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Application of Reaction Additives to Suppress Degradation Reactions in a Hydrothermal Pretreatment in a Wheat Straw Biorefinery

by

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Abstract

For the development of biorefineries and the production of bio-based products, lignocellulosic biomass constitutes the largest source of renewable organic feedstock. The fractionation of its main components (hemicellulose, cellulose and lignin) is a promising approach for the production of platform chemicals.

At the Institute of Thermal Separation Processes (TVT) at the Hamburg University of Technology (TUHH), a second-generation biorefinery approach is being investigated and Liquid Hot Water (LHW) pretreatment is conducting in a batch screening reactor. LHW pretreatment can remove up to 80% of the hemicellulose and enhances the enzymatic digestibility of the material. Nevertheless, sugar degradations to furfural and others is unwanted and to be avoided.

Application of citrate and acetate pH-buffers as reaction additives to suppress degradation reactions was studied. Different buffer concentrations were tested in a range of 0.005 M to 0.05 M. Highest buffer concentrations had an undesirable effect in hemicellulose yield and citrate buffers resulted to be noneffective to minimize furfural yield. As a consequence, they are not useful to reduce xylose degradation.

Acetate pH-buffer was used with two different pH: 4 and 5. Unfortunately, the use of pH 4 buffer did not have a positive effect on the reduction of degradation reactions. However, it was found that pH 5 buffers can be used to reduce furfural yield (but not selectivity) and to keep the pH more basic after the LHW pretreatment in order to make suitable the consecutive enzymatic hydrolysis for the production of glucose and a highly pure solid lignin.

Symbols

Latin symbol	Unit	Meaning
C	mol/L	Concentration
Ea	kJ/mol	Activation energy
Ka	mol/L	Acid constant
m	g	Mass
P	bar	Pressure
pH		pH value
pKa		pKa value
R		Recovery
S		Selectivity
t	min	Treatment duration
T	K	Temperature
V	L	Volume
Y		Yield

Greek symbol	Unit	Meaning
σ	-	Standard deviation

Indices	Meaning
A-	Acid anion
HA	Protonated acid
L	Aqueous phase
N	Sample size
S	Solid phase
0	Initially

Abbreviations

Abbreviation	Meaning
AE	**Activation energies**
AFEX	**Ammonia fiber explosion**
C5	**Basic sugar unit with five carbon atoms**
C6	**Basic sugar unit with six carbon atoms**
DA	**Dilute acid**
DP	**Degradation products**
DS	**Degree of solubilization**
DSC	**Differential Scanning Calorimetry**
EH	**Enzymatic hydrolysis**
HC	**Hemicellulose**
HMF	**Hydroxyl-methyl-furfural**
HPLC	**High Performance Liquid Chromatography**
ILs	**Ionic Liquids**
LHW	**Liquid Hot Water**
L/S	**Liquid-to-solid ratio**
SAA	**Soaking in aqueous ammonia**
TVT	**Thermal Separation Processes**
TUHH	**Hamburg University of Technology**

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1 Introduction and objectives

1.1 Introduction

In the current 21st century, the management of resources is an important task for sustainable development. In this way, it is essential to convert the global economy into a sustainable economy based on the biological basis whose pillars are bioenergy, biofuels and bioproducts. The development of biorefineries could be a main tool for the production of feed, chemicals, materials, goods and fuels of the future [1]. Biorefineries must achieve the optimization of their technologies, raw materials and products and be integrated in the industrial cluster.

At the Institute of Thermal Separation Processes (TVT) at the Hamburg University of Technology (TUHH), a second-generation biorefinery approach is being investigated, using lignocellulosic biomass as raw material. Wheat straw is one of the most abundant lignocellulosic biomasses and its utilization reduces the dependence on fossil resources and avoid pollution from straw combustion.

The fractionation of wheat straw and the Liquid Hot Water (LHW) pretreatment are investigated for being a promising approach for the environmentally friendly and economical productions of mainly lignin, glucose and xylose. Pretreatment is an essential prerequisite for the fractionation of wheat straw into hemicellulose, cellulose and lignin. LHW pretreatment, at temperatures of 170-230ºC, is able to make cellulose accessible to the enzymatic hydrolysis, minimizing the formation of degradation products and hydrolyzing hemicellulose into extractable sugars.

Degradation of the sugars in the hydrothermal reactor is a challenge regarding full-fractionation of the biomass. Therefore, suppression of degradation reactions is being investigated.

1.2 Objectives

The objective of this work is the investigation of the extraction of hemicellulose from wheat straw in a high yield, with the requirement that the hemicellulose is removed from the solid in order to produce high-purity lignin. Additionally, the main challenge is to minimize the formation of degradation products through the investigation of the effect of pH on hemicellulose solubilization and xylose degradation. For this approach, different pH-buffers and organic acids shall be utilized in order to observe the effects on the process speed and its products. A batch screening reactor is used to conduct the Liquid Hot Water (LHW) pretreatment of wheat straw.

The work is divided into three parts. The first part focus on the evaluation of the standards experiments using water as medium, where the effect of the residence time will be observed. The last two parts are based on the use of buffers. In the second part, a concentration series of experiments will be carried out: citric acid and acetic acid are the chosen types of buffers. Last part consists in a pH series of experiments, where the same type of buffer is used with different initial pH, based on the previous results.

Different output parameters have to be measured and studied in every part of the whole work: hemicellulose, glucose, acetic acid, furfural and HMF concentrations and hydrolysates pH values. In this way, the evaluation and calculation of the degree of solubilization and the conversion, selectivity and recovery of hemicellulose could be analyzed and compared. In addition, the yield of furfural has to be investigated since it is speculated in literature that there is no furfural production if the pH is kept above a value of 4 [2].

The main purpose of this thesis is the detailed analysis of all the mentioned parameters in order to evaluate and consider the use of buffers effectivity in the LHW pretreatment of wheat straw on the suppression of degradation reactions.

2 Fundamentals and state of the art

This chapter summarizes the state of knowledge of hemicellulose hydrothermal fractionation and autohydrolysis during lignocellulosic biomass pretreatments. Reaction pathway and kinetics of hemicellulose hydrolysis are presented. The fundamentals of the biorefinery approach as well as the different methods of pretreatment on industrial scale are discussed.

2.1 Lignocellulosic biomass

Lignocellulose is the most abundant and cheapest form of biomass, constituting the largest source of renewable organic material on Earth [3]. Its composition is cellulose (40-50%), hemicellulose (25-35%) and lignin (10-30%), and minor compounds [4]. There are variations in the amounts of each structural components depending on the plant species. In Table 1 the range in composition for various lignocelluloses are displayed.

The main beneficial properties of lignocellulosic material are the renewability and the richness worldwide. The amount of lignocellulose produced on earth is 2×10^{11} tons per year. Therefore, bioethanol production from lignocellulosic waste materials are being considered as an alternative to fossil fuels in order to overcome the uncontrolled emission of greenhouse gases due to the utilization of fossil fuel which lead to increase the threat of global warming [5].

Table 1: Composition of different lignocellulosic biomass types [3].

General Biomass Classification	Lignocellulosic Biomass Type	Cellulose	Hemicellulose	Lignin
Hardwood	American sycamore	37.2–41.8	17.6–19.6	25.0–27.3
	Black locust	39.3–42.6	16.6–18.9	24.4–28.6
	Eucalyptus	46.6–50.3	12.7–14.4	26.9–28.2
	Hybrid poplar	40.3–47.3	16.6–22.6	15.5–16.3
	Willow	42.4–45.3	20.6–22.9	16.9–18.9
	Oak	40.4	35.9	24.1
Softwood	Pine	42.0–50.0	24.0–27.0	20.0
	Spruce	45.5	22.9	27.9
Agricultural/ agroindustrial residues	Sugarcane bagasse	31.9–43.4	12.2–25.5	23.1–27.6
	Brewer's spent grains	16.8–26.0	19.2–29.6	16.9–27.8
	Spent coffee grounds	11.6–13.2	37.2–41.0	22.2–25.6
	Rice straw	29.2–34.7	23.0–25.9	17.0–19.0
	Rice husks	28.7–35.6	12.0–29.3	15.4–20.0
	Corn stover	30.6–38.1	19.1–25.3	16.7–21.3
	Corn cobs	33.7–41.2	31.9–36.0	6.1–15.9
	Corn stalks	35.0–39.6	16.8–35.0	7.0–18.4
	Wheat straw	35.0–39.0	23.0–30.0	12.0–16.0
	Barley hull	34.0	36.0	13.8–19.0
	Barley straw	36.0–43.0	24.0–33.0	6.3–9.8
	Oat straw	31.0–35.0	20.0–26.0	10.0–15.0
	Ray straw	36.0–47.0	19.0–24.5	9.9–24.0
	Sorghum straw	32.0–35.0	24.0–27.0	15.0–21.0
Herbaceous crops	Big bluestem	29.0–37.2	20.5–25.8	17.1–23.8
	Sericea lespedeza	32.7–39.4	15.7–19.4	24.1–31.9
	Tall fescue	23.4–26.4	18.2–20.4	10.9–14.8
	Switchgrass	26.8–37.5	22.4–28.8	13.2–22.5
	Miscanthus	35.0–40.0	16.0–20.0	20.0–25.0
	Tobacco chops	22.0–30.0	15.0–20.0	15.0–25.0
Other waste	Cellulose sludge	31.4	9.8	15.3
	Agave whole residue	30.7	16.9	16.9
	Waste papers from chemical pulps	60.0–70.0	10.0–20.0	5.0–10.0
	Solid cattle manure	1.6–4.7	1.4–3.3	2.7–5.7

Due to the lignocellulosic resources is an alternative to fossil fuels, production technology advances are being developed and the use of lignocellulosic biomass is expanding. In addition to energy purposes, it also had architectural and medical uses, and applications in the paper and textile areas. Moreover, departing from the concept of a single product, and moving to the concept of lignocellulose biorefinery integration process, it is based on the idea of fractional utilization and component separation [5].

Research in recent years is focusing in the full-fractionation of the substrate and lignocellulose has to be separated in order to isolate fractions and break into its individual building blocks [4]: lignin is made up of linked aromatic units, hemicellulose of C5 sugars and cellulose of C6 sugars. Nevertheless, cell-wall components such as lignin, hemicellulose, and proteins can cross-link with each other to create a complex matrix,

which increases the biomass recalcitrance [6]. Therefore, lignocellulose matrix and crystallinity of cellulose make difficult to separate into their compounds that requires long reaction times for the hydrothermal and enzymatic hydrolysis or strong reagents. As a result, degradation on non-cellulosic fractions as well as large volumes of effluent occurs [4].

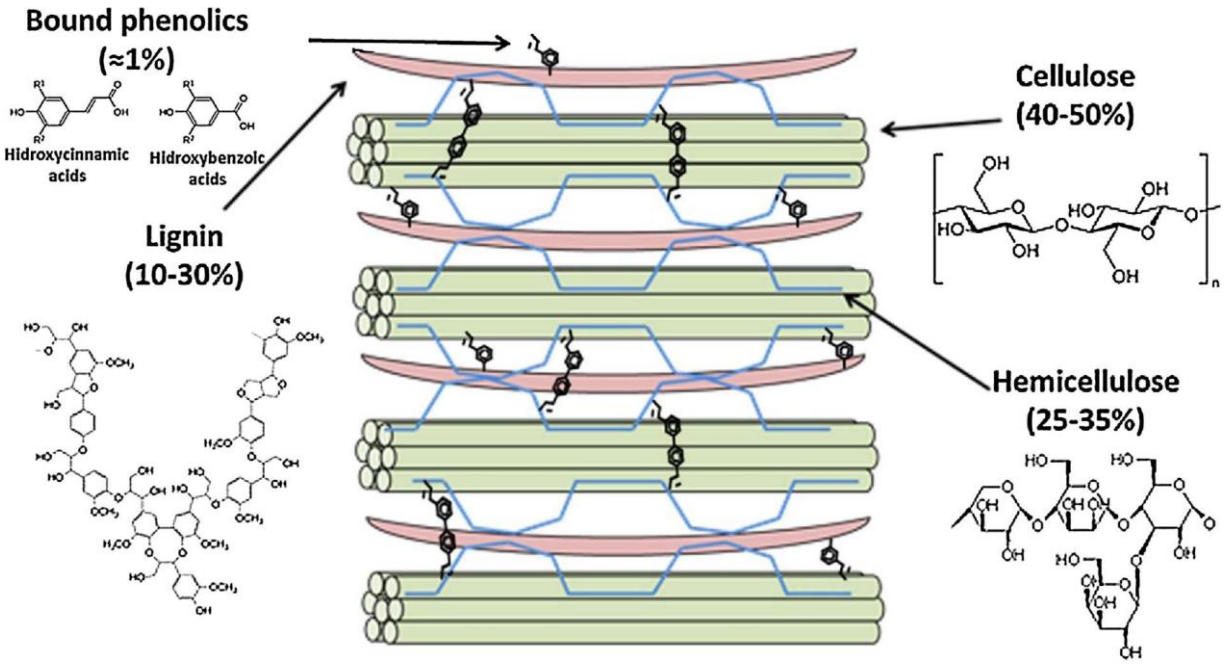

Figure 1: Lignocellulosic biomass structure. [4]. Cellulose (green fibers); hemicellulose (blue threads) and hemicellulose (pink sheets).

2.1.1 Hemicellulose

Hemicellulose in lignocellulosic materials connects the fibers of cellulose and the lignin.

Hemicellulose chains interact with cellulose fibrils forming non-covalent cross-links between cellulose bundles. However, hemicellulose is easier to remove than the other components during pretreatment under acidic conditions [6]. Its structure is amorphous, and it is composed of a group of complex heterogeneous polysaccharides composed of 5-carbon sugars or pentoses (xylose and arabinose), 6-carbon sugars or hexoses (glucose, mannose, and galactose), and some acids (acetic acid, D-glucuronic acid and D-galacturonic acid), with a chain length between 200 and 300 monomeric sugars [3].

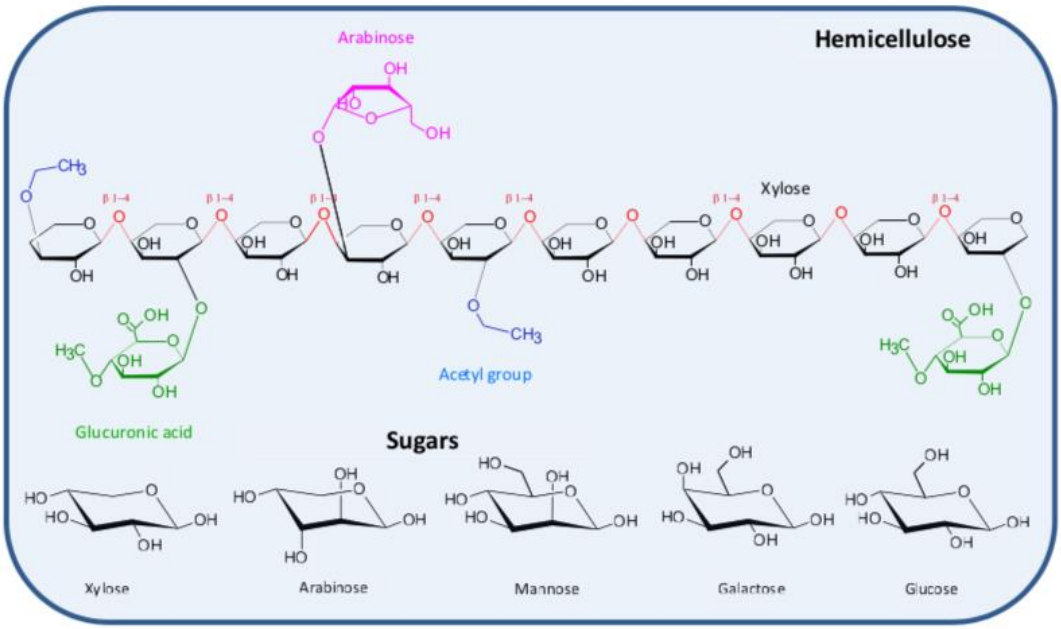

Figure 2: Structure of hemicellulose [3]. Monomers found in hemicellulose: xylose; arabinose; mannose; galactose and glucose

Hemicellulose can be liquefied with a lower process temperature compared to cellulose. Thereby, hydrothermal extraction that only requires water and mild temperatures (an operational temperature around 180ºC), can recover 60% of the initial hemicellulose as oligomers and sugars. With higher temperatures, degradation reactions and products appear [4].

2.1.2 Cellulose

Cellulose is a linear-chain polysaccharide consisting on units of glucose linked by β-1,4 glucosidic bonds. The hydrogen bond network between OH groups in its structure is the reason why cellulose chains form fibrils, which are insoluble and forms the plant cell walls. Cellulose is associated with another polysaccharide, hemicellulose and both are sealed with lignin [7].

The cellulose microfibrils are hydrophobic and crystalline, which contributes to the recalcitrance behavior [6].

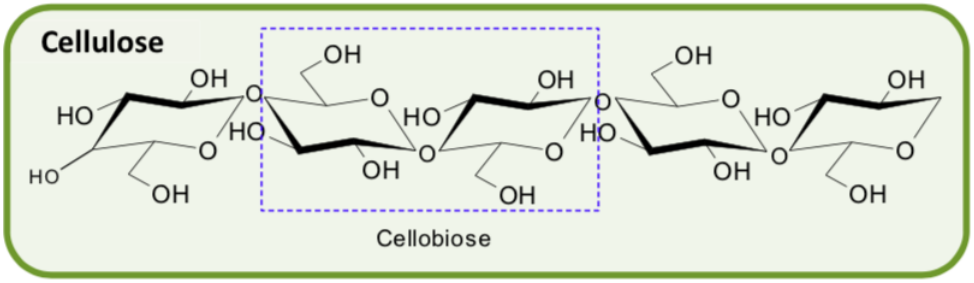

Figure 2: Structure of cellulose [3]. D-glucose units attached through β(1→4)-glycosidic bonds

2.1.3 Lignin

Lignin is another compound contributor to the recalcitrance of lignocellulosic biomass [3]. Lignin has an amorphous and branched structure which comes from the polymerization of three phenylpropane monomer units: coniferyl, synapyl and p-comaryl alcohol. Lignin has a great number of functional groups and linkages, which are various between different species of lignocellulosic materials. Lignin can be used as an energy source but also has higher-values uses despite its non-well known structure [4].

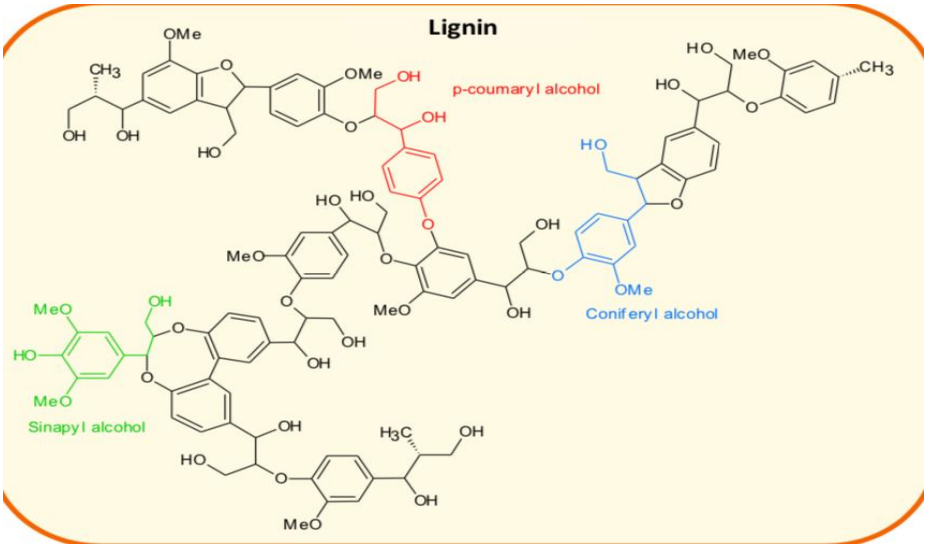

Figure 3: Structure of lignin polimer [3]. Chemical structures of the phenylpropanoid alcohols.

Hemicellulose and cellulose are also associated with lignin by covalent bonds through ester, ether, and glycosidic linkages.

Due to the lignin reticulation, lignin is insoluble in most solvents, unless it undergoes degradation [3].

2.2 Biorefinery

In the current 21st century, the management of resources is an important task for sustainable development in order to use less and cleaner energy, thus reducing the environmental footprints and resulting in an overall reduction in production costs. In that way, it is essential to change the global economy into a sustainable biobased economy with its pillars are: bioenergy, biofuels and biobased products. The development of biorefineries could be the main tool to the production of feed, chemicals, materials, good and fuels of future [1].

One approach for the definition of the term "biorefinery" was published by The American National Renewable Energy Laboratory: "A biorefinery is a facility that integrates biomass conversion processes and equipment to produce fuels, power and chemical from biomass. The biorefinery concept is analogous to today's petroleum refineries, which produce multiple fuels and products from petroleum. Industrial biorefineries have been identified as the most promising route to the creation of a new domestic biobased industry".

There are four different biorefinery systems [1]:

1. "Lignocellulosic feedstock biorefinery", using cellulose biomass and waste as raw material.
2. "Whole crop biorefinery", which uses cereals or maize.
3. "Green biorefineries", using green grass, alfalfa…
4. "Biorefinery two platforms concept" which includes the sugar and the syngas platform

Bio-based products, such as chemicals, lubricants and solvents offer the most development perspectives. Products made entirely or partially from biomass present an alternative to products solely produced by conventional and non-renewable resources and thus present the potential for a long-term shift away from fossil-based towards a bio-

based economy. Therefore, biorefinery is the key of a sustainable bio-economy, with viable small-scale options.

At TUHH- Institute of Thermal Separation Processes, second generation biorefinery is being developed and different lignocellulosic plant residues are being used. First step is a Liquid Hot Water pretreatment process for the lignocellulose hydrolyzation, which allows a water throughput at 200ºC and 50 bar with a residence time about 30 min. Batch and fixed-bed reactors of 3 L and 40 L are in operation. A detailed description of LHW pretreatment process is given in chapter 2.3.2. In a second step, the solid residue from the LHW is subjected to an enzymatic hydrolysis for the separation of cellulose and remaining lignin, treated in an atmospheric stirred tank 10 L reactor at 50ºC for 42 hours and a pH of 5. Finally, the slurry is centrifuged in order to separate lignin from the glucose solution [8].

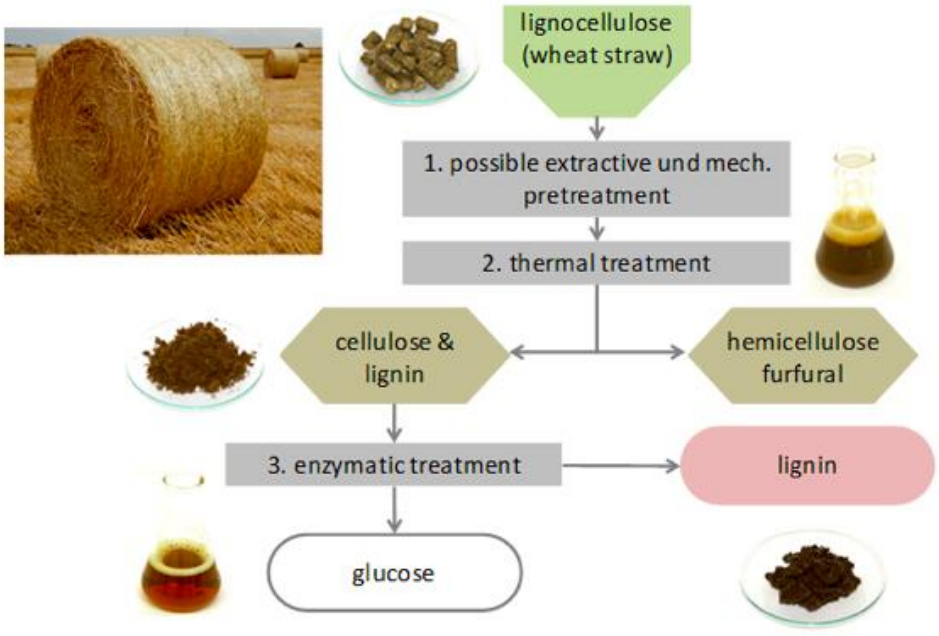

Figure 4: Fractionation of added value streams for the model substrate wheat straw [9]

The aim is to isolate the compounds with a process cascade, utilizing water and enzymes and convert these into platform chemicals such as glucose, xylanes, furfural and lignin, which is currently gaining more attention in the institute.

The suppression of degradations reactions in the LHW pretreatment is being investigated in order to extract hemicellulose in a high yield.

2.3 Methods of pretreatment of lignocellulosic feedstocks

Enzymatic hydrolysis does not work without pretreatment because of the mentioned recalcitrance of the lignocellulosic feedstock materials. Pretreatment processes take part in the separation of the interlinked fractions of lignocellulosic feedstock materials, increasing the accessibility of each individual component [10]. The main goal for pretreatment is to achieve the highest product yields at the lowest costs obtaining high hemicellulose recovery.

In order to prepare cellulosic biomass for enzyme or acid catalyzed reactions to release sugars, several pretreatment methods are being developed by researchers. The pretreatment should meet some requirements [3]:

1) Deconstructing lignocellulosic biomass structure and decreasing the cellulose crystallinity
2) Increasing the surface area and porosity
3) Generating digestible solids and promoting high sugar yields
4) Avoiding the formation of fermentation inhibitor compounds (acetic acid, furfural, HMF and phenolic compounds)
5) Allowing hemicellulose and lignin recovery
6) Requiring a low demand of post pretreatment operations
7) Requiring minimal energy, water and chemicals and low-cost reactors

Current pretreatment approaches favored for catalytic processing employ dilute acid to remove hemicellulose with high sugar or furfural yields. On the other hand, aqueous pretreatment can avoid the high costs of enzymes opening up the biomass structure to make cellulose accessible to enzymes, achieving high yields from the pretreated solids and recovering sugars released in pretreatment with high yields [6].

2.3.1 Industrial processes

Dilute acid (DA), lime, soaking in aqueous ammonia (SAA), sulfur dioxide-impregnated steam explosion (SO_2), ammonia fiber explosion (AFEX) and LHW are the pretreatments which have been applied in demonstration plants with more success [11].

Normally, combinations of methods are necessary. In general, physical pretreatments aim to reduce the particle size and crystallinity of cellulose and chemical pretreatments are more focused on lignin removal [3].

Ibicon/Dong Energy (Denmark), Chemetex (Italy), Clariant (Germany), and Stolz (Spain) are some examples of industrial facilities in Europe which use hydrothermal processes for the pretreatment of wheat straw, sugarcane bagasse, and *Arundo donax* [12].

2.3.2 Liquid Hot Water (LHW) pretreatment process

This type of pretreatment method uses water at high temperature (170-230ºC) and pressure above boiling point of water (up to 5 MPa). At this high temperature conditions, the concentrations of H3O+ and OH- ions are increased [13]. Hydrolysis of hemicellulose occurs, and lignin is removed making cellulose more accessible [10]. A detailed description of hemicellulose hydrolysis which happens in LHW pretreatment process is given in chapter 2.4.

LHW pretreatment removes up to 80% of the hemicellulose and enhances the enzymatic digestibility of the material [11]. However, LHW pretreatment liberates oligomeric form of sugars requiring an enzymatic hydrolysis step to then produce fermentable monomeric sugars.

During LHW pretreatment, hemicellulose is mostly depolymerized, and its degradation products are dissolved in the liquid phase, while cellulose is retained in the solid phase. On the other hand, lignin suffers from depolymerization and repolymerization reactions and most of insoluble lignin is retained in the solid residues [11].

Regarding to the operational conditions used in Liquid Hot Water biomass pretreatment, several studies have examined a variety of operational conditions. The reaction time may

vary from seconds up to hours, depending on the temperature. Liquid-to-solid ratio (L/S) ranges between 2 and 100 (w/w) and common values are around 10 [12].

The concentration of solids may also affect the catalyst concentration in the reaction media because the decrease of the L/S triggers to a higher concentration of acetic acid, which favors depolymerization. Finally, the relationship between temperature and time also influences. However, empiric parameters such as the severity factor (Log R_0) can group both process parameters [12].

LHW pretreatment can be performed in three types of reactor configurations: cocurrent, countercurrent and flowthrough. The most widely studied is cocurrent pretreatment processes conducted in a batch reactor where biomass and water together is heated and held at a desired temperature and time [13].

In addition, different reactor types have been investigated and hemicellulose extraction has been performed in different systems.

Fixed-bed reactors are a promising approach and the flowthrough treatment in these reactors allows high solids loads and the recovery of the hemicellulose fraction. However, the permeability of the fixed-bed, the viscosity of the biomass and the hydrolysate cause a considerable pressure drop which can lead to an irregular distribution of the fluid. In this way, the pressure drop and the compressibility of the bed limit the expansion of fixed-bed reactors for the use of LHW pretreatment [14].

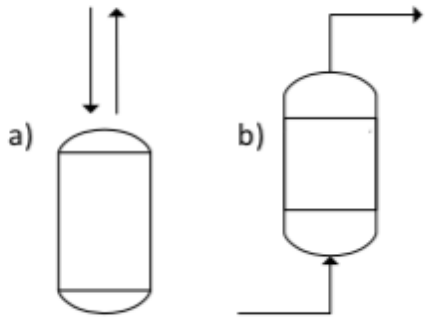

Figure 5: Reactor types for LHW process: a) Batch reactor; b) Fixed-bed reactor

Liquid hot water (LHW) pretreatment could be considered as a weak acid technology pretreatment. The aim of LHW pretreatment is to maximize the enzymatic advantage and minimizing the severity of pretreatment [6].

In summary, this aqueous pretreatment fractionates the biomass into an aqueous phase that contains hemicellulose oligosaccharides and some hemicellulose-derived monosaccharides, and a solid phase that is mostly cellulose and lignin. Pentose sugars from hemicellulose can be converted with the glucose obtained from cellulose, or the pentose sugars can be separated by filtration or centrifugation to process biofuels or co-products [6].

The advantages of LHW pretreatment are a minimum formation of inhibitory compounds and the low cost of solvent. Nevertheless, although large amount of energy for high-temperatures is required [10], a combined process producing ethanol and high value-added products would make LHW pretreatment suitable in the industrial application [11].

2.4 Hemicellulose hydrothermal fractionation fundamentals

Hemicellulose cleaving occurs due to high temperatures breaking the bonds between the monomeric sugars and producing shorter oligomers. In addition, oligomer dissolution and oligomer cleaving are present at the same time. Temperature, molecular weight and deacetyl affect hemicellulose solubility and its extraction [4].

The cleaving can produce monomeric sugars and operational conditions imply sugar degradation too. These degradation products can be also useful, like furfural which can be used as fungicides or lubricants. However, focusing on xylose production, sugar degradations to furfural and others are undesirable. Sugar degradation depends on the operating temperature and pH as well as the volumetric flow (less residence time, lower degradation) [4].

2.4.1 Hemicellulose autohydrolysis

In parallel to the mentioned cleaving, hemicellulose deacetylation takes place releasing acetic acid. Acids are a source of protons that catalyze the cleaving and degradation reactions. Thus, deacetylation has to be considered [4].

When water is at high temperatures (150-230ºC) autoionization of water occurs, increasing the acidic hydronium ions (H3O+) concentration, which leads to a release of acetic acid and consequently a drop of the pH [13].

The hydronium ions initially cause xylan depolymerization and cleavage of acetyl groups from the hemicellulose. Lately, the hydronium ions generated from acetic acid autoionization catalyze the hydrolysis of hemicellulose. As a conclusion, the hydrolysis of glycosidic linkages in hemicellulose and in lignin are catalyzed by water and by acetic acid formed at high temperature from acetyl groups present in hemicellulose [13].

The evolution of proton concentration during the hydrolysis follows complex behavior depending on the acetyl group and ash content of biomass, initial acid concentrations and temperature [15].

There are competing pathways governing proton evolution and neutralization. Four primary reactive pathways can be considered, assuming they follow elementary kinetics [15]. In Figure 7 these pathways are shown:

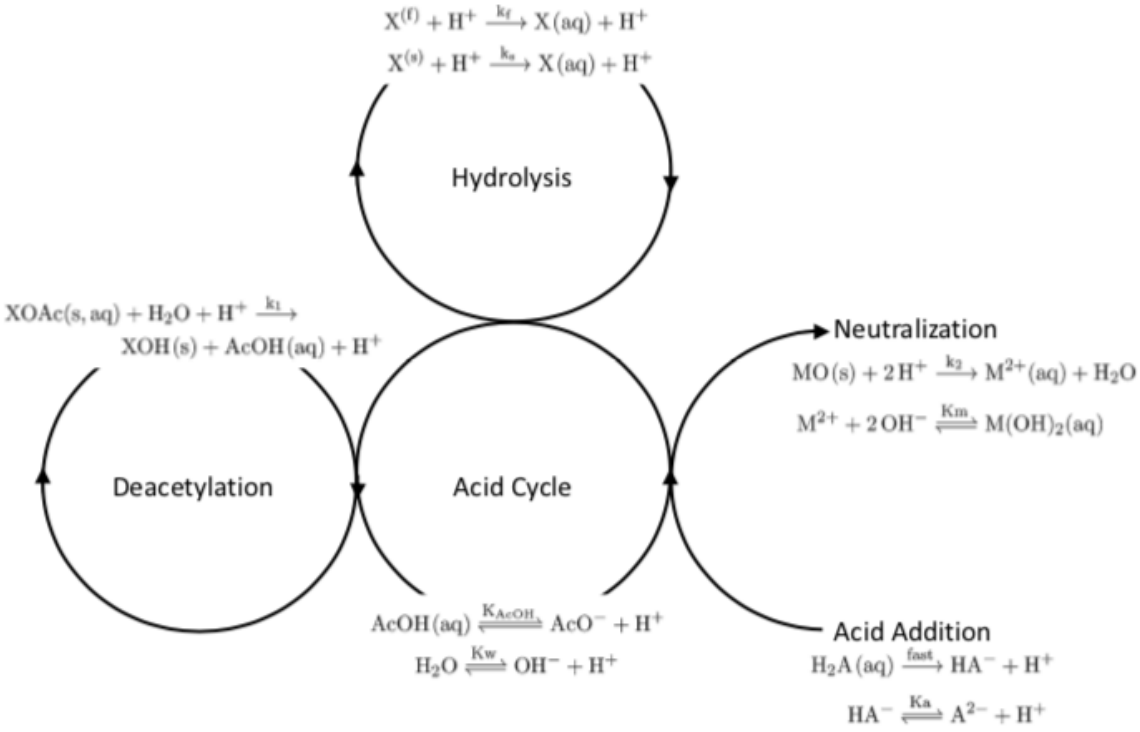

Figure 6: Reaction scheme [15]

On the left of the Figure 7 is shown the deacetylation where Ac is cleaved from the hemicellulose though an acid hydrolysis of the ester. As the product AcOH(aq), acetic acid adopts an equilibrium, behaving as a weak acid. Another additional source of H+ is the water disassociation and due to these equilibria, H+ is available for both the neutralization and hydrolysis reactions [15]. Based on equilibrium constants, the contribution of H_3O^+ ions derived from acetic acid is much higher than from water autoionization [12]. Additionally, if acid is added, more protons will be also available. The neutralization of the protons is the final aspect to consider and it is related with the hypothetical oxide (MO) by the ash [15].

However, due to the accumulation of organic acids and hydrogen-ions, monitoring and control of the pretreatment conditions, must prevent the acid-catalyzed degradation of the monosaccharides [7]. A detailed description of how pretreatment conditions affect to the reaction pathway of hemicellulose hydrolysis is given in the following chapter 2.5.

2.5 Reaction pathway and kinetics of hemicellulose hydrolysis

Kinetics models have been developed describing the complex reactions that occur in hemicellulose hydrolysis: parallel reactions that hydrolyze sugar polymers. In Figure 8 are shown the reaction pathways of every hemicellulose and cellulose sugar component.

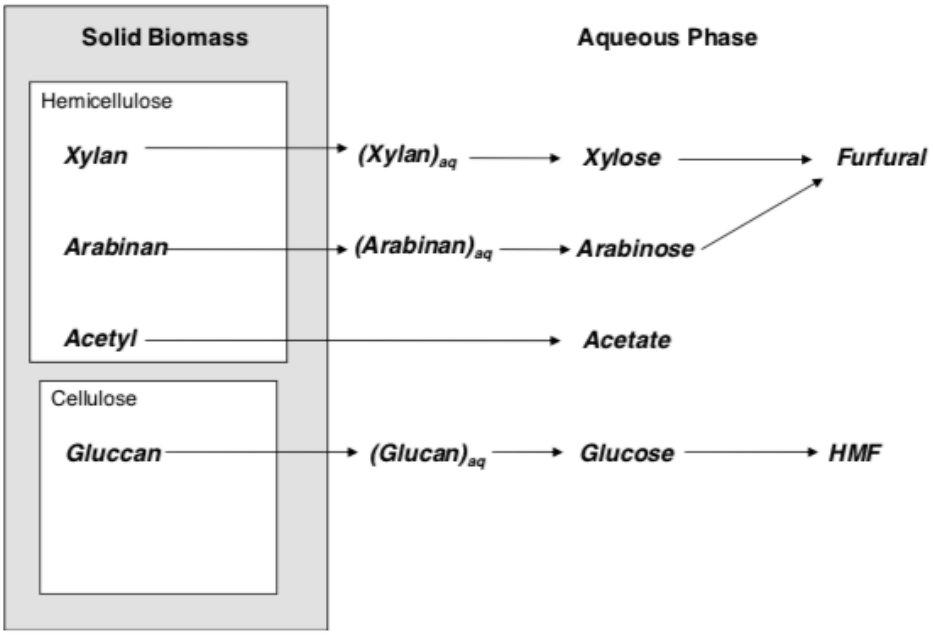

Figure 7: Kinetic model of aquous acid hydrolysis of lignocellulosic biomass [6]

Models study the depolymerization of the xylan of the hemicellulose, the release of other sugars and organic acids. Xylan is the anhydro xylose constituent of hemicellulose. These models for hemicellulose hydrolysis follow the convention described by Saeman for cellulose hydrolysis [6]:

glucan → glucose at rate constant k; defined k:

$$k = k_0[Acid]^n e^{-Ea/RT} \quad (1)$$

where k_0 is the pre-exponential Arrhenius constant; n is the acid power factor; Ea is the activation energy; R is the universal gas constant; and T is absolute temperature (K).

In that way, hemicellulose hydrolyses is a consecutive first-order steps: xylan is first hydrolyzed to xylose, and the xylose is degraded to furfural once it is liberated to the acidic solution. Xylose degradation is also strongly increased by the presence of ions, such as halide ions, which might be present in biomass and released during pretreatment [6].

$$\text{Xylan} \xrightarrow{k_f} \text{Xylo-oligomers} \xrightarrow{k_1} \text{Xylose} \xrightarrow{k_2} \text{Furfural}$$

Figure 8: Monophasic hemicellulose hydrolysis model [11]

Considering an homogeneous, first-order reaction, the following kinetic model is proposed [11]:

$$C_X = \frac{C_{H_0} k_f k_1}{(k_1 - k_f)(k_2 - k_f)}(e^{-k_f t} - e^{-k_2 t}) - \frac{C_{H_0} k_f k_1}{(k_1 - k_f)(k_2 - k_1)}(e^{-k_1 t} - e^{-k_2 t}) \quad (2)$$

where C_H, C_O, C_X, C_D and C_{H0} are the concentrations of xylan, xylo- oligomers, xylose, furfural and the initial concentration of xylan, respectively. k_f, k_1 and k_2 are kinetic constants.

Activation energies (AE) ratios for steps of Ea_f/Ea_1 and Ea_1/Ea_2 is more than 1, indicating that there is high selectivity of xylo-oligomers formation and monomer degradation at higher temperatures [11].

The aim is the formation of xylan oligosaccharides, and the design of aqueous pretreatments must optimize the first reaction (insoluble xylan to soluble xylan) and minimize the following reaction (hydrolysis of soluble xylan to xylose) in order to reduce the formation of furfural (third reaction). This could be possible controlling the reaction conditions: time, temperature and pH during the pretreatment [6].

Different types of pretreatments involve different results in the reaction pathway and in sugars yields:

2.5.1 SUPERCRITICAL WATER

Supercritical water is water at temperature and pressure values above the critical points (Tc = 374.8ºC and Pc = 22.1 MPa) [16].

The reaction pathway is shown in Figure 10:

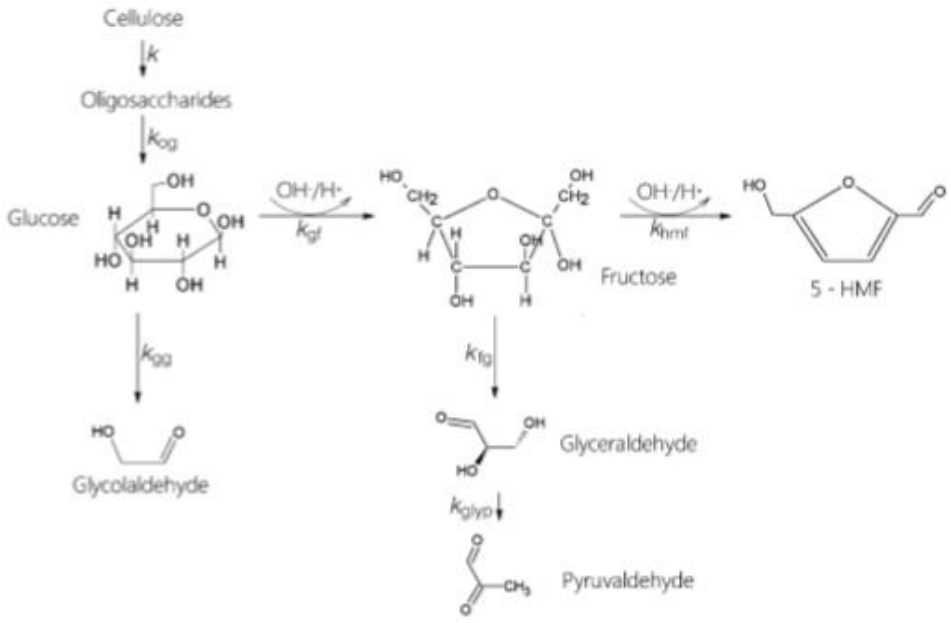

Figure 9: Reaction pathways of cellulose hydrolysis in supercritical water

As it can be observed in Figure 10, fructose can follow two main reaction pathways: fructose dehydration or retroaldol condensation. The second reaction obtains glyceraldehyde as the main product from fructose [16].

The yield of sugars is enhanced by using continuous supercritical water reactors at high temperature for short reaction times. The combination of these two parameters is essential for obtaining high yields of sugars. Long reaction times involve the derivation of sugars and, on the other hand, at low reaction temperatures, several side reactions take place [16].

In particular, it is observed that the formation of 5-HMF is practically avoided at super-critical conditions, being highly dependent on the reaction temperature and on the ion concentration [16].

To conclude, it was found that low H^+ concentration in the reaction medium is determining in the selectivity. In this way, under supercritical conditions, the retroaldol condensation pathway is enhanced, instead of the dehydration pathway [16].

2.5.2 LHW

An alternative solvent for hydrolysis of biomass is water below its critical point, requiring mild temperatures (160-210 ºC). High yields of oligomers and sugars can be obtained [4]. This pretreatment enable high recoveries of C5 oligosaccharides and solvent free lignin as well as high yields in enzymatic saccharification [14]. However, undesired degradation product formation, such as furfural, from C5 monomers appear, leading to a decrease of the selectivity.

In addition, the phenomenon of deacetylation is also related with the hemicellulose extraction selectivity. Acids are a source of protons, catalyzing the cleaving and degradation reactions. On the other hand, operational pH also affects selectivity, reducing up degradation if pH is maintaining above 4-5 [4].

Nevertheless, there are some ways to overcome degradation in LHW pretreatment:

DEACETYLATION: NaOH wash to remove acetic acid

Biomass deacetylation prior to pretreatment is an efficient strategy in order to increase monomeric xylose yields from pretreatment and enzymatic hydrolysis as well as improving cellulose digestibility [17].

However, deacetylation requires high process costs through increases in chemical (NaOH) and more water consumption because of the solid-liquid separation after alkaline extraction [17].

NEUTRALISATION WITH KOH

Several authors have proposed the monitoring and control of pH during hydrothermal pretreatment in order to maximize hemicellulose hydrolysis and avoid degradation products.

A continuous pH-monitoring system was developed through the addition of base (KOH) to the pretreatment vessel in order to prevent the drop in pH that will promote the degradation of the monosaccharides. This system enables a base addition profile to keep the pH within a desirable range, minimizing the hydrogen-ion concentration during the pretreatment [7].

SLIGHTLY ALKALINE WATER TO END WITH NEUTRAL pH

Another effective pH pre-corrected liquid hot water pretreatment was developed by employing a small amount of NaOH. This new pretreatment technology accelerates hemicellulose deacetylation and correct the acid hydrolysate, leading to a reduction of degradation of hemicellulose and an increase in the recovery of sugars [18].

As a conclusion, main variables on extraction selectivity are the operational time and the operating temperature: the higher temperature or time is, the bigger yield is obtained. However, if these are too high, sugars start to degrade, reducing the yield. It has been demonstrated that temperatures around 180ºC and high volumetric flows (residence time lower than 4 min) promote high hemicellulose selectivity. Furthermore, as it was mentioned, degradation can be reduced up if pH is maintained above 4 [4]. Thus, buffers can also be used to keep pH constant in order to overcome degradation in LHW pretreatments.

2.6 Theory of selected pH buffers

A pH buffer is a solution that can maintain a nearly constant pH when a small amount of strong acid or base is added [19].

pH buffers consist of a mixture of a weak acid and its conjugate base but there are different ways to produce this mixture [19]:

- Weak acid and a salt of its conjugate base.
- Weak acid and enough strong base to neutralize some of the weak acid.
- Weak base and enough strong acid to neutralize some of the weak base.

For prediction the p[H3O+] of a buffer solution, there is an expression called the *Henderson–Hasselbalch equation* which can approximate it when concentrations of the weak acid and the base are high enough with a high buffer capacity [19]:

$$\mathrm{p[H3O+]} = \mathrm{pKa} + \log\left(\frac{\mathrm{CA}-}{CHA}\right) \qquad (3)$$

The buffer capacity is expressed as the amount of strong acid or base that must be added to 1 liter of the solution to change its pH by one unit. Thus, the desired solution pH should be within 1 of the pK_a of the acid used in the buffer system [19].

2.6.1 Citric acid and citrate buffer

Citric acid as a product can be either in the anhydrous form or as the monohydrate[20]. Citric acid is a polyprotic acid. Polyprotic acids are those that possess more than one acidic proton [19]. The pKa values of citric acid, with its three dissociation steps, are 3.13, 4.78, and 6.43 [20]. Thus, citric acid is quite useful in buffer systems because of the widest buffering capacity as compared to other organic acids [21], serving over broadened pH ranges. In the pH 2.6–7.6 range where this buffer is applied, one, two and tri-charged citrate anions exist in different proportions, depending on pH values, that is, all three steps of citric acid dissociation are involved [21].

Ka is the equilibrium constant for the dissociation reaction of a weak acid. The Ka values decline as protons are expelled from a polyprotic acid in light of the fact that each subsequent proton is progressively hard to expel as the molecule becomes increasingly more electronegative. However, the mentioned rule of the buffer capacity is valid also for polyprotic acids and the buffer action is ±1 from the pKa [19]and the maximum buffer capacities, as expected, are located for pH of solutions which are close to pKi values of citric acid [21].

In respect of thermal behavior of citric acid, its decomposition is preceded by its melting. The melting point was determined by DSC (Differential Scanning Calorimetry) measurements being at 433.9 K [22].

The lowest thermal stability is displayed by cis-aconitic acid experiencing dehydration at the first phase of decomposition, which promotes to the formation of cis-aconitic anhydride. Cis- aconitic anhydride then experiences isomerization into trans- aconitic anhydride. In perspective of the absence of exothermic effects on the DSC curves of citric acid and trans-aconitic acid, it may be expected that thermal transformations of the two

acids lead directly to the formation of trans-aconitic anhydride and finally leading to the formation of citraconic anhydride or itaconic anhydride or the mixture of both isomers [22].

2.6.2 Acetic acid and acetate buffer

Acetic acid, in aqueous solution, has a pKa value of 4.74. Therefore, an acetate buffer will have an effective pH range of 3.74-5.74, which can be observed in titration curves. These curves show how the pH of weak acid solutions reacts in different ways. When a strong base is added to the weak acid solution, there is a slight increase in pH and afterward it rises more gradually. The level curve from the titration curves is the buffer region and at the equivalence point, the acid is neutralized leaving only the acidic ion in solution. After the equivalence point, the point at which the moles of acid are equivalent to the moles of base, the weak acid behaves as a strong acid and the pH will be defined by the excess of base [19].

On the other hand, in respect of the decomposition studies of acetic acid, high activation energies and temperatures above 320ºC are required, being thermally-stable for lower temperatures [23].

3 Materials and methods

The following chapter summarizes the analytical methods and experimental procedures applied in this work, including required chemicals and laboratory materials.

3.1 Materials

3.1.1 Cut wheat straw

Cut wheat straw is used as biomass for the experiments. Material source is *Weizenshoh einstreu* from Cordes Grasberg Germany. The material is washed in a 40 L fixed-bed reactor at 20ºC for 30 minutes (180 kg/h) and then it is dried at 40ºC for 24 hours in a convective oven. Moisture content was measured being approx. 12.6 wt%.

The composition of the dried and cut wheat straw is shown in Table 3. The compositional analysis was carried out at TUHH central laboratory.

Table 2: Composition of wheat straw

Lignin [%]	**Hemicellulose [%]**			**Cellulose [%]**	**Others [%]**
	Xylose	Arabinose	Other sugars	Glucose	
22.4	24.8	2.7	2.0	39.2	8.9

3.1.2 Chemicals

Table 3: Information of used chemicals

NAME	FORMULA	PURITY	PRODUCER
Citric acid	$C_6H_8O_7$	>99,5%	AppliChem GmbH
Acetic acid	CH_3COOH	>99%	Fluka Chemie GmbH
Sodium hydroxide	NaOH	>99%	Roth

3.1.3 Citrate buffers

Three different buffers are prepared: 0.05 M, 0.01 M and 0.005 M citrate buffers.

The solutions are made up to 200 mL of distilled water requiring different amounts of anhydrous citric acid (1.921 g for 0.05 M; 0.384 g for 0.01 M and 0.192 g for 0.005 M) and the pH is adjusted to 4 with NaOH. The solutions are stored in the fridge.

3.1.4 Acetate buffers

For the preparation of a 1 molar acetate buffer, used as stock solution, 1.2 g of acetic acid in 20 mL is required. This solution is diluted to 0.05 M, 0.01 M and 0.005 M. Then, the pH of these solutions is adjusted to 4 with NaOH. Additionally, half of the 0.005 M acetate buffer solution is corrected to a pH value of 5 in order to obtain a different buffer pH. All of the buffer solutions are stored in the fridge.

3.2 Experimental part

In this work, a previously established batch process for liquid hot water (LHW) pretreatment is used. The system consists in six magnetically stirred stainless-steel high-pressure batch reactors with an effective volume of 30 mL, pressurized to 50 bar with N_2. Reactors are detachable and each reactor has a corresponding reactor lit.

Temperature control was performed with electrical heating jackets and a temperature sensor is placed inside the reactors. The jacket temperature is controlled by a PID cascade controller operated with a computer.

Experiments were carried out at 200ºC in duplicates (2 repetitions). Table 5 shows an overview of all executed experiments.

Table 4: Overview of all executed experiments

No	Name of experiments	Residence time [min]	Buffer type	Buffer concentration [M]	Buffer pH	Purpose
1	Standards series	5-50 min	NO buffer	-	-	Compare latter results
2	Concentration series	20 min	Acetate buffer; Citrate buffer	0.05 M; 0.01 M; 0.005 M	4	Investigate the effect of the buffer concentration and which concentration is needed at least to see an effect
3	pH series	5-50 min	Acetate buffer	0.005 M	4; 5	Investigate the effect of the pH on the production of degradation products

The samples are weighed into the inlays directly on the lab scale, adding 0.55 g of dry biomass. According to a moisture content of 12.6 wt%, necessary solid mass weight was calculated to be 0.627 g. Consecutively, deionized water is added to reach 30 g of total mass.

Corrosion resistant PTFE inlays are entered into the reactors, placing a rubber O-ring onto the reactor and adding the magnetic bar inside. Then, the prepared reactors are screwed into the corresponding reactor lit and control enclosure and computer must be turned on.

The reaction was stopped immediately in iced water after the set duration. When the temperature is low enough (approx. 40ºC), depressurization must be carried out by opening the outlet valves of the reactors.

For the filtration, a metal tissue (mesh size = 250 μm) is used as a filter. The filter is folded to from a cone and placed in funnel in 50 mL Falcon tube. The reaction mixture is poured on the filter. Consequently, funnel and filter are transferred onto a waste container and the solids are washed with deionized water onto the filter. Finally, solids are transferred into an aluminum pan using a spatula.

In order to do the filtration again for another sample, the filter and the funnel must be clean with deionized water and dried with the air gun.

The fines were recovered by centrifugation. Centrifugation of the 50 mL Falcon tubes is required to separate fines from hydrolysate. The samples are centrifugated at 4500 rpm for 10 minutes at 25ºC. After centrifugation, the supernatant is transferred into 15 ml tubes. The falcons are stored in the fridge at 8ºC to bring them to the central laboratory for the compositional analysis.

The solids from the aluminum pans must be dried in a convective oven at 40ºC for at least 24 hours in order to be correctly analyzed. Apart from that, the 50 mL falcon tubes are placed into the oven at 105ºC for more than 6 hours in order to weight the fines.

3.3 Analytical methods

3.3.1 HPLC

At the central laboratory of Hamburg University of Technology (TUHH), High Performance Liquid Chromatography (HPLC) were used to analyze hydrolysate composition.

Aqueous samples are centrifuged and directly analyzed by an HPLC system coupled to a refractive index detector to determine free carbohydrates such as cellobiose, glucose, xylose, arabinose and degradation products like HMF and furfural.

After determinizing the monomers concentration and in order to detect oligomers, bound carbohydrates are extracted with acid hydrolysis (4% H_2SO_4) beforehand. Separation is performed with an ion exclusion HPLC phase column.

Thus, monomers concentration is considered to be the concentration of xylose plus the concentration of arabinose in the first sugars determination, before the acid digestion. On the other hand, the second sugars determination is used to quantify the whole concentration of hemicellulose sugars (xylose and arabinose) in the hydrolysates.

3.3.2 pH measurement

For the determination of the hydrolysate's pH value, a handheld pH-meter (*WTW pH340*) was used. Measurements were conducted twice. Additionally, pH measurements were required for the preparation of the buffers.

3.3.3 Analytical balance

The lab balance is designed to measure small mass in the miligram range.

The samples are weighted into the inlays directly on the lab scale with an accuracy of +/- 0.01 g. The scale is also used to weigh the dried vessels and the fines from the hydrolysates in order to calculate the degree of solubilization of the samples.

3.4 Parameters calculation

The degree of solubilization *DS* is based on the solid mass after the LHW pretreatment m_S, including the fines in the aqueous phase in the Falcon tubes, and the initial mass of dry biomass m_{0_dry}. The dry biomass m_{0_dry} is calculated considering the moisture content x_{H2O} (12.6 %wt) of the initial weighed biomass m_0, see equations 4 and 5:

$$DS = \frac{m0\_dry\ -\ m_s}{m0\_dry} \quad (4)$$

$$m0\_dry = m_0 * \left(\frac{1\ -\ xH2O}{1}\right) \quad (5)$$

For the evaluation of the hydrolysates, the yield of furfural *Y_furfural* and the yield of the formed acetic acid *Y_acetic acid* during the LHW pretreatment are calculated following equations 6 and 7. *V* refers to the total volume of the reactor in L (0.03 L).

When acetate buffer was used, the concentration of the formed acetic acid *C_acetic acid (formed)* in the hydrolysates has to be defined according equation 8. The buffer concentration is *C_acetate buffer.*

$$Y\_furfural = \frac{V\ *\ C\_furfural}{m\_dryBM} \quad (6)$$

$$Y\_acetic\ acid = \frac{V\ *\ C\_acetic\ acid\ (formed)}{m\_dryBM} \quad (7)$$

$$C\_acetic\ acid\ (formed) = C\_acetic\ acid\ -\ C\_acetate\ buffer \quad (8)$$

The furfural selectivity *S_furfural* in the hydrolysates was evaluated according to the concentration of furfural *C_furfural* relative to total hemicellulose concentration *C_HC*, see equation 9. The sum of xylose and arabinose concentrations were considered as hemicellulose concentration *C_HC.*

$$S\_furfural = \frac{Y\_furfural}{Y\_HC} = \frac{C\_furfural}{C\_HC} \quad (9)$$

The recovery of hemicellulose *R_HC* is also calculated according to the mass of hemicellulose (HC) in the hydrolysates m_{HC^L} relative to the initial mass of HC in the

biomass $m^{S}_{HC,0}$, see equation 10. The initial composition of HC $w_{HC,0}$ is 28 %wt. Furthermore, the yield of HC *Y_HC* is defined according equation 11.

$$R\_HC = \frac{m^{L}_{HC}}{m^{S}_{HC,0}} = \frac{V * C\_HC}{m\_dryBM * w_{HC,0}} \quad (10)$$

$$Y\_HC = \frac{V * C\_HC}{m\_dryBM} \quad (11)$$

Additionally, the pH values of the hydrolysates can be predicted and calculated (equation 12) in order to be compared with the measurements of pH values. In the hydrolysate solution acetic and formic acid are found. Most weak acids barely dissociate in solution and the H^+ concentration can be calculated with the following equation 13 [24]. The acid constants (*K_aAA* for acetic acid; *K_aFA* for formic acid), which can be calculated with the pKa values according equation 14, and concentrations of both acids (*C_AA*; *C_FA*) in the hydrolysates are required to calculate H^+ concentration.

$$pH = -log[H+] \quad (12)$$

$$[H+] = \sqrt{K\_aAA * C\_AA + K\_aFA * C\_FA} \quad (13)$$

$$K\_a = 10^{-pKa} \quad (14)$$

3.5 Error calculation

Multiple determinations and experiments have been conducted for the calculation of the parameters as well as for the sugar analysis. The empirical standard deviation and mean values were calculated using equations 15 and 16. For the calculation of the statistical error of dependent factors, the standard deviation for the independently calculated values were used.

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n} \tag{15}$$

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}} \tag{16}$$

Error bars on the plots of this work represent the uncertainty or variation of the corresponding coordinate of the point, communicating how spread the data are around the mean value and how accurately the mean value represent the data.

For standard experiments hydrolysates, a duplicate of the same conditions was analyzed. By contrast, for concentration series, only one hydrolysate was analyzed. As a consequence, there is no possibility to provide real error bars. All the experiments in this block were conducted for a duration of 20 minutes, using a buffer of pH 4. For this reason, for each parameter it will be assumed the same relative error than the standard experiments of 20 minutes of residence time.

As the previous block of experiments, only one hydrolysate for each experiment of pH series was analyzed by the central laboratory and no error bars are provided in the plots.

4 Results and discussion

In this chapter, solids are weight in order to calculate the degree of solubilization in the different samples. The hydrolysates analysis is based on the discussion of the hemicellulose, acetic acid and furfural concentrations and hydrolysates pH.

4.1 Standards

For the same conditions of temperature (200ºC) and pressure (50 bar), the HPLC results are shown in Table 5.

Table 5: HPLC results for standards

		Xylose	Arabinose	Xylose	Arabinose	Acetic acid	Furfural
	RESIDENCE TIME	Free sugar	Free sugar	Hydrolyses	Hydrolyses		
Nr.	min	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
1	5	110	170	2900	380	190	54
2	5	100	180	3000	360	180	52
3	10	350	150	3300	280	330	150
4	10	340	170	3400	300	330	140
5	20	470	110	2500	200	400	170
6	20	720	140	3200	230	430	270
7	30	1200	86	1800	100	570	610
8	30	1100	120	2200	150	500	440
9	50	710	<50	740	<100	650	890
10	50	840	61	1100	<100	540	620

Some sugars concentrations are under detention limits. It means the concentration is quite small that it can be considered that there is no production of this sugar. In these cases, in order to calculate monomers and oligomers concentrations, it will be considered to be zero.

Standards experiments were conducted four times. Nevertheless, there are two hydrolysates samples that are been analyzed for each experiment. These experiments results will be compared to previous standards experiments, used as a reference. However, in the reference experiments, the material was pelleted wheat straw and, on the other hand, in the experiments of this work, the material was cut wheat straw. Cut wheat straw is a material to be investigated at the institute. On the other hand, pelleted wheat straw has been investigated for long time. It is meaningful to compare both materials for same operational conditions in order to observe the effect of different materials on hemicellulose yield and recovery.

In Figure 10, the monomers concentration is plotted against residence time. It is suspected that using cut wheat straw as material, the monomers concentration is higher than using pelleted wheat straw. Nevertheless, the tendency of both curves follows the same pattern: there is a maximum of monomers concentration for a residence time of 30 minutes, which means that the hydrolyzation to monomers is increasing until a point (residence time of 30 minutes) where the monomers start to degradate.

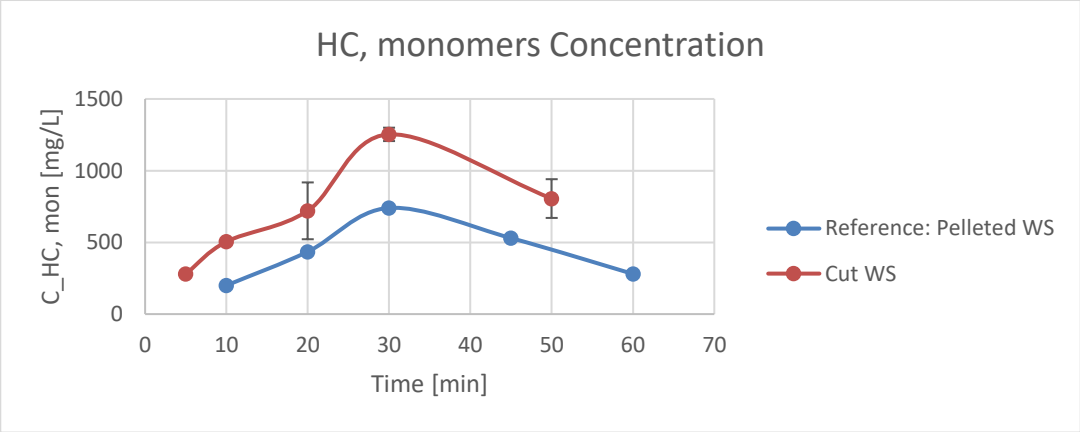

Figure 10: Monomers concentration against time in standards experiments

Nevertheless, there is no a significant difference in oligomers concentration if the used material is cut wheat straw instead of pelleted. In figure 11, oligomers concentration is displayed for different residence times. For both materials, there is a maximum at 10 minutes of residence time. For lower residence times, the oligomers formation is increasing and after this maximum at 10 minutes, oligomers start to hydrolysate to monomers.

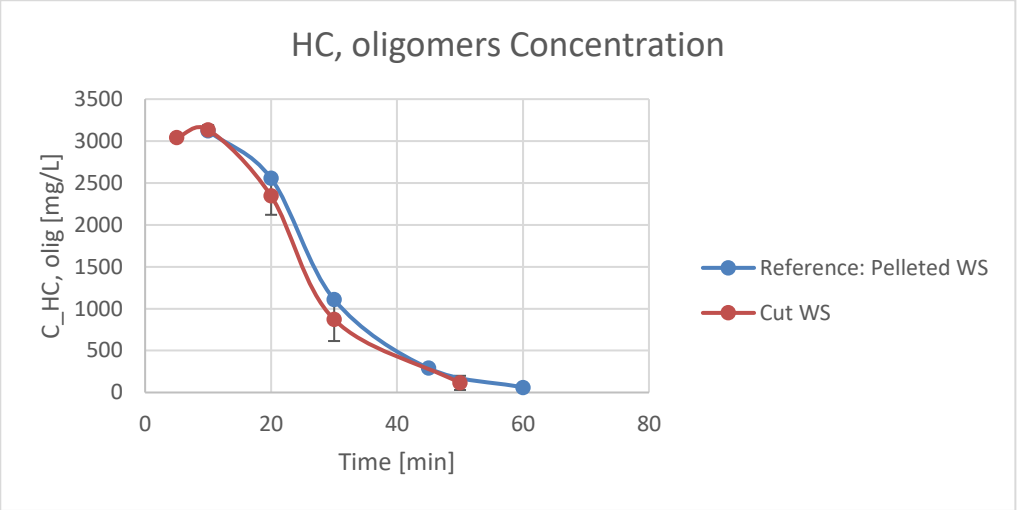

Figure 11: Oligomers concentration against residence time in standards experiments

In the following Figure 12, it is shown how the yield of hemicellulose decreases with time. However, the values for cut wheat straw experiments are slightly higher. The maximum corresponds to a residence time of 10 minutes, with almost 20% of yield. By contrast, the value of the reference maximum is around 16%. Before 10 minutes practically all hemicellulose sugars are newly formed oligomers.

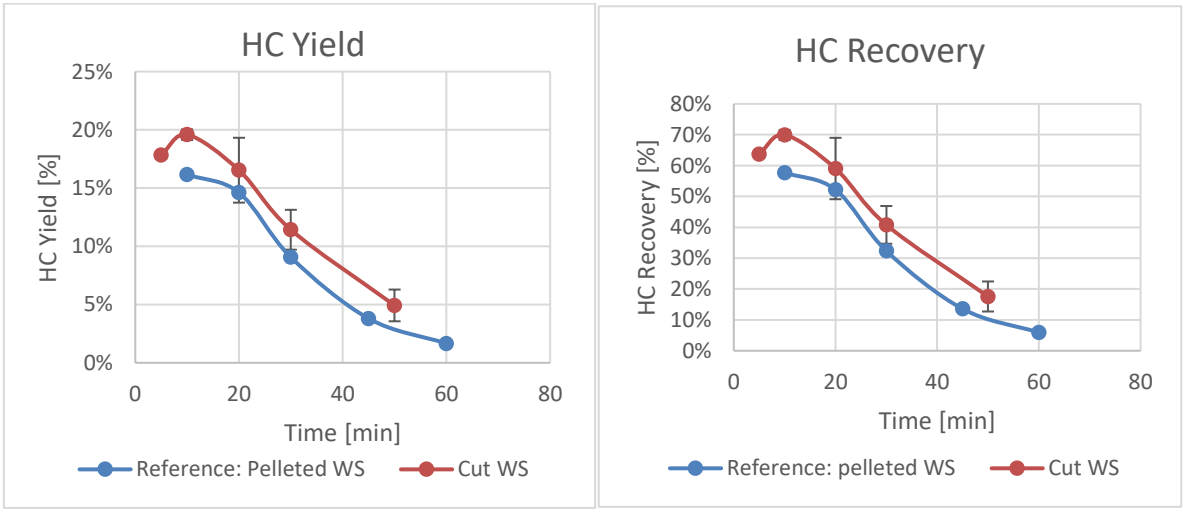

Figure 12: Hemicellulose yield against residence time in standards experiments (left); Hemicellulose recovery against residence time in standards experiments (right)

With cut wheat straw the recovery of hemicellulose after the LHW pretreatment reaches a maximum value of 70% and almost 60% for pelleted wheat straw. Therefore, cut wheat straw may be a better material to obtain more hemicellulose recovery than pelleted.

The release of acetic acid during the hemicellulose deacetylation is measured as the increase of acetic acid concentration that HPLC detects.

In figure 13, acetic acid yield is plotted against residence time. Considering error bars, the curve follows the same tendency as the reference: there is a point, around a residence time of 50 minutes, where the value of acetic acid yield remains constant, approx. 3% of yield.

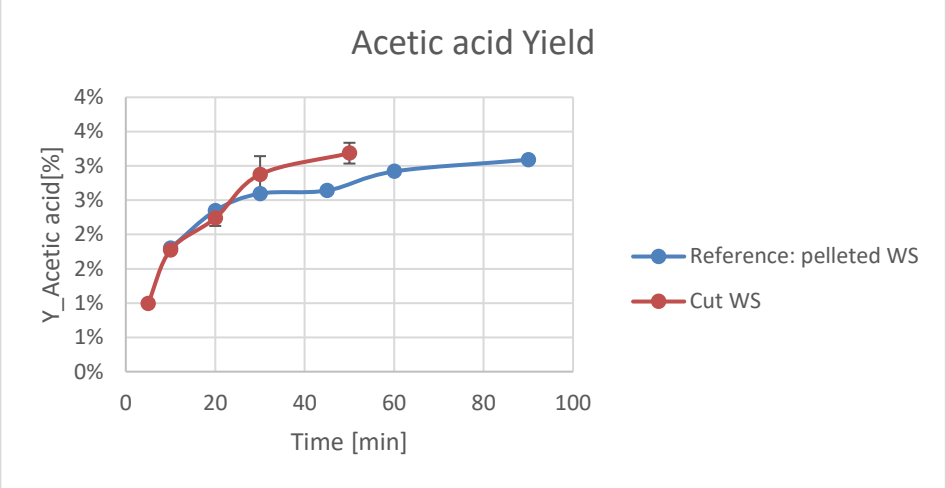

Figure 13: Acetic acid yield for standards experiments

Furfural is a degradation product and the measurement of the concentration in the hydrolysates is an important parameter to compare in order to, afterwards, check the effectivity of the buffers on the suppression of degradation reactions. Calculation of the yield and selectivity of this compound and the comparison of different experiment conditions is one of the main objectives of the work.

Furfural yield for standards experiments has been compared to the reference in Figure 14. Error bars are large enough and, as a consequence, the differences in values between the standards and reference experiments are not considerable enough. However, tendencies of the curves are similar: furfural yield increases with residence time because of the monomers degradation.

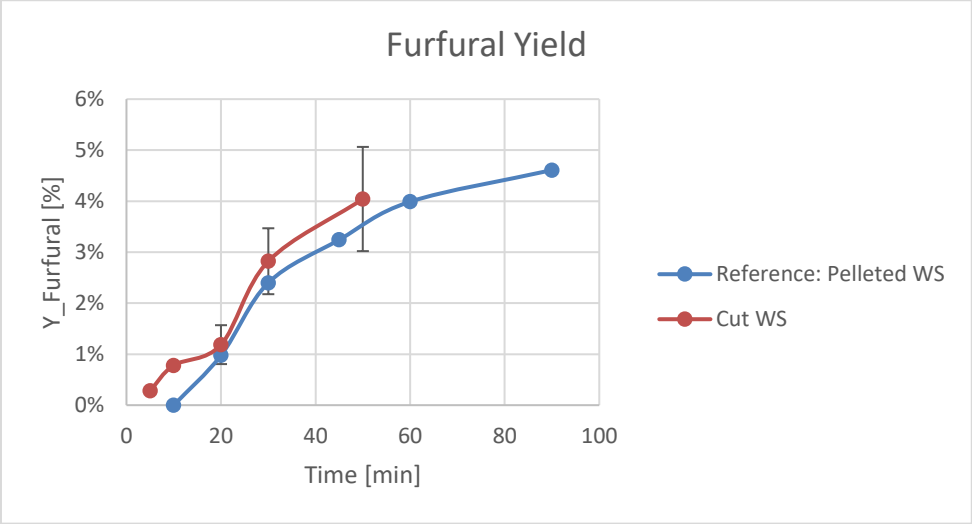

Figure 14: Furfural yield against residence time for standards experiments

A primary goal of this process is to completely solubilize hemicellulose and separate it from the rest of the solid material. Both hemicellulose and part of the lignin are solubilized by LHW pretreatment. For this reason, degree of solubilization has to be analyzed.

In Figure 15, degree of solubilization (DS) is plotted against residence time for both different materials. Considering the error bars, there is no a significant difference from the values of the reference material DS. Both curves follow the same tendency: DS increases with residence time until around 50 minutes of residence time where it is suspected there is no change in DS value for higher residence time, reaching a maximum value of approx. 40%.

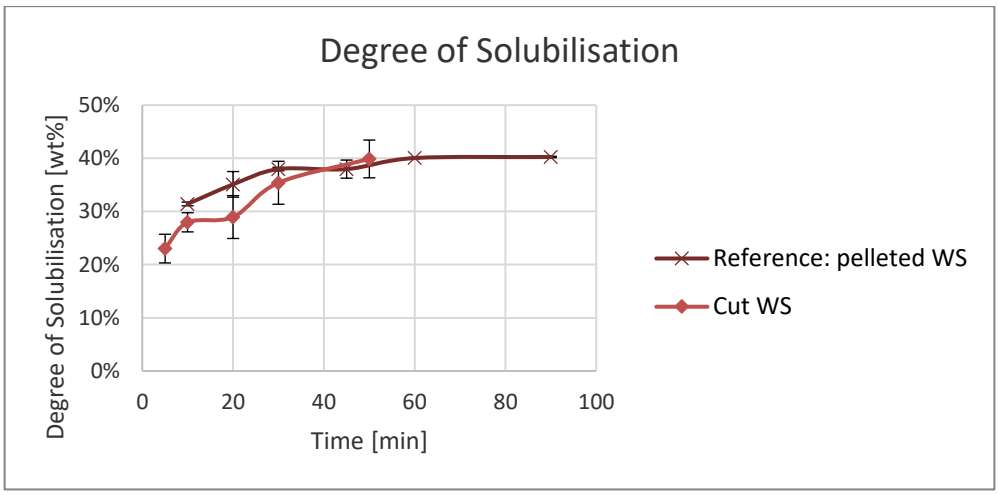

Figure 15: Degree of solubilisation of standards experiments against residence time.

Figure 16 shows a series of solid samples for different residence times. The increase in color intensity is observed. Solid sample of 50 minutes of residence (right) time is much darker than solid sample of 5 minutes (left).

Figure 16: Photograph of standards solid samples for different residence times: 5 mins (left); 10 mins (middle-left); 20 mins (middle); 30 mins (middle-right); 50 mins (right)

In order to know the exact value of the components concentration of the hydrolysates for a specific value of its yield, Figure 17 displays this data:

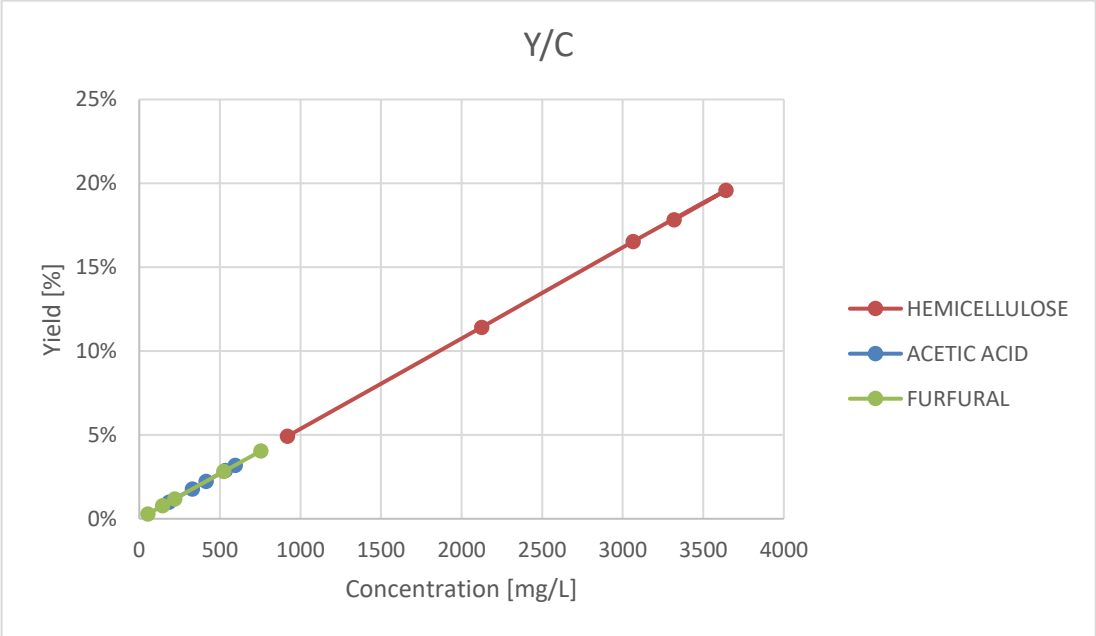

Figure 17: Proporcional relationship in relation to the concentration and the yield of the main components of the hydrolysates

4.2 Concentration series

For the same conditions of temperature (200ºC), pressure (50 bar) and residence time (20 minutes), concentrations of hemicellulose sugars, acetic acid and furfural are shown in Table 6.

Table 6: HPLC results for concentation series

		Xylose	Arabinose	Xylose	Arabinose	Acetic acid	Furfural
	BUFFER TYPE AND BUFFER CONCENTRATION	Free sugar	Free sugar	Hydrolyses	Hydrolyses		
Nr.		**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**
1	Citrate buffer (0.05 M)	220	<50	470	<100	790	510
2	Citrate buffer (0.01 M)	160	<50	2400	150	110	94
3	Citrate buffer (0.005 M)	760	110	2700	150	500	390
4	Acetate buffer (0.05 M)	340	<50	1800	100	3500	280
5	Acetate buffer (0.01 M)	610	110	2700	160	1000	290
6	Acetate buffer (0.005 M)	480	97	2800	140	760	230

Hemicellulose sugars concentration is plotted against the buffer concentration in Figure 18 (monomers concentration) and in Figure 19 (oligomers concentration). For both buffers it is observed that, in general, sugar concentration decreases with the concentration of the buffer. Therefore, higher concentration of acids (buffers) leads to catalyze hydrolyzation to monomers, and consequently, degradation reactions.

To see an effect using citrate buffer in order to compare the sugars concentration with the standards it is necessary at least 0.01 M of buffer concentration: monomers concentration is stabilized in a minimum value, while oligomers concentration keep on decreasing with buffer concentration, even faster than with acetate buffer.

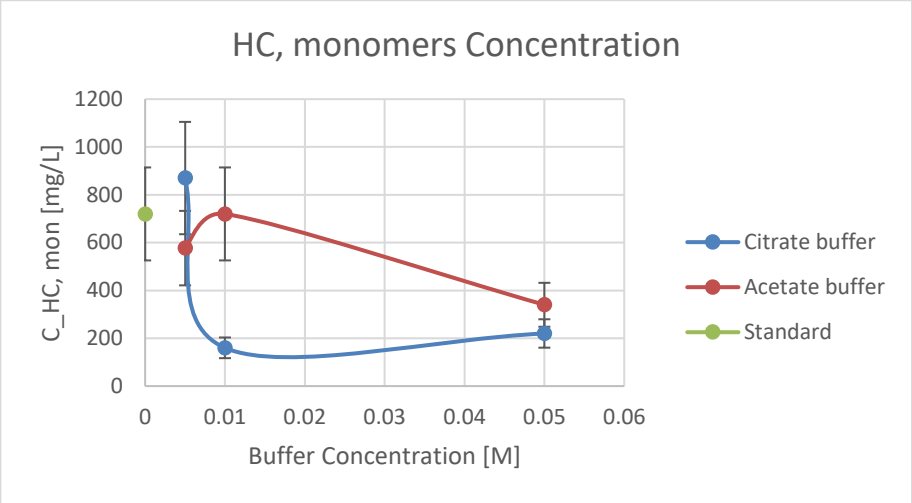

Figure 18: Monomers concentration against buffer concentration

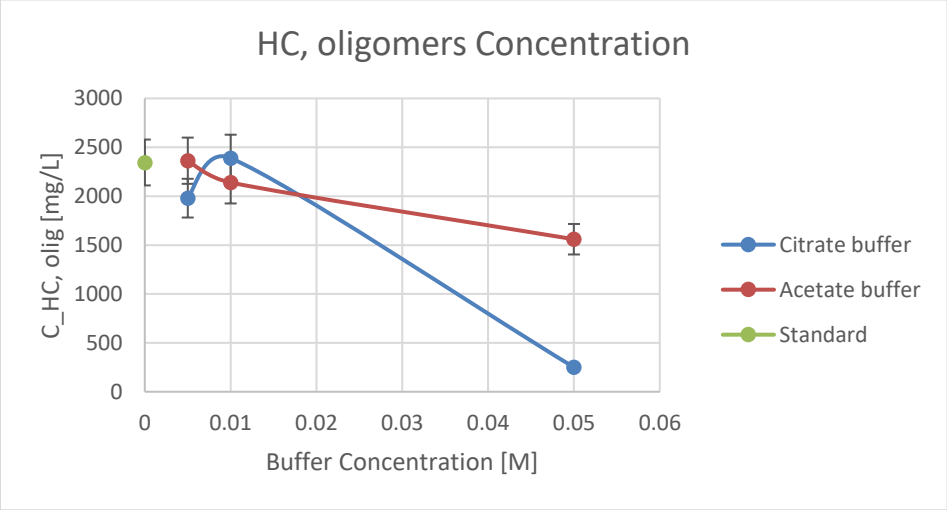

Figure 19: Oligomers concentration against buffer concentration

Similary, hemicellulose yield decreases with the buffer concentration. The lowest yield (less than 5%) is obtained using 0.05 M citrate buffer, the highest buffer concentration which has been investigated. See Figure 20. As a first conclusion for this block of experiments, the lowest concentration of buffer has to be chosen for the next block of experiments since higher concentrations leads to less hemicellulose yield.

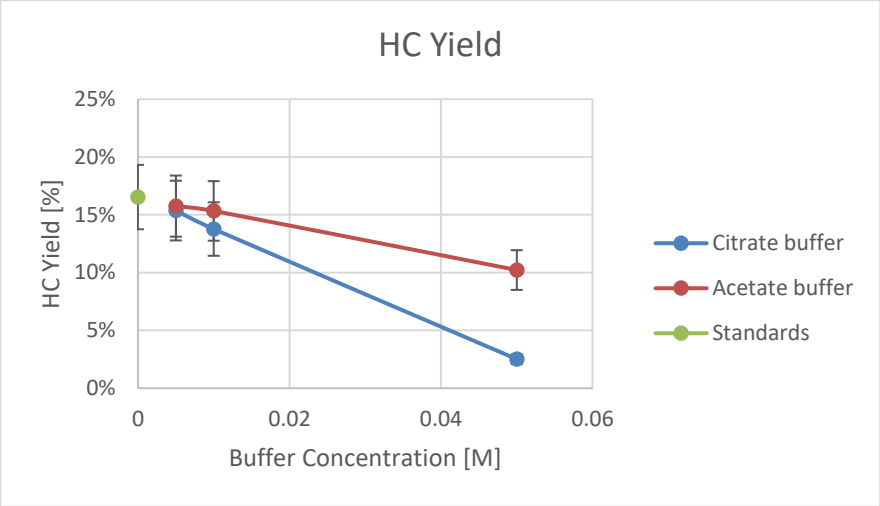

Figure 20: Hemicellulose yield against buffer concentration

The effect of both different buffers in the production of acetic acid is compared in Figure 21. Acetic acid yield using an acetate buffer there is a small relative change for different buffer concentrations, similar to the yield obtained without using any buffer. By contrast, the behavior of the citrate buffer is different: there is a drop in the yield until a concentration of buffer of 0.01 M, then the acetic acid yield increases with buffer concentration. However, the curve for citrate buffer is confusing in order to find an explanation.

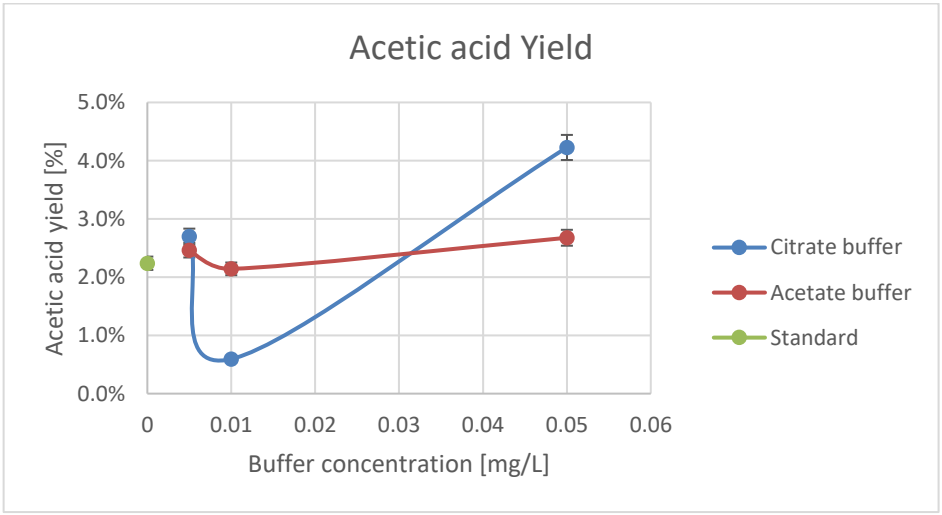

Figure 21: Acetic acid yield against buffer concentration

In order to evaluate the dependence of furfural formation with buffer concentration, Figure 22 displays the selectivity of furfural against buffer concentration for the used buffers in this work: acetate and citrate buffers.

For acetate buffer, there is no dependency with the buffer concentration and selectivity has the same value than the standard experiment. On the other hand, experiments conducted with citrate buffer show a drop in furfural selectivity between 0.005 M and 0.01 M concentrations, afterwards, a rise until the highest buffer concentration (0.05 M) is observed. If furfural selectivity is higher than the standard leads to none suppression of degradation reactions, which is not achieving the objectives of this work.

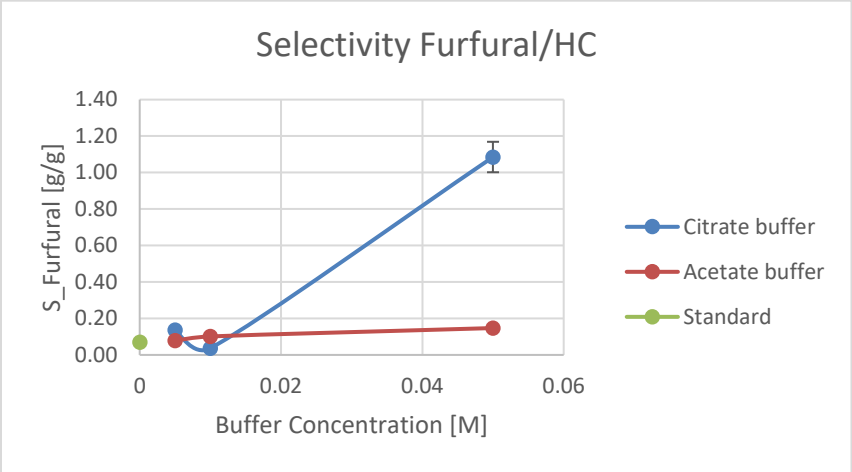

Figure 22: Selectivity of furfural against buffer concentration

The effect of buffer concentration in the degree of solubilisation is compared in Figure 23. It is suspected that the concentration in acetate buffer did not affect. Nevertheless, using higher concentrations of citrate buffer, the degree of solubilisation tends to decrease.

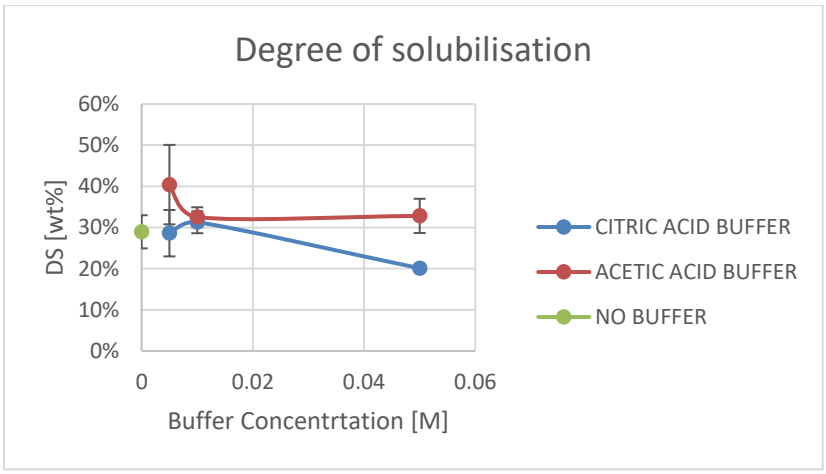

Figure 23: Degree of solubilisation against buffer concentrations

4.3 pH series

For this block of experiments, acetate buffer with a concentration of 0.005 M was the chosen type of buffer. All the experiments were carried out at 200ºC and 50 bar. In table 7, a summary of the most important compounds concentrations of HPLC results are shown.

Table 7: HPLC results for pH series

			Xylose	Arabinose	Xylose	Arabinose	Acetic acid	Furfural
	RESIDENCE TIME	pH	Free sugar	Free sugar	Hydrolyses	Hydrolyses		
Nr.	min	-	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
1	5	4	120	140	2900	310	520	<50
2	10	4	200	140	3100	300	570	92
3	30	4	900	76	1800	<100	860	500
4	50	4	620	<50	810	<100	820	610
5	5	5	<50	<50	1600	210	1700	<50
6	10	5	<50	<50	1700	160	1600	<50
7	20	5	100	<50	2100	<100	1800	98
8	30	5	120	<50	1700	<100	1700	85
9	50	5	160	<50	390	<100	1800	280

Hemicellulose sugars concentration curve (which is plotted against residence time in Figure 24 for monomers and in Figure 25 for oligomers) for pH 5 buffer behaves differently than the standards. With an initial pH of 5 (using the pH 5 buffer), the reactions speeds seem to be slower, and the max peak of oligomers concentration is later (at 20 minutes of residence time) than with the standards. Less concentration of monomers is found, being practically constant for residence times higher than 20 minutes. It is suspected that monomers degradation is small.

By contrast, using pH 4 buffer, the curves tendencies for monomers and oligomers concentrations are similar to standards. With an initial pH of 4, the speed of the monomer degradation is faster, which explains that the monomer concentration is lower than the standards while the oligomers concentrations are similar.

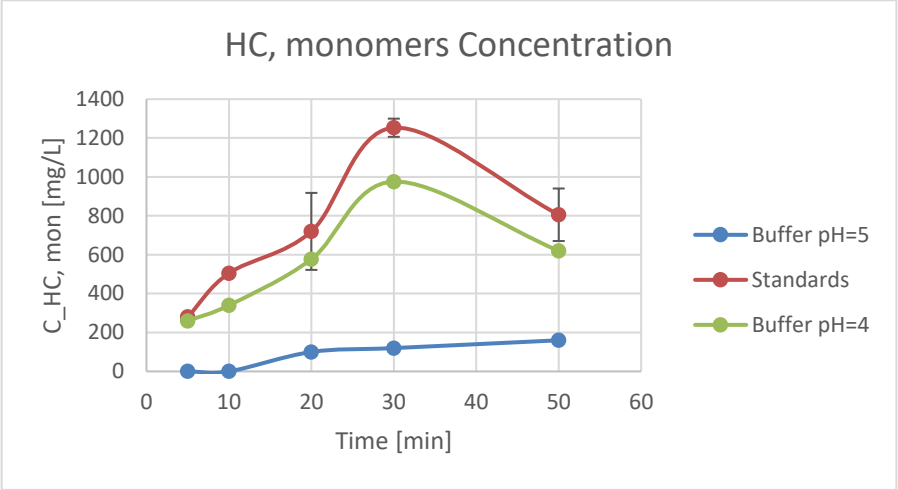

Figure 24: Monomers concentration against residence time for different pH buffers

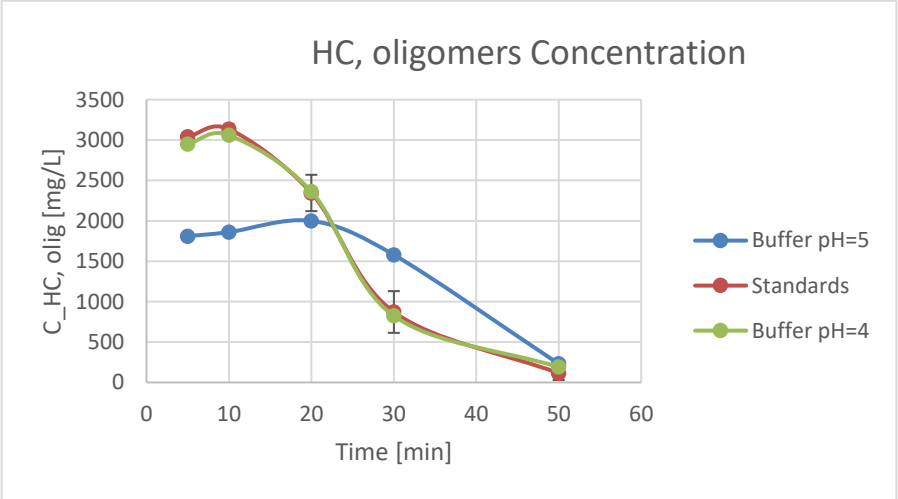

Figure 25: Oligomers concentration against residence time for different pH buffers

In this way, when the hemicellulose yield is displayed against the residence time, shown in Figure 26, it is observed that the yield is slightly lower when the pH 4 buffer is used, but much lower if the pH 5 buffer is used instead, mostly for times under 30 minutes. Since 30 minutes, oligomers concentration is higher for experiments with the use of pH 5 buffer, compared to the standards, but the hemicellulose yield is not higher because of the low concentration of monomers.

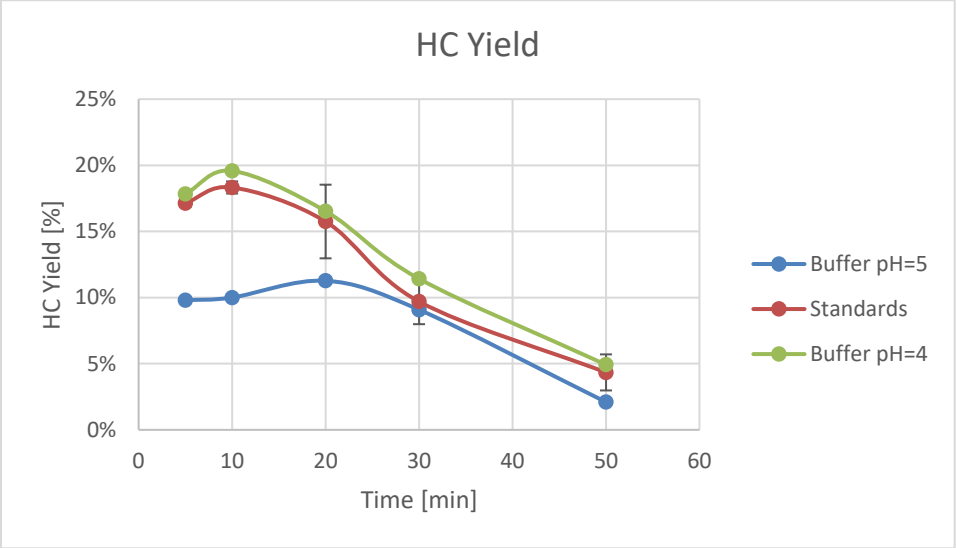

Figure 26: Hemicellulose yield against residence time for different pH buffers

When an acetate buffer of pH 5 is used insted of a pH 4 buffer, acetic acid yield is, unexpecetdly, much higher and constant with residence time, around 8%, as it is shown in Figure 27.

On the other hand, the tendency of the curve that respresents the dependency of acetic acid yield with residence time shows that the acetic acid yield increases with residence time until a value of around 3% at 30 minutes of residence time, and then remains constant for higher residence times. Same behaviour as standards experiments.

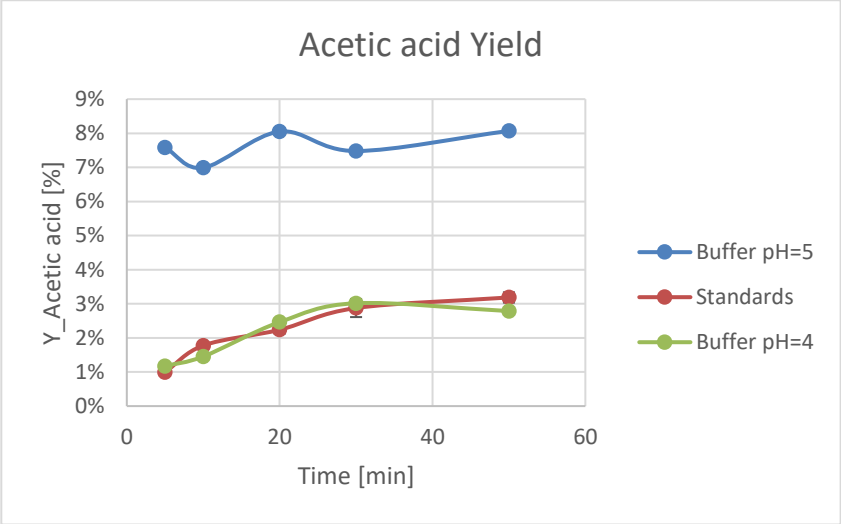

Figure 27: Acetic acid yield against residence time for different pH buffers

Furfural yield is reduced with the use of pH 5 buffer as it is shown in Figure 28. By contrast, pH 4 buffer seems to not have an effect in the formation of furfural. However, furfural selectivity with the use of pH 5 does not appear to show significant differences compared with standards experiments. See Figure 29.

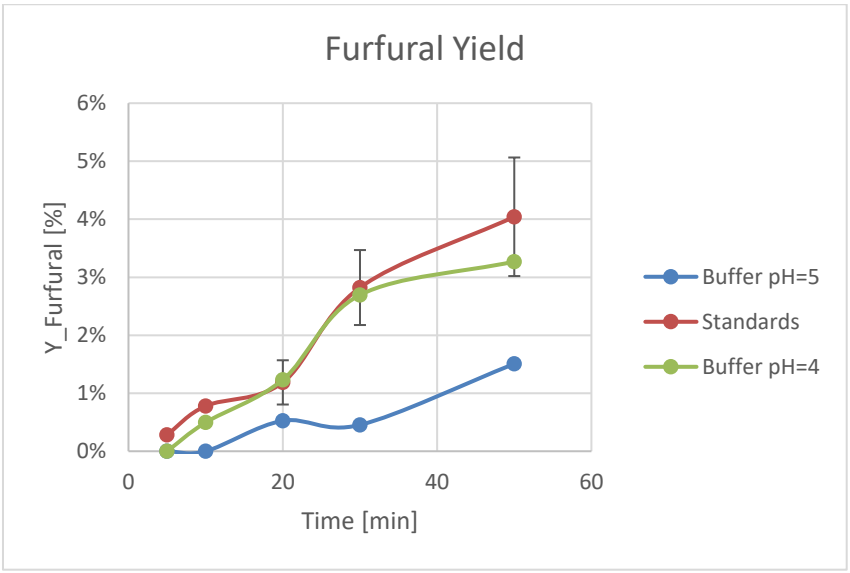

Figure 28: Furfural yield against residence time

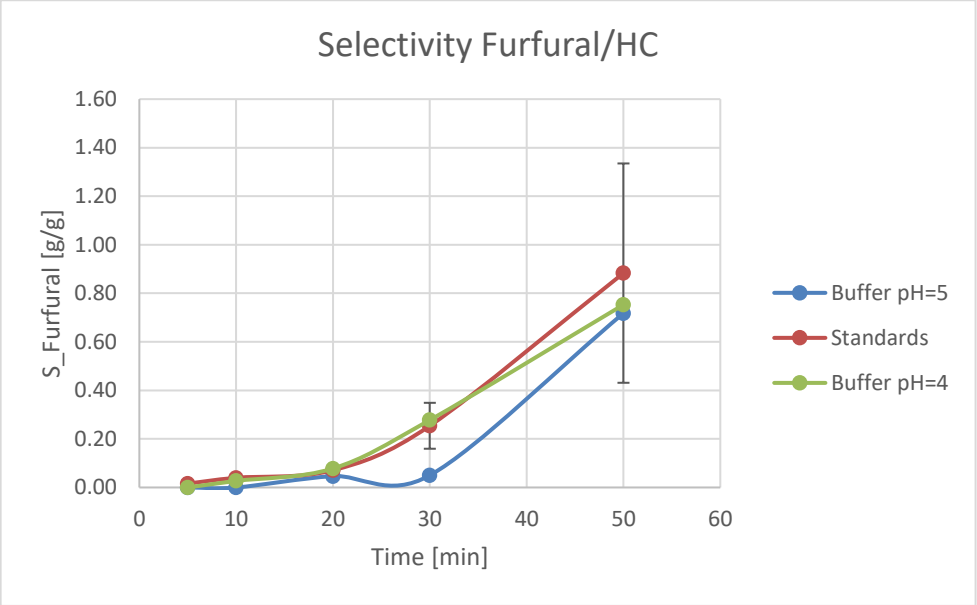

Figure 29: Furfural selectivity against residence time

The resulting degrees of solubilisation of the different experiments are shown in Figure 30. Nevertheless, considering the error bars, there can be no assurance that pH-buffers affect in the degree of solubilization.

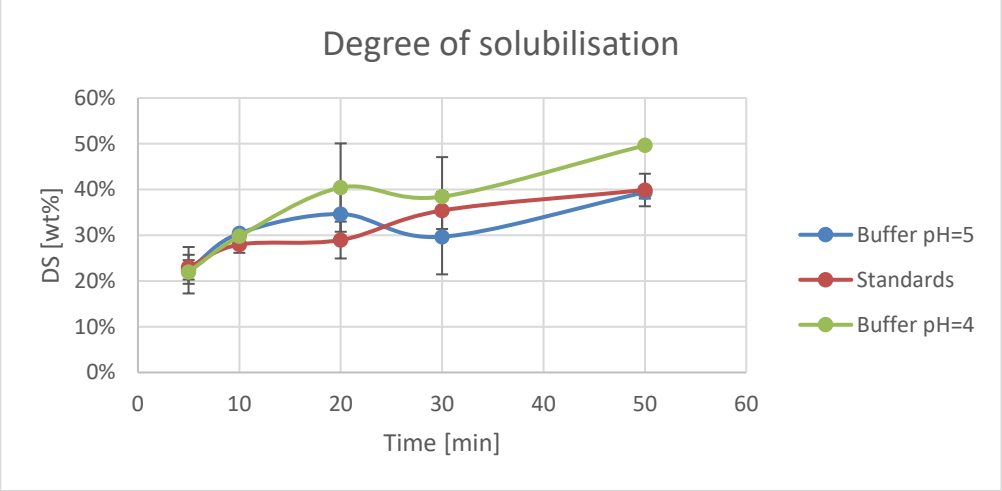

Figure 30: Degree of solubilisation against residence time using buffers

4.3.1 Hydrolysates pH values

After LHW pretreatment, filtration and centrifugation of the samples, pH of the hydrolysates is measured.

In Figure 31, these pH values are plotted against the residence time.

Regarding to standards pH values, which the initial pH is neutral (pH of pure water), these values decrease with residence time. On the other hand, experiments with buffers as medium are expected to keep the pH constant. Nevertheless, although the pH drop is not that sharp like with the standards, it is appreciated that the pH drops with time for both buffers systems. However, using pH 5 buffer (exact pH=5,17), pH values are more different from the standards than using pH 4 buffer (exact pH=4,25), achieving a higher pH of the hydrolysates even for high residence times.

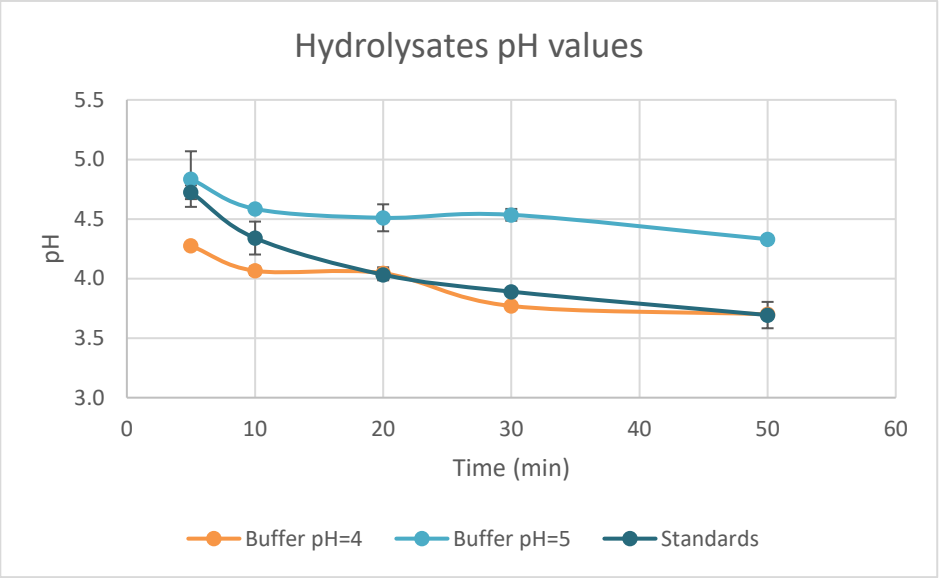

Figure 31: Hydrolysates pH values against residence time

Furthermore, pH values were predicted and calculated using the equation 13 from the previous chapter. Acetic and formic acid concentrations data from HPLC analysis are needed in the equation.

Predicted pH values are compared to real values in the following Table 9. It is observed that predicted values are always lower than measured ones. It can be explained by the fact of the presence of minerals, ash and impurities in the biomass material that could neutralize the acids, and as a consequence, the pH will be slightly higher.

Table 8: Predicted and real hydrolysates pH values

Experiment conditions	pH	Predicted pH
	[-]	[-]
Acetate buffer (pH=4); t=5 min	4,28	3,20
Acetate buffer (pH=4); t=10 min	4,07	3,16
Acetate buffer (pH=4); t=20 min	4,05	3,10
Acetate buffer (pH=4); t=30 min	3,77	3,01
Acetate buffer (pH=4); t=50 min	3,70	3,06
Acetate buffer (pH=5); t=5 min	4,84	3,07
Acetate buffer (pH=5); t=10 min	4,59	3,06
Acetate buffer (pH=5); t=20 min	4,51	2,95
Acetate buffer (pH=5); t=30 min	4,54	2,96
Acetate buffer (pH=5); t=50 min	4,33	2,87
Standard; t=5 min	4,73	3,25
Standard; t=10 min	4,34	3,14
Standard; t=20 min	4,03	3,12
Standard; t=30 min	3,89	3,06
Standard; t=50 min	3,69	3,08

5 Conclusions

Subject of this thesis was the selection of pH-buffers systems to minimize the formation of degradation products during LHW pretreatment. The selection was based on published theorical studies about the effect of pH on hemicellulose solubilization and xylose degradation.

Acetate and citrate buffers were chosen and investigated for different experiments conditions, differing in the buffer concentration and pH.

Standards experiments were conducted in order to compare cut wheat straw material to a reference material (pelleted wheat straw). All the experiments carried out using cut wheat straw resulted in favorable hemicellulose yield and recovery, due to an increase of hemicellulose monomers concentration in the hydrolysates. The maximum concentration of hemicellulose monomers for both materials takes place at 30 minutes of residence time. On the other hand, the max peak for the hemicellulose yield (19.6 % for cut WS; 14.6 % for pelleted WS) and recovery (69.9 % for cut WS; 57.7 % for pelleted WS) take place at 10 minutes of residence time.

The use of buffers is not feasible for hemicellulose yield. Furthermore, high buffer concentration affects unfavorably: the highest tested buffer concentration (0.05 M) led to a hemicellulose yield of 10.2 % for acetate buffer, and a value of 2.5 % for citrate buffer. On the other hand, at the same conditions, a standard experiment reached a 16.5 % of hemicellulose yield.

It was found that high concentrations of citrate buffer also have an effect on acetic acid yield and furfural selectivity: these both parameters are much higher than without the use of buffer, which proves that this type of buffer is noneffective to achieve the objectives of this work. Moreover, the degree of solubilization using a citrate buffer (0.05 M) resulted lower (20.1 wt%) than the standard experiment at the same conditions (28,9 wt%).

In the third block of experiments (pH series), different pH buffers were tested. Due to the lower furfural yield between 0 % and 1.5 %, it is suspected that 0.005 M acetate buffer with a pH of 5, may minimize the xylose degradation in furfural. By contrast, without the use of buffer, furfural yield reached a value of 3.3 % for the highest residence time (50 minutes). For this reason, pH 5 acetate buffers, in low concentrations, have to be considered as a reaction additive to minimize degradation reactions in order to optimize LHW pretreatments. However, furfural selectivity did not decrease enough in order to affirm this conclusion. In addition, acetic acid yield was significantly and especially high, around 7 %, with this type of buffer, while the maximum acetic acid yield for standard conditions was around 3 %.

LHW pretreatment is normally followed by an enzymatic hydrolysis. The treatment must result in a solid fraction suitable for the enzymatic hydrolysis for the production of glucose and a highly pure solid lignin. For its optimization, the pH has to be more basic. Acetate buffer of pH 5 was also used for this aim and it was proved that more basic hydrolysates are expected.

6 Future prospects

The results of this work represent an additional step towards the search and development of suitable reactions additives (pH-buffers, bases or acids) and process parameters to extract hemicellulose in a high yield without the formation of degradation products. Additionally, kinetic modelling using Arrhenius need to be done and different reactors must be tested.

This process can be used as a reference for approaches with the use of different pH-buffers since, to include the use of buffers in the further development, more experimental data is needed. In this research, it was found that high buffer concentration has a negative effect on the suppression of degradation reactions. In this way, considering low buffer concentrations, more and different type of pH-buffers should be investigated in order to find the optimal pH that minimize the degradation reactions.

Broadening the view to further methods than analyzed in this work, alternative methods may be considered. An extensive research is still required for the development of new and more efficient reactor configurations. Moreover, particle size of biomass plays an important role on the overall sugar recovery [10]. Consequently, it could be an interesting parameter to vary in order to investigate the effect on monomers degradation.

This approach might help to find optimal conditions of LHW pretreatment in order to improve its efficiency in the industrial cluster.

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