

HYDROTHERMAL TREATMENT OF BIOMASS IN HOT-PRESSURIZED WATER

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ABSTRACT

HYDROTHERMAL TREATMENT OF BIOMASS IN HOT-PRESSURIZED WATER

The aim of this study is to observe conversion of cellulose, which is the main compound of biomass, into its building-block chemicals in hot-pressured water as reaction medium with no addition of organic solvents. Water in liquid state under temperature and pressure above boiling point and below the critical point (374.15 °C and 22.1 MPa) is called as hot-pressurized water (or sub-critical water).

Nowadays the biomass has great attention across the World as renewable source of energy, at the background of the quickly growing energy demand, since it is widely available and cheap. This technology is totally environmentally friendly and uses water as a reaction medium. As well known, since plant biomass contains up to 50% cellulose, it was decided to use it as a model compound in this study. Decomposition of cellulose leads to formation of various compounds. Among them levulinic acid is the most attractive chemical. Moreover, this acid marked as “Top 12 Building Blocks” of most perspective chemicals and obtaining from biomass by hydrothermal treatment is not widely studied.

During this study, different reaction parameters such as temperature, pressure, reaction time and external oxidizer addition were studied to clarify their effects on cellulose decomposition and product yields to achieve the highest selectivity of the desired product. Addition of H₂SO₄ led to increase cellulose conversion up to 73% at 200 °C with a H₂SO₄ concentration of 125 mM at 60 min reaction time. Under same conditions, the yield of levulinic acid was successfully achieved to 38% after 60 min.

ÖZET

SICAK-BASINÇLI SUDA BİYOKÜTLENİN HİDROTERMAL BOZUNMASI

Bu çalışmanın amacı, biyokütlenin ana bileşeni olan selülozun, herhangi bir organik çözücü eklenmeden, sıcak-basınçlı suda organik yapıtaşlarına ayrılmasını gözlemlemektir. Kaynama noktasının üstünde, kritik noktanın altında basınç ve sıcaklığa sahip sıvı haldeki suya sıcak-basınçlı su (ya da kritik altı su) denilir. Suyun kritik noktası 374.15 °C ve 22.1 MPa'dır.

Günümüzde biyokütle, kolayca erişilebilir ve ucuz olduğu için hızla büyüyen enerji talebine karşılık yenilenebilir bir enerji kaynağı olarak büyük ilgi görmektedir. Biyokütlenin enerjiye dönüştürülmesinin tasarım aşamasında birçok uygulaması vardır. Biyokütlenin hidrotermal bozunması, bu uygulamalar arasında en muhtemel olanlardan biridir. Bu uygulama tamamen çevre dostudur ve tepkime ortamı olarak suyu kullanır. İyi bilindiği üzere, bitki biyokütlesinin %50 kadarını selüloz oluşturur ve bu nedenle selüloz bu çalışmada model bileşik olarak kullanılmak üzere seçilmiştir. Selülozun ayrışması birçok değişik bileşiğin oluşmasına yol açar. Bunlar arasında en çekici olan levulinik asittir. Ayrıca, bu asit "12 En Önemli Yapıtaşı" kimyasallarından biri olduğu için ve biyokütlenin hidrotermal bozunması üzerinde çok çalışılan bir alan olmadığı için yeşil kimya alanına bu konuda katkıda bulunmaya karar verdik.

Araştırma sırasında sıcaklık, basınç, tepkime süresi, oksidan eklenmesi gibi reaksiyon parametrelerinin selüloz bozunması ve ürün verimi üzerindeki etkileri, en yüksek hedef ürünün üretilmesi amacıyla çalışılmıştır.

*To my grandmothers Ozipa Abdulkarimova
and Darkhan Atabekova*

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CHAPTER 1

INTRODUCTION

We live in the period, when whole of the world have a problem with quickly growing energy demand, and in company with this, we understood that natural resources which are becoming expensive day by day could be finished in the future (Kruse et al. 2003). Furthermore, we perceived that to leave clean environment as legacy to the next generation we cannot go by our ancestors left path of energy production and consumption. All this problems lead us to find out alternative energy sources which must be clean in case of nature. Therefore, environmentally friendly technologies and fields of sciences are becoming new growing trend. To do this, governments of all countries trying to change their economics and all their industries to ecologically sustainable ways; sciences' academies and institutions are looking for “green” solutions to almost all problems, and new inventions in the first place must be environmentally friendly. In this case, importance of biomass as a renewable energy resource is growing every day (Yanik et al. 2007)

1.1. The Aim and the Importance of the Study

The present study is aimed to observe decomposition of cellulose as a model compound of biomass into its building blocks in subcritical water as a reaction medium without addition of any organic solvent. Subcritical water can be any temperature, pressure and time combination that is above the boiling point and below the critical point. In the case of water, the critical temperature is 374.15 °C and the critical pressure is 22.1 MPa. Since water is chemically stable, safe, harmless to environment, easily available, and cheap subcritical water is chosen as a reaction medium to degrade biomass into high-value chemicals (levulinic acid, 5-HMF, etc). During the degradation of cellulose, the effects of various reaction parameters such as temperature, pressure, acid concentration, reaction time, etc on the cellulose conversion, the product distribution and yields are being investigated.

Production technologies of high-value chemicals are well defined and mostly these technologies are very harmful in case of human being and nature. During these commercial processes expensive, hazardous substances are used and mostly highly-toxic byproducts are produced. Byproducts make problems with their utilization or disposition (e.g. ammonium sulfate in formic acid production) (Reutemann, W. and Kieczka, H. 2000). Therefore, other cheap and safer production methods are needed in designing stage and intensive research to develop a practicable production method.

Significance of this study is that biomass conversion in subcritical water could be one of those alternative green production methods. Renewable feedstock and safe, cheap reaction media make this method attractive not only in case of valuable organics production but also in the utilization and detoxification of organic residues and wastes. It is an ancillary advantage of subcritical water conversion of biomass. Generically, it helps to “kill two birds with one stone” – utilize organic waste, produce valuable chemicals in a harmless production method to nature.

We would like to make a contribution to this important sphere of green sciences and technology by optimization of reaction conditions and clarification of the reaction mechanisms in order to obtain high selectivity of desired product from biomass without using any organic, toxic solvents.

1.2. Biomass as a Renewable Energy Source

Biomass due to its organic content can be used as a raw material in many applications. Biomass energy or “bioenergy” is a field of science and industry that aims to obtain any kinds of energy and valuable products from biomass. There are many technologies and techniques, which can deal with converting biomass into different needs, from fuel for engines to electricity for daily consumption (Baskar C., Baskar S., Dhillon R.S. 2012). These technologies are kept developing, and trying to reach to their improvements by designing new supplements or new methods.

1.2.1. General Description and Classification of Biomass

The term “biomass” commonly refers to renewable organic substance produced by plants through photosynthesis process. As well known, during photosynthesis, plants

combine carbon dioxide from air and water from soil to form carbohydrates that are biochemical building blocks of biomass. The solar energy drives this process is accumulated in the chemical bonds of carbohydrates and other molecules included in the biomass. If biomass cultivated and harvested in a way such that allows subsequent growth with no draining nutrients and water, it is a renewable resource, with almost no additional contributions to greenhouse gas emissions on the Earth.

Biomass is a scientific term for living or recently dead (non-fossilized) organic substance. Additionally, the word biomass is also used to mark wide range of products derived from living organisms – plant parts such as stems, leaves, twigs, aquatic plants and wood from trees, as well as animal wastes. Materials, which have organic combustible stuff, are also referred under the term of biomass (Jenkins et al.1998)

Biomass can be used as a fuel directly or can be transformed through different ways into valuable form of fuel. Energy of biomass or “bioenergy” comprehends any solid, liquid or gaseous fuel, or useful chemical product, or any electric power derived from, organic matter, which comes from plants directly or plant-derived agricultural and forestry, urban, commercial or industrial wastes. It means that bioenergy can be produced by various methods and from wide range of raw materials. Bioenergy is generally considered as chain of many different feedstock-technology mixed combinations because there are wide range of potential feedstocks and the different technologies to produce and process them.

Biomass of all the Earth concentrated in a thin surface layer called the biosphere. It seems that represents only a tiny fraction of the total mass of the Earth, but in case of human it is a big store of energy, to be used as food and fuel. More essentially, it is a store that is continuously replenishing. The source that supplies the energy is solar energy and it is continuously converting into biomass. The yearly amount of biomass world is estimated at 146 billion metric tons, mostly from uncontrolled plant growth (Biomass Conversion. The Interface of Biotechnology, Chemistry and Materials Science; Editors: Baskar C., Baskar S., Dhillon R.S. 2012). Only 6% of the global production of biomass can provide energy that equal to current world need for oil and gas. Biomass is important heating fuel, and in some part of the world it is the widely used for cooking. This source of energy is beneficial in case of air pollution; it is not add any net carbon dioxide to the atmosphere.

Biomass has the following advantages:

- ✓ It is widely available.

- ✓ Technology of production and conversion is clearly understood.
- ✓ It is pretty appropriate for small or large applications.
- ✓ Production requires low light and utilization comparatively low temperature (535°C)
- ✓ Storage and transportation comparatively cheap.
- ✓ There is almost no pollution.

Biomass can be classified as:

- Agricultural and forestry remainders.
- Herbaceous crops.
- Marine and aquatic biomass (algae, aquatic plants, coral reefs etc.).
- Wastes (municipal sewage, solid wastes, and industrial wastes) (Baskar C., Baskar S., Dhillon R.S. 2012).

Biomass can be wet and dry. The moisture content depends on origin of biomass and condition of its storage. Dry biomass mainly has low moisture content (about 30 wt %). Wood, straw, or other sun dried wastes are examples of dry biomass. Other types of biomass can be classified as wet. For instance: algae suspensions, sugar solutions, sewage sludge, waste streams from biorefineries, and from biomass processing (Jenkins et al. 1998). 95% of total biomass has high humidity (Kruse et al., 2003).

1.2.1. Structure of Biomass

As it is previously mentioned, although biomass has different types, it is mainly plant derived. The plant-derived biomass or lignocellulosic biomass makes up approximately 50% of total biomass and its chemical composition strongly depends on its source (Thompson et al., 1979). Generally lignocellulosic biomass consists of 38-50% of cellulose, 23-32% of hemicelluloses and 15-25% lignin.

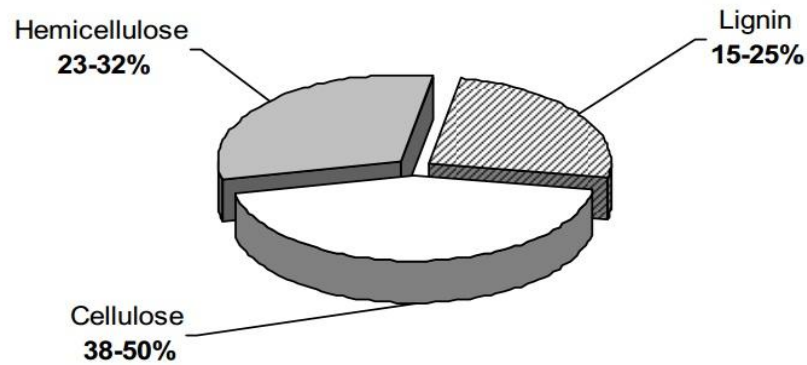


Figure 1.1 Biomass content
(Source: Girisuta 2007)

Cellulose is a polysaccharide made up of glucose units linked by β (1, 4)-glycosidic bonds (Figure 1.2.).

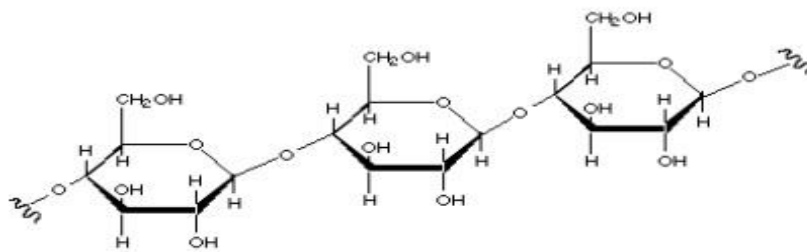


Figure 1.2 The structure of cellulose
(Source: Perez et al., 2002)

Cellulose is the most teeming biopolymer synthesized by nature and its amount is approximately 2×10^9 tons year (Sasaki et al. 2003). Hemicellulose is a polymeric material consisting hexoses (glucose, galactose and mannose) and pentoses (principally arabinose and xylose), and its molecular weight lower than cellulose (Figure 1.3)

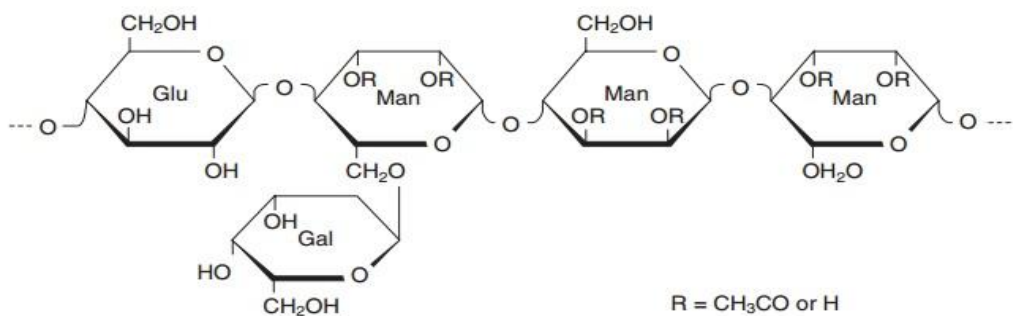


Figure 1.3 The structure of hemicelluloses
(Source: Perez et al., 2002)

Lignin is cross-linked polymer made from substituted phenylpropene units, as shown in Figure 1.4, and its function is to hold the cellulose and hemicellulose fibers together.

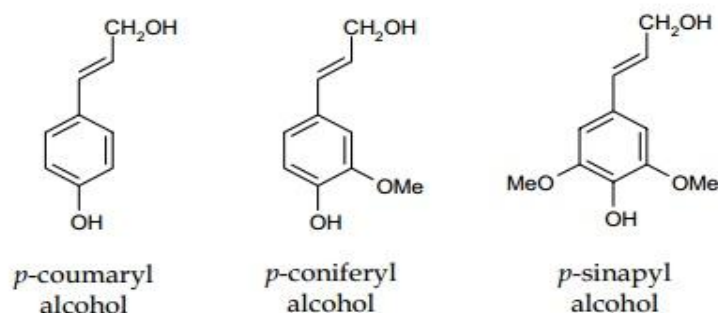


Figure 1.4 Phenylpropene units of lignin
(Source: Toor et al. 2011)

Biomass due to its nature has a various correlation of three main compound described before. The content of some types of biomass are displayed in Table 1.1.

Table 1.1 Typical biomass and waste compositions (% dry basis)
(Source: Toor et al. 2011)

Lignocellulosic materials	Cellulose	Hemicelluloses	Lignin
<i>Hard woods</i>			
White poplar	49.0	25.6	23.1
European birch	48.5	25.1	19.4
White willow	49.6	26.7	22.7
<i>Soft woods</i>			
White spruce	44.8	30.9	27.1
Monterey pine	41.7	20.5	25.9
Douglas fir	42.0	23.5	27.8
<i>Agricultural residues</i>			
Corn stover	37.1	24.2	18.2
Sugarcane bagasse	39.0	24.9	23.1
Wheat straw	44.5	24.3	21.3
<i>Other wastes</i>			
Newspaper	40–55	25–40	18–30
Swine waste	6.0	28	–
Switch grass	45	31.4	12.0
Dried distillers grains with solubles (DDGS)	–	–	–
Slaughterhouse waste	–	–	–

According to this data, it is understood that cellulose is the main compound of most of the plant-derived biomass, which allows us to use it as a model compound in experiments during this study.

1.3. Properties of Subcritical Water

The point at which the substance has indistinguishable phase called as critical point, and it happens under the certain condition such as temperature, pressure or both of them. Every substance due to its physical and chemical properties has a different critical point. When substance reaches its critical point properties of liquid and gas phases converge and make one – homogeneous supercritical fluid phase. The critical pressure is equal to vapor pressure at critical temperature. Also, there is critical molar volume that is molar volume of one mole of substance at critical point. The state of substance below the critical point called as “subcritical liquid”, whereas above the critical point it is referred as “supercritical fluid” (Cengel, Y. A., Boles, M.A. 2002). Some of substances’ critical temperatures and pressures are listed in Table 1.2.

Table 1.2 Substances and their critical temperature and pressure
(Source: Cengel, Y. A., Boles, M.A. 2002).

Substance	Critical temperature (°C)	Critical pressure (atm)
NH ₃	132	111.5
O ₂	-119	49.7
CO ₂	31.2	73.0
H	-239.95	12.8
C ₂ H ₅ OH	241	62.18
CH ₄	-82.3	45.79
H ₂ O	374	217.7

As it is well known, water can be in solid, liquid and gas phases. It means that physico-chemical properties of water such as dielectric constant, density, surface tension, enthalpy, etc. are changing depending on temperature or pressure. In addition to that water has high specific heat capacity. This phenomenon makes water useful in several applications. As it is mentioned before, the critical point of water is 374 °C and 22.1 MPa and below this point water has subcritical liquid state while above this point it is referred as supercritical fluid state (Hendry 2012). The main regions of water states along with temperature and pressure are shown in Figure 1.4.

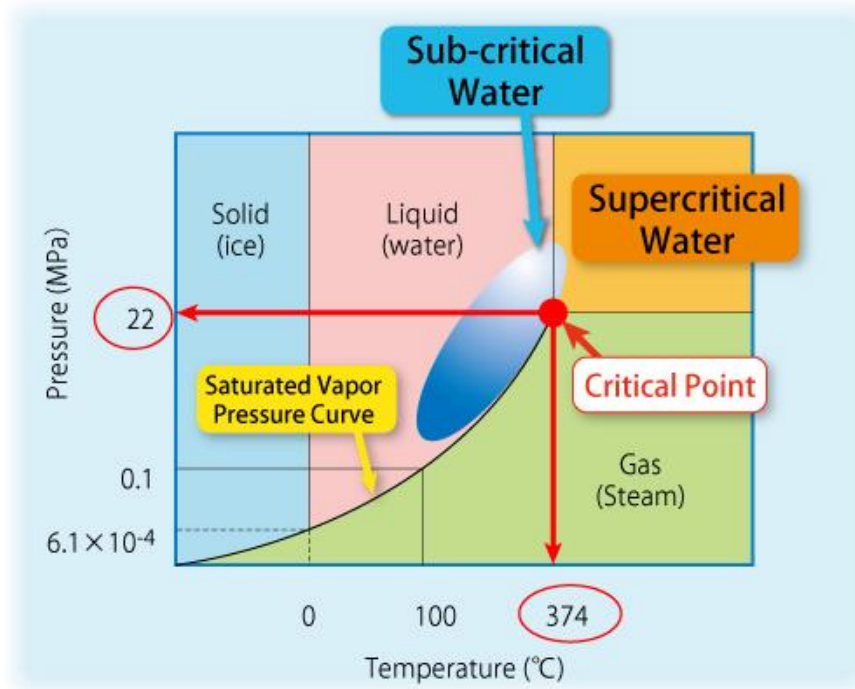


Figure 1.5. Critical point of water and its phases in different temperature and pressure (Source: <http://www.asiabiomass.jp/>; 15 March 2015).

Subcritical and supercritical water extensively used in many applications from supercritical fluid extraction, hydrothermal processing to degradation of hazardous wastes. Uniqueness of supercritical fluids is mainly arising from their gas-like and liquid-like properties. The properties like high diffusivity and low viscosity are gas-like, while high density which is abnormal to gaseous compound is liquid-like behavior. These characteristics changes are visible when we compare supercritical and ambient water. Values are given in Table 1.3.

Table 1.3. Characteristics of ambient and supercritical water
(Source: Hendry 2012)

Characteristics	Ambient Water	Supercritical Water
Dielectric constant	78	<5
Solubility of organics	Very low	Fully miscible
Solubility of gases	Very low	Fully miscible
Solubility of inorganics	Very high	Not soluble
Diffusivity (cm ² /s)	1x10 ⁵	1x10 ⁻³
Viscosity (g/cm-s)	1x10 ⁻²	1x10 ⁻⁴
Density (g/cm ³)	1	0.2-0.9

Substantial changes in the properties of water happen in the vicinity of critical point. For instance, near critical point water is highly compressible. Its density falls nearly two orders with no change in phase from liquid-like to dense gas-like when temperature increases from 300 to 450 °C. These changes reflect other properties like viscosity, polarity, solvation energy, degree of hydrogen bonding, dielectric strength, etc. (Peterson et al. 2008). The changes in thermodynamic properties of water with temperature is illustrated in Figure 1.5.

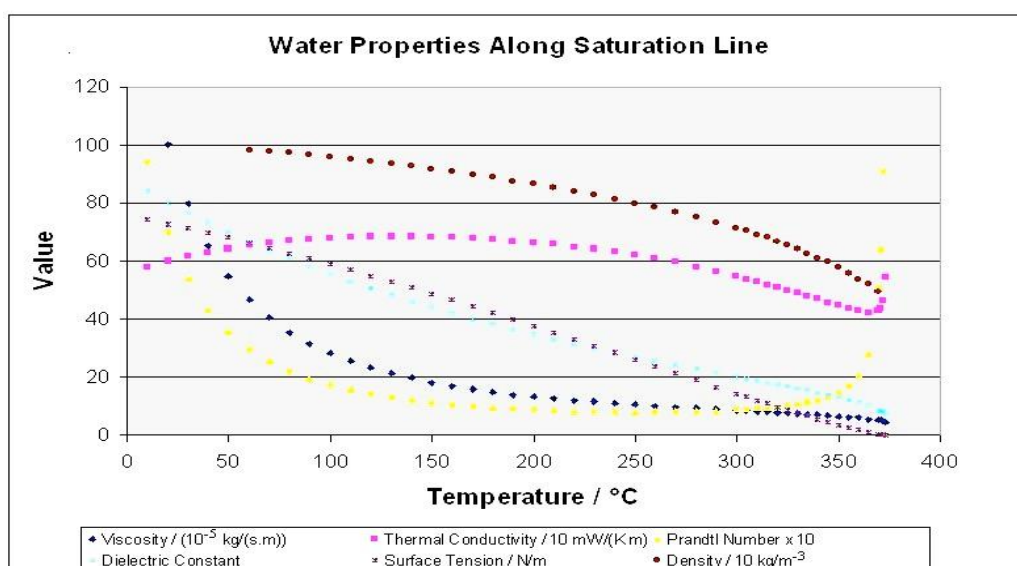


Figure 1.6. Changes of waters properties at different temperature
(Source: <http://www.criticalprocesses.com/>; 25 March 2015).

The characteristics of water such as hydrogen bonding make it a good non-organic solvent. Most of organic matters hardly make hydrogen bonds and that is why they do not dissolve easily in water. Polarity of water also plays an initial role. Charge differential within water molecule, one end has a positive charge and other end has a negative charge, has ability to attract other polar molecules, then helps to dissolve other polar substances. However, most of organic molecules are nonpolar so they cannot be dissolved in water. Hydrogen bonding is also important in heat capacity of water. If temperature of water is rising, the extensive hydrogen bonding interactions break down and extra energy is needed to put in to break the bonds. For example, at a constant pressure specific heat capacity changes from 4.8183 kJ/ (kg K) at 20° C to 8.138 kJ/ (kg K) at 350° C. When critical point is approached specific heat capacity is rising to infinity at constant pressure (Peterson et al. 2008). Graph of specific heat capacity is shown in Figure 1.6.

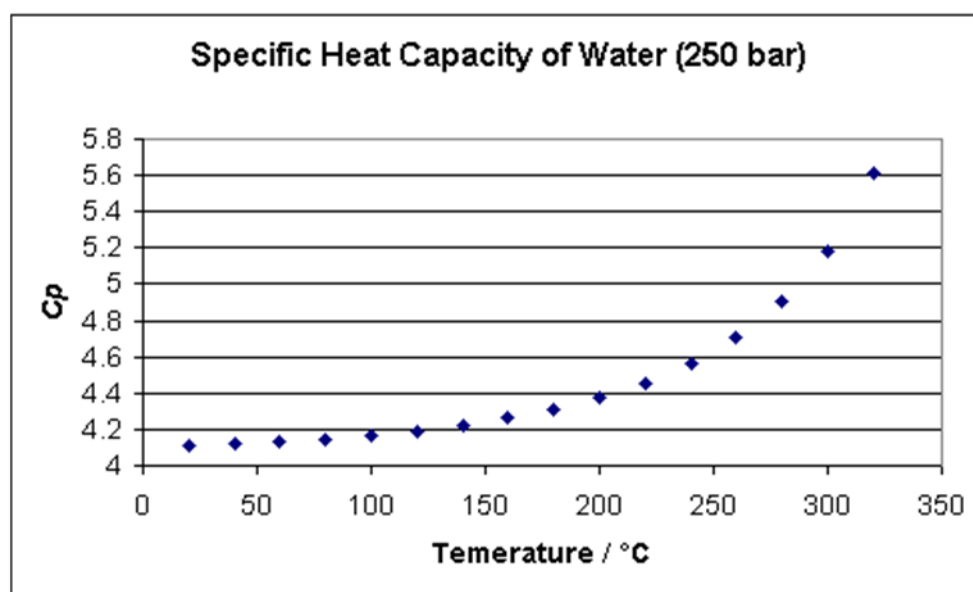


Figure 1.7. Specific heat capacity of water at constant pressure (250 bar)
(Source: <http://www.criticalprocesses.com/>; 25 March 2015)

Properties of subcritical and supercritical water are shown in Table 1.4. Thereby, water in subcritical and supercritical states show different characteristics. Both of them have their own special application depending on desired product.

Table 1.4. Some characteristics of supercritical and subcritical water
(Source: Ehara et al. 2005)

State	Temperature (°C)	Pressure (MPa)	Density (g/cm ³)	Ionic product log K_w (mol ² /l ²)	Dielectric constant
Supercritical water	400	40	0.5	-12.8	9.6
Subcritical water	280	40	0.8	-10.9	25.2

1.4. Levulinic Acid and 5-Hydroxy Methyl Furfural (5-HMF)

Levulinic acid, also known as 4-oxopentanoic acid, γ -ketovaleric acid or β -acetylpropionic acid which has formula C₅H₈O₃, is white crystalline, water soluble and polar organic solvent, member of keto acids. It has two reactive functional groups, have the ketone (-C=O) group at the third carbon from the carboxylic acid (-COOH) (Figure 1.7.).

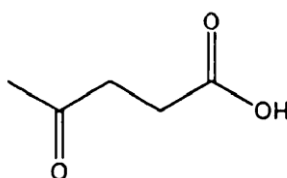


Figure 1.8. Levulinic acid
(Source: Rosatella et al. 2011)

The first time Levulinic acid was synthesized by Dutch scientist G.J. Mulder in 1840, by heating sucrose with mineral acids at high temperature (Kamm et al. 2006). Some physical properties are given in Table 1.5. Levulinic acid is a platform chemical, has a wide range of applications which are shown in Figure 1.8.

Table 1.5. Physical properties of Levulinic acid
(Source: Girisuta 2007)

Property	Value
Dissociation constant (pK_a)	4.59
Density	1.14 kg L ⁻¹
Melting point	37 °C
Boiling point	249 °C
Heat of vaporization	0.58 kJ mol ⁻¹
Heat of fusion	79.8 kJ mol ⁻¹

The secret of having great number of applications that levulinic acid could be used is hidden in the structure of it. It was reported that various reaction pathways are available to use levulinic acid including methyl, carbonyl and carboxylic functional groups transformation and oxidation and reduction reactions (Girisuta 2007).

- Reactions including the methyl group:

Bromide or chloride used to halogenate methyl group of levulinic acid to form organic halides. Bromination in methanol is used to obtain 5-bromolevulinic acid. 5-bromolevulinic acid is a precursor for δ -aminolevulinic acid which is an active ingredient of a biodegradable herbicide. It is also used in pharmaceutical industry, in photodynamic detection to visualize bladder cancer in photodynamic cancer treatment. (MacDonald, S. F. 1974)

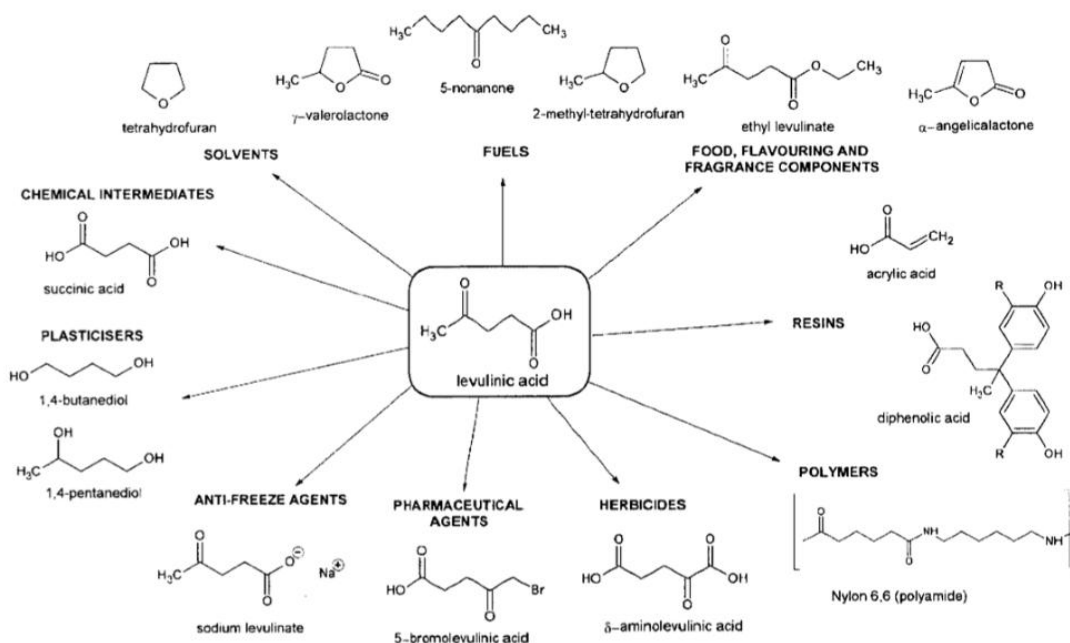


Figure 1.9. Levulinic acids potential application
(Source: Girisuta 2007).

- Reaction including carbonyl group:

In the presence of hydrogen gas and metal catalyst, levulinic acid can react with ammonium hydroxide or ammonia to obtain 5-methyl-2-pyrrolidone that is a useful agent for pharmaceutical industry. Also by acid-catalyzed condensation reaction with aromatic or heterocyclic alcohols levulinic acid can form diphenolic acid that used in production of different polymers, lubricants, paints and fire-retardant materials (Hayes et al. 2006).

- Reactions involving the carboxylic group:

Carboxylic group of levulinic acid is involved to produce various esters of levulinic acid by esterification. Usually this reaction needs acid catalyst such as sulphuric acid, phosphoric acid etc. By esterifying levulinic acid with ethanol ethyl levulinate can be produced. This chemical can be used as an oxygenate additive in diesel engines, in flavouring and fragrance industries, and potentially can replace kerosene as fuel for the direct firing of gas turbines (Hayes et al. 2006).

- Oxidation reactions:

Levulinic acid can be oxidized to various derivatives and chemo-selectivity of this reaction highly depending on type of oxidant. Oxidation using oxygen at high temperature (365-390° C) with catalyst V_2O_5 gives succinic acid with yield of about

80%. . (Willke et al.2004). Succinic acid by-turn has derivatives such as γ -butyrolactone which intermediate for agrochemicals and pharmaceuticals; 1, 4-butanediol which is starting material for production of essential polymers such as polyethers, polyesters and polyurethanes. Polybutylene terephthalate that produced from 1, 4-butanediol is used for plastic engineering, fibers adhesives, films and solvent for polyvinyl chloride (Hayes et al. 2006).

- Reduction reactions:

Levulinic acid may be reduced by platinum oxide, copper-chromite, Raney nickel etc. during the catalytic hydrogenation to form γ -valerolactone. One of derivatives of γ -valerolactone is methyltetrahydrofuran that has potential as a gasoline oxygenate (Girisuta 2007).

By evaluation of all these properties Pacific Northwest National Laboratory, National Renewable Energy Laboratory and Office of Biomass Program of the United States Department of Energy added levulinic acid to “Top 12 Building Blocks” that is accessible from lignocellulosic biomass, on the basis of estimated selling price, estimated processing cost, and market potential (Werpy and Petersen 2007).

The conversion of biomass to levulinic acid is shown in Figure 1.9.

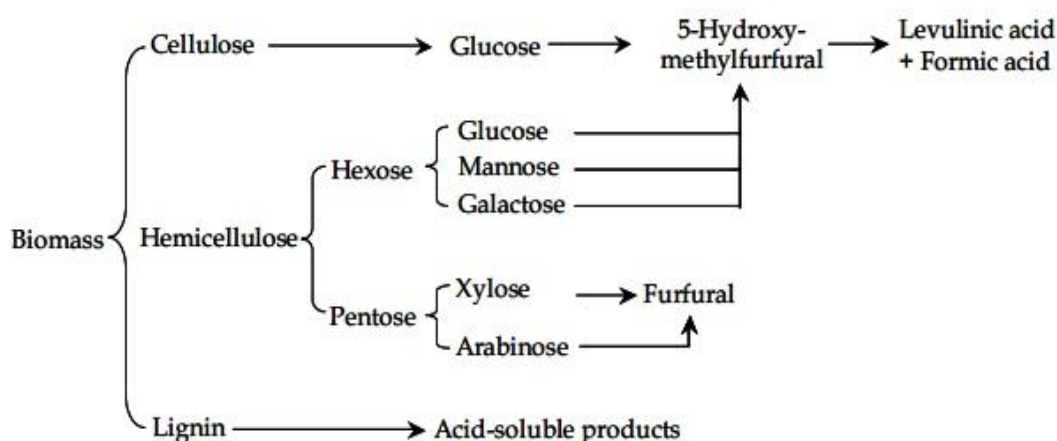


Figure 1.10. Simplified reaction scheme of biomass conversion to levulinic acid (Source: Girisuta 2007)

As it is seen from reaction scheme of levulinic acid production, monomer sugars by dehydration reaction form 5-hydroxy-methylfurfural which by-turn form levulinic acid. 5-hydroxy-methylfurfural (5-HMF) is one of the building block chemicals derived from biomass. Molecular formula is $C_6H_6O_3$. Structural formula is given in Figure 1.10. The

molecule consist furan ring which has both alcohol and aldehyde functional groups. It is yellow low-melting solid highly water-soluble (Huber 2006).

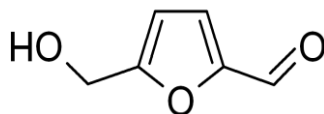


Figure 1.11. 5-hydroxy-methylfurfural
(Source: Rosatella et al. 2011)

5-HMF was first reported by Dull et al., who obtained by heating inulin with oxalic acid solution under pressure, at the end of 19th century. 5-HMF is very useful not only as intermediate chemical for the production of our desired product – levulinic acid but also important for biofuel dimethylfuran and other chemicals such as 2,5-furandicarboxylic acid, 2,5-diformilfuran, dihydroxymethylfuran and 5-hydroxy-4-keto-2-pentenoic acid productions which are illustrated in Figure 1.11. (Rosatella et al. 2011).

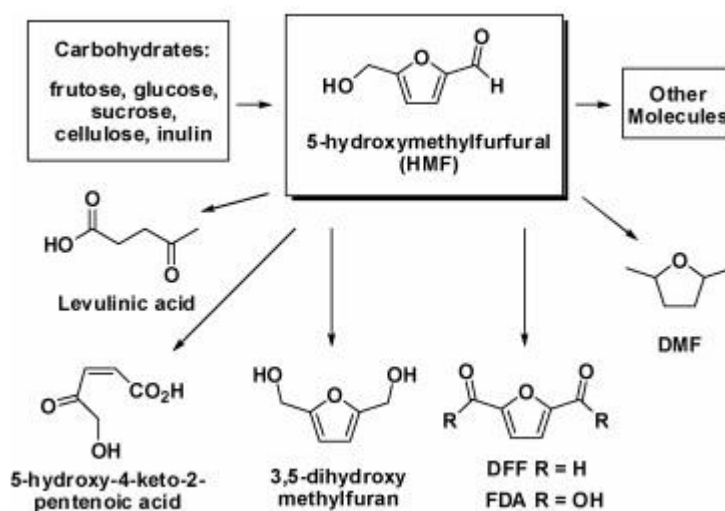


Figure 1.12. Potential derivatives of 5-hydroxy-methylfurfural
(Source: Rosatella et al. 2011)

In this study we investigated the degradation of cellulose in subcritical water in order to produce high value chemicals such as 5-HMF, levulinic acid, etc. For this purpose, different reaction parameters (temperature, pressure, acid amount and kind, reaction time, etc.) were used to obtain higher yields of desired products.

CHAPTER 2

LITERATURE REVIEW

2.1. Biomass Conversion Processes

There are a number of biomass conversion technologies and they are still under development for both wet and dry types of feedstocks (see Figure 2.1 and Figure 2.2). These technologies aim to release the energy directly such as heat or electricity, or can convert into other useful form like biogas or biofuel.

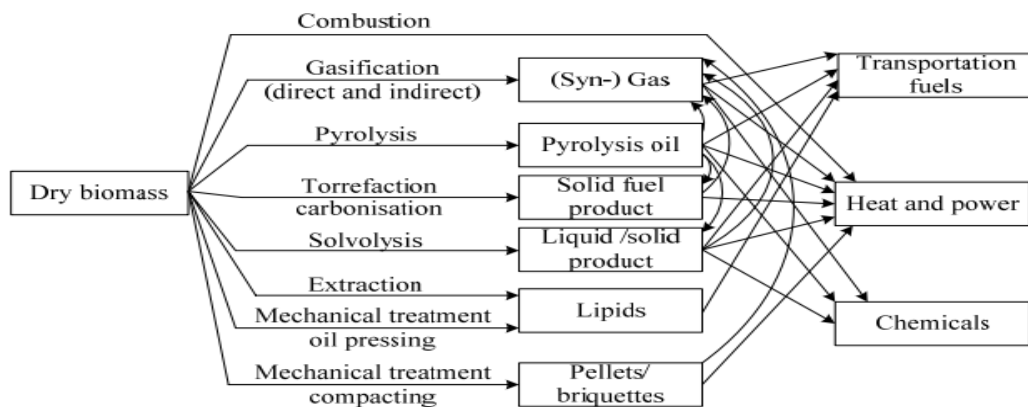


Figure 2.1. Dry biomass conversion and utilization technologies
(Source: Knežević 2009)

Direct combustion process. Mainly feedstocks of this process are residues such as woodchips, bark, straw, sawdust, bagasse, municipal solid waste and food industry waste. Furnaces work in two stages. The first is drying and partial gasification, the second is completely combustion. This method can be used to produce either steam or heat.

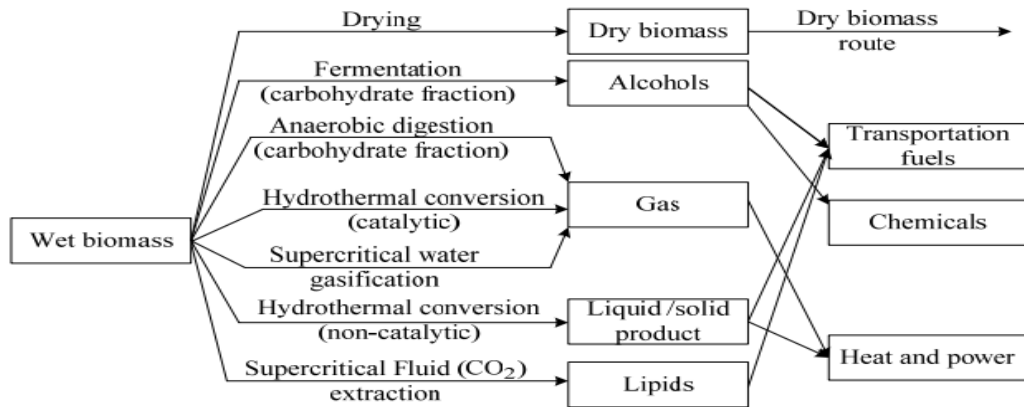


Figure 2.2. Wet biomass conversion and utilization technologies (Source: Knežević 2009)

The general comparison of biomass conversion processes in terms of reaction conditions is illustrated in Figure 2.3.

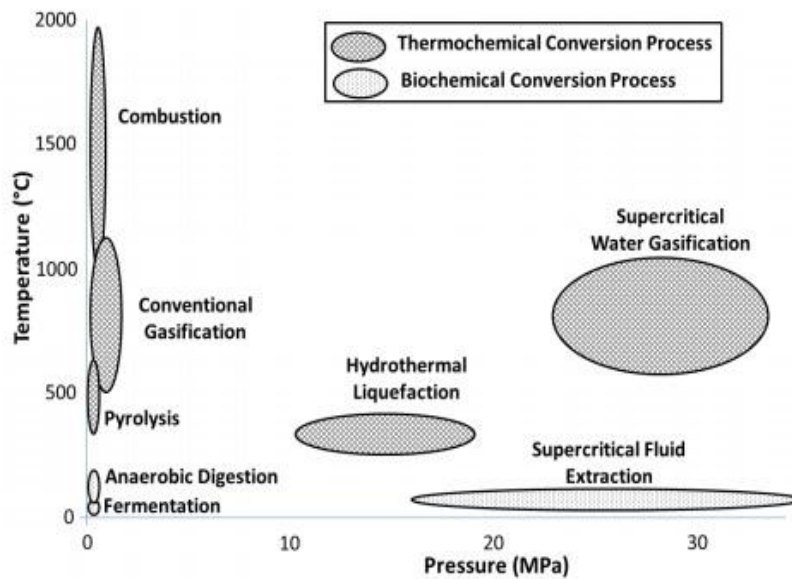


Figure 2.3. Comparison of temperature and pressure of biomass conversion processes (Source: Hendry 2012)

The brief explanations of all these techniques are as follows:

2.1.1 Thermochemical processes

This type of biomass conversion includes pyrolysis, torrefaction, carbonization, conventional gasification, and hydrothermal liquefaction. *Pyrolysis* occurs in absence of

oxygen, under pressure and at operating temperatures above 480 °C. During the pyrolysis of organic materials gas and liquid products are generated, and it leaves carbon rich solid residue. Ultimate pyrolysis that leaves mostly carbon is called **carbonization**. This process is very old and also called as “dry wood distillation”. The main idea of this process is charcoal production by reducing levels of oxygen and hydrogen in the wood. Another thermochemical process is **Torrefaction** which is a mild form of pyrolysis, operating at 200-320 °C in presence or absence of oxygen. During this process water and superfluous volatiles contained in biomass are removed and cellulose, hemicellulose and lignin emit different types of volatiles. The final product of torrefaction is solid, dry torrefied biomass or bio-coal. One of the most commonly used thermochemical process for the conversion of biomass is **gasification**. In the gasification process, it is aimed to obtain a gaseous product and it takes place in two stages: In the first stage gas and charcoal are produced by partially combustion from biomass. In the second stage charcoal is chemically being reduced to H₂O and CO₂, which are produced in the first stage, forming H and CO. The gas contains 8-10% CO₂, 2-3% CH₄, 18-20% H₂ and 18-20% of CO and rest is the nitrogen. These stages are separated in a gasifier. Gasifier design depends on feedstock properties. Gasification occurs at temperatures nearly 800 °C and can be operated at atmospheric pressure or higher (Kurchania 2012).

2.1.2. Biochemical conversion processes

Anaerobic digestion and fermentation processes are the two most important biochemical conversion processes used in biomass treatment. *Anaerobic digestion*, also known as methane fermentation or biomethanation aims to produce a combustible gaseous fuel with naturally occurring microorganisms in the absence of air. The gaseous mixture is generally termed as “biogas”, and comprising primarily of methane (CH₄) and carbon dioxide (CO₂) and vestiges of other gases such as nitrogen (N₂) and hydrogen sulphide (H₂S) (Shinya Y. and Yukihiro M. 2008). Whole process includes three steps as schematically shown in Figure 2.4: The first step is the conversion of organic solid complex by the enzymatic hydrolysis into soluble compounds. The second step is acidogenesis in which formed soluble organic material converted into mainly short-chain acids and alcohols. The last step is methanogenesis, in which the products of

second step are converted into gases by different species of strictly anaerobic bacteria which called “metanogenic bacteria”. This process is a microbiological process; therefore, occurs under normal temperature (5-70° C) and pressure; require neutral pH (6.4-7.2) (Capareda 2011).

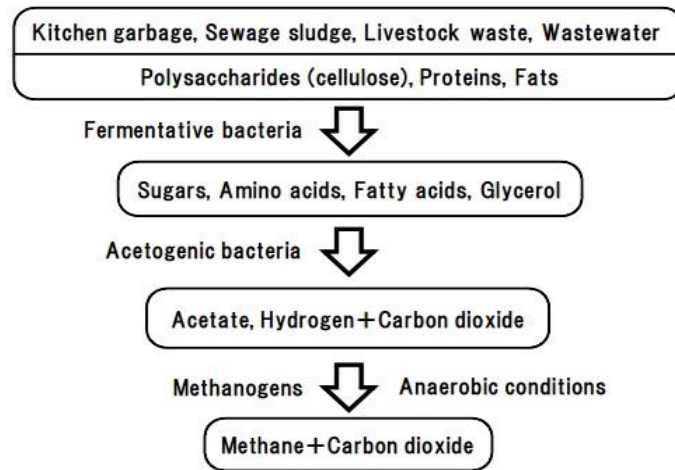


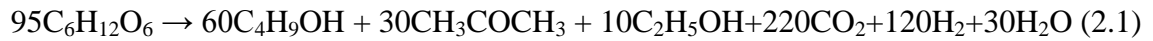
Figure 2.4. Diagram of biomethanation process
(Source: Shinya Y. and Yukihiro M. 2008)

In anaerobic digestion variety of biomass can be used as a substrate, this not allowed in fermentation processes due to activities of complex microflora. Another biochemical conversion process is *Fermentation*. Fermentation of biomass includes ethanol fermentation, acetone-butanol fermentation, hydrogen fermentation, lactic acid fermentation, silage and composting (Shinya Y. and Yukihiro M. 2008). The ethanol fermentation process will be described widely in section 2.1.4.

Acetone - butanol fermentation, which was industrialized in period of World Wars, to produce smokeless powder (raw material is acetone) and to produce fuel for war planes (raw material is butanol) but due to the development of petroleum chemistry, further development in this industry was fell out. Nowadays this industry got its second wind. Butanol may be an additive to both gasoline and diesel fuels and has more affinity than ethanol when it is added to gasoline (Grabbe et al. 2001). In this process, *Clostridium*, a microorganism that is widely present in soil, uses glucose. There are mainly two strains of this microorganism, the first strain produces butanol from starch and called as Weizmann-type, the second one is Saccro-type and its raw material is sucrose. *Clostridium* secretes enzymes amylase, xylanase, protease and lipase. During the

fermentation ethanol is also produced that sometimes this process called as ‘acetone-butanol-ethanol (ABE) fermentation’.

The reaction pathway is shown in Figure 2.5., and stoichiometric equation is given below:



During the process glucose via EMP pathway is decomposing to pyruvate, acetyl-CoA and acetoacetyl-CoA and at the end of reaction butanol, acetone and ethanol are produced. As it seen from equation above, from 95 mol glucose (273 MJ) are produced 60 mol butanol (170 MJ), 30 mol acetone (54MJ), 10 mol ethanol and 120 mol hydrogen (Ishizaki et al. 1999).

Lactic acid fermentation is a biological process in which sugars are converted to lactic acid by bacteria or fungi. Poly-lactate is a biodegradable plastic and its demand increasing day by day. Chemically produced lactic acid has yield D-lactic and L-lactic acid half by half, and its optical purity is zero. That is why, only lactic acid produced by fermentation is used for poly-lactate production. Lactic acid bacteria uses only sugars as energy source and more 50% of consumed sugars can be converted into lactic acid. *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* are species of lactic acid bacteria which used in industrial scale (Morichi et al. 1990).

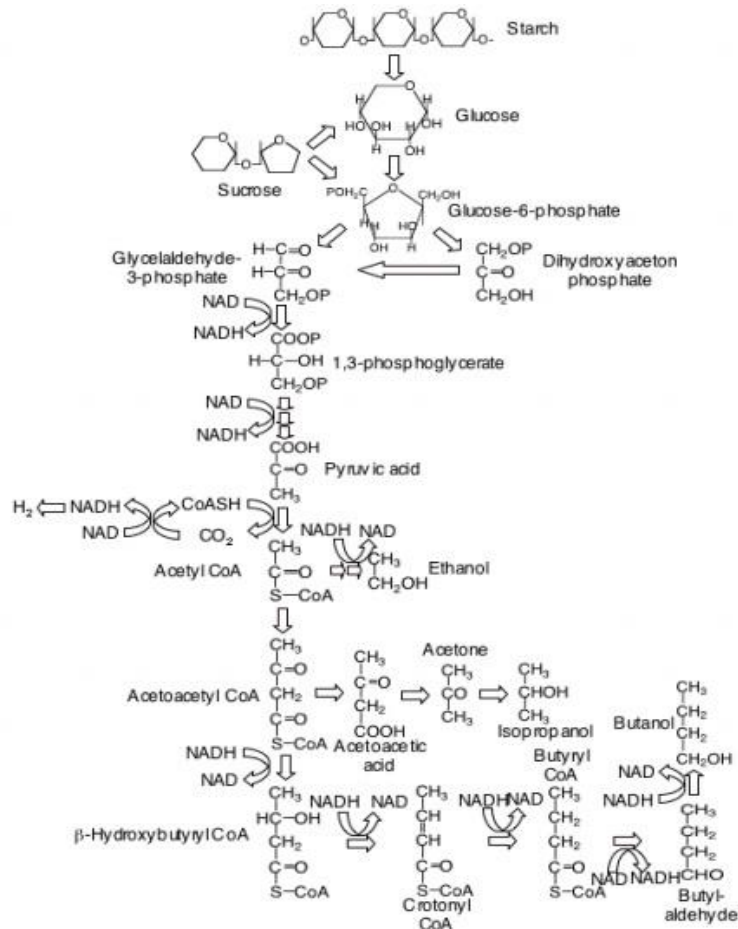


Figure 2.5. Acetone-Butanol fermentation
(Source: Shinya Y. and Yukihiro M. 2008)

Lactic acid fermentation can be divided into two groups: The first group is homo-lactic fermentation, in which from one mole of monosaccharide two moles of lactic acid and two moles of ATP can be produced, has almost 100% yield. The second one is hetero-lactic fermentation in which lactic acid and other compounds such as ethanol and carbon dioxide or acetic acid can be produced. Lactic acid bacteria (LAB) have both D and L lactate-dehydrogenases, which mean both D-lactic acid and L-lactic acid, can be produced. For poly-lactate production *Lactobacillus rhamnosus* is used that can produce only L-lactic acid with almost 100% optical purity (Morichi et al. 1990).

Also, lactic acid bacteria play initial role in silage fermentation process. It can be prepared from forage grasses and crops and suitable moisture content should be about 50-70%. The epiphytic lactic acid bacteria, which are a major component of forage crops can convert sugar into lactic acid in ensiling process. The LAB growth with other plant-associated microorganisms and they generally characterize properties of silage. As

a result of water-soluble carbohydrates transformation into organic acids pH is reduced and forage is preserved (McDonald, P., Henderson A. R. and Heron S. J. E. 1991).

2.1.3 Supercritical Fluid Extraction

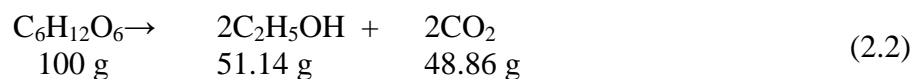
Supercritical fluid extraction is the process of separating of one particular component (the extractant) from another (the matrix) by using supercritical fluids as the extracting solvent. Supercritical fluids can produce a product with no solvent remaining (Sapkale et al. 2010). This process can be used as a sample preparation step for analytical aims or in larger scale either to remove unwanted material from a product (to produce products such as decaffeinated coffee, cholesterol-free butter, and low-fat meat) or to collect a desired product (essential oils, flavor and fragrance agents, squalene from shark liver, etc) (Capuzzo et al.2013). *Carbon dioxide (CO₂)* is the most commonly used solvent and sometimes in use can be mixed with co-solvents such as methanol or ethanol. For Supercritical CO₂ (SC-CO₂) extraction conditions are above its critical points, which are 31 °C and 7.38 MPa. CO₂ is inert, tasteless, odorless, non-toxic, non-flammable, inexpensive, and in addition to this it leaves no residue behind. Due to its low critical temperature it is widely using in food, essential oils, aromas and nutraceutical industries. Additionally, SC-CO₂ is also commonly used to extract high value products from natural materials (Sapkale et al. 2010).

2.1.4 Production of Ethanol from Biomass

Ethanol (C₂H₅OH) or ethyl alcohol can be produced from a different sugar containing material by fermentation process lead by yeast strains through glycolysis reaction pathway (Embden-Meyerhof Pathway). In this reaction, one molecule glucose and two molecules of ATP are used as energy for yeast cells growth. Ethanol fermentation is a biological reaction that occurs at room temperature and atmospheric pressure. Humankind used ethanol fermentation for brewery of alcohol drinks, in the fermented food manufactory, bakery for several thousands of years. In the19-20th century due to great technological progress in fermentation and distillation, ethanol became available in the field of different chemical industries, beverage and food industry, medical use and fuel. As an alternative to fossil fuel and to prevent global

warming, large amount of fuel ethanol has been produced from corn in USA and from sugarcane in Brazil (Kurchania 2012).

Yeast is one of the most commonly used feedstock in fermentation. Yeast cells were first isolated from beer in Denmark in 1883. *Saccharomyces cerevisiae* is type of yeast that has perfect ethanol fermenting ability and ethanol tolerance, widely used for industrial and fuel ethanol production. Next to *S. cerevisiae* bacterium *Zymomomas mobilis* is excellent to ferment limited ranges of carbohydrates of glucose, fructose and sucrose. Fermentation rate and fermentation yield are better than *S. cerevisiae* but tolerance to ethanol is not so good (Saiki et al. 1999). *S. cerevisiae* can ferment many sugars such as glucose, fructose, galactose, mannose, sucrose, maltose but cannot ferment pentoses such as xylose and arabinose. As pentose fermenting yeasts *Pichia stipites* and *Pachysolen tannophilus* can be used but their tolerance to ethanol is not good as *S. cerevisiae*. Many laboratories are kept working on construction of pentose fermentable *S. cerevisiae* strains and other types of microorganisms such as *Escherichia coli* and *Corynebacterium glutamicum* by DNA recombination technology. Other ethanol fermenting microorganisms such as hetero-lactic bacteria *Lactobacillus*, cellulose degrading *Clostridium*, anaerobic thermophilic *Thermoanaerobacter* are producing relatively low concentration of ethanol and byproducts like organic acids. That is why, *Saccharomices serevisiae* is the best ethanol fermenter for now. During the reaction of glucose conversion to ethanol, as it shown in equation below, yeast strains produce from 100 g of glucose about 51. 14 g of ethanol.



In this reaction nearly 50% of glucose weight is lost as carbon dioxide but about 91% of energy concentrated in glucose (2.872 MJ/mol) held in ethanol. This makes ethanol fermentation reaction a perfect biological process to transform biomass to liquid fuel ethanol (Shinya Y. and Yukihiro M. 2008). (2.2)

The production technology of bioethanol in industrial scale is depending on raw materials. Producers such as Brazil and India mainly use molasses of sugar cane. Molasses is a liquid byproduct of sugar production; sugar concentration is about 50% and contains glucose, fructose and sucrose. These saccharine materials are good

substrate for yeasts and *Zymomonas* to ethanol fermentation (Saiki et al. 1999). Melle-Boinot process is common ethanol production process in Brazil. Scheme of Melle-Boinot fermentation process is indicated in Figure 2.6. It is continuous or semi-continuous fermentation process in which yeast cells are recovered from beer by centrifugation and recycled to fermentation tank after sterilization of contaminated microflora by dilution of sulfuric acid at pH₃. From cane juice ethanol fermentation at high concentration of yeast cells can make beer comprising 6-8% of ethanol in about 15 hours of fermentation time. Molasses diluted two-times or mixed with cane juice or cane juice. If fermentation yield is 82% of total sugar, and sugars level of molasses is 55%, 3.3 t-wet molasses amount is required to produce 1m³ of 95% ethanol (Shinya Y. and Yukihiro M. 2008).

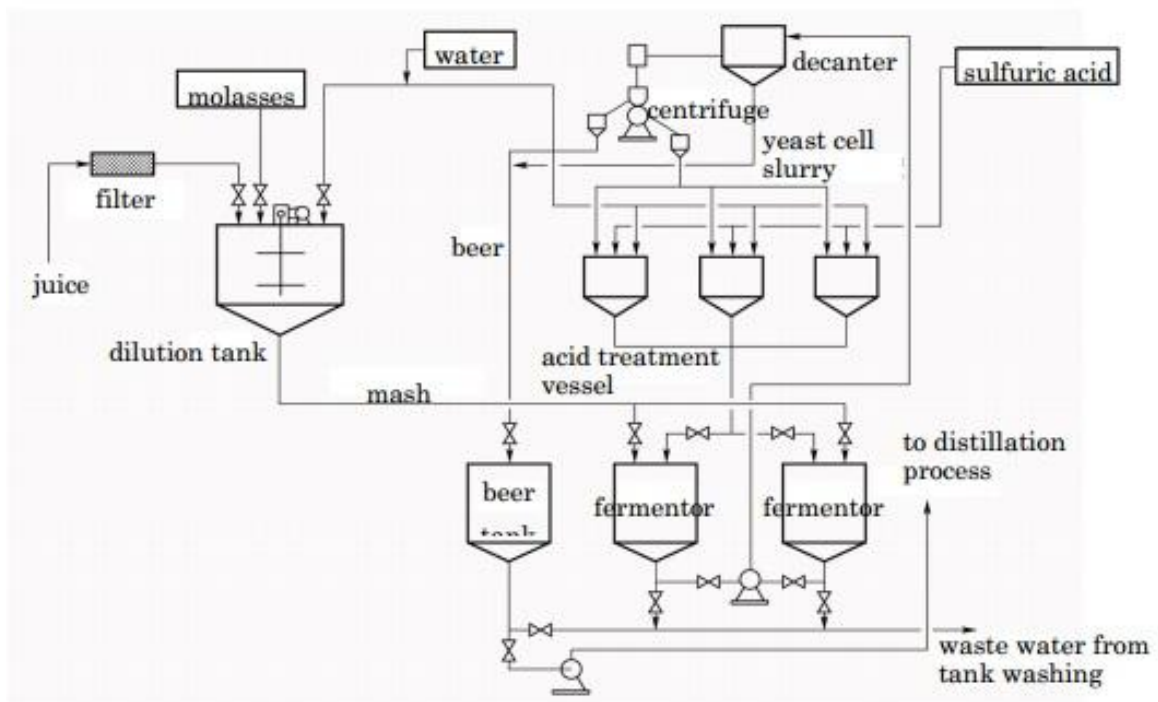
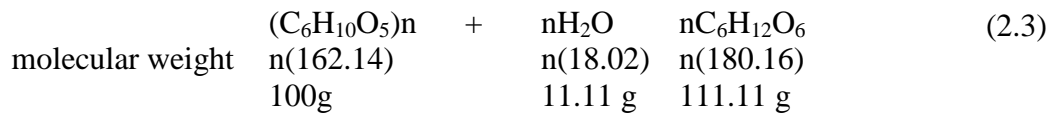


Figure 2.6. Melle-Boinot process of ethanol fermentation
(Source: Shinya Y. and Yukihiro M. 2008)

A large amount of ethanol is produced from starchy materials, from corn in USA and sweet potato in China. First starchy raw material is hydrolyzed by amylase enzyme to glucose.

The reaction is shown in equation below:



In USA that is largest producer of fuel ethanol, mostly used corn as a feedstock. Commonly, wet mill process, continuous fermentation process, and batch-wise process are used. Wet mill process started from immersing of corn in dilute sulfite solution where it is fractioned into starch, germ, gluten and fiber. Starch fraction is hydrolyzed with amylases to glucose and then yeast fermentation begins. Scheme of wet mill process is illustrated in Figure 2.7. 2.4 t-wet of corn is needed to produce 1m³ (kL) of 95% ethanol if starch value is 63% and fermentation yield is 90%. In this process also high fructose syrup is produced (Saiki et al. 1999).

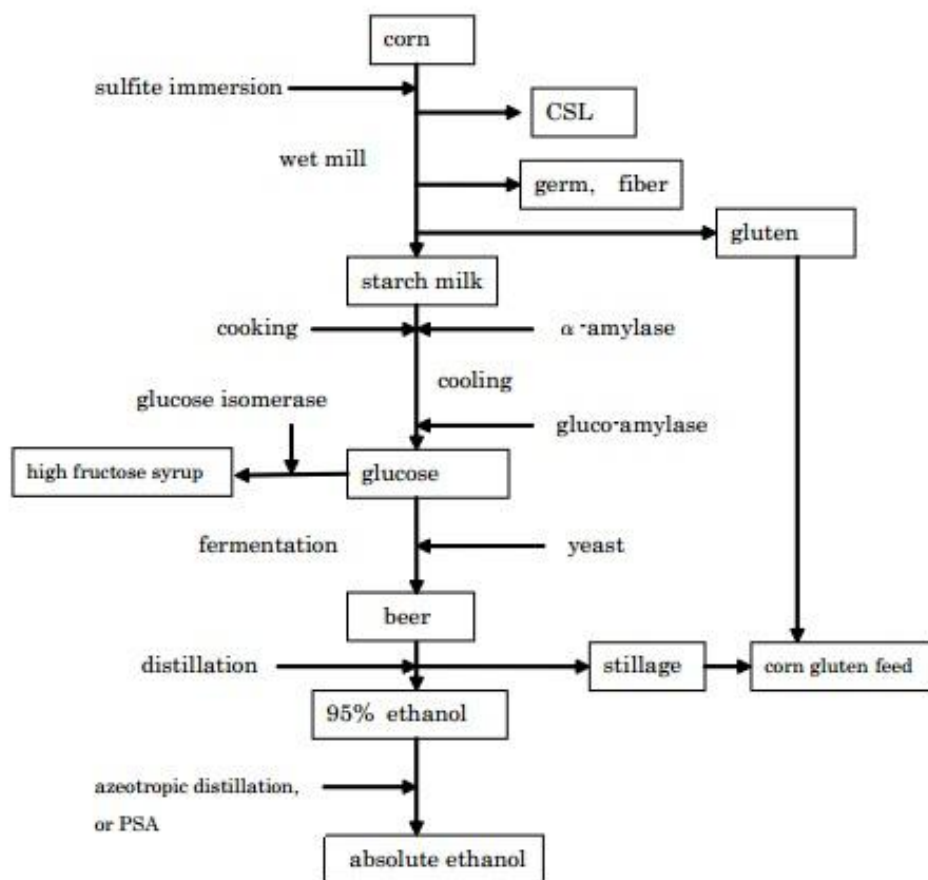


Figure 2.7. Wet mill fermentation process of ethanol and high fructose syrup

(Source: Shinya Y. and Yukihiro M. 2008)

2.1.5 Supercritical Water Gasification

Supercritical water gasification (SCWG) is a method of biomass conversion that uses special properties of supercritical water and aims to obtain hydrogen rich gaseous products (Guo et al, 2007). Chemical conversion of this process can be displayed by the simplified reaction divided into two steps:

1. Steam reforming $\text{CH}_x\text{O}_y + ((1-y)\text{H}_2\text{O}) \rightarrow \text{CO} + (1-y+x/2) \text{H}_2$
2. Water-gas shift $\text{CO} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + \text{H}_2$ (Zhang 2008).

SCWG has several advantages over conventional gasification and pyrolysis in normal pressure:

- ✓ Most organic materials contained in biomass can be dissolved in supercritical water.
- ✓ There is high gasification efficiency and a high molar fraction of hydrogen. No char and tar occurs. Volumetric ratio of H_2 can be greater than 50%. High pressure of the gaseous product makes easier usage, transportation, carbon capture and further purification of produced gas via pressure swing adsorption or steam reforming.
- ✓ In SCWG of biomass drying is not required. It means higher energy efficiency can be achieved.
- ✓ The reaction temperature is much lower than conventional gasification and pyrolysis. If in SCWG glucose gasification can be reached at 650°C and 34.5 MPa, the temperature of conventional gasification is always above 1000°C .
- ✓ The final product can be very clean. Since no NO_x and SO_x were generated in SCWG and concentration of CO is very low, especially when to enhance water-gas shift reaction catalyst is used (Momba M. and Bux F. 2010).

For the first time SCWG was described by Modell in 1977. This process mainly suitable for wet types of biomass; for instance, wastewaters from different production processes

(sewage sludge, black liquor from pulping process, wheat straw and olive oil wastewaters) which sometimes have toxic content. Since reactivity of water is high under supercritical condition biomass can be gasified quickly and almost completely. The main products are usually H_2 , CO_2 and CH_4 , with small portions of CO and C_2 (Shinya Y. and Yukihiko M. 2008). The reactor designed depends on raw material and to enhance the reaction catalysts such as metal, alkali or carbon catalysts can be added. Supercritical gasification of anaerobic wastewater from wheat straw was investigated with flow tube continuous reactor system. This wastewater contained organic acids such as acetic acid, butyric acid and ethanol. During this study at 775 °C 100% gasification efficiency (GE) was achieved and carbon conversion efficiency (CE) together with total gas yield raised when temperature was raised from 700 °C to 775 °C. Results are illustrated in Figure 2.8. Authors concluded that supercritical gasification of wheat straw wastewater can be used not only to obtain hydrogen but also to reduce contamination (Guo et al, 2007).

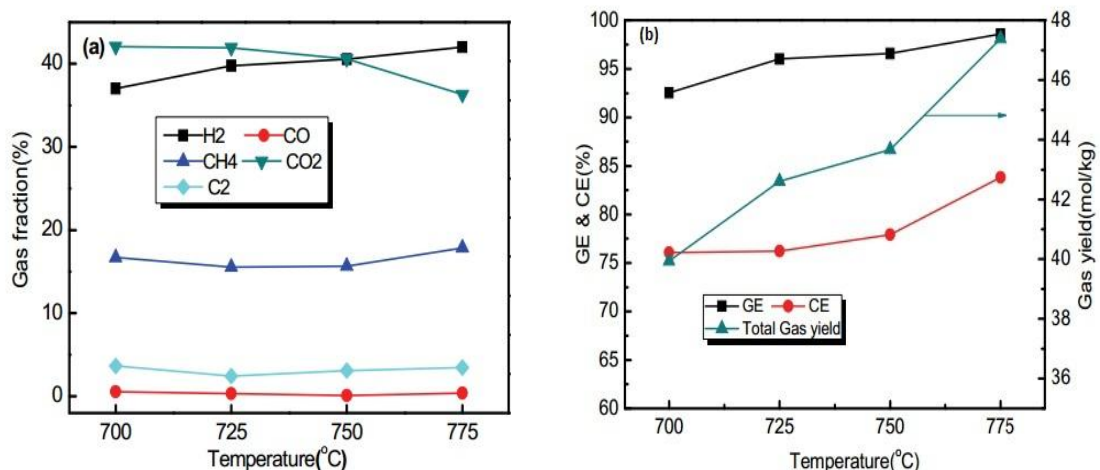


Figure 2.8. The effect of temperature in Supercritical Water Gasification of wheat straw wastewater at 25 MPa; (a) Fractions of produced gases, (b) GE, CE and total gas yield (Source: Momba M. and Bux F. 2010).

Despite important advantages of this technique still in stage of development and several implementations are designed in laboratory scale to avoid following problems:

Plugging; during the gasification process salt and if heating rate of feedstock at the inlet of the reactor is low char can be formed. Usually to avoid this, pre-heater is used. However, it may happen at a high feed rate or a high biomass ratio that could result in expensive solution. Inorganic compounds contained in biomass have low solubility in water can accumulate into a large particle. As a result, tubes of reactor can be plugged

(Guo et al. 2007). Corrosion; in supercritical state water can form OH which by-turn in presence O_2 can form H_2O_2 which amplify corrosion ability of supercritical water. In addition to this, metals, alkali and chlorine in the biomass enhance corrosion of the reactor material.

Economy; during the process to minimize plugging pre-heating of the biomass suspension and the reactor is needed. High temperature is required to provide 100% conversion. This could mar profitability of this method (Zhang 2008). This kind of technological challenges require long time research. Because of high cost presently no commercial plants have been built. In industrial scale there are three pilot SCWG plants on stream, VARENA Plant in Germany, TEES Process in USA and Energia Co. plant in Japan (Shinya Y. and Yukihiro M. 2008).

2.1.6. Supercritical Water Oxidation

Supercritical water oxidation (SCWO) is an oxidation process that uses supercritical water as a reaction medium. Distinctiveness of this process are in the unique solvating properties to provide increased solubility of organic agents and permanent gases, to be a single-phase environment which does not have inter-phase mass transfer limitation, faster reaction kinetics and increased selectivity of complete oxidation products. This process was developed as a result of looking to wet oxidation under more severe conditions such as higher temperature and pressure. Due to other destruction methods based on oxidation of liquid wastes such as activated carbon treatment, biological treatment, wet air oxidation and incineration were insufficient in terms of economic effectiveness or ecological regulations (which needs complete destruction of some wastes) (Veriansyah et al. 2006). Simplified diagram of SCWO is given in Figure 2.9. It contained preheater which function is recovery of heat from the reactor.

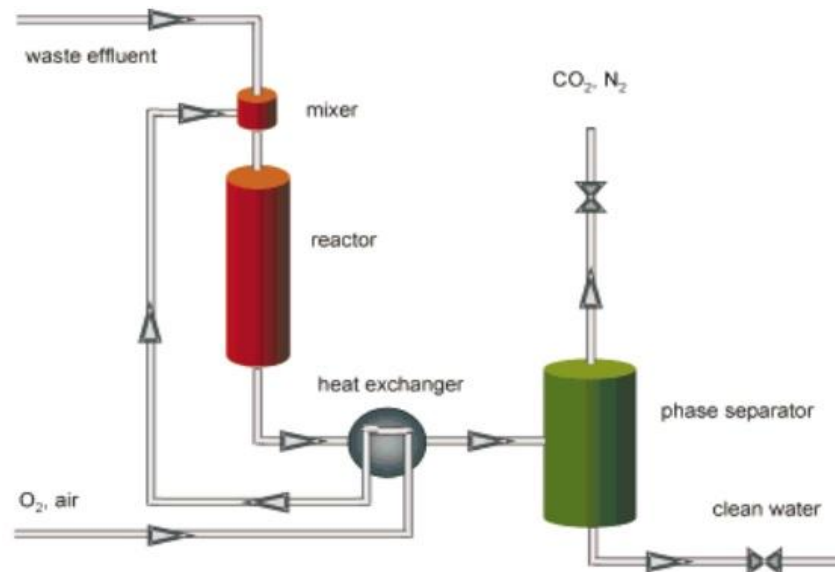


Figure 2.9. Simplified diagram of Supercritical Water Oxidation Process (Source: Schmieder et al. 1999).

SCWO process has following advantages:

- High oxidation efficiency via higher reaction temperature and density;
- Low residence time;
- One process step for gaseous and aqueous effluents (no additional process steps).

It has disadvantages from engineering point of view:

- Increased corrosion of materials, especially reactor and heat exchanger;
- Fouling and plugging of reactor and feed preheater caused by drastically decreased solubility of salts resulting in precipitation (Schmieder et al. 1999).

2.1.7. Autohydrolysis

Another method of biomass treatment with water assistance is autohydrolysis, that is also called as hot water extraction, steam explosion or stream treatment. The main idea of this method is to involve intermediates, which occurs during the process from substrate, to further hydrolyze the rest content of substrate. According to Yoon (1998) the autohydrolysis is the method in which biomass heated (above 150 °C) by pressurized water and form acids via de-esterification of ester groups and solubilization of acidic components in hemicelluloses, pH value of liquor drops.

The formed acids such as formic acid, acetic acid, glucuronic acid etc. are unsubstantial and cannot hydrolyze cellulose. That is why, autohydrolysis is mainly used as a supplementation to lignocellulosic processing methods. As it is known, hemicellulose in biomass may be an obstacle during the enzymatic hydrolysis of cellulose. Thus makes autohydrolysis useful in terms of hemicelluloses recovery and total cellulose hydrolysis (Yoon 1998). After the pretreatment almost all hemicellulose and some lignin are dissolved and extracted wood chips could be easily delignified and increase bleachability of pulp (Li et al. 2013). Autohydrolysis pretreatment can increase efficiency of enzymatic hydrolysis of poplar chips up to 90% when efficiency of untreated ones is 15% (Kumar et al. 2009). In Figure 2.10., autohydrolysis pretreatment of biomass is illustrated.

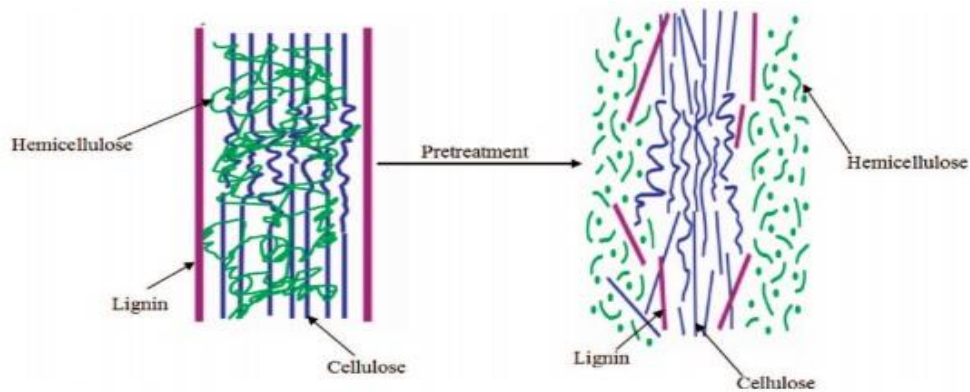


Figure 2.10. Diagram of autohydrolysis pretreatment of lignocellulosic biomass (Source: Li et al. 2013).

Autohydrolysis pretreatment temperature range is from 120 °C to 240 °C and reaction time can differ from several minutes to several hours. Depends on temperature and reaction time level of degradation of lignocellulosic biomass and produced organics can change. The high temperature (>200 °C) and longer reaction time can lead to complete decomposition of biomass which by-turn results in decreasing sugar content in liquor (Li et al. 2013).

Microwave irradiation was tested to increase efficiency of autohydrolysis and shows good results. Also, it was reported that addition of sulfuric acid, hydrogen peroxide, inorganic ions can enhance efficiency of autohydrolysis by decreasing temperature and reaction time (Grundas S. 2011)

2.2 Biomass Treatment in Subcritical Water

Hydrothermal liquefaction is a type of thermo-chemical process that uses high-pressure and comparatively medium-temperature to convert wet biomass and waste streams. In short, it uses subcritical water as a reaction medium and final product is mainly solid and liquid. During this process, hydrolysis or degradation of macromolecules leads to obtain smaller molecules. Considerable part of oxygen in the biomass is removed by dehydration or decarboxylation (Toor et al. 2011). Amount, type and chemical properties of final product depend on biomass content and reaction conditions.

As supercritical gasification, which was explained in the section 2.1.5, leads to total degradation of biomass to mainly gaseous products, whereas the main point of subcritical water treatment is in obtaining valuable chemicals from biomass. So, their functions are totally different and it signifies that depends on desired product treatment of biomass should be chosen. In Figure 2.11. comparison of two types of treatment of carbohydrates consisting of biomass and possible final products are displayed.

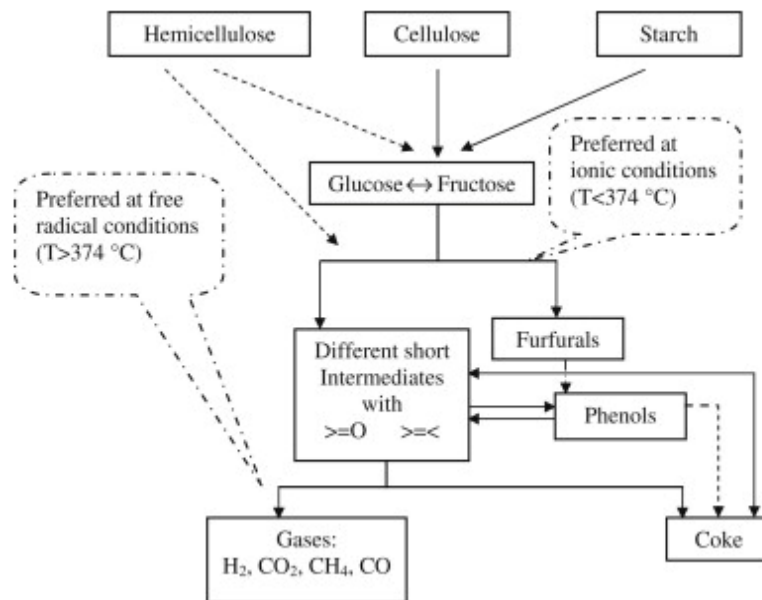


Figure 2.11. Simplified reaction mechanisms of degradation of carbohydrates in sub- and supercritical conditions (Source: Toor et al. 2011).

Many studies to understand idea of final products formation and influence of conditions have been done. Kruse et al. (2003) studied degradation of biomass at 330-410 °C and

30-50 MPa. As a substrate they used phytomass from baby food. They aimed to characterize the chemistry of biomass degradation process, to compare sub- and supercritical conditions and identify key intermediate compounds of biomass. Obtained final products were compared with final products of previous studies in order to identify reaction pathway. As a result, it was concluded that by increasing temperature, pH value is changing from acidic to basic, meaning that the production of organic acid decrease. Also, it was reflected in final products, in conditions above critical point gaseous products, below liquid organics amounts were high.

Results of studies about hydrothermal treatment of agricultural residues from different countries (about 17 countries) were collected and screened by Cardenas-Toro et al. (2014). It was concluded that hydrothermal liquefaction produces valuable products, which can be used in different applications, from raw materials in food, cosmetic and pharmaceutical industry to intermediates in chemical industry. Also, based on studies it was understandable that for conversion of key compounds of agricultural residues such as components of hemicellulose and starch preferable temperature less than 200°C, for cellulose higher than 200°C. The schemes of main reaction pathways of hemicellulose and cellulose hydrolysis are respectively given in Figure 2.12 and Figure 2.13.

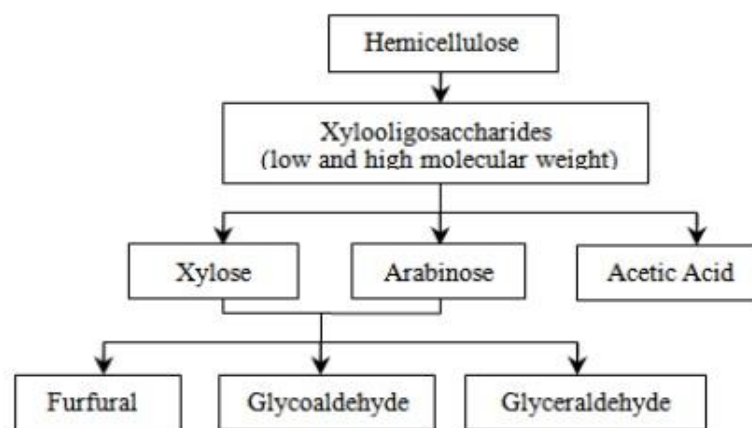


Figure 2.12. Hemicellulose degradation pathway
(Source: Cardenas-Toro et al. 2014)

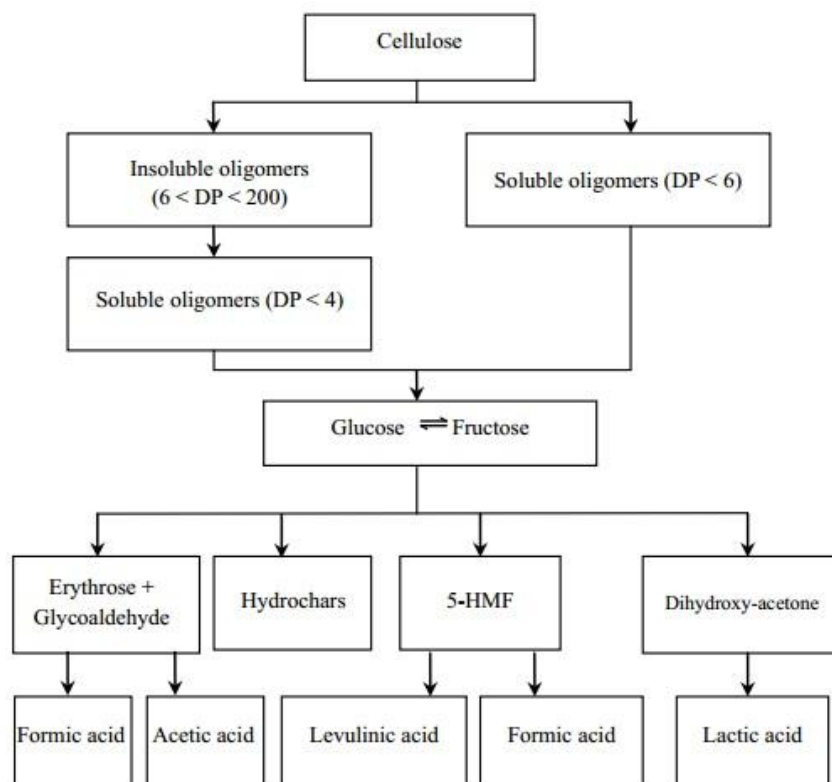


Figure 2.13. Cellulose degradation pathway
(Source: Cardenas-Toro et al. 2014)

As cellulose is a main compound of plant biomass and its amount can reach up-to 55% we use it as substrate in our study (Toor et al. 2011). There are many studies about cellulose treatment in supercritical and subcritical water. According to Ehara et al., supercritical water treatment has shown the best result to obtain high yields of hydrolyzed products. However, it was observed that in subcritical water cellulose was liable to more dehydration (Ehara et al. 2005).

A new way of lactic acid production by conversion of carbohydrates in subcritical water with adding metal salts as catalysts to was proposed by Bicker et al. (2005). It was concluded that the best results were obtained from Zinc sulfate ($ZnSO_4$) and yield of lactic acid was high in fructose decomposition following by glucose decomposition (Figure 2.14). Based on data they gave possible scheme of Zn-catalyzed conversion of carbohydrates in subcritical water as illustrated in Figure 2.15.

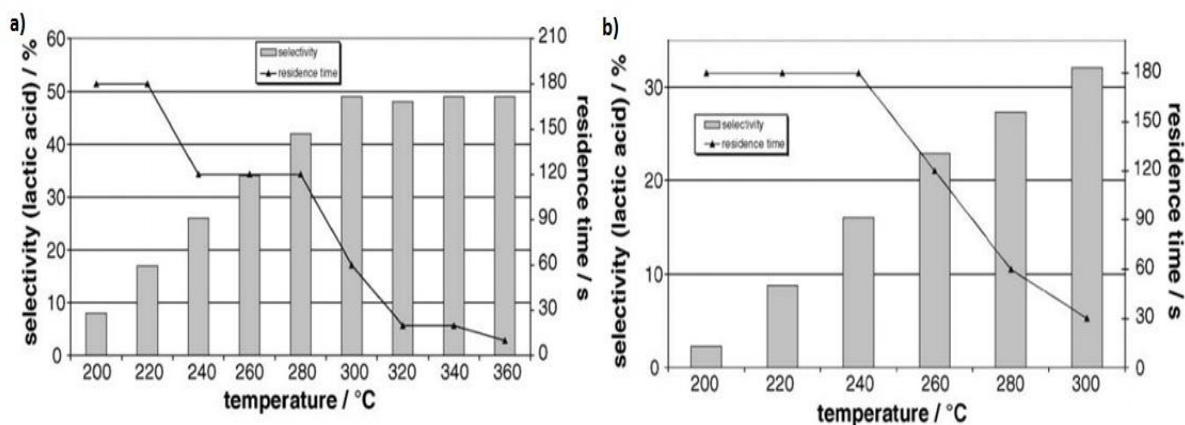


Figure 2.14. Lactic acid selectivity (columns) with respective reaction times (triangles) in catalytic fructose (a) and glucose (b) decompositions at 200-360 °C, ZnSO₄ concentration is 400 ppm (Source: Bicker et al. 2005).

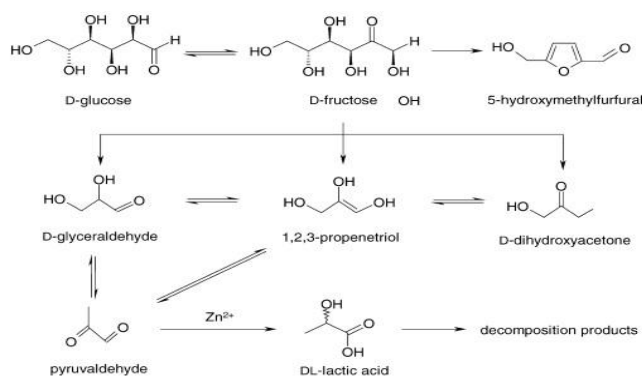


Figure 2.15. Scheme of Zn catalyzed degradation of carbohydrates (Source: Bicker et al. 2005).

Decomposition of cellulose in subcritical water, supercritical water and their combined treatment were investigated (Ehara et al.2005). The highest yield of water soluble portion was obtained in combined treatment. Results are given in Table 2.1.

Table 2.1. Yield of fractionated portions of cellulose as treated in supercritical water, subcritical water, and their combined treatments (Source: Ehara et al.2005).

Treatment	Time (s)	Yield (%)		
		Water soluble		Water insoluble
		Water soluble	Precipitates	
Supercritical water (400°C, 40 MPa)	0.1	41.2	49.0	9.8
	0.2	58.3	41.7	0.0
	0.3	93.9	6.1	0.0
Subcritical water (280°C, 40 MPa)	120.0	49.5	0.0	50.5
	180.0	58.7	0.0	41.3
	240.0	82.7	0.0	17.3
Combined (400°C, 40 MPa and 280°C , 40 MPa)	15.1	81.9	18.1	0.0
	30.1	88.9	11.1	0.0
	45.1	100.0	0.0	0.0

2.3. Biomass Conversion into Levulinic Acid and 5-HMF

As it was noticed in introduction part of this thesis, Levulinic acid is one of those organics which has a significant attention across the world as it is multifunctional platform chemical and has many applications to be used. There are many papers about technologies of the production of levulinic acid. Among them production from biomass interested us a lot. During the literature investigation we noticed that mainly studies had been done on acid-catalyzed hydrolysis and biofine (bio-refining) process was developed and used in industrial scale. Bio-refining process is a fast chemical conversion process that was developed by Fitzpatrick S.W. in 1980s. This process use mainly cellulose and starch as a substrate and depends on region and biomass source could have supplementation for the necessary pretreatment. As a catalyst sulfuric acid is mainly used (Rackemann et al.2011).

Acids that have high catalytic activity such as H_3PO_4 , HCl, and H_2SO_4 are traditionally used in production process of levulinic acid. However, their drawbacks such as pollution to environment and corrosion to equipment force to search another ways of production (Wang et al. 2013).

Among the organics that have been obtained from biomass in subcritical water presence of levulinic acid was also noticed by many scientists. As in chemical method, hydrothermal production of levulinic acid is based on rehydration of 5-HMF with two

molecules of water (Application of Hydrothermal Reactions to Biomass Conversion. Edited by Jin F. 2014).

5-HMF by-turn is formed by dehydration of glucose or fructose. Formic acid is a by-product of levulinic acid and rehydration of 5-HMF gives the same amount of them (Asghari et al. 2010). Reaction pathway of levulinic acid production from D-glucose is illustrated in Figure 2.16.

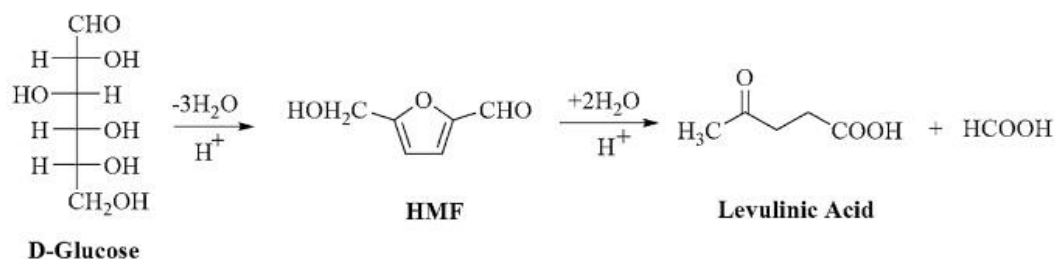


Figure 2.16. Reaction pathway of levulinic acid production from d-glucose (Source: Zeng et al.2010).

Reaction condition has an initial effect on levulinic acid yield. Kruse et al. 2003 reported that levulinic acid yield falls down when temperature was above critical point (Figure 2.17.).

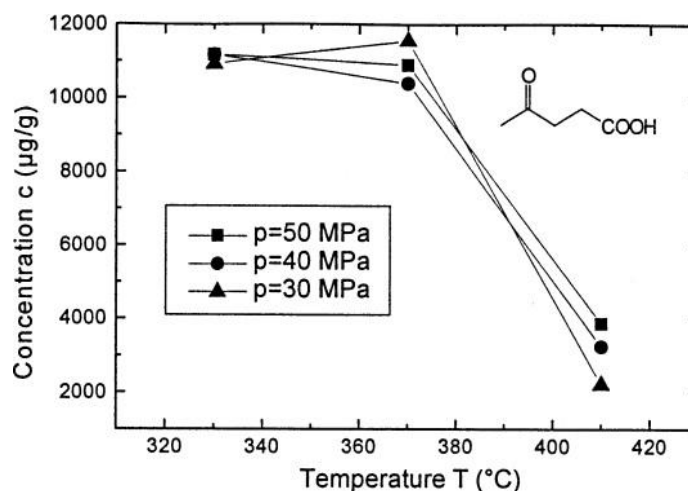


Figure 2.17. Temperature and pressure dependence of levulinic acid concentration (Source: Kruse et al. 2003)

Many types of biomass were treated in subcritical water. During the degradation of rice bran it was noticed that levulinic acid and other acids such as formic, glycolic were obtained at temperatures above 190 °C (Pourali et al. 2009). Treatment of Japanese red pine wood was done in non-catalytic and catalyzed conditions in order to compare yield

of valuable products. It was observed that addition of catalyst (phosphoric acid) leads to high yield of organic acids included levulinic acid (Asghari et al. 2010).

Based on this literature review, we decided to choose levulinic acid as a desired product of our study due to the wide application areas of this organic acid (see Figure 1.8 in introduction section) and insufficient information about its production from cellulose in subcritical water. We would like to understand the effect of reaction conditions such as temperature (150, 200, 250 and 280 °C), residence time (30, 60, 90 and 120 min) and initial pressure (0, 5, 10 and 15 bar). Moreover, different oxidizing agents (H_2SO_4 and H_2O_2) with different amounts (0, 5, 25, 50, 75, 100 and 125 mM) were tested to obtain higher yield and results were compared with oxidizer free way of treatment.

CHAPTER 3

EXPERIMENTAL

3.1. Chemicals

In this study all chemicals were used in their analytical standards and no purification and other methods were performed. All information about chemicals used during the experiments is given in Table 3.1. Also, de-ionized water was used during the preparation of solutions and cleaning processes.

Table 3.1. List of chemicals used during the study and manufacturers from where they were purchased

Name	The product code	Producer
Cellulose	31,069-7	Aldrich Sigma
Furfural	8.04012.0500	Merck
Sulfuric Acid	84721	Fluka
Lactic Acid	141034,1211	Panreac
Acetic Acid	1,00063,2500	Merck
Glucose	1,08337,0250	Merck
Formic acid	1,00264,2500	Merck
Fructose	F0127-1006	Sigma Aldrich
Glycolic Acid	124737-256	Sigma
Glycolaldehyde dimer	G6805-1G	Aldrich
Pyruvic Acid	107360-25G	Aldrich
5HMF	W501808-25G-K	SAFC
Levulinic Acid	L2009-50G	Aldrich
DL-Glycerolaldehyde	G5001-500MG	Sigma
Glycerol	141339,1212	Panreac

3.2. Experimental Apparatus (Batch Reactor)

All experiments in this study were performed in batch-type Parr 5500 High Pressure Compact reactor developed by Parr Instrument Company. This reactor has such characteristics: the vessel is rated for a maximum working of 3000 psi, the maximum operating temperature is 350 °C, and volume is 300 ml. The reactor equipped with gas inlet and outlet valves, liquid sampling valve, pressure gage, safety rupture disc, internal stirrer and internal thermocouple. The reactor is constructed from stainless steel type 316. Experimental setup contained the reactor, cooler and controller are illustrated in Figure 3.1.

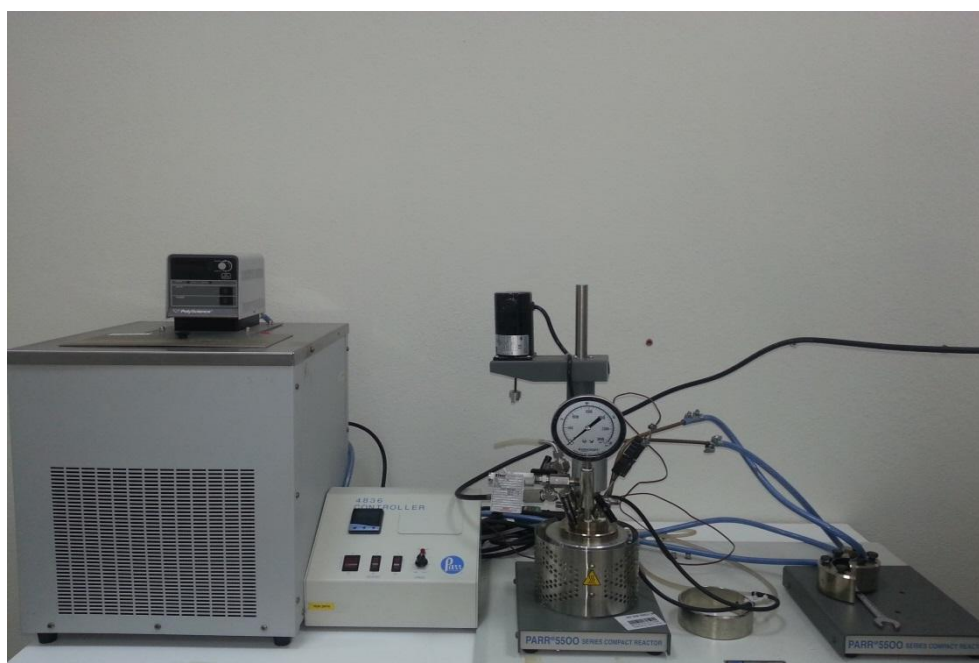


Figure 3.1. Experimental setup included the reactor, controller and cooler.

3.3. Experimental Procedure

For solution preparation each time 4 grams of cellulose were weighed and added to 100 ml of de-ionized water. The pH value of the solution was measured and it was equal to 6.82 (at 21 °C) in non-oxidizer reaction. This value was taken as initial pH and used to compare with pH of produced liquor. In each experiment with adding oxidizer, initial pH was re-measured. As oxidizing agents sulfuric acid (H_2SO_4) and

hydrogen peroxide (H_2O_2) were used in order to compare reaction results. Due to operation safety of the reactor volume of solution was adjusted to 100 ml. During the reaction, temperature and pressure were recorded in every 5 minutes before and after reaching set reaction conditions. After reaching the set values, reaction time was initialized which means the reaction is started. The temperature-pressure profile for 280 °C and 60 min is given in Figure 3.2.

At the end of reaction time, heater was turned off. In general temperature ranges of reaction were 150-280 °C, final pressure vary from 5 to 64 bars, respectively. Reaction times were 30, 60, 90 and 120 minutes. Initial pressure was applied (5, 10 and 15 bars) in order to see influence on the desired product. Experimental data is given in Section 3.5. at the end of this chapter.

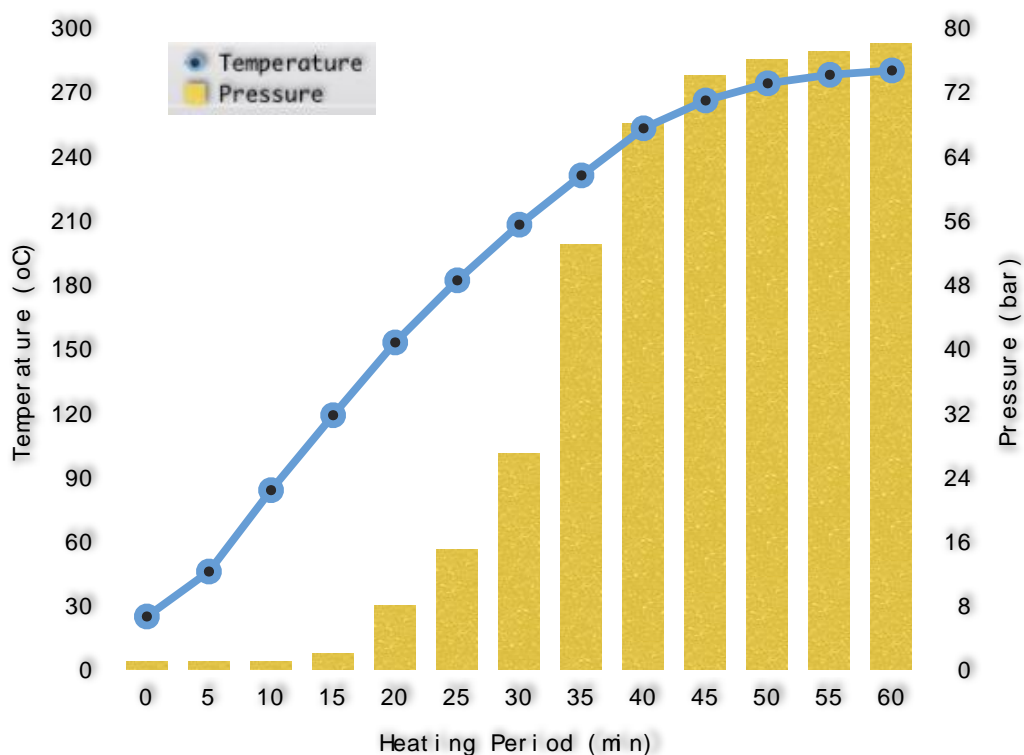


Figure 3.2. Temperature-pressure profile (280 °C and 60 min)

As well known from literature, the final product could contain gaseous, liquid and solid portions. Each portion was separated at the end of each experiment. Gaseous products were collected to special gas collecting bags through gas output valve. Solid and liquid parts were separated via filtration paper. pH value of the liquid portion was measured. Solid portion was put into vacuum oven with set conditions as 40°C for 48 hours. After

drying water insoluble portion was weighted to compare with the initial amount of cellulose. Differences between weights were considered as dissolved in water and to calculate cellulose conversion in “%” the equation was used:

$$x = \frac{\text{Cellulose}(\text{initial}) - \text{Cellulose}(\text{after_rxn})}{\text{Cellulose}(\text{initial})} \times 100\% \quad (3.1)$$

This data was used for further calculation of liquid products yield. In early stage of the study, solid portion was examined in terms of hexane solubility but since there were no significant differences in the product, it was stopped to analyze hexane soluble portion. Solid products were taken for further analysis such as SEM-EDX, FTIR, elemental analysis (CHNS), XRD. Liquid products were analyzed by GC-MS and HPLC and the gaseous products were monitored by GC-TCD. The general scheme of procedure is given in Figure 3.3.

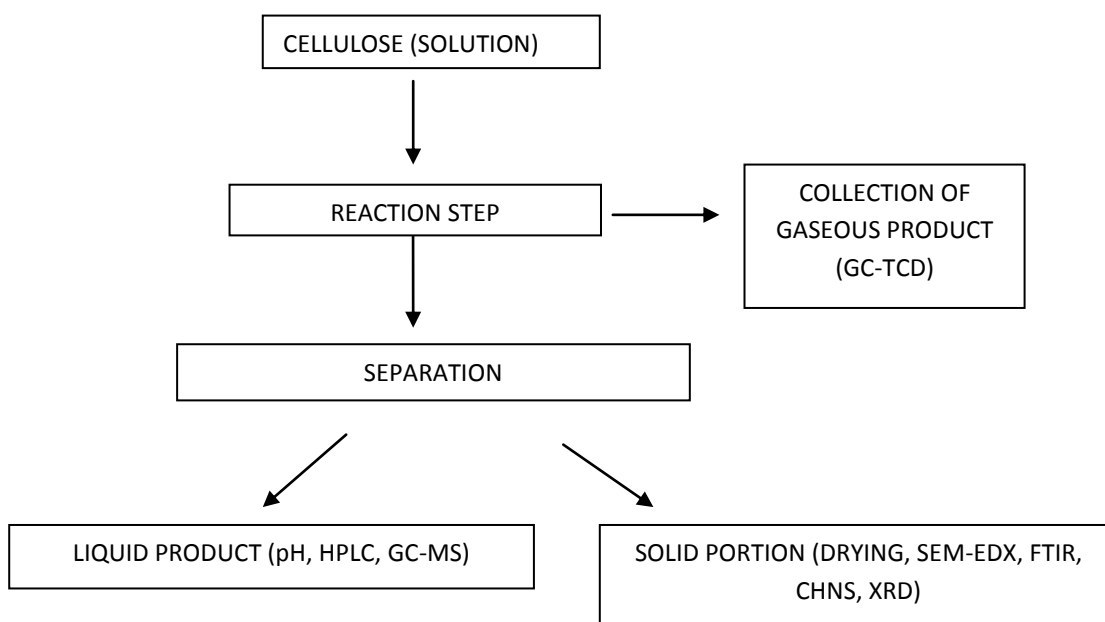


Figure 3.3. Scheme the Steps of Experimental Procedure

The yield of each product contained after reaction liquor obtained by HPLC was measured with this equation:

$$\text{Yield}_{-A} = \frac{A_{-concentration}(ppm)}{\text{Degraded}_{-cellulose}(ppm)} \times 100\% \quad (3.2)$$

In this equation, to calculate yield of “A” product the concentration obtained from HPLC analysis (ppm) is divided to degraded cellulose (ppm) and multiplied to 100%.

3.4. Product Analysis

In total about 50 experiments were performed and after each, solid, liquid and gaseous products were analyzed with different equipment as listed below.

3.4.1. Analysis of the Solid Products

The solid portion of sample was analyzed with SEM-EDX, FTIR and Elemental (CHNS) analyses. These analyses were performed to confirm results and support each others. A scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDX) was done with a model of Philips XL 30 FEG in the Materials Research Center at Izmir Institute of Technology (IZTECH) to see visual changes in the structure and determine carbon and oxygen content of solid portion. Samples were sputter-coated in gold and were zoomed in 1000, 2000, 5000, 20000 and 50000 times (100 μ m, 50 μ m, 20 μ m, 5 μ m and 2 μ m respectively). Additionally, Fourier transform infrared spectroscopy (FTIR- Shimadzu-8400S) and Elemental (CHNS) analysis (LECO CHNS) were performed in Chemical Engineering Department and the Department of Chemistry at IZTECH, respectively.

3.4.2. Analysis of the Liquid Products

To identify content of liquid product high-performed liquid chromatography (HPLC) was used. This analysis was done in Environmental Development Application and Research Center IZTECH by chromatograph Agilent 1200 with Refractive Index Detector. Shodex SH 1011 Sugar column was used which has operating pressure about 30 bar and it was heated up to 50°C and 65°C. As a mobile phase 3.75 mM solution of sulfuric acid in water was used.

To check and support HPLC results gas chromatography – mass spectrometry (GC-MS) was also performed in the same research center. The instrument was Agilent GC

6890N/MS5973 and Restek Stabilwax-DA column was used. Inlet temperature was set to 240 °C.

3.4.3. Analysis of the Gas Products

To determine content of gaseous product gas chromatography with thermal conductivity detector (GC-TCD) was used. It is also located at Environmental Development Application and Research Center at IZTECH. The instrument is Agilent 6890N Network GC System with Agilent 7683 injector.

3.5. Cellulose Degradation Experiments in Subcritical Water

The study started with oxidizer-free conditions. Different temperatures (150-280 °C) and reaction times (30-120 min) were applied in order to compare the product distribution, yields and cellulose conversion. As it was mentioned before, during the study two oxidizing agents at different concentrations were tested to see the differences in the final products yield. The first oxidizer was sulfuric acid (H_2SO_4). The concentration of H_2SO_4 in experiments were 5, 25, 50, 75, 100 and 125 mM. During the most of experiments concentration of H_2SO_4 was 50 mM. Analyses results were compared with oxidizer-free experiments. Then, it was decided to test hydrogen peroxide (H_2O_2) to see the effect of this oxidizer. After getting analyses results and comparing them with H_2SO_4 , since the yield of levulinic acid was so low with H_2O_2 , the rest of the experiments were carried in the presence of H_2SO_4 . Additionally, initial pressure such as 5, 10 and 15 bar was loaded at the beginning of the reaction to see influence on the final products distribution at temperatures of 200°C and 250°C for 60 mins. The gaseous product formation was observed usually at temperatures higher than 200°C. Data of the performed experiments is given in Table 3.2. Experiments are numbered according to oxidizing agent concentration and were performed in different period in order to make easier comparison of results.

Table 3.2. Experimental data of study

Experiment number	Oxidizer amount (H₂SO₄) (mM)	T (°C)	t (min)	Initial P (bar)
1	0	150	60	----
2	0	200	60	----
3	0	250	60	----
4	0	150	30	----
5	0	150	90	----
6	0	150	120	----
7	0	200	30	----
8	0	200	90	----
9	0	200	120	----
10	0	250	30	----
11	0	250	90	----
12	0	250	120	----
13	5	150	60	----
14	5	150	120	----
15	5	200	60	----
16	5	200	90	----
17	5	250	30	----
18	5	250	60	----
19	5	250	90	----
20	5	250	120	----
21	25	200	60	----
22	50	150	30	----
23	50	150	60	----
24	50	150	90	----
25	50	150	120	----
26	50	200	30	----
27	50	200	60	----
28	50	200	90	----

(Cont. on next page)

Table 3.2. (Cont.)

29	50	200	120	----
30	50	250	30	----
31	50	250	60	----
32	50	250	90	----
33	50	250	120	----
34	50	280	30	----
35	50	280	60	----
36	50	280	90	----
37	50	280	120	----
38	50	200	60	5
39	50	200	60	10
40	50	200	60	15
41	50	250	60	5
42	50	250	60	10
43	50	250	60	15
44	75	200	60	----
45	100	200	60	----
46	125	200	60	----
	H₂O₂ (mM)			
47	50	200	60	----
48	75	200	60	----
49	100	200	60	----
50	125	200	60	----

CHAPTER 4

RESULTS AND DISCUSSION

During this study, we carried out experiments at various reaction temperatures (150, 200, 250 and 280 °C), initial pressures (0, 5, 10 and 15 bars), reaction times (30, 60, 90 and 120 mins) oxidant concentrations (0, 5, 25, 50, 75, 100 and 125 mM) and oxidant types (H_2SO_4 and H_2O_2). After each experiment, we observed the effects of these parameters on the degradation of cellulose, the product distribution and yields. Although, we monitored the gaseous, liquid and solid products, we focused on the liquefaction of cellulose. So that gaseous product distribution was only mentioned for one run which was done at higher reaction temperature (280 °C). After the hydrothermal degradation of cellulose with an acid, pyruvic acid, glycolaldehyde, glyceraldehyde, formic acid, lactic acid, acetic acid, levulinic acid, 5-HMF, glycerol, furfural, were formed as liquid products. In addition to them, some oligomers (cellobiose, cellotriose, etc.) and monomers (glucose, fructose) were also identified by HPLC in the liquid product solution (Figure 4.1)

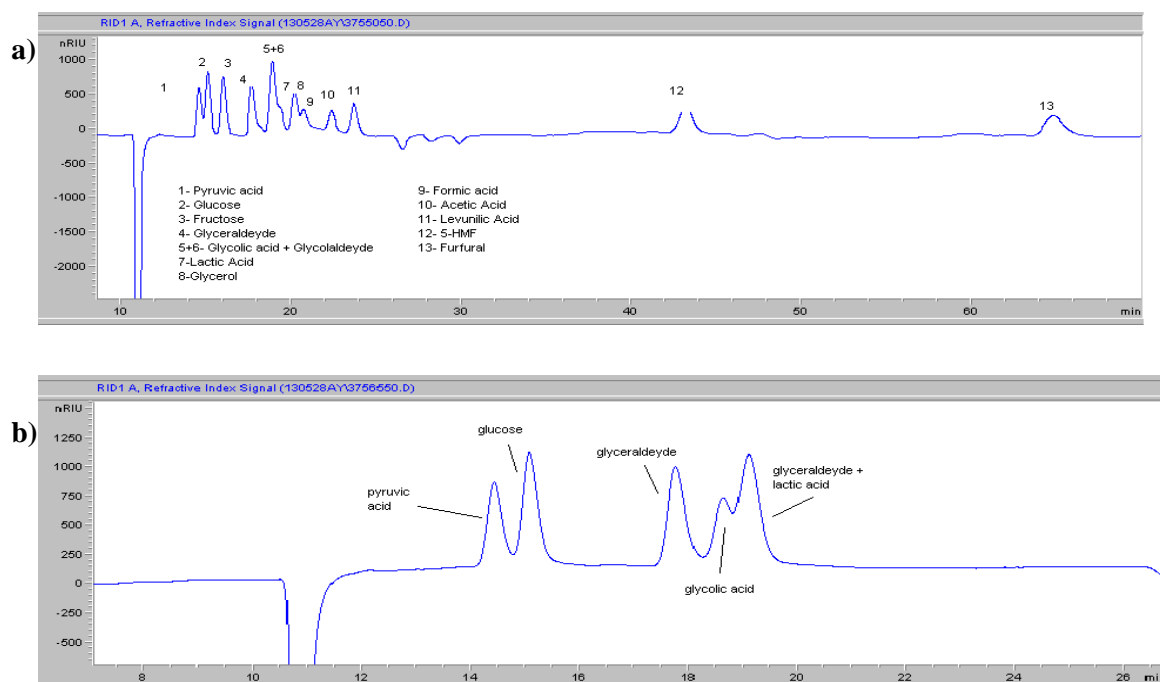


Figure 4.1. Refractive index spectral data of standard compounds at a) 50°C and b) 65°C

Since there were many products in the liquor, the identification of all of them was not possible. Therefore, by using GC-MS, we tried to re-observe the same products identified by HPLC and one of the GC-MS chromatogram is given in Figure 4.2.

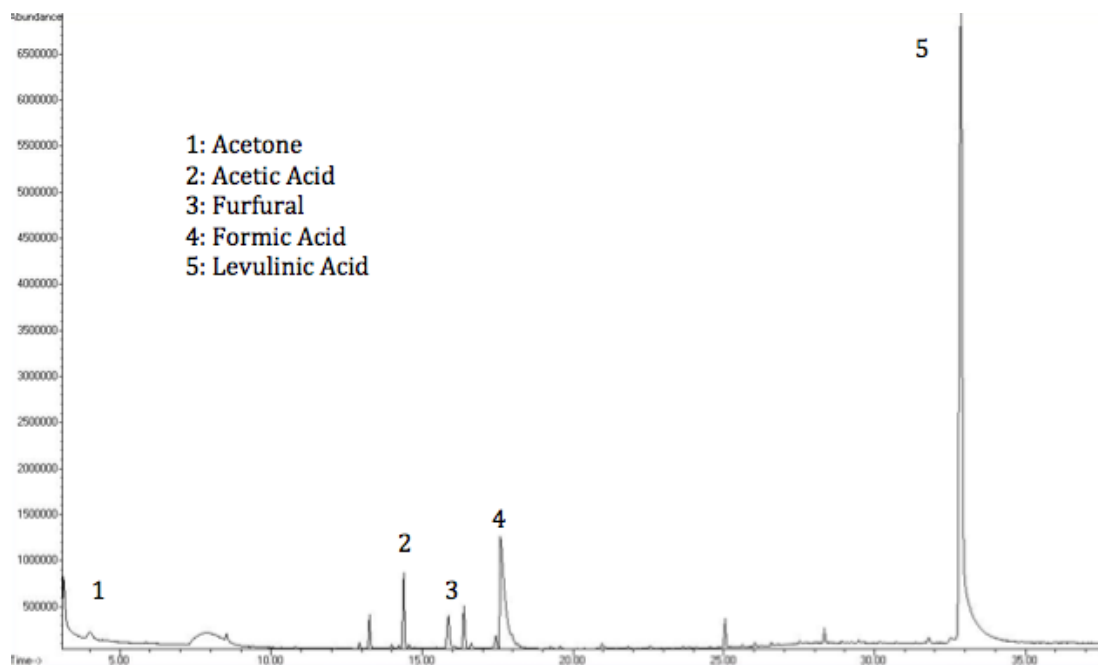


Figure 4.2. GC-MS chromatogram of the liquid phase recovered after treatment with 125 mM H₂SO₄ at 200 °C, 52 bar, 60 min.

4.1 Effect of Reaction Temperature on Cellulose Degradation and Product Yields

As it was mentioned in previous sections of this thesis, the effects of reaction conditions such as temperature, pressure, oxidizing agent and reaction time were the main aim of our study. As it was expected, the temperature was a main factor that effects cellulose conversion that was also mentioned in literatures (Ehara et al. 2005, Williams et al. 2006, Cardenas-Toro et al. 2014). As it was mentioned, reactions were carried out at 150, 200, 250 and 280 °C. The amount of converted cellulose was measured by weighting remained solid portion, and calculated with respect to the initial amount of cellulose (4 grams).

In acid concentration of 50 mM, the highest cellulose conversion value in different residence time was seen at 280 °C with a value of 73.1%, whereas the least amount of conversion was observed at 150 °C with a value of 10.9% at the same conditions. The comparison of cellulose conversion levels at different temperatures and reaction times is given in Figure 4.3. The second highest result of cellulose conversion was observed at 250 °C in all reaction times but starting from 60 mins, the differences of cellulose conversion level between 250°C and 200°C are slight.

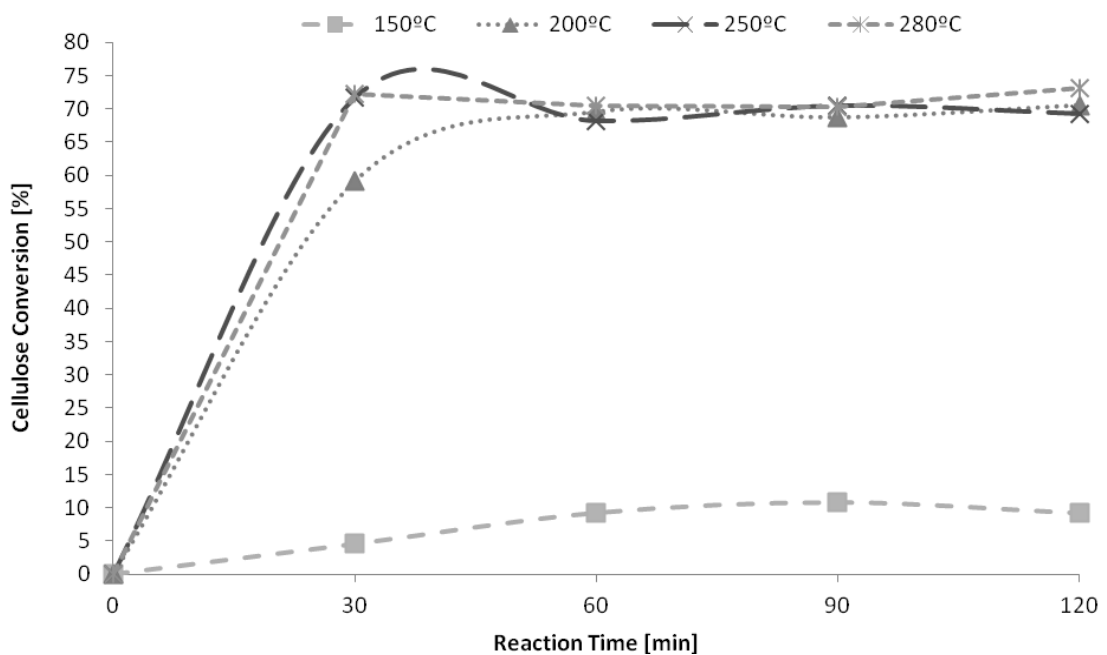


Figure 4.3. Cellulose Conversion at different reaction times (0-120 mins) and temperatures (150-280 °C)

Even after 120 mins, level of cellulose conversion was around 70.5% at 200 °C and 69.3% at 250 °C respectively. As well known, the thermodynamic properties of water play a key role during the hydrothermal treatment and increasing temperature and correspondingly pressure directly affect the treated raw material. Ehara et al., studied decomposition behavior of cellulose and highlighted that under different conditions of water, mechanism of cellulose conversion would also be different (Ehara et al. 2005). As well known from literature (Cardenas-Toro et al. 2014) the main idea of subcritical condition is to create favorable environment for breakdown glycosidic bonds of cellulose that have high chemical resistance. It leads to cellulose decomposition to oligomers. These oligomers have various degree of polymerization (DP) and could be

water-soluble (DP<6) and water-insoluble (DP<200). The addition of oxidizing agent increases the hydrolysis of cellulose and formation of products (Bicker et al. 2005). Those results were confirmed in our study. Liquid product contains a high number of various compounds. Among them, we decided to give the results of levulinic acid, formic acid, acetic acid and 5-HMF. Percentage values of cellulose decomposition and products yields at different temperatures observed during HPLC analysis are given in Figure 4.4.

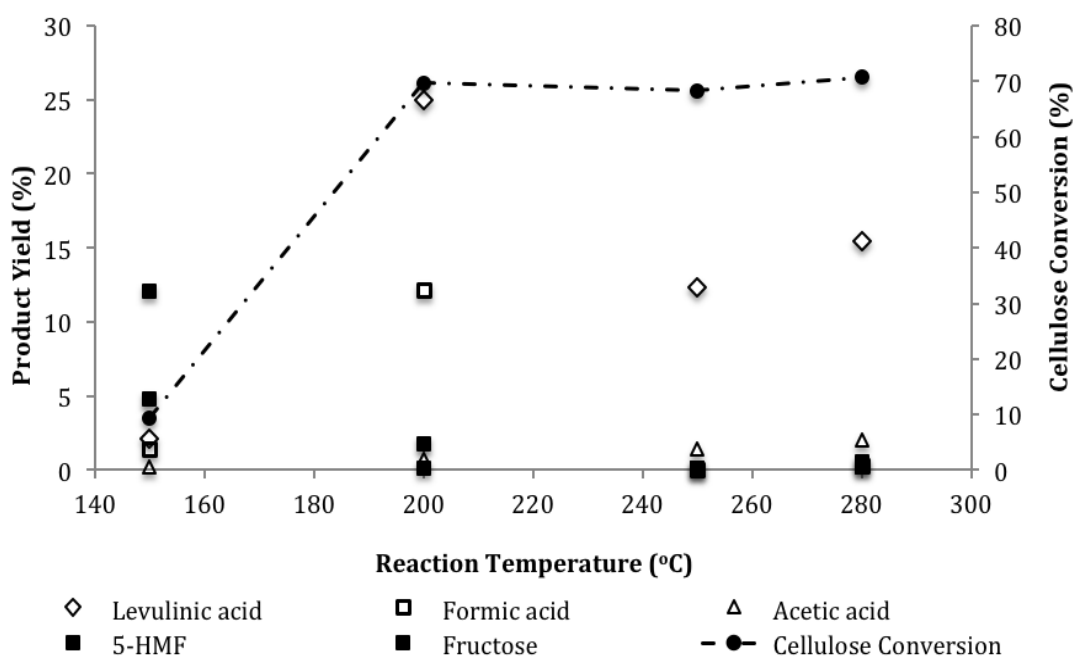


Figure 4.4. Conversion of cellulose and distribution of the liquid products with 50 mM H₂SO₄ at different reaction temperatures

In Figure 4.4, it was seen that at 150 °C, the level of cellulose conversion and yield of levulinic acid were low but at the same time yield of 5-HMF was comparatively high. At 200 °C, the amount of 5-HMF in liquid product dropped while the percentage of cellulose conversion and yields of levulinic acid and formic acid were higher. It might be as proposed from the literature that during the conversion of cellulose in the first stage glucose was formed. Glucose isomerized to fructose, which by-turn formed 5-HMF (Rosatella et al 2011). 5-HMF was the main intermediate and could form various final compounds including levulinic acid and its by-product formic acid at higher temperatures (Zeng et al. 2010, Rosatella et al. 2011, Cardenas-Toro et al. 2014). This information from literature is totally reflected in our results. As it can be seen from Figure 4.4, when levulinic acid and formic acid concentrations were high, the

concentration of 5-HMF was low which means that 5-HMF was dehydrated. According to Kruse et al. (2003), acetic acid was one of the minor acid formed from hydrothermal degradation of cellulose and the effect of temperature on the amount of acetic acid was low, which could also be seen from Figure 4.4.

In the case of levulinic acid yield as the desired product, the effect of the temperature at different reaction time is given in Figure 4.5. From this diagram it was seen that the yield of levulinic acid reached to 24.9% at 200 °C within 60 mins.

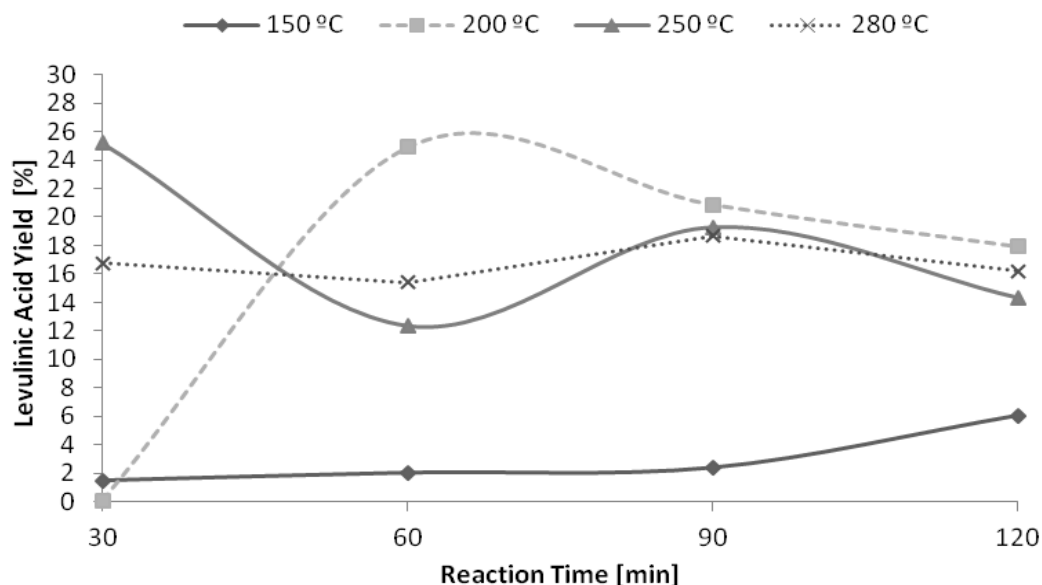


Figure 4.5. Levulinic acid yield with 50 mM H₂SO₄ from 30 min to 120 min reaction time at various reaction temperatures of 150, 200, 250 and 280 °C

From Figure 4.5, it could be seen that for the first 30 min, the yield of levulinic acid was high at 250 °C. In the case of 200 °C, there was no levulinic acid detected at 30 min, but after 60 mins it reached to 25.21 and 18% respectively. In general, as the reaction time was longer, the yield of levulinic acid decreased, except for 150 °C for which it increased by time. The temperature-time correlation is an essential factor in terms of levulinic acid formation. According to Girisuta et al. (2006), the best result of levulinic acid yield was obtained at higher reaction temperature but at shorter reaction time. At the same study, it was concluded that high yield of levulinic acid resulted in less amount of 5-HMF produced in the liquor. By the increase in temperature, the structure of solid part was also changing as shown by SEM images in Figure 4.5.

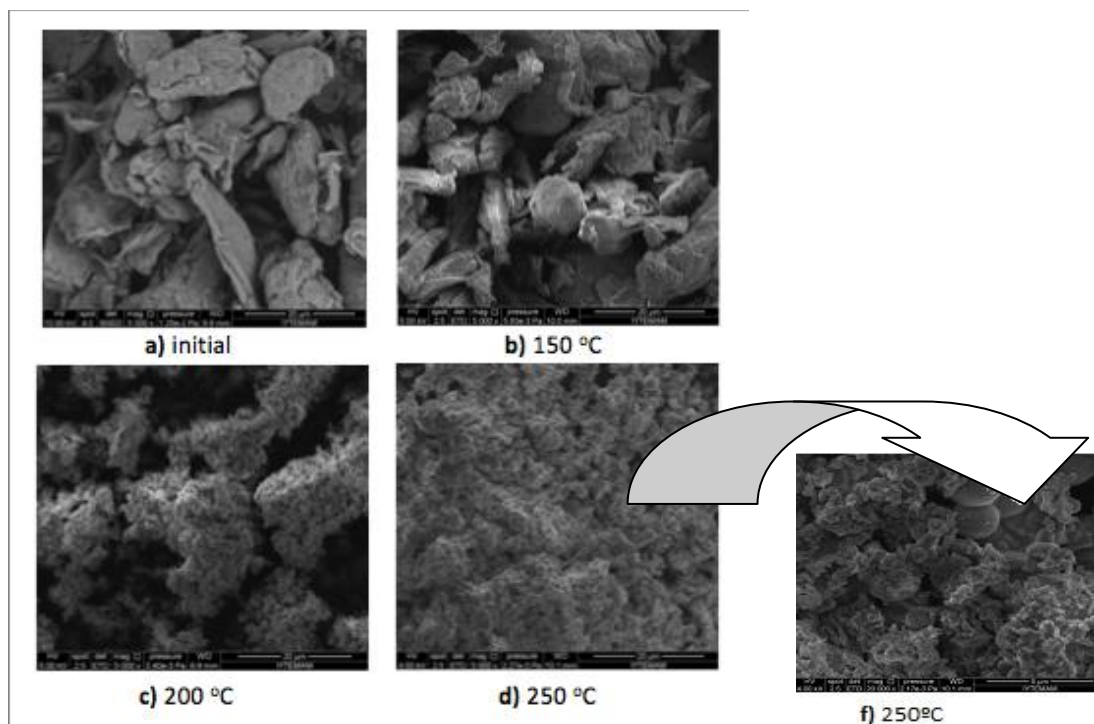


Figure 4.6. SEM images (same scale-1000X) of a) initial cellulose and solid products after the treatment at b) 150 °C; c) 200 °C; d) 250 °C; f) humins at 250 °C (magnitude 20000, 5 micrometer)

Micrystalline cellulose was taken as an initial (Figure a) to see differences after reaction solid part. As it is seen from pictures by increasing the temperature of reaction there are visual changes in structures of solid part. At high temperatures (here is 250°C) and with high acid concentrations, formation of black, insoluble particles known as ‘humins’ were observed which is clearly seen in picture f. Clearly visible typical round shaped, agglomerated humin particles have particle sizes in the range of 5–15 μm .

According Kruse et al. (2003) after hydrothermal treatment the dark brown deposit was formed and it was not possible to quantitatively identify the content of it. In this study we tried to describe the content of solid product by FTIR and CHNS analyses. FTIR spectrum of formed solid portions at 150 °C, 200 °C, 250 °C and 280 °C is given in Figure 4.7.

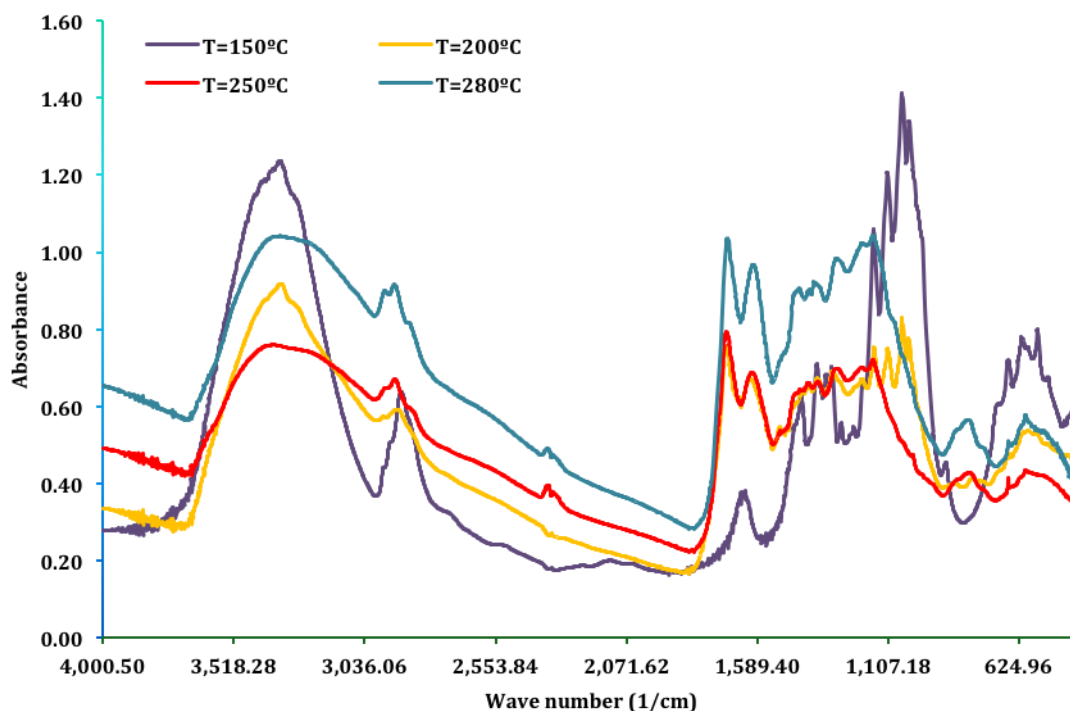


Figure 4.7. FTIR spectrum of solid portion at 150, 200, 250 and 280 °C.

In ranges from 3335 cm^{-1} broad O-H stretching vibrations observed which is highest at 150°C , indicates presence of the functional alcohols, phenols groups. Peak around $2750\text{--}2950\text{ cm}^{-1}$ of shown C-H stretching vibrations, which indicates presence aromatic hydrocarbons. The presence of aromatic hydrocarbons in solid product was also observed in literature (Cardenas-Toro et al. 2014). The peak around $1685\text{--}1775\text{ cm}^{-1}$ which is higher at 280°C , belongs to C=O stretching vibrations which indicates the presence of aliphatic groups. The peak of 935 cm^{-1} belongs to medium bonded O-H shows presence of carboxylic acids functional group. The CHNS results also have shown the difference between the reaction temperatures of 150°C and 250°C . The CHNS analysis results are displayed in Table 4.1.

Table 4.1. Elementary composition of solid product formed at 150°C and 250°C with 50 mM of H_2SO_4 for 60 min

	C	H	N	S
150°C	56.14	6.17	0	0.407
250°C	72.27	4.17	0	1.326

As it is seen from this table, at 250 °C comparatively higher level of loss in hydrogen amount was observed. The dehydration occurred during the process of cellulose conversion, which was also noticed in Kruse et al (2003). The importance of temperature in cellulose conversion is demonstrably seen in liquid, solid products formation. Corresponding with HPLC results GC-MS analysis results are given in Figure 4.8.

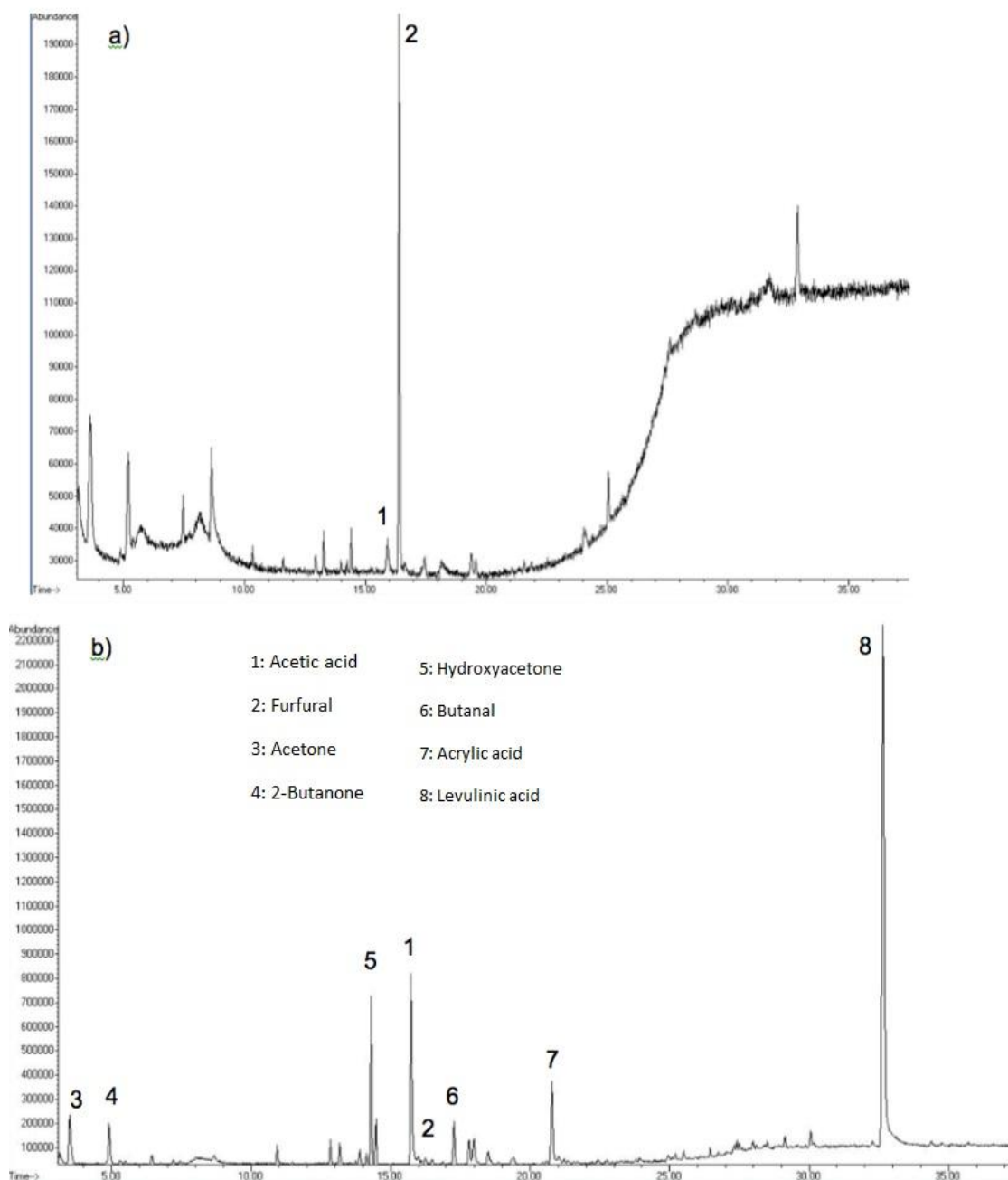


Figure 4.8. GC-MS spectrum of the liquid products after the treatment with 50 mM H_2SO_4 for 60 min at a reaction temperature of a) 150 °C and b) 280 °C

The importance of temperature in cellulose conversion is demonstrably seen in liquid, solid products formation. Corresponding with HPLC results GC-MS analysis results are given in Figure 4.8.

The distribution of gaseous product is higher at higher temperature and longer reaction time (Kruse et al. 2003, Girisuta et al. 2005, Cardenas-Toro et al. 2014). In our study the maximum value of temperature and reaction time were 280 °C and 120 mins, respectively. As gaseous product by GC-TCD analysis, mainly H₂, O₂, CO, CH₄, CO₂, C₂H₂ and C₂H₄ were observed as illustrated in Figure 4.9. Similar observations were obtained in Kruse et al. (2003).

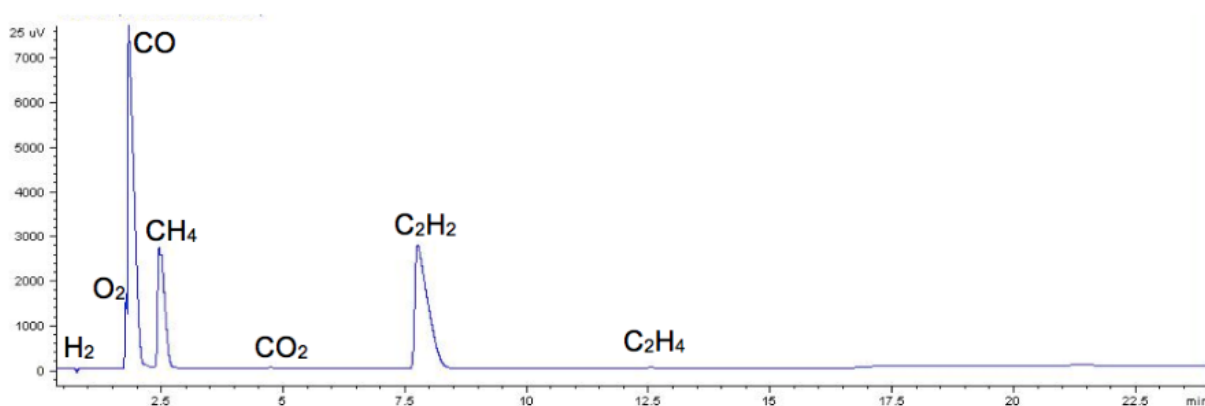


Figure 4.9. Gas products identified after the treatment with 50 mM H₂SO₄ at 280 °C for 120 mins

4.2 Effect of Reaction Pressure on Cellulose Degradation and Product Yields

In our study the reaction pressure range from 5 to 64 bars. Also, in order to see the effect of initial pressure on cellulose conversion and product distribution, initially pressure in values of 5, 10 and 15 bars were loaded into the reactor vessel. In Figure 4.10, the influence of initial pressure on cellulose conversion and acidity of liquid product was illustrated.

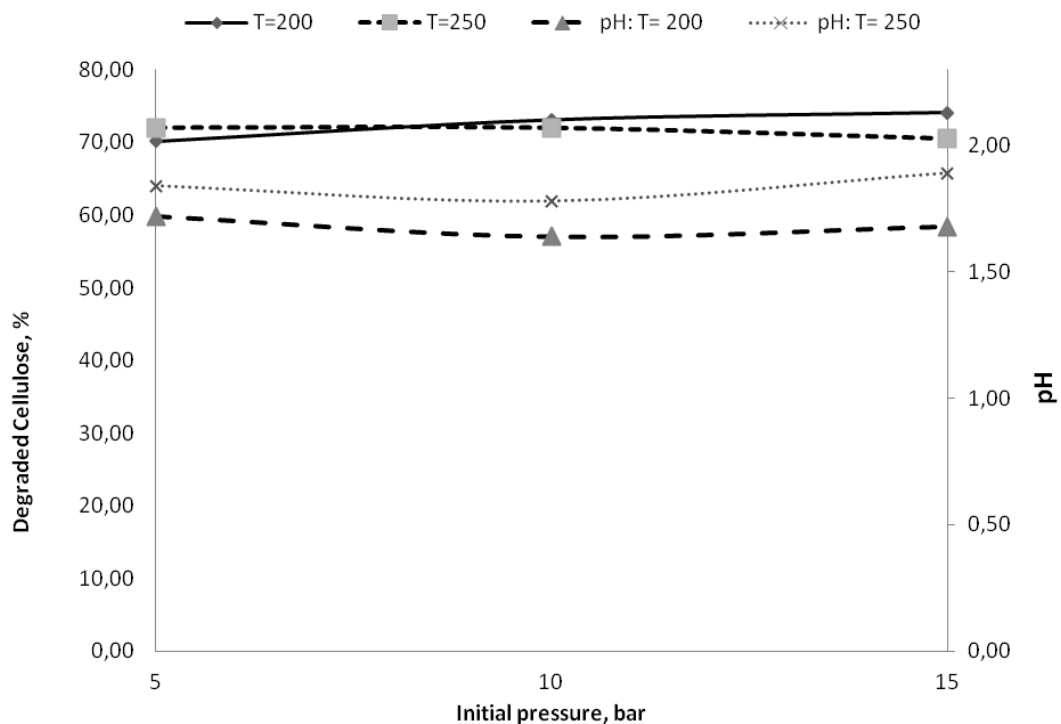


Figure 4.10. Influence of initial pressure (5, 10 and 15 bars) on the degradation of cellulose and acidity of the liquor after hydrothermal treatment with 50 mM H₂SO₄ for 60 mins

As it is seen from the figure above, there were no significant changes in cellulose conversion and pH value of liquid product with the increase in the initial pressure. Kruse et al. (2003) described the influence of pressure. It was explained that the absence of significant changes in cellulose conversion and product distribution was also hidden in water properties. As well known, in subcritical water conditions the pressure of reaction is equal to vaporized water and its function is to keep water in liquid state, to control density of environment. Decomposition of substrate will run in two reaction pathways such as ionic and free-radical. Ionic pathway dominates if there is high density and below the critical temperature or in supercritical fluid state when pressure is very high. Free-radical reaction pathway occurs if there is high temperature and low density. For instance, below critical point of water reaction runs in liquid phase and there is low dependence of water properties on the pressure, whereas above supercritical point, water is supercritical and its properties are strongly pressure-dependent. Since our study was carried out at temperatures below the critical point of water, pressure influence was not observed.

4.3 Effect of Acid Addition on Cellulose Degradation and Product Yields

One of the aims of our study was to compare the oxidizer-free and oxidizer-added conversion of cellulose and to see the influence of oxidizing agent on the product yields. In literature (Girisuta et al. 2005, Toor et al. 2011) many types of acids were described to degrade various types of biomasses. It was also highlighted that using oxidizer agent can improve gasification efficiency. In our study, as an oxidizing agent sulfuric acid was mainly used. The results of reaction were compared with oxidizer-free experiments results for the conversion level of cellulose is given in Figure 4.11.

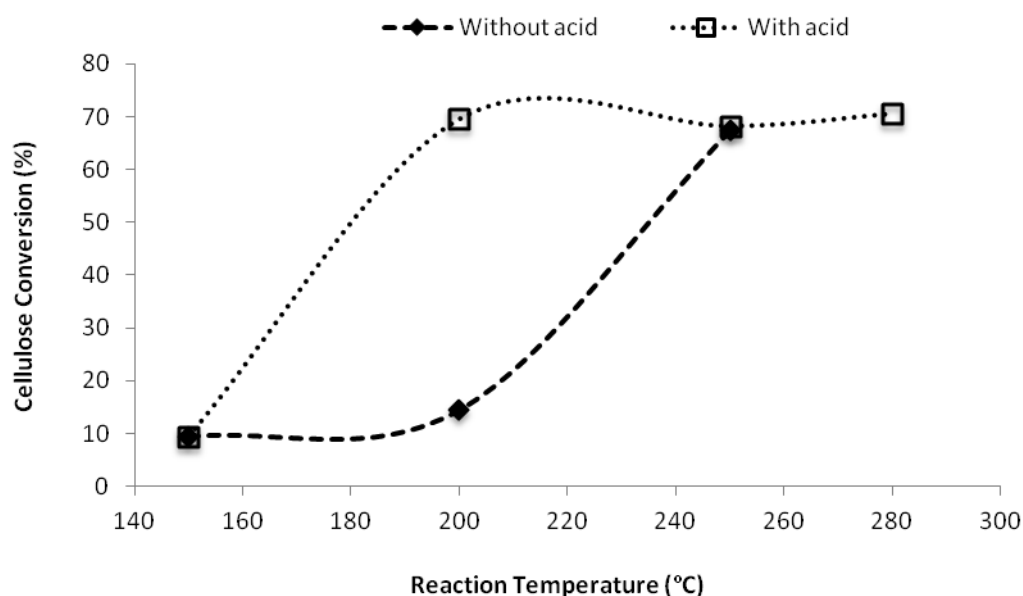


Figure 4.11. Effect of oxidizer addition (50 mM H₂SO₄) on the conversion of cellulose at various reaction temperatures

From Figure 4.11, it can be easily understood that presence of oxidizer gives better result compared with the experiments carried out in the absence of oxidizing agent. At low temperature (150 °C) the conversion of cellulose in both processes were nearly the same. However, at 200 °C the differences were perceivable, The cellulose conversions were recorded as 14.5 % in oxidizer-free reaction and 69.5% in oxidizer-added process. At 250°C the cellulose conversion levels were very close to each other.

In the case of product yields, it was observed that addition of oxidizing agent had a great influence on the production of organic acids including our intended product- levulinic acid, as

illustrated in Figure 4.12.

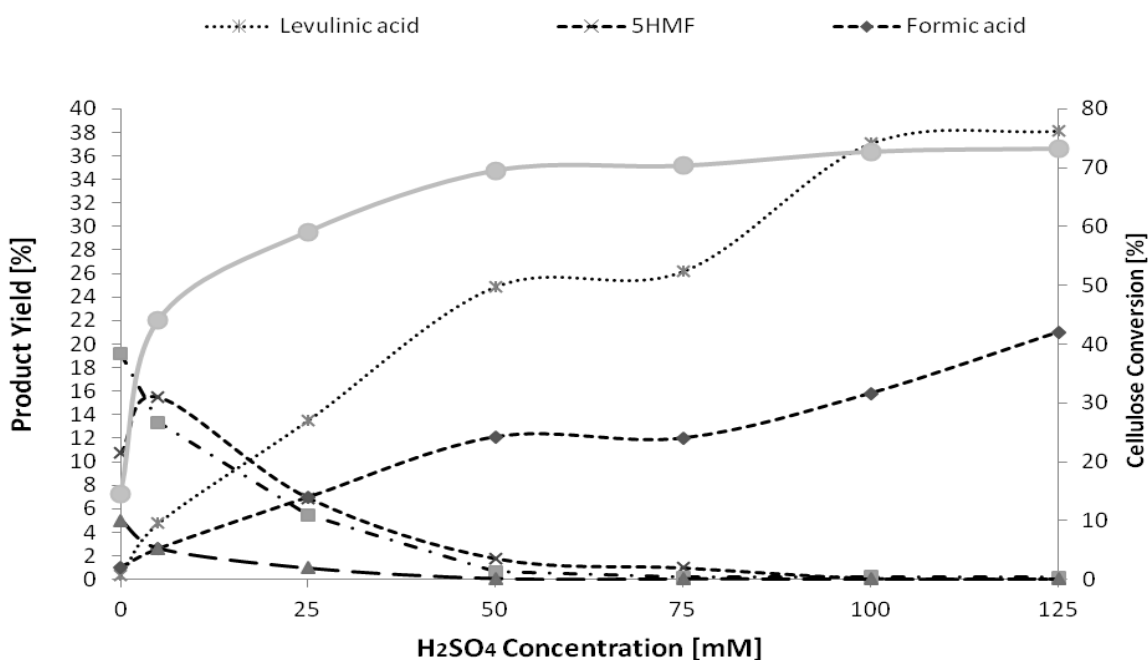


Figure 4.12. Effect of H₂SO₄ concentration on the conversion of cellulose and product yields (reaction time: 60 min; reaction temperature: 200 °C)

In our study H₂SO₄ was mainly used in concentration of 50 mM but as it seen from Figure 4.12, to compare the product distribution 5, 25, 75, 100 and 125 mM were also tested. In the same figure, yield of levulinic acid increased with increasing concentration of added H₂SO₄ and reached from 4.8 % (with 5 mM H₂SO₄) to 38% with the addition of 125 mM H₂SO₄. Similar trend was observed in the yield of formic acid. The yields were 2.6% and 21% after the treatment with 5 and 125 mM H₂SO₄, respectively. However, the yields of other products such as glucose, fructose, and 5-HMF were decreasing. This could be explained by the reaction mechanism of cellulose degradation in hot-compressed water. First glucose and fructose were formed as simple sugars from cellulose. Then they were further converted to 5-HMF as the main intermediate product. Finally, 5-HMF was decomposed into levulinic acid and formic acid (Zeng et al. 2010, Rosatella et al. 2011, Cardenas-Toro et al. 2014).

In Figure 4.13. the yield of levulinic acid in the presence and absence of oxidizing agent at 150 °C and 250 °C is shown. From this data we can confirm that effect of H₂SO₄ on the degradation of cellulose to produce levulinic acid was significant, which was also observed in literature (Toor et al. 2011).

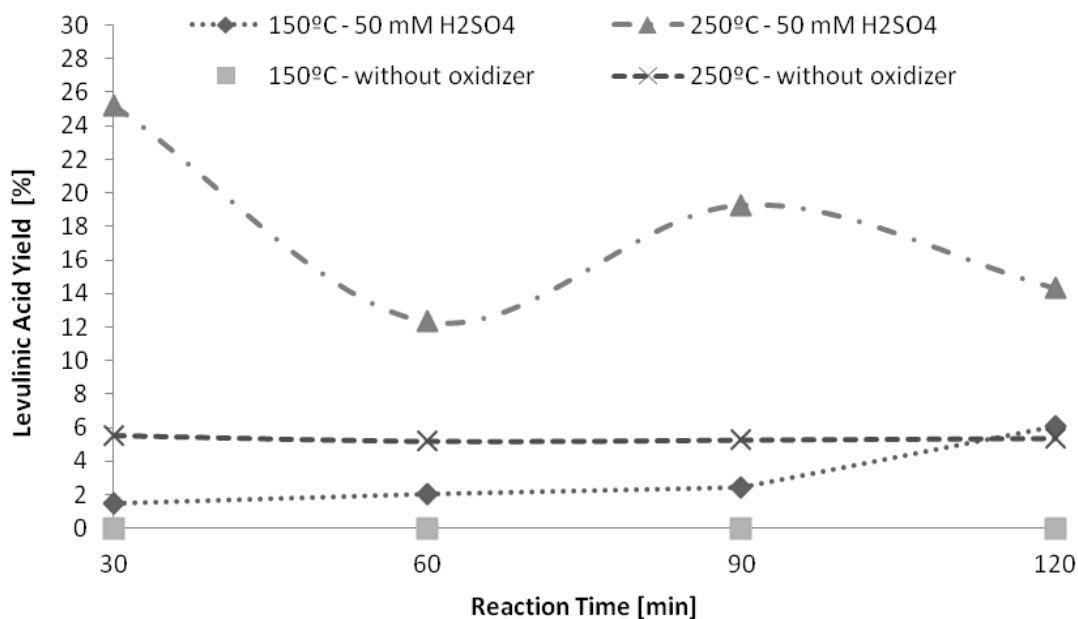


Figure 4.13. Levulinic acid yield with and without acid at 150 and 250 °C

Influence of oxidizing agent on solid product was visually observed by SEM analysis. The SEM images of solid portions obtained after the treatment with different amount of H₂SO₄ are given in Figure 4.14.

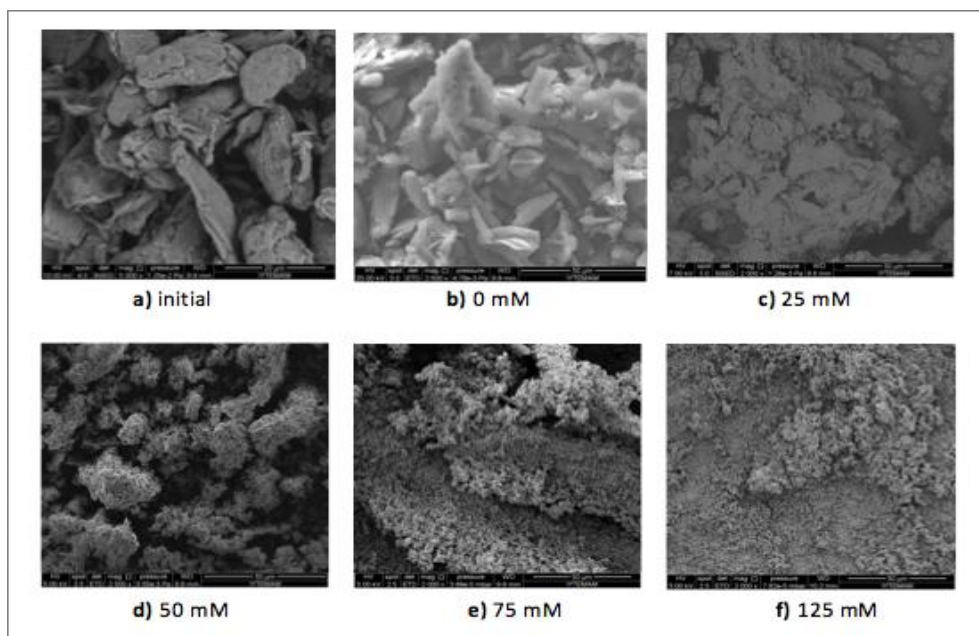


Figure 4.14. SEM images (same scale-1000X) of a) initial cellulose and solid products after the treatment with acid at a concentration of b) 0 mM; c) 25 mM; d) 50 mM; e) 75 mM; e) 125 mM

The effect of H₂SO₄ can clearly seen in pictures “b” (without any acid) and “f”(with 125 mM acid). Under the effect of 125 mM of oxidizing agent, agglomerated-round shaped

humins, which had similar structure obtained at high temperature treatment (280 °C), were observed. These changes can be considered as indications of significant effect of H₂SO₄ that was mentioned in literature (Toor et al. 2011).

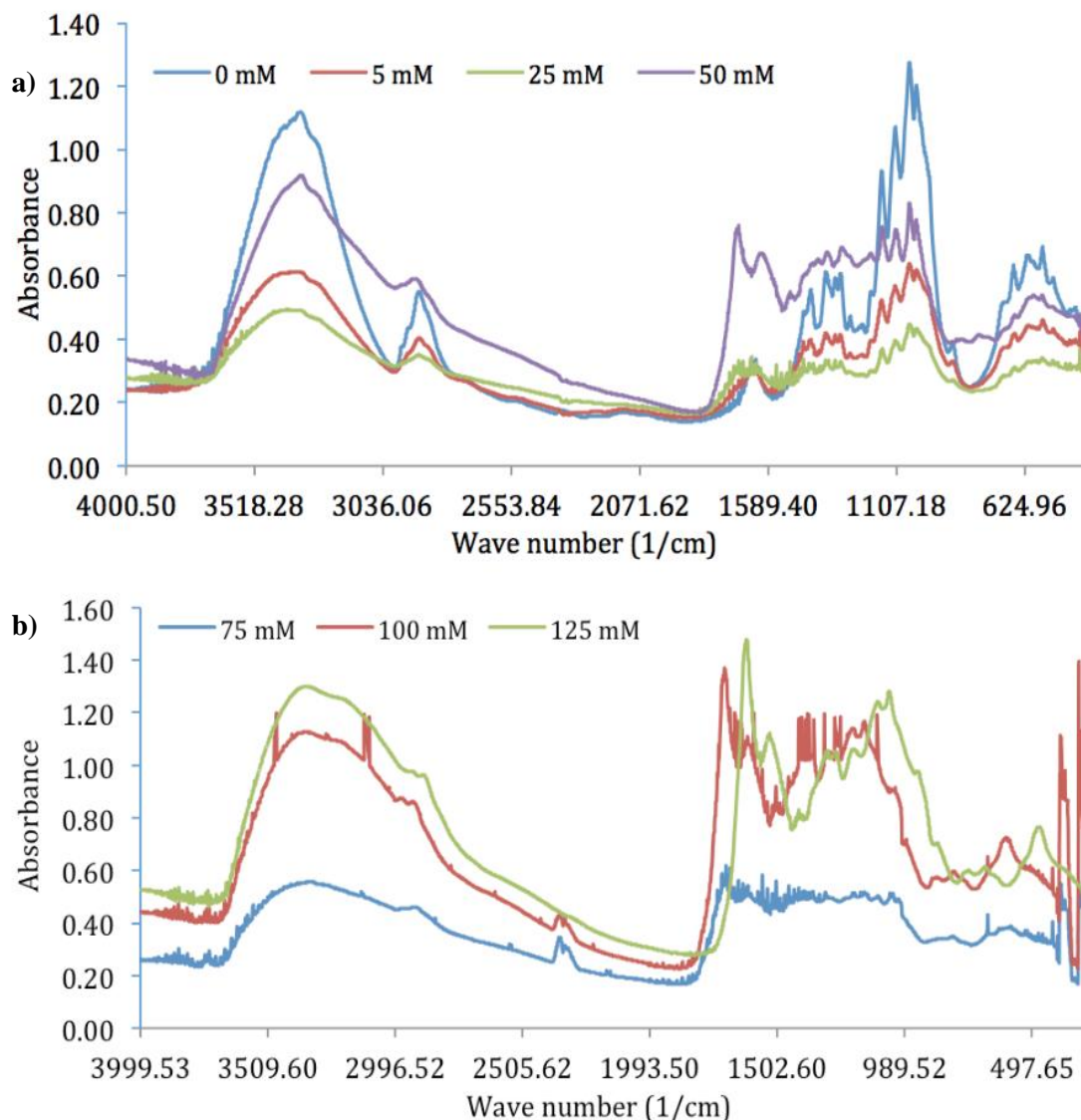


Figure 4.15. FTIR spectrum of solid portion with a) 0, 5, 25 and 50 mM H₂SO₄ and b) 75, 100 and 125 mM H₂SO₄ at 200 °C and 60 min

In Figure 4.15, the results of FTIR analysis after the treatment with the presence of various acid concentrations is shown. In this figure, the concentration of H₂SO₄ with 0, 5, 25 and 50 mM were grouped as “a”; 75, 100 and 125 mM were grouped as “b”.

In group “a” observed peaks at 3370 cm⁻¹ shows medium N-H stretch bonds indicating the presence of amides and amines. The peak at 2840 cm⁻¹ corresponds to medium C-H

stretch bond refers to alkanes. The peak at 1670 cm^{-1} shows weak-medium C=C stretching vibration also indicates the presence of alkenes. The peak at 1050 cm^{-1} shows strong C-O bonds that corresponds to carboxylic acids and alcohols. In group “b”, peak seen at 3400 cm^{-1} indicates strong O-H bond, which may refer to carboxylic group. The peak at 1650 cm^{-1} shows C=C stretching vibration indicating the presence of alkenes. The peak at 1200 cm^{-1} shows strong C-O stretching vibration refers to acetates. The peak at 990 cm^{-1} shows weak C-H rocking vibrations relating to ethers.

4.4 Effect of Oxidizer Type on Cellulose Degradation and Product Yields

As it was discussed before, the main idea of using oxidizing agent is to improve conversion of cellulose and increase the product yields by reducing the reaction temperature and time which leads to energy saving and saving the time. In the literature it was reported that many catalysts such as H_2SO_4 , KHCO_3 , ZnCl_2 (Bicker et al.2005), H_3PO_4 , oxalic acid, HCl , HI (Girisuta et al. 2005), $\text{SiO}_2/\text{Al}_2\text{O}_3$ (Zeng et al. 2010) and CO_2 (Cardenas-Toro et al. 2014) showed good result in hydrothermal treatment of biomass. After literature investigation we decided to test H_2O_2 in order to compare the results with H_2SO_4 . H_2O_2 was added to reaction solution with amount of 50, 75, 100 and 125 mM at temperature $200\text{ }^\circ\text{C}$ for 60 mins treatment. The influence on cellulose conversion and product yield was examined and the results are shown in Figure 4.16 .

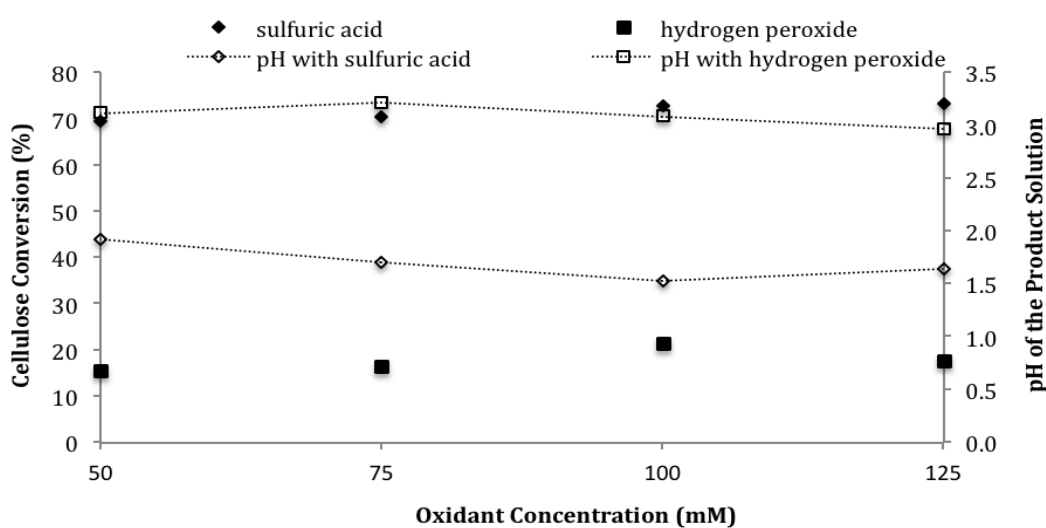


Figure 4.16. Effect of sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) addition on the conversion of cellulose at $200\text{ }^\circ\text{C}$ after 60 mins

As it is seen from Figure 4.16, the conversion values were totally different in the presence of H_2O_2 and H_2SO_4 . Level of cellulose conversion with 125 mM of H_2SO_4 was 73.2 %, whereas with the same amount of H_2O_2 it was only 17.6%. pH values with the same amount of acid addition were 1.64 and 2.97, respectively. It was expected that the product distribution in the presence of H_2O_2 and H_2SO_4 would also be different, was confirmed by HPLC analysis and the results are illustrated in Figure 4.17.

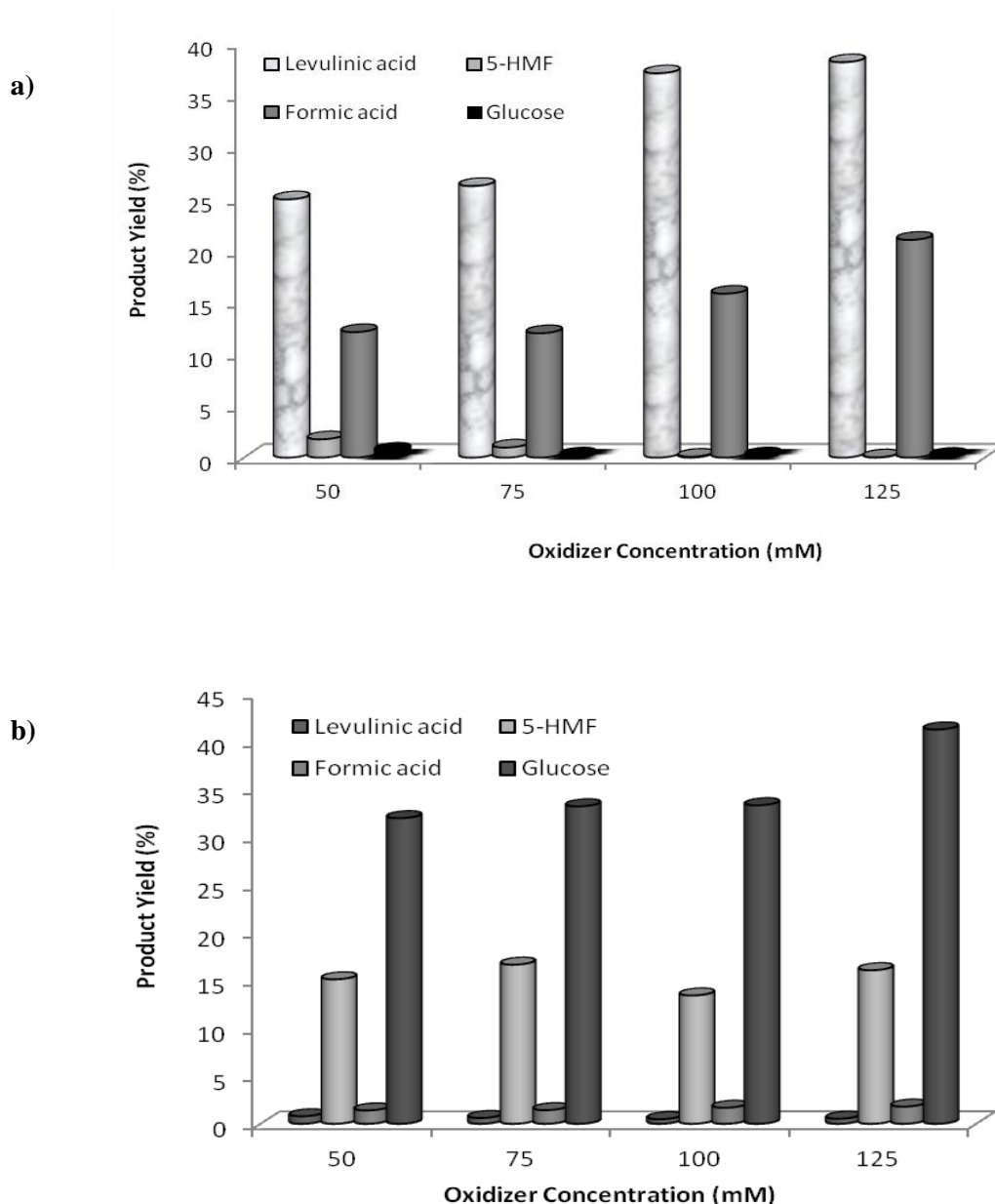


Figure 4.17. Effect of a) sulfuric acid (H_2SO_4) and b) hydrogen peroxide (H_2O_2) addition on the yields of levulinic acid, 5-HMF, formic acid and glucose at 200 °C after 60 mins

After HPLC, the liquid product was screened by GC-MS analysis. The chromatograms are given in Figure 4.18

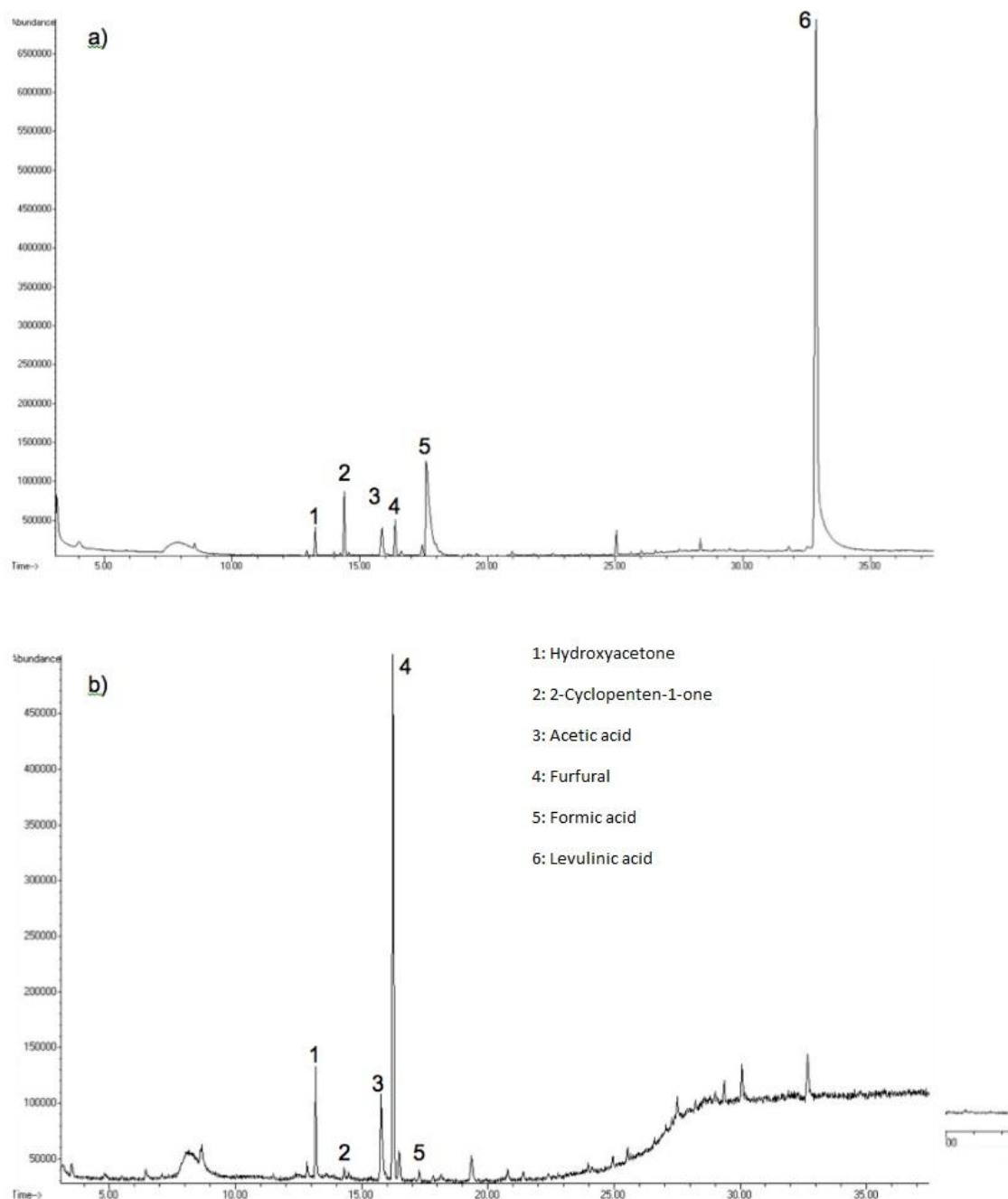


Figure 4.18. GC-MS spectrum of the liquid products after the treatment with a) 125 mM H_2SO_4 and b) 125 mM H_2O_2 for 60 min at a reaction temperature of $200^\circ C$

The HPLC and GC-MS analyses showed that among these two oxidizing agents, H_2SO_4 was favorable in terms of levulinic acid production. Totally different final liquid products of two oxidizing agents allow us to conclude that they can be useful in studies with different objectives. Bicker et al. (2005) proposed a new way of lactic acid

production through hydrothermal decomposition of carbohydrates. The main source was the glucose, which is monomer of most of carbohydrates. In this case H_2O_2 can be used to decompose cellulose. After obtaining the results of analyses and further discussion, it was decided not to use H_2O_2 in rest of the experiments due to low level of cellulose conversion and yield of the desired product.

4.5 Effect of Reaction Time on Cellulose Degradation and Product Yields

Correlation of all conditions can help us to understand the reaction mechanism to achieve high selectivity of the desired product. In our study, reactions were carried out for 30, 60, 90 and 120 mins. That means, almost all reaction parameters such as acid concentration, pressure, and temperature were tested in those ranges of reaction time. However, it should be noted that the experiments mainly were carried out in 60 mins. In literature (Kruse et al. 2003, Bicker et al. 2005, Girisuta et al. 2005, Ehara et al. 2005) different reaction times for the degradation of biomass were used depending on desirable product and objectives of the studies. In our study, time also affected the cellulose degradation and product yields depending mostly on added amount of oxidizing agent and reaction temperature. In Figure 4.19, the product yields were given at different reaction times with 50 mM oxidizing agent at 200 °C.

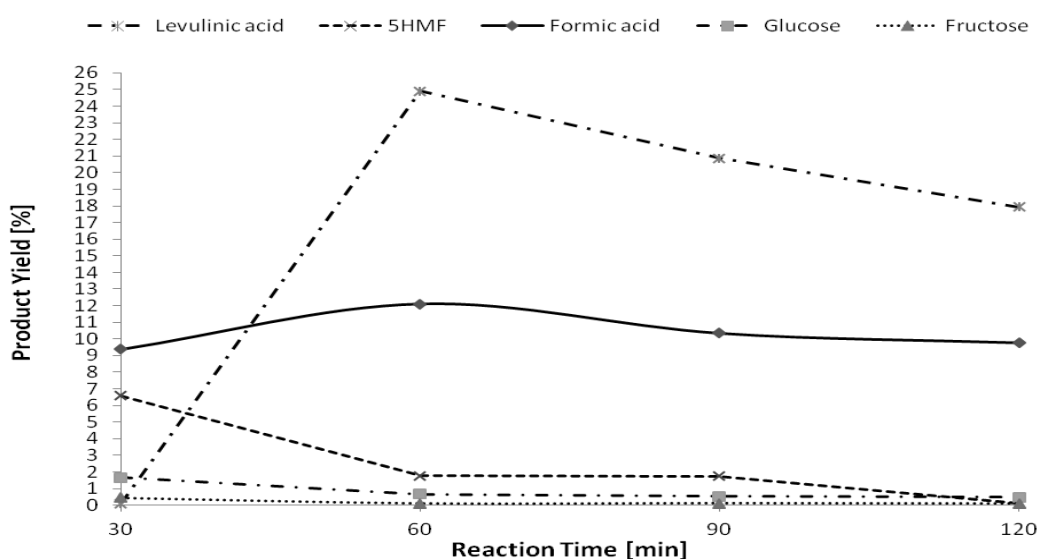


Figure 4.19. Effect of reaction time on the product distribution with the addition of 50 mM H_2SO_4 at 200 °C

As it can be seen from Figure 4.19, after 60 min treatment, the products yields started to decrease by the reaction time. For instance, at 30 mins the yield of levulinic acid drastically raised and achieved higher yield (25%) after 60 mins. Then, yield of levulinic acid exhibited a tendency to decrease. Formic acid yield was also decreasing but not as much as levulinic acid. Other products yield such as glucose, fructose and 5-HMF, as expected, dropped by the time. That is an evidence of decomposition of main carbohydrates and 5-HMF to other products including levulinic and formic acids. (Zeng et al. 2010, Rosatella et al. 2011, Cardenas-Toro et al. 2014). Peng et al. (2010) reported similar trend in levulinic acid yield decreasing from 10 wt % to 15 wt % as the reaction time increased. The reason for this effect might probably be related to insufficient amount of catalyst, feedback inhibition and decrease in reactivity.

4.6 Statistical Analysis by Analysis of Variance (ANOVA)

In order to obtain significance of experimental results, full factorial design of experiment was investigated via Minitab 16 software program. Factors such as reaction temperature, time and, concentration of H₂SO₄ affect the responses of analyzed design. In this design responses of experiments are the concentration of products. Therefore, ANOVA analysis quantifies the interaction between responses and factors by considering the couple affects of factors such as temperature and acid concentration.

ANOVA analysis were applied by accepting the p-value lower than 0.1. The p-values of factors differ accordingly to the responses. Therefore, same factors can exhibit different p-values in comparison between responses. ANOVA analysis gives the distribution of the residual in graphical representation in normal probability plot. The distribution indicates the applicability of analysis of variance to the response. A small (<0.1, a level of significance) p-value indicates that the two main factors (reaction temperature and acid concentration) and coupled interactions have statistically significant effect on the response.

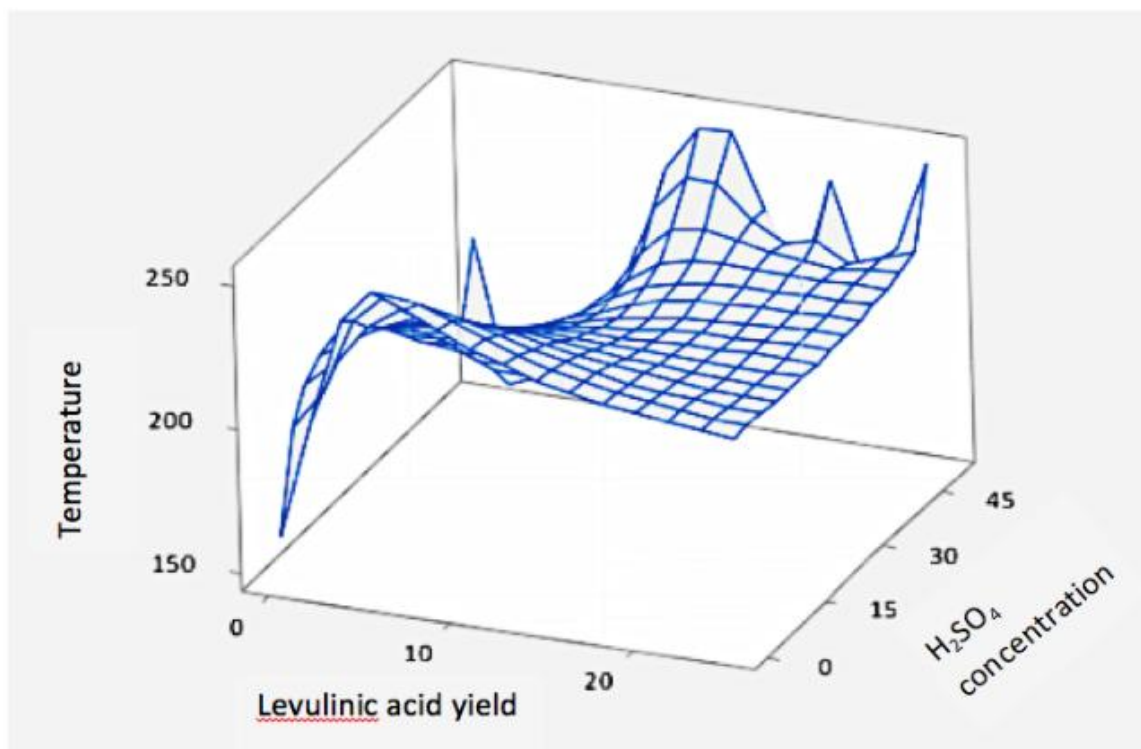


Figure 4.20. Surface plot of temperature (150, 200 and 250 °C) vs acid concentration (0, 15, 30 and 45 mM H₂SO₄) for Levulinic acid yield at 60 mins

Figure 4.20 shows the surface plot for levulinic acid to analyze the effect of temperature and acid concentration separately and their coupled effect. P-values for temperature effect and acid effect were 0.003 and 0.00, respectively. In the case of coupled effect, it was 0.061 (<0.1) indicating that both temperature and acid concentration have great influence on the yield of levulinic acid, which was also the case for the coupled effect.

Figure 4.21 shows the surface plot for 5-HMF to analyze the effect of temperature and acid concentration separately and their coupled effect. When we checked p-value for acid concentration, we noticed that by itself only, the H₂SO₄ concentration was not strongly affected on the yield of 5-HMF since the p-value was 0.206 which was greater than 0.1. However, when temperature and acid concentration were analyzed together, we can say that they have a great influence on 5-HMF yield with a p-value of 0.001.

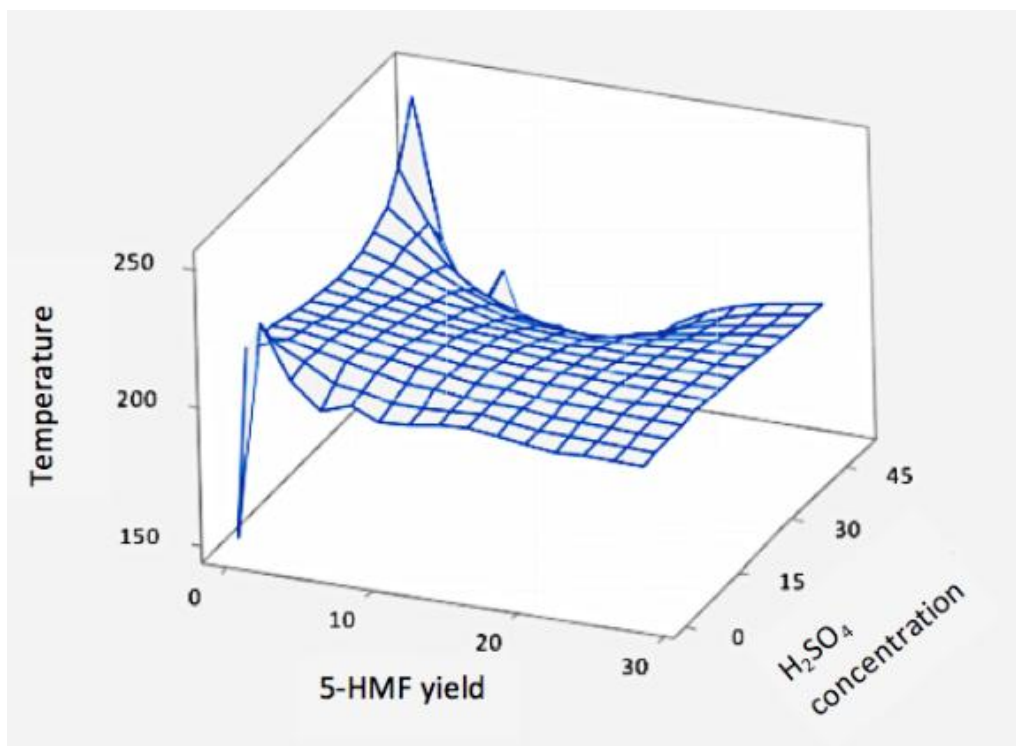


Figure 4.21. Surface plot of temperature (150, 200 and 250 °C) vs acid concentration (0, 15, 30 and 45 mM H₂SO₄) for 5-HMF yield at 60 min

Normal probability plots for both levulinic acid and formic acid were almost linear but in the case of 5-HMF, there was some deviation from the linearity. This might be caused by the effect of pressure. In the parameter evaluation by ANOVA, we did not count pressure since we could not observe significant effect on the degradation of cellulose in the experimental section. However, there might be some coupled effect (pressure-temperature or pressure-acid concentration) on the yield of product. This missing information may cause some deviations in the normal probability plots.

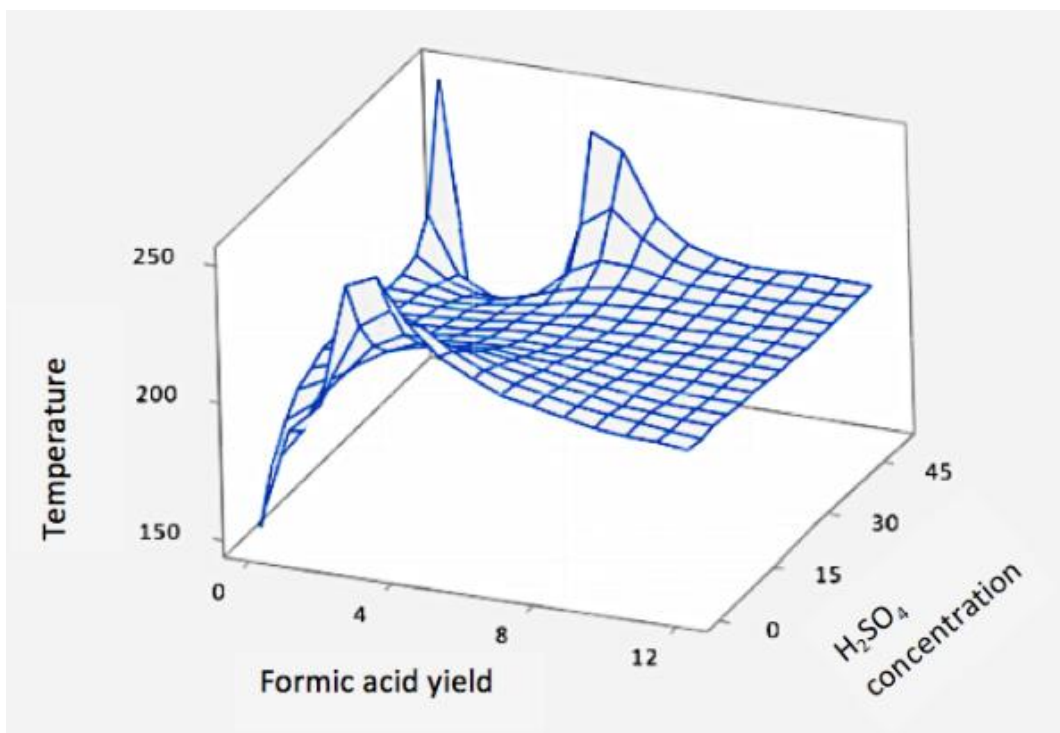


Figure 4.22. Surface plot of temperature (150, 200 and 250 °C) vs acid concentration (0, 15, 30 and 45 mM H₂SO₄) for Formic acid yield at 60 mins

Similar explanations with the levulinic acid on the result of p-values for formic acid could be done (Figure 4.22). The acid concentration and reaction temperature could greatly enhance the production of formic acid with p-values of less than the level of significance.

CHAPTER 5

CONCLUSION

The degradation of cellulose as a model compound of biomass in hot-pressurized water was investigated without addition of any organic solvent. In order to clarify the effects of reaction parameters on the degradation of cellulose and the yield of desired liquid products, series experiments were carried out at different reaction temperatures (150, 200, 250 and 280 °C), pressures (5-64 bars), oxidizer concentration (0, 5, 25, 50, 75, 100 and 125 mM), oxidizer type (H_2SO_4 and H_2O_2) and reaction times (30, 60, 90 and 120 mins). After the hydrothermal degradation of cellulose with H_2SO_4 in hot-pressurized water, levulinic acid and 5-HMF were produced in the liquid phase. Additionally, small amounts of organic acids (formic acid, glycolic acid, lactic acid, etc.) aldehydes, monomers and oligomers were generated as minor products. Addition of H_2SO_4 led to a great increase in the degradation of cellulose with a percentage of 73% at 200 °C with a H_2SO_4 concentration of 125 mM at the end of 60 min reaction time. Under same conditions, the yield of levulinic acid was successfully achieved to 38% after 60 min. To clarify the coupled-effect of these parameters, obtained results were statistically analyzed by ANOVA and surface plots were drawn. According to these results, temperature and acid concentration greatly influenced the yield of products and the degradation of cellulose in hot-pressurized water without any organic solvent addition.

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