were in the range of 61,5-70,6% except for the material pretreated at 210°C for 10 minutes for which the glucan recovery was only 46,8%. This sample is most likely over treated. However the composition analysis of this sample was only performed once and the results for this sample are therefore not as reliable as the other samples.

6.3 Enzymatic hydrolysis of steam pretreated JAS

The amount of sugar hydrolysed from the solids during EH was calculated by measuring the sugar concentration after the EH compared with the sugar content in the liquid fraction after pretreatment.

When taking several samples of the hydrolysate its composition changed. This is due to the difficulty to take a homogeneous sample from it, especially at the beginning of the hydrolysis when most of the sugars were still in the solid fraction. Therefore the hydrolysis was run two times, first with several samples taken over time and a second with samples only taken at 72 and 96 hours. The mean values of the measured concentration of glucose during the first hydrolysis is displayed in Figure 6.1. The samples taken from the second hydrolysis were used to calculate the total amount of glucose hydrolysed during the EH.

The top graph in Figure 6.1 shows the EH for the samples that were pretreated for 5 minutes. Among these samples the sample pretreated at 200°C was the one that reached the highest glucose concentration (25,4 g/l). It seems as this is the optimal temperature for pretreatment when the duration of the pretreatment is 5 minutes. The sample that was pretreated at 210°C for 5 minutes reached a lower concentration which should correlate to a higher amount of inhibitors because of a higher amount of degradation of sugars. However the results of the EH for the samples that were pretreated for 10 minutes suggests otherwise (bottom graph in Figure 6.1). Among these samples the one pretreated at 210°C is in fact the sample that reached the highest glucose concentration (23,1 g/l). If the amount of inhibitors in the 210°C 5 minutes sample was too high for the enzyme to work properly it would have been the same for the sample pretreated at the same temperature for 10 minutes but looking at the results displayed in 6.1 this doesn't seem to be the case.

The samples from the second EH run were analysed to calculate the total amount of glucose and xylose released and lost during pretreatment and EH for the different pretreatments. According to the analysis the glucose yield obtain in EH was significantly higher than 100% of the glucose content in the pretreated material for the sample pretreated at 210°C for 10 minutes. This might be due to underestimation of glucose in the composition analysis of this sample. This sample was therefore excluded from the results of overall sugar yield over pretreatment and EH. The result of the sugar yield is illustrated in Figure 6.2 for glucan and in Figure 6.3 for xylan.

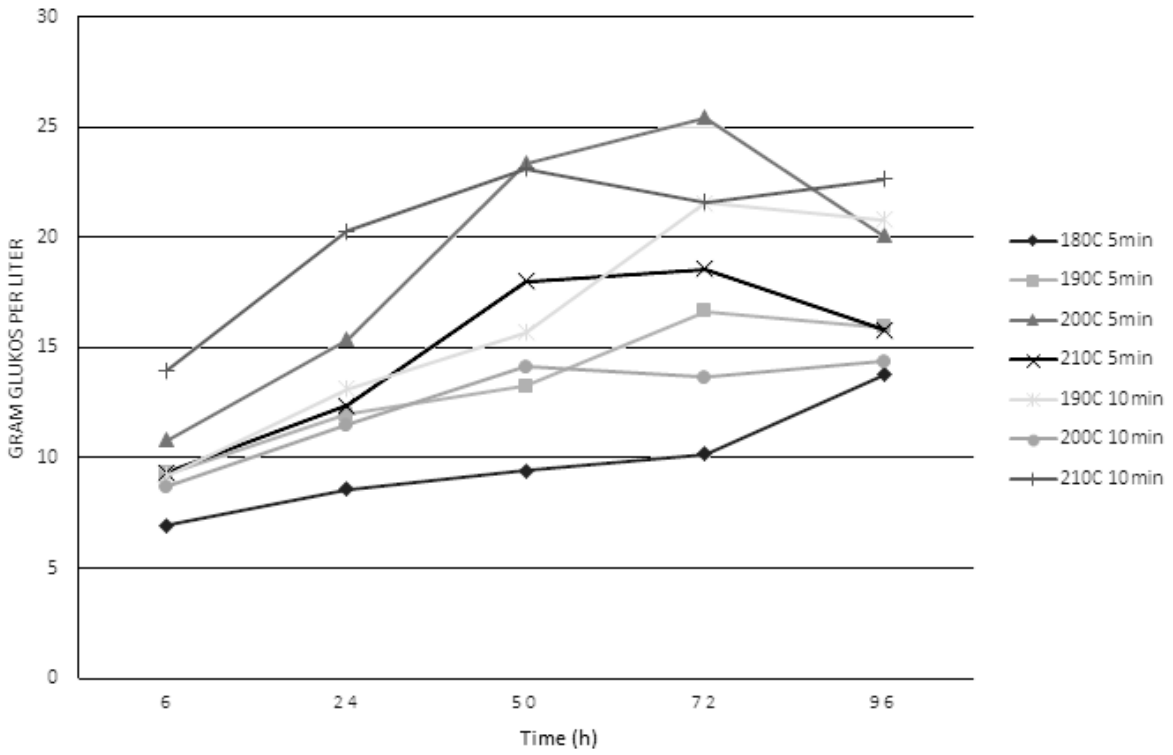

Figure 6.1: Glucose concentration in the EH of JAS that was steam pretreated in the 4-l pretreatment unit, (4% WIS, 10 FPU/g WIS enzyme loading, 45°C, PH 4,8)

From the results of the overall sugar yields there was no clear relation between the hydrolysability and the temperature which the samples were pretreated at nor the time which the samples were pretreated. There where neither any correlations between the pretreatment temperature or the time of pretreatment and the amount of sugars obtained. The sugars left in the solid fractions after EH did not show any pattern either considering time and temperature of the pretreatment. The amount of sugars hydrolysed was expected to have an optima at one set of parameters and the amount of degraded sugars was expected to be higher when the time and temperature was increased. This was not the case for the samples. The lack of correlations was probably due to analytical errors and other uncertainties.

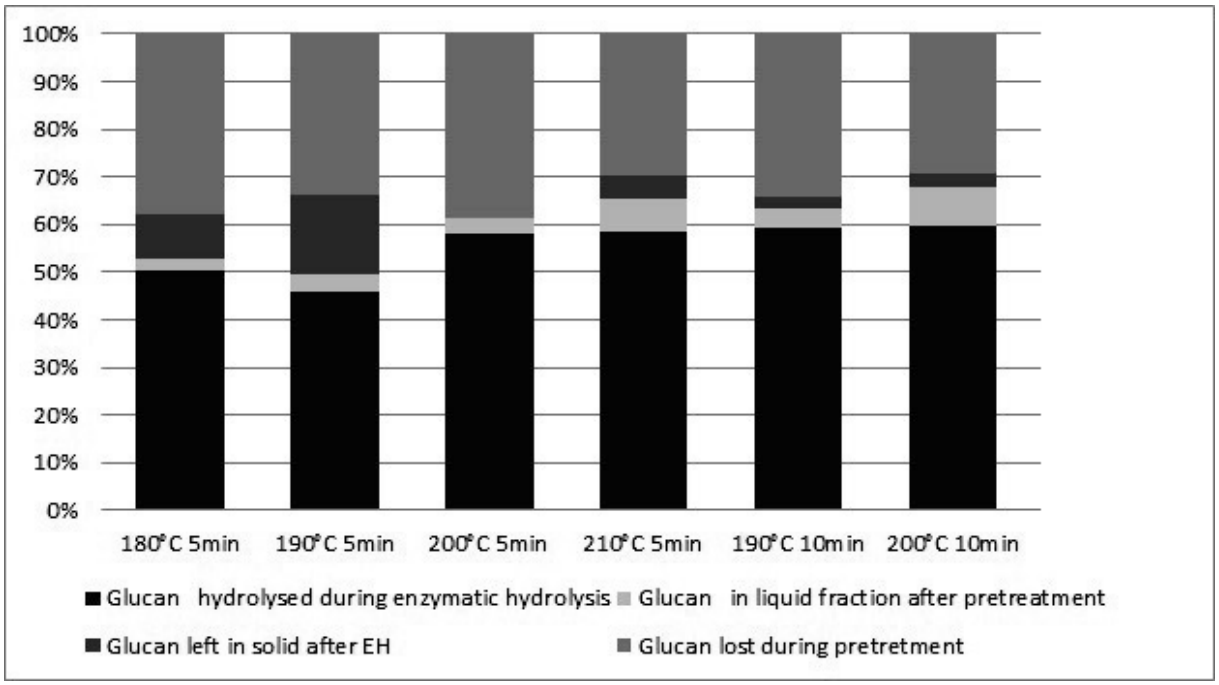

Figure 6.2: Amount of glucan that ended up in the solid and liquid fraction and the amount of glucan lost after JAS was pretreated and hydrolysed for the different sets of parameters.

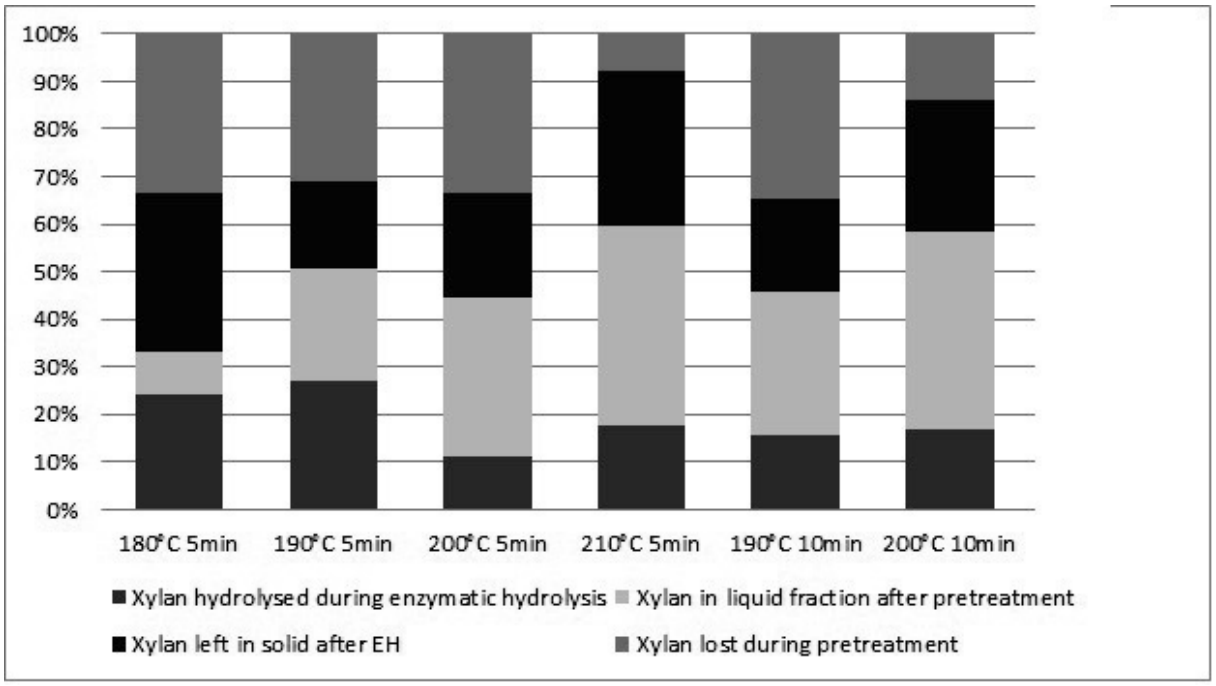

Figure 6.3: Amount of xylan that ended up in the solid and liquid fraction and the amount of xylan lost after JAS was pretreated and hydrolysed for the different sets of parameters.

To further investigate the samples the amount of inhibitors in the samples was measured. As mentioned before the less good EH for the sample pretreated at 200°C for 10 minutes should co-relate with a higher degradation of sugars which means a higher amount of inhibitors in the

end product which would explain the decrease of efficiency of the enzyme. However it does not seem to be a higher degradation of sugars. Looking at Figure 6.2 and 6.3 it seems as though the loss of sugars actually is higher in the sample that was pretreated at 190°C for the same amount of time. The same goes for the sample pretreated at 210 °C for 5 minutes where the loss of sugars seems to be greater in the sample pretreated at 200°for the same amount of time. When looking at the measurements of the inhibitors for the different samples, the amount of HMF (Figure 6.6), formic acid (Figure 6.4) and levulinic acid (Figure 6.5) the measurements suggest the same thing. The amount of inhibitors should increase with increasing severity of pretreatment. Looking at the amount of the solids that contains of inhibitors for the different samples it is clear that the severity of the pretreatment was lower than what would be expected for the sample pretreated at 210°C for 5 minutes and the sample pretreated at 200°C for 10 minutes when comparing with the other samples. This further indicates that these two samples were not pretreated properly which was suspected from the result of the composition of the pretreated material in general and the result of xylan composition in particular. This might be due to:

- The distribution of the material in the reactor was not optimal. It might have been placed in a way so the steam could not access all the material. E.g. the samples might have been too compact.
- For some reason the set temperature was not reached. The steam that controls the temperate might not have been hot enough, the steam accessed the thermometer better than the material or leakage of steam from the reactor could be explanations.

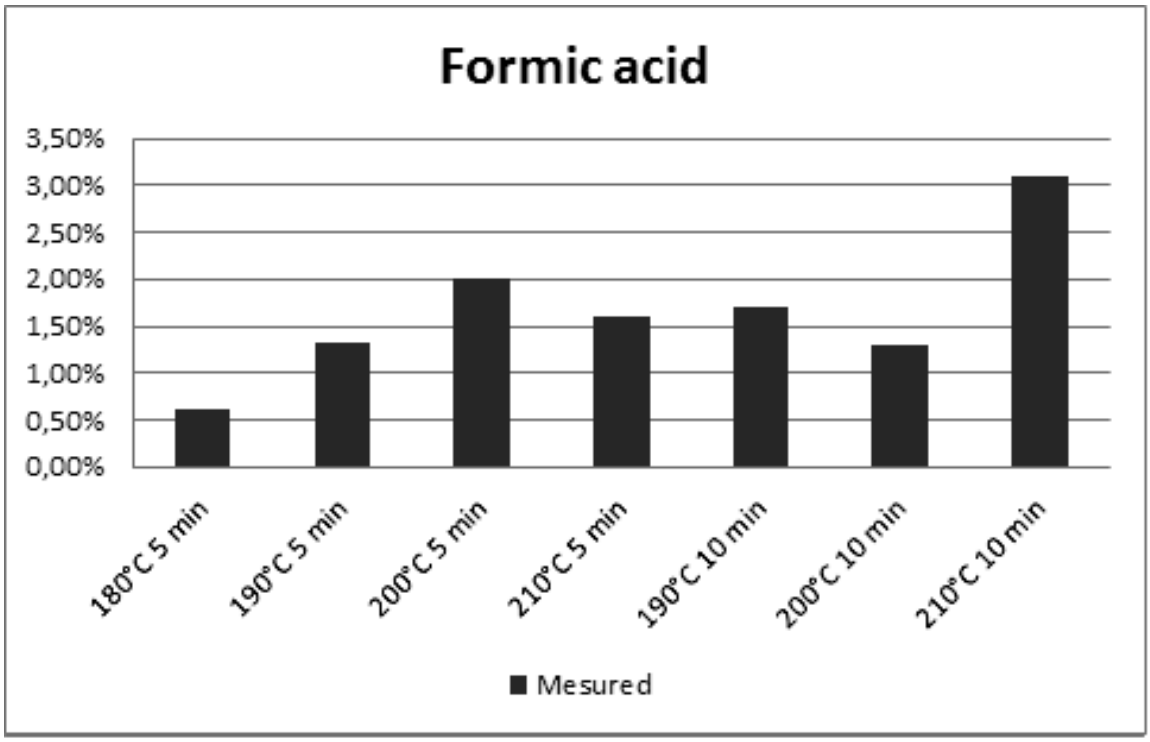

Figure 6.4: Measured quantities of formic acid for pretreated material, % of Total solids

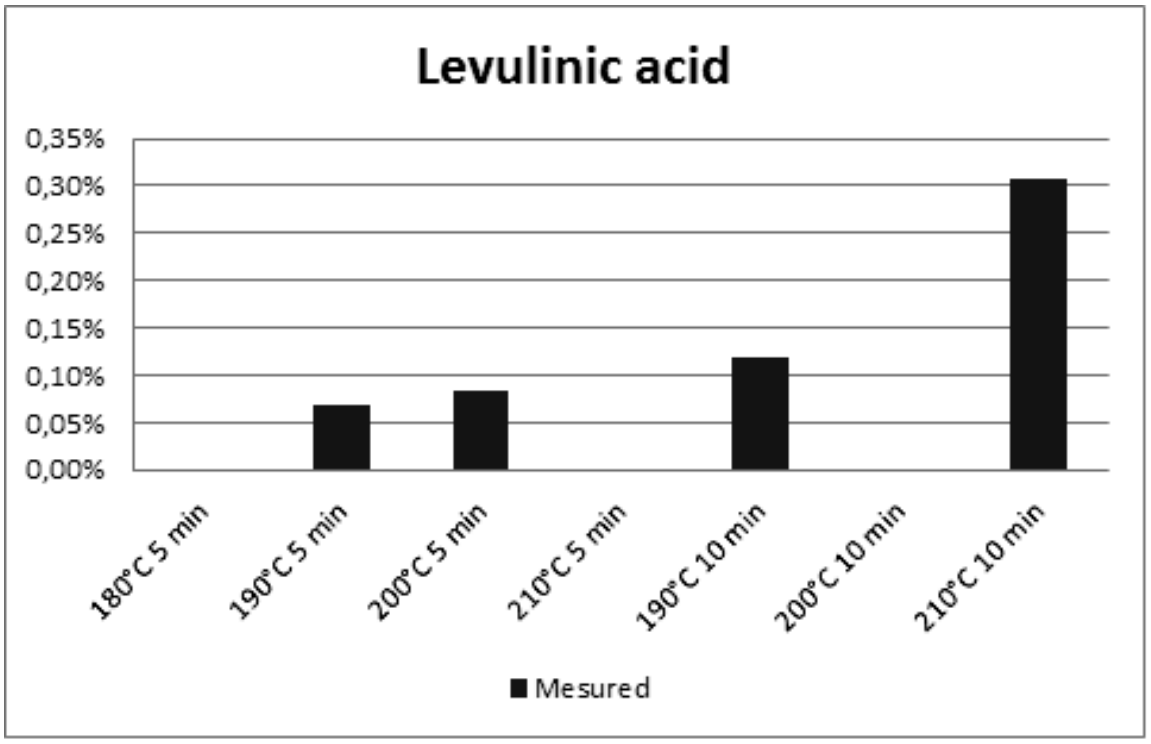

Figure 6.5: Measured quantities of levulinic acid for pretreated material, % of Total solids

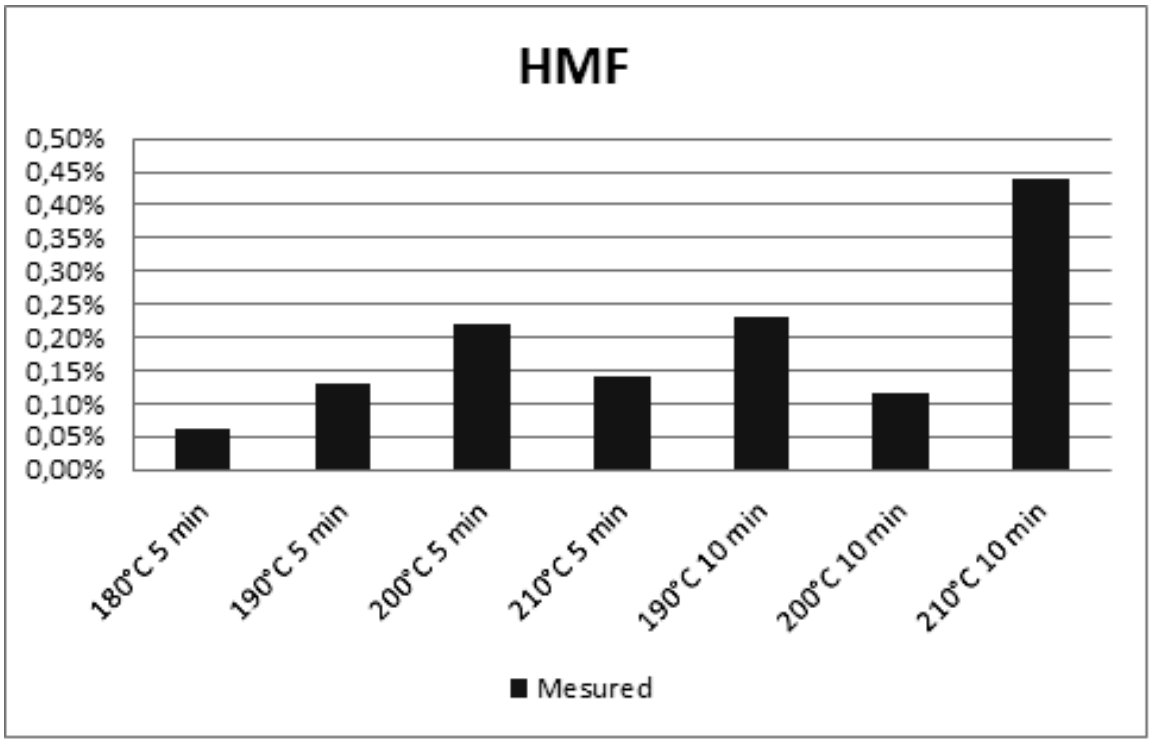

Figure 6.6: Measured quantities of HMF for pretreated material, % of Total solids

6.4 Steam pretreatment of JAS in the 10-l unit

Based on the composition, glucose recovery, amount of sugar hydrolysed during EH and amount of inhibitors, parameters were to be chosen as parameters to be used when pretreating the larger batch in the 10 liter pretreatment unit. In Figure 6.2 and 6.3 the total amount of hydrolysed glucose and xylose in the different samples can be seen. The samples seem to have about the same glucose content in the liquid fraction after EH. According to the analysis made on the hydrolysate after EH the sample pretreated at 200°C for 5 minutes had the highest glucose concentration.

It was concluded that two samples were not pretreated properly (200°C, 10 min and 210°C, 5 min). Due to these irregularities it was not totally clear which parameters that would be optimal for steam pretreatment of JAS. The sample that was pretreated at 200°C for 5 minutes seemed to be treated enough for all glucan to hydrolyse and significantly lower glucose recovery was achieved in the pretreatment for the sample pretreated at 210°C. The sample that reached the highest concentration of sugars during the EH also was the 200°C 5 minutes sample. Therefore it was decided to further investigate this set of parameters. Due to the insufficient results of the sample pretreated for 10 minutes at the same temperature it was decided to also include this set of parameters.

There were two parameter sets that were chosen to be investigate further (200°C, 5 min and 200°C, 10 min). A larger batch was prepared in a 10 liter pretreatment unit. The results from the earlier experiments formed the basis for the decision of which pretreatment parameters to use for the SSF.

After pretreatment the composition of the pretreated materials (Table 6.8) was analysed along with the glucose and xylose recovery (Figure 6.7 and Figure 6.8). Both the table and the figure show the corresponding sample from the small batch. It is obvious from the table and the figure that the materials are not equally pretreated. This was expected for the samples run at 200°C for 10 minutes because of the conclusion that it was not pretreated properly in the small pretreatment unit. It seems as though the samples run at 200°C for 5 minutes also differ. The glucose and the lignin content differ with almost 10% for these samples. The glucose recovery was more than 20% higher and the xylose recovery was 30% lower. The material pretreated in the different units even looked different. The samples run in the small unit contained a higher amount of water and some parts of the structure of the raw material still looked intact whereas the samples pretreated in the large unit looked more homogeneous.

The change in recovery of glucan can be explained as a result of less percentile loss of material in the large steam pretreatment unit or by an underestimation of glucan in the small batches which already was suspected from looking at the result from the hydrolysis and composition of the pretreated samples. But the decrease in xylan recovery suggests that the material pretreated in the lager pretreatment unit was more harshly treated then the material pretreated in the small unit. The WIS recovery was higher in the sample pretreated in the larger unit for the sample that was pretreated at 200°C for 5 minutes (Table 6.9). This also suggest that the percentile loss is less in the big unit. The decrease in WIS between the small and the large batch for the samples

pretreated at 200°C for 10 minutes is probably due to that the sample pretreated in that small unit was not properly treated.

Table 6.8: Composition of JAS pretreated at 200°C for 5 and for 10 minutes, large (800g, 10 liter unit) and small batch (200g, 4 liter unit)

	Lignin	Galactose	Glucose	Xylose	Mannose	Ash	Total
			Small				
5 min	28,2%	1,5%	36,7%	8,5%	2,0%	1,9%	78,8%
st div	0,4%	0,0%	0,7%	0,0%	0,0%	0,1%	1,2%
10 min	26,2%	1,9%	31,0%	8,9%	1,9%	1,1%	71,1%
st div	1,4%	0,2%	3,6%	1,6%	0,2%	0,0%	4,2%
			Large				
5 min	36,8%	1,5%	46,2%	6,3%	2,5%	2,5%	95,9%
st div	1,4%	0,1%	1,5%	0,2%	0,0%	0,2%	1,9%
10 min	39,6%	1,4%	47,9%	5,1%	2,3%	2,4%	98,7%
st div	4,5%	0,1%	2,1%	0,2%	0,0%	0,1%	5,8

Table 6.9: Weight of WIS and WIS recovery of JAS pretreated at 200°C for 5 and for 10 minutes in the large(800g, 10 liter unit) and in the small batch (200g, 4 liter unit)

	Tot out (g)	WIS%	WIS out (g)	WIS recovery
			Small batch	
200°C 5min	1269,8	7,0%	89,1	44,6%
200°C 10min	1770,8	4,6%	113,9	56,9%
			Large batch	
200°C 5min	3838	10,5%	403,0	50,3%
200°C 10min	4325	8,5%	367,6	45,9%

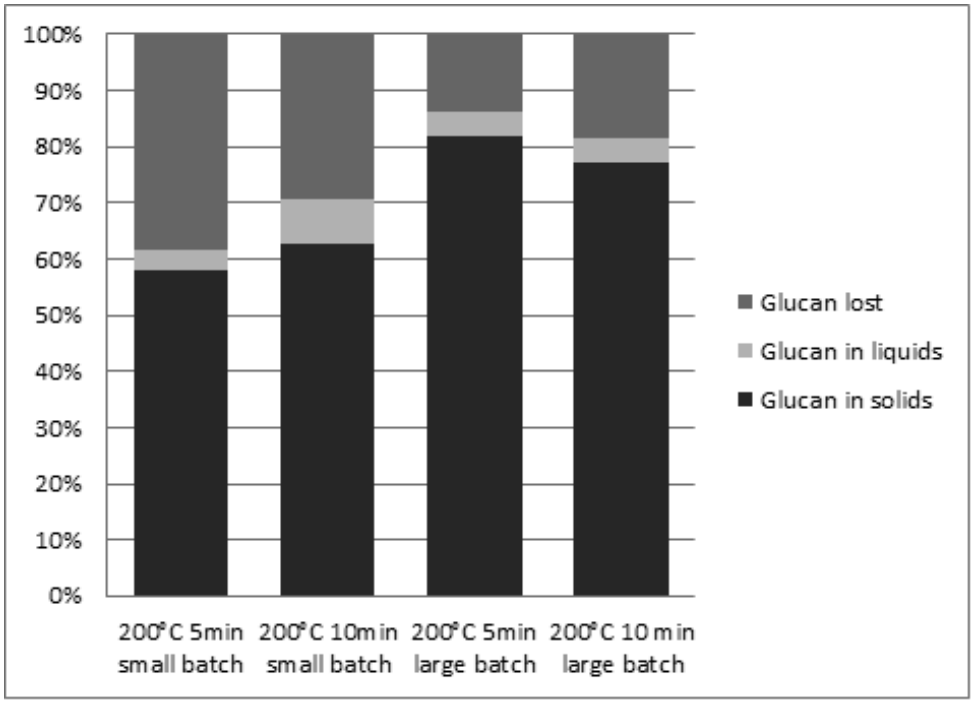

Figure 6.7: Recovery of glucan in JAS pretreated at 200°C for 5 and for 10 minutes, large (800g, 10 liter unit) and small batch (200g, 4 liter unit)

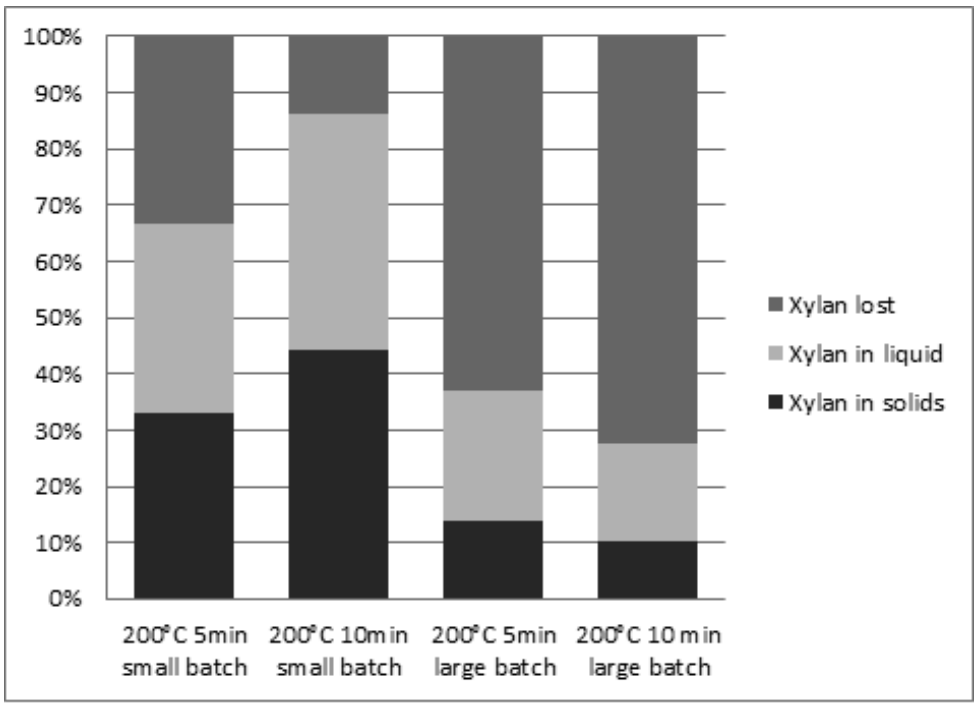

Figure 6.8: Recovery of xylan in JAS pretreated at 200°C for 5 and for 10 minutes, large (800g, 10 liter unit) and small batch (200g, 4 liter unit)

6.5 Simultaneous saccharification and fermentation

After the steam pretreatment the samples pretreated in the 10-l unit were subjected to SSF and the amount of ethanol was analysed. The ethanol concentration over time can be seen in Figure 6.9 and Figure 6.10. The sudden increase in ethanol yield after the the sample taken after 56h may be a result of the pH adjustment. If the pH was adjusted continuously the curve would be smoother. The drop in ethanol at the last samples may be due to growth of microbes able to digest ethanol [40].

The amount of fermentable sugars, maximal ethanol concentration after fermentation, theoretical maximal ethanol concentration and ethanol yield of pretreatedd JAS can be seen in Table 6.10.

Table 6.10: Amount of fermentable sugars, maximal ethanol concentration after fermentation, theoretical maximal ethanol concentration and ethanol yield of JAS pretreated at 200°C for 5 and for 10 minutes.

	5 min sample	10 min sample
Glucose in solids (g/l)	23,11	23,94
Glucose in liquid (g/l)	1,37	1,14
Mannose in solids (g/l)	1,27	1,17
Mannose in liquid (g/l)	0,93	0,79
Fructose in liquid (g/l)	0,44	0,35
Sucrose in liquid (g/l)	0,83	0,31
Total fermentable sugars (g/l)	27,94	27,71
Ethanol consentration (g/l)	9,10	10,77
Theoretical max (g/l)	14,25	14,13
Yield %	64	76

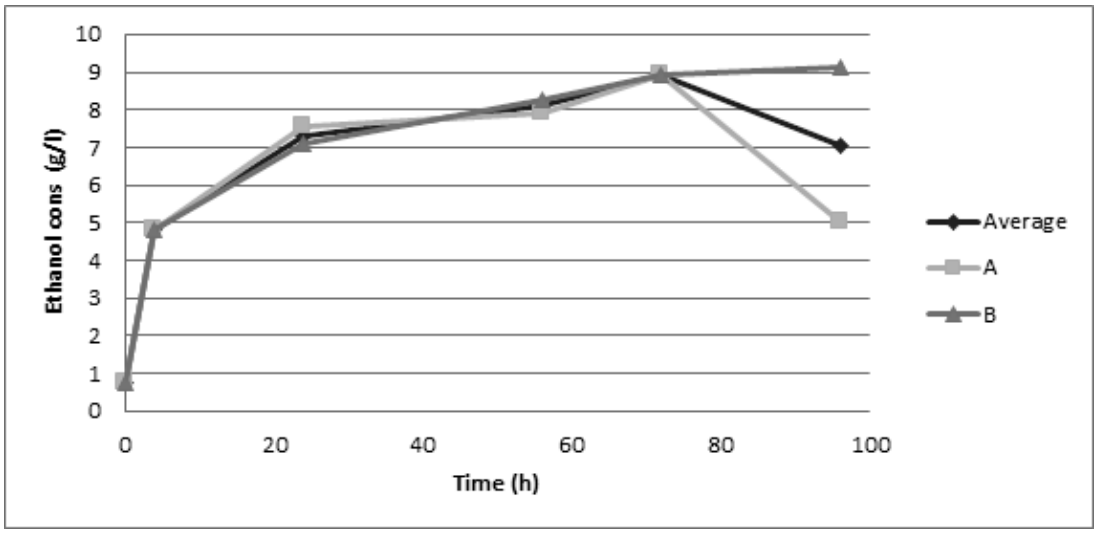

Figure 6.9: Ethanol concentration measured over time during fermentation for JAS pretreated at 200°C for 5 minutes (5% WIS, 5 g yeast/l)

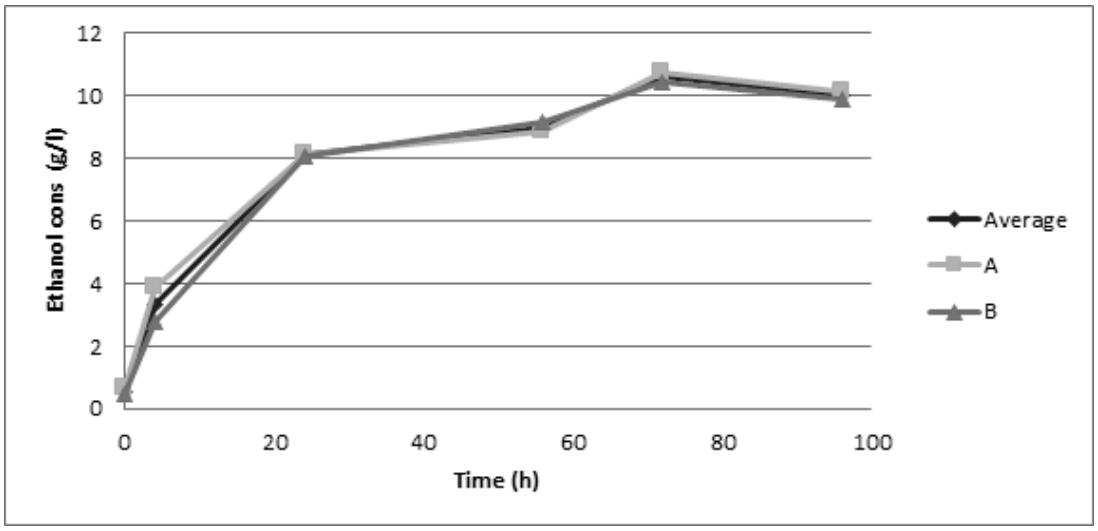

Figure 6.10: Ethanol concentration measured over time during fermentation for JAS pretreated at 200°C for 10 minutes (5% WIS, 5 g yeast/l)

The ethanol yields received was lower compared to other studies on similar materials [22] but compared to the article on ethanol production on JAS the ethanol yields are in the same range [41]. However the fermentation in this research was run at significantly lower WIS (5% to 20%). However there is still much room for improvements of the procedure and there are several changes that could be made to further increase the total yield without changing the parameters for the steam pretreatment.

- Addition of hop extract eg. VitaHop to repress bacterial growth.
- Longer fermentation time.
- Larger batch for pretreatment to minimize the amount of material lost in the pretreatment unit.

The highest ethanol concentrations received was 10,77g/l. This is to low to get an economic process due to the costs of distilling the ethanol. It is desirable to reach a concentration of at least 40g/l [42] and preferably as high concentrations as possible. The low considerations is due to the low amount of solidus in the fermented material. To receive a higher concentration the amount of solids on the fermentor has to be increased (higher WIS). As can be seen from Table 6.10 the corresponding theoretical maximum was 14,13g/l for this sample.

6.6 Inulin and fructose

To measure the content of inulin in the process samples the same standard methods used to analyze the other carbohydrates was used [33] [32] [34]. It was first assumed that this method would be sufficient for this analysis. When analysing the results it was obvious that the fructose was degraded to a much higher extent during the acid hydrolysis used in the standard methods [34] [33] then the other sugars measured. This resulted in that it was not possible to measure any amounts of inulin that could be left in the solids or oligomers of fructose left in the liquid fraction of pretreatment and the extractives. Therefore it was instead assumed that all inulin dissolved into the liquid fraction of the pretreatment samples and to the extractives during the extraction of the raw material leaving no inulin in the solids. It was also assumed that the inulin was fully hydrolysed to fructose during pretreatment and extraction.

According to another study [43] inulin is fairly soluble in water and observations from results of the extraction (high amount of fructose in the extractives Table 6.1) suggests the same. About the hydrolysis of inulin, according to study [44] the inulin is hydrolyzed to 80% after boiling for 7.5 minutes at pH 3. However this study also shows that just 2.5% of the inulin was hydrolyzed after 55 minutes of boiling at pH 7. It would therefore be safe to assume that the inulin in the pretreated material is fully hydrolysed due to the harsh treatment of the material but for the amount of inulin in the raw material the assumption is not as good which probably results in that the inulin content of the raw material is a bit underestimated which also seems to be the case when comparing the composition results (Table 6.1) to other analysis made [18] [9] [10].

The total loss of fructose during impregnation and pretreatment was way high-er compared to the other sugars (Figure 6.11). Therefore, to get a better conversion from carbohydrates to ethanol, there would be good to extract the inulin prior to pretreatment to prevent the loss of fructose and formation of HMF and formic acid. Some of the inulin is probably lost when the material is filtered after being soaked in acid prior to pretreatment.

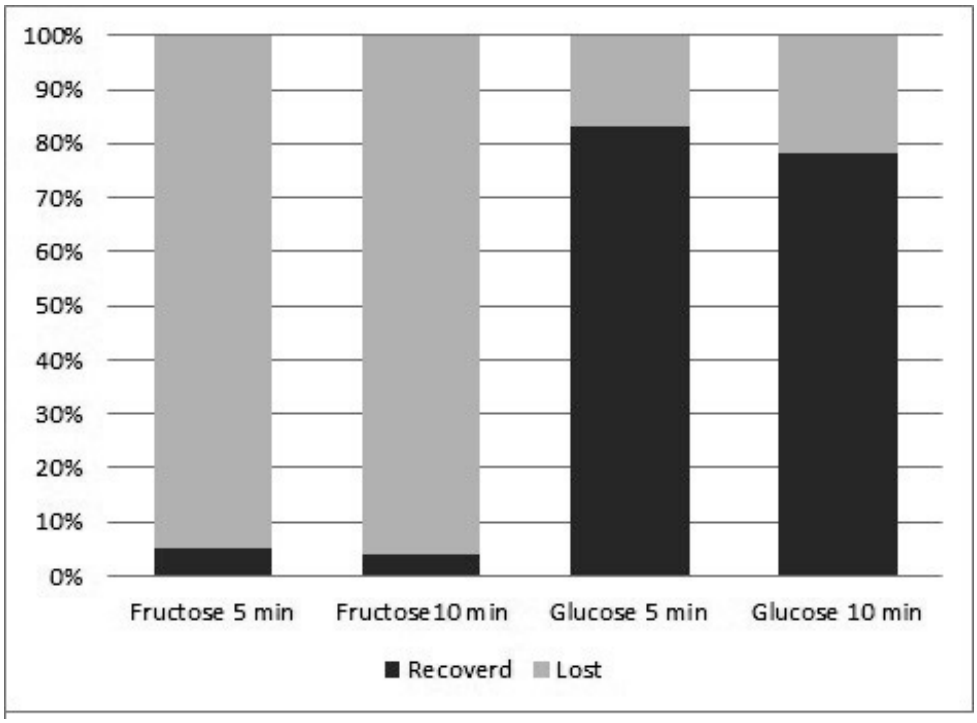

Figure 6.11: Fructose and glucose recovery for the large batch pretreated at 200°C for 5 and 10 minutes

7 Conclusions

Steam pretreatment, enzymatic hydrolysis and Simultaneous saccharification of stem from jerusalem artichoke was investigated in this study. The aim was to find at which conditions the steam pretreatment should run for maximal sugar conversion, and to evaluate the ethanol potential of the material.

As discussed in the results some of the samples obtained were not pretreated as harshly as would have been expected for the set of parameters used. Due to this it was not possible to reach the goals for this project. It was not possible to validate the set of parameters at which the pretreatment should be run.

To be able to draw a final conclusion about which parameters to run the pretreatment at, more experiments have to be preformed. The fact that the samples pretreated in the 10-l unit do not show the same results as the corresponding pretreatments in the 4-l unit suggests that the batch size impacts the extent of pretreatment and further proves that the results from this study are not enough to draw a final conclusion about which set of parameters is best to use. Due to the fact that the pretreatment was harsher in the large pretreatment unit it is safe to assume that 210°C is a too high temperature to run the steam pretreatment at.

For the two pretreated material that were investigated in SSF the yield from the pretreated samples to ethanol was 64% and 76% for 5 respectively 10 minutes of pretreatment which could be considered acceptable yields for a process that is not yet fully optimized. The corresponding

over all glucan to glucose conversion for these samples was 86 and 81% which gives a total glucan to ethanol conversion from the raw material of 55 and 62%

The degradation/loss of fructose is significantly higher than that of the other sugars during the pretreatment. Therefore, to get a better conversion from carbohydrates to ethanol, it would be preferable to extract the inulin prior to pretreatment to prevent the loss of fructose and formation of inhibitors.

8 Future work

There are several aspects that could be investigated further:

- First and foremost, due to the inconsistent results, more pretreatments have to be carried out to get a statistical reliable result for which set of parameters should be used.
- Because of the divergent results of corresponding parameter sets in the large and the small batch the impact of batch sizes would need to be investigated.
- Other parameters should be investigated. The time the material is soaked in acid before pretreatment, particle size of raw material etc.
- Investigate other yeasts. Yeast able to digest xylose, better heat resistance etc.
- Investigate composition of JAS from different harvests times and the impact of harvest time on the process.
- Investigate the possibility of pretreatment of extracted or washed material and fermentation of the extractives. If the material was to be washed before pretreatment the extractives and the solids could be fermented separately. There are yeasts able to digest inulin directly [45] and from the measurements of the extractives the inulin seems to be washed out from the raw material during extraction. Fermenting the fructose separately could produce better yields due to less degradation of sugars when not pretreating the inulin.
- Take the inulin more into consideration. Find a better way of measuring the amount of inulin/fructose in the process samples and raw material [46].
- Investigate the possibility to increase WIS content of the material fermented to receive higher ethanol concentrations after fermentation.

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