

## HYDROTHERMAL TREATMENT OF CELLULOSE IN HOT-PRESSURIZED WATER FOR THE PRODUCTION OF LEVULINIC ACID

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Received: 28.05.2016; revised: 29.11.2016; accepted: 15.12.2016

**Abstract:** In this paper, hot-pressurized water, operating above boiling point and below critical point of water (374. 15 °C and 22.1 MPa), was used as a reaction medium for the decomposition of cellulose to high-value chemicals, such levulinic acid. Effects of reaction temperature, pressure, time, external oxidant type and concentration on the cellulose degradation and product distribution were evaluated. In order to compare the cellulose decomposition and yields of levulinic acid, experiments were performed with and without addition of oxidizing agents (H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub>). Analysis of the liqueur was monitored by HPLC and GC-MS at different temperatures (150 - 280 °C), pressures (5-64 bars) and reaction times (30 - 120 mins). Levulinic acid, 5-HMF and formic acid were detected as main products. 73% cellulose conversion was achieved with 38% levulinic acid yield when 125 mM of sulfuric acid was added to the reaction medium at 200 °C for 60 min reaction time.

**Keywords:** Cellulose, levulinic acid, 5-HMF, hot-pressurized water

### Levulinik Asit Üretimi için Selülozun Sıcak-Basınçlı Suda Hidrotermal Muamelesi

**Öz:** Bu makalede selülozu değerli kimyasallara, özellikle levulinik asite, parçalamak amacı ile suyun kaynama noktası üzerinde ve kritik noktası altında (374. 15 °C ve 22.1 MPa) çalışılan sıcak-basınçlı su, reaksiyon ortamı olarak kullanılmıştır. Reaksiyon sıcaklığı, basıncı, süresi, oksitleme ajanı çeşidi ve miktarının selülozun parçalanmasına ve ürün dağılımına olan etkileri incelenmiştir. Selüloz bozunma yüzdesi ve levulinik asit oluşum verimini kıyaslamak amacı ile deneyler hem oksitleme ajanı (H<sub>2</sub>SO<sub>4</sub> ve H<sub>2</sub>O<sub>2</sub>) kullanarak, hem de kullanmayarak gerçekleştirilmiştir. Farklı sıcaklık (150 - 280 °C), basınç (5-64 bar) ve tepkime sürelerinde (30 - 120 dk.) oluşan likit ürünlerin analizleri HPLC ve GC-MS ile gerçekleştirilmiştir. Ana sıvı ürünler olarak levulinik asit, 5-HMF ve formik asit tespit edilmiştir. 125 mM sülfürik asit eklenerek 200 °C sıcaklıkta ve 60 dk boyunca gerçekleştirilen tepkime sonucunda, %73 selüloz bozunmuş ve %38 verimle levulinik asit elde edilmiştir.

**Anahtar Kelimeler:** Selüloz, levulinik asit, 5-HMF, sıcak-basınçlı su

## 1. INTRODUCTION

There has been an increasing attention to the processes that utilize biomass to energy sources and platform chemicals. Levulinic acid is listed as the most important platform bio-based chemicals by the Energy Department of United States. While governments are changing the energy politics to the sustainable and renewable energy sources as an alternative to petroleum sourced energy, levulinic acid is a key chemical for the production of variety of chemicals that derived from bio-based sources. Levulinic acid has been used in different kinds of sectors such as plastic goods, personal care products, coatings and resins, solvents, detergent,

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food industry, pharmaceutical products, fuel additive and food packaging applications. However, there is no production facility of levulinic acid in Turkey, hence it is imported. Therefore, utilization of an abundant waste in both countries and developing applicable processes carry a great economical value for the purpose of producing key platform chemicals such as levulinic acid.

Biomass is a heterogeneous mixture of organic substances including cellulose, hemicellulose, lignin and a small amount of inorganic substances included in the ash. Although the composition of lignocellulosic biomass changes with respect to biomass type, tissue type, growth stage and growing conditions, it is generally composed of 38-50% cellulose, 23-32% hemicellulose and 15-25% lignin (Thompson and Grethlein 1979; Toor et.al. 2011).

Hydrothermal treatment of cellulose is one of the most attractive techniques. In such treatment technologies, water is chosen because it is cheap, nontoxic, nonflammable, nonexplosive, and offers essential advantages compared to other organic solvents, particularly in the field of "green chemistry." Tunable properties of water with temperature and pressure as it approaches critical point, transfer hot-pressurized water into a good solvent with unique characteristics (Savage 1999). These thermodynamic properties of water including dielectric constant, density, viscosity, ion product, and solvating power, etc. show great effect on cellulose decomposition. When cellulose was used as a model compound under hydrothermal conditions, different high-value products were obtained. Saito et al. (2009) treated cellulose in hot-pressurized water (200-240 °C, 15-20 MPa, 50-120 sec.) and found that 2-furfural, 5-HMF, glycolic acid, lactic acid and formic acid were obtained in high yields after 120 secs. As model compounds, Willams and Onwudili (2006) used cellulose, starch, glucose and cassava waste in sub- and supercritical water to obtain gaseous products. They found that together with oil and char, carbon dioxide, carbon monoxide, hydrogen and methane were produced as gaseous products. Depending on temperature and the starting material, different product distribution and gas yields were obtained. For instance, when cellulose was used as a starting material, high yield of char, carbon monoxide and C<sub>1</sub>-C<sub>4</sub> hydrocarbons were produced (Willams and Onwudili 2006). In another study cellulose treatment in supercritical water, subcritical water and their combined system were examined and it was observed that yield of organics were different in each system. In combined treatment, no precipitates were formed and the authors concluded that reaction rate and decomposition mechanism of cellulose strongly depended on water conditions (Ehara and Saka 2005). Moreover, catalyst function in the degradation of cellulose to lactic acid in sub- and supercritical water was investigated by adding various metal ions (Co(II), Ni(II), Cu(II) and Zn(II)) to the reaction environment achieving 42% (g/g) of lactic acid yield at 300 °C and 25 MPa (Bicker et. al. 2005).

Levulinic acid, including methyl, carbonyl and carboxylic functional groups, has wide range of applications due to its structure. Therefore, this acid is included in the list of Top Value Added Chemicals from Biomass. The available information implies that hexose sugars initially dehydrate to form the intermediate product 5-hydroxymethylfurfural (5-HMF), which is subsequently hydrated to give the final products levulinic acid and formic acid in the same stoichiometric amount. The conversion of 5-HMF into levulinic acid is the result of water addition to the C2 – C3 bond of the furan ring to give the final products levulinic acid and formic acid (Horvat J. 1985; Asghari and Yoshida 2010).

Reaction condition has an important effect on the degradation of biomass. Chan et al. (2014) reported the influence of temperature and pressure on the liquefaction of three different oil palm biomass (empty fruit bunch, palm mesocarp fiber and palm kernel shell) in subcritical and supercritical water. They stated that cellulose and hemicellulose degraded nearly at 220 °C and 340 °C, respectively; whereas the degradation of lignin took place in a wide temperature range according to differential thermo-gravimetric curve. Chemical product compositions varied with respect to biomass type because each biomass has different amount of cellulose, hemicellulose and lignin content. Accordingly, more phenolic compounds were produced from

palm mesocarp fiber than that of empty fruit bunch and palm kernel shell under supercritical water conditions since having higher amount of lignin. Also, alcohol and ester compounds were only formed from the decomposition of palm kernel shell.

In literature, there were few studies focused on the production of levulinic acid from cellulose and some lignocellulosic biomass. Kruse et al. (2003) reported that when temperature was increased above critical point of water, levulinic acid yield decreased. Therefore, in the researches that focused on the liquid products, subcritical water was selected as a reaction medium. When rice bran degradation was investigated under hydrothermal conditions, levulinic acid and other acids such as formic, glycolic acid were obtained at temperatures above 190 °C (Pourali et. al. 2009). In another study, Asghari and Yoshida, 2010 treated Japanese red pine wood in non-catalytic and catalyzed conditions to compare yields of valuable products. It was observed that addition of catalyst (phosphoric acid) resulted in the production of high yield of organic acids including levulinic acid). They also found that higher sugar yield was obtained with acid treatment and the rehydration of HMF was determined as a main levulinic acid formation pathway from Japanese red pine wood (Asghari and Yoshida 2010). The identification of possible reaction pathways of biomass phytomass (cooked carrots and potatoes) degradation in sub- or supercritical water were also evaluated (Kruse and Gawlik 2003). In subcritical water, formation of levulinic acid via acid-catalyzed reaction and production of 1,2,4-benzenetriol pathways were favored. On contrary, degradation of HMF to furfural was dominant beyond the critical point,; hence, levulinic acid concentration decreased.

During the literature investigation of hydrothermal treatment of cellulose, it was noticed that there was lack of information about the production of levulinic acid. Hence, in this study, it was aimed to investigate the degradation of microcrystalline cellulose in hot-pressurized water to produce high-value chemicals such levulinic acid. All reactions were carried out in subcritical water (hot-pressurized water) without using any toxic solvents. Different reaction parameters such as temperature (150, 200, 250 and 280 °C), pressure (5-64 bars) and reaction time (30, 60, 90 and 120 mins) were evaluated on the degradation of cellulose and yields of levulinic acid and other high value products (5-HMF, etc). Moreover, although there is no need to use catalyst since all reactions were carried out in subcritical water, separate experiments were done to investigate the effect of external oxidizer addition. For this purpose, various concentrations (0, 5, 25, 50, 75, 100 and 125 mM) of H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub> were added to the reaction solution and results were compared in terms of cellulose conversion and product distribution.

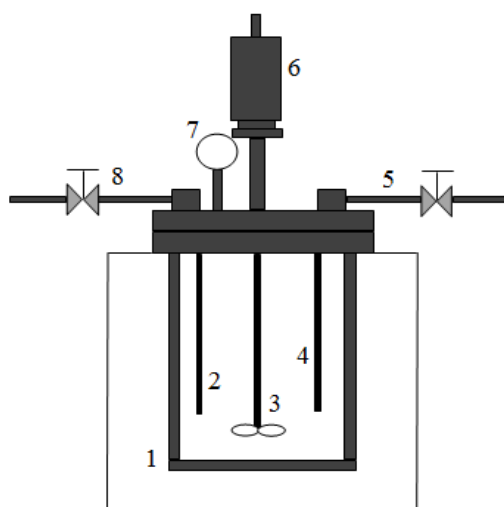
## **2. EXPERIMENTAL PROCEDURE AND ANALYSIS**

### **2.1 Chemicals**

All chemicals were used as analytical grade without further purification. Chemicals used in this study were microcrystalline cellulose, fructose (Sigma-Aldrich), furfural, acetic acid, glucose, formic acid (Merck), sulfuric acid (Fluka), lactic acid, glycerol (Panreac), glycolic acid, DL-glycerolaldehyde (Sigma), glycolaldehyde dimer, pyruvic acid, levulinic acid (Aldrich) and 5-HMF (SAFC). Deionized water was used to prepare various solutions.

### **2.2 Equipment and Procedure**

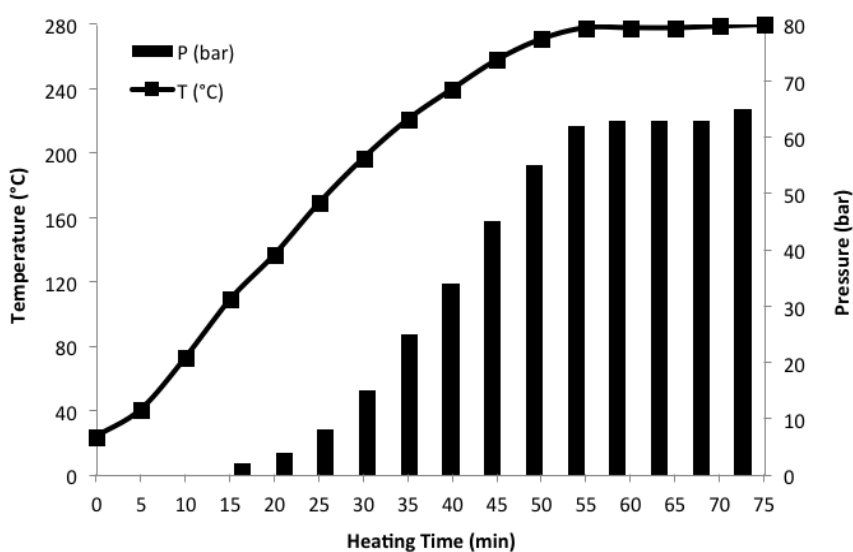
In this study, experiments were carried out in 300 mL of an autoclave made of 316 stainless steel (Parr Instrument Company, USA). The reactor equipped with gas inlet and outlet valves, liquid sampling valve, pressure gage, safety rupture disc, magnetic-driven impeller, internal thermocouple and cooling loop. The schematic diagram of the experimental apparatus is illustrated in Figure 1.



**Figure 1:**

*Hydrothermal conversion reactor: (1) stainless steel beaker, (2) thermocouple, (3) stirring impeller, (4) gas inlet, (5) input nitrogen gas, (6) magnetically driven stirrer, (7) pressure gauge, (8) gas sample collector.*

The reactor was initially loaded with 4 g of cellulose and then the volume is adjusted to 100 ml by adding de-ionized water. All the pins were tightened and then the reactor was purged with an inert gas ( $N_2$ ) to remove air inside the autoclave. After that, the reaction temperature was set to the desired reaction temperature with stirring rate of 200-250 rpm throughout the experiment. Temperature and pressures were recorded at every 5 minutes until the temperature reached the desired operating conditions. During heating period, temperature and internal pressure increased until reaching desired reaction temperature. When the set conditions were reached, the reaction was started for a certain reaction time. At the end of reaction time, the heater was turned off and the system was cooled to 40 °C to collect the final products. The typical temperature-pressure profile of cellulose degradation process at 280 °C and 75 min is given in Figure 2.



**Figure 2:**

*Typical temperature-pressure profile (280 °C and 75 min).*

For the present study, 4 g of microcrystalline cellulose and varying concentrations of H<sub>2</sub>SO<sub>4</sub> (0, 5, 25, 50, 75, 100 and 125 mM) were used as feed materials. Reaction temperature and pressure ranges were 150-280 °C and 5-64 bars, respectively. Reactions were carried out for 30, 60, 90 and 120 mins. Moreover, initially the system was pressurized by 5, 10 and 15 bars to investigate the influence of the pressure loading on the product distribution. Finally, to compare the product distribution and yields, separate experiments were carried out with the addition of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) as oxidizing agents.

Since there were many parameters investigated, A<sup>1/2</sup> fractional factorial design with 2-level of three factors; reaction time (30 and 120 min), temperature (150 and 250 °C) and H<sub>2</sub>SO<sub>4</sub> (0 and 50 mM) concentration were built and responses (cellulose conversion and product yields) were statistically analyzed via Analysis of Variance (ANOVA). In this study, acceptable p-values of each parameter were determined as p≤0.1.

### 2.3 Product Analysis

After the reaction, gas products, which were produced at higher reaction temperatures (T>200 °C) were collected into gas sampling bags and analyzed by gas chromatography equipped by thermal conductivity detector (GC-TCD). Liquid and solid products were separated with a filter paper. Remained 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. The overall conversion of the cellulose was calculated relative to the initial amount of cellulose loaded to the reactor by the following Equation (1):

$$\text{Overall conversion (wt. \%)} = \frac{\text{initial cellulose amount} - \text{solid residue}}{\text{initial cellulose amount}} \times 100 \quad (1)$$

Solid products were analyzed by Fourier Transform Infrared Spectroscopy (FTIR) (Shimadzu IRPrestige-21 FTIR-8400S) and the morphology of the solid product was monitored by Scanning Electron Microscope with an EDX detector (SEM-EDX) (Philips XL 30S FEG). High Performance Liquid Chromatography (HPLC) and Gas Chromatography Equipped with a Mass Spectroscopy (GC-MS) were used for the analysis of liquid products. Since the columns used in both analyses are suitable for water containing samples, the liquid products were directly analyzed by HPLC and GC-MS without any pretreatment. The yield of each product in the liqueur identified by HPLC was determined by the following Equation (2):

$$\text{Product yield (\%)} = \frac{\text{concentration of compound in the liquid product (ppm)}}{\text{degraded cellulose (ppm)}} \times 100 \quad (2)$$

The HPLC and GC-MS analysis conditions are given in Table 1.

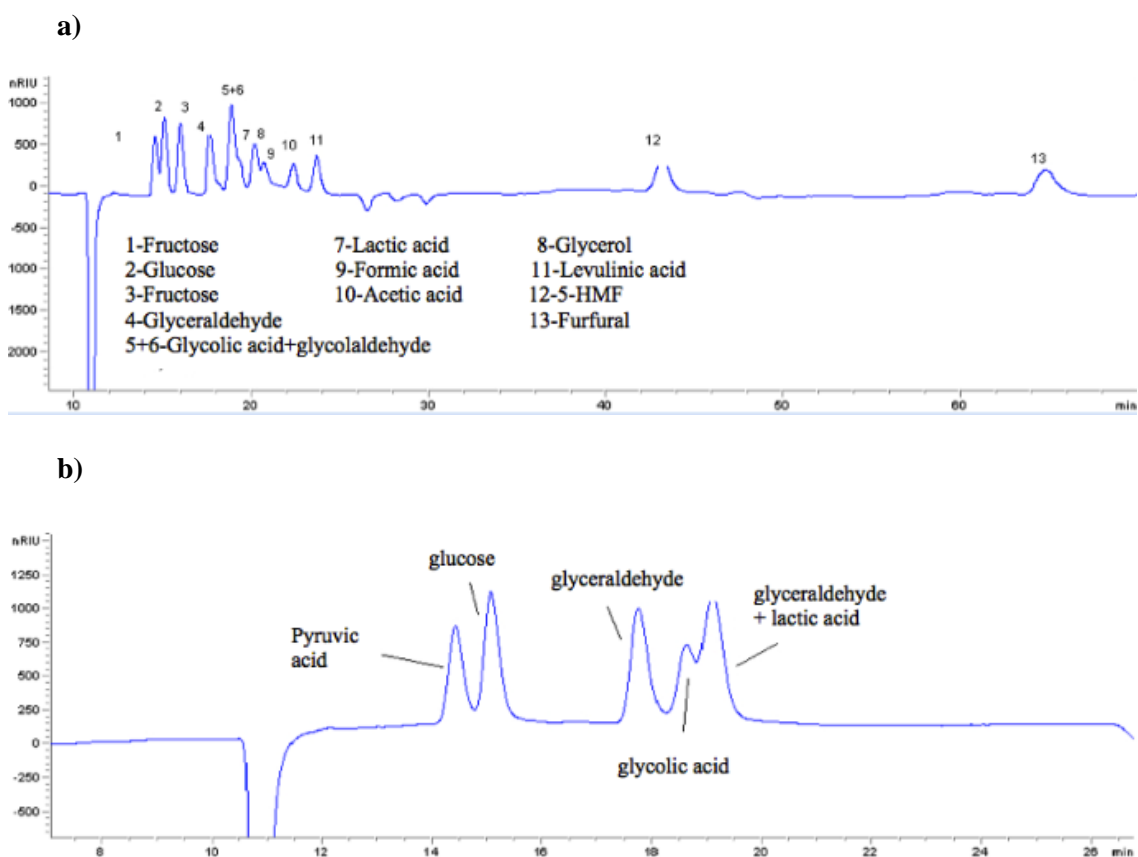
**Table 1. Conditions for the analysis of HPLC and GC-MS.**

	HPLC	GC-MS
Column	Sugar SH1011 (Shodex)	Restek Stabilwax-DA
Detector	Refractive Index (Jasco)	Agilent 5973 Mass Selective Detector (S/SL inlet)
Eluent/carrier gas	3.75 mM H <sub>2</sub> SO <sub>4</sub>	He gas
Eluent/carrier gas flow rate	0.5 ml/min	1.3 ml/min
Operating temperature	50 °C and 65 °C	240 °C (inlet)
Sample injection	20 µL	0.1 µL

Split ratio	---	1/10
Oven programme	---	40 °C (2 min with an increase of 8 °C/min), 140 °C (5 min with an increase of 10 °C/min), 220 °C (10 min)

### 3. RESULTS AND DISCUSSION

During this study, experiments were carried out 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), external oxidant concentrations (0, 5, 25, 50, 75, 100 and 125 mM) and external oxidant types (H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub>). After each experiment, effects of these parameters on the degradation of cellulose, product distribution and product yields were evaluated. After degradation of microcrystalline cellulose with H<sub>2</sub>SO<sub>4</sub> in hot-compressed water, pyruvic acid, glycolaldehyde, glyceraldehyde, formic acid, glycolic acid, lactic acid, acetic acid, levulinic acid, 5-HMF, glycerol, and 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. The HPLC chromatograms of the standard solutions are shown in Figure 3 at different column temperatures.



**Figure 3:** Refractive index data of standard compounds obtained from HPLC analysis at the column temperatures of **a)** 50 °C and **b)** 65 °C.

For the product identification, in addition to HPLC, GC-MS was also used and typical GC-MS products point of interest is given in Table 2.

**Table 2. GC-MS data of hydrolysis products of cellulose**

Species	Molecular Formula	Retention Time (min)
Glycolaldehyde	C <sub>2</sub> H <sub>4</sub> O <sub>3</sub>	13.05
Glyceraldehyde	C <sub>3</sub> H <sub>6</sub> O <sub>3</sub>	13.05
Acetic acid	C <sub>2</sub> H <sub>4</sub> O <sub>2</sub>	14.34
Furfural	C <sub>5</sub> H <sub>4</sub> O <sub>2</sub>	15.81
Formic acid	CH <sub>2</sub> O <sub>2</sub>	17.21
Acrylic acid	C <sub>3</sub> H <sub>4</sub> O <sub>2</sub>	20.83
Lactic acid	C <sub>3</sub> H <sub>6</sub> O <sub>3</sub>	30.21
Glycerol	C <sub>3</sub> H <sub>8</sub> O <sub>3</sub>	31.15
Levulinic acid	C <sub>5</sub> H <sub>8</sub> O <sub>3</sub>	33.24
5-HMF	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	36.09

### 3.1 Effect of reaction temperature on cellulose degradation and product yields

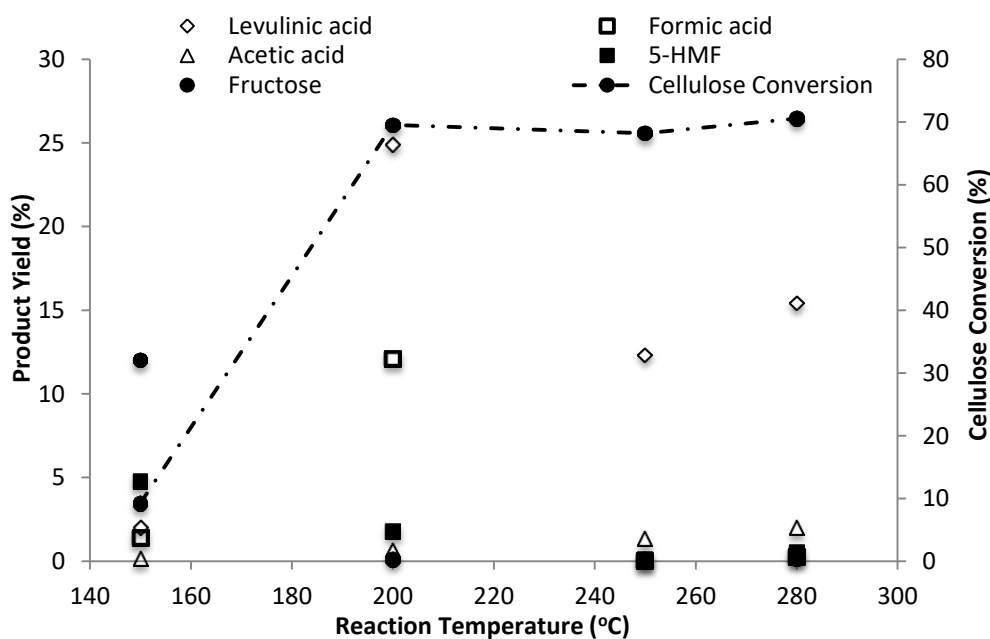
In order to understand the effect of reaction temperature on the degradation of cellulose and the product yields, experiments were performed with 4 g of microcrystalline cellulose and 50 mM H<sub>2</sub>SO<sub>4</sub> within a temperature range from 150 to 280 °C (pressure range: 5-64 bar), for 30-120 mins. The results are given in Table 3.

**Table 3. Cellulose conversion at different reaction times (0-120 mins) and temperatures (150-280 °C) with 50 mM of H<sub>2</sub>SO<sub>4</sub>.**

Cellulose Conversion (%)				
Reaction Time (min)	Reaction Temperature (°C)			
	150	200	250	280
30	4.57	59.07	71.67	72.28
60	9.27	69.51	68.21	70.55
90	10.85	68.65	70.50	70.28
120	9.22	70.49	69.29	73.10

As listed in Table 3, conversion of cellulose increased with increasing reaction temperature. For the first 30 min, at 150 °C, conversion was 4.57%, which increased to 59.07% after increasing the reaction temperature to 200 °C. Conversion recorded at 250 and 280 °C were almost same (73%) after 30 min. From 60 to 120 mins, the conversion profile at 200, 250 and 280 °C had similar trend. After 120 mins, maximum cellulose conversion was recorded as 73.10% at 280 °C. As well known, differences in the thermodynamic properties of water must play a key role on the reactions in the aqueous media. Ehara and Saka (2005) studied decomposition behavior of cellulose at different water densities, ionic products and dielectric constants in sub- and supercritical regions. They found that reaction time in supercritical water was much more shorter than in subcritical water. After the treatment of cellulose in supercritical water, the authors found that fragmented products yields were much higher compared to degradation in subcritical water. Additionally, there was no difference in glucose and fructose yields in sub- and supercritical water treatments. However, in the case of dehydrated products, subcritical water was the right reaction medium (Ehara and Saka 2005).

The main idea of using subcritical water as a reaction medium is to create favorable environment for the breakdown of the glycosic bonds in cellulose that have high chemical resistance (Cardenas-Toro et. al. 2014). This disruption in the glycosic bonds leads to depolymerization of cellulose to form oligosaccharides and monosaccharides (glucose). At further stage of the reaction, hydrolysis and rearrangement/decomposition of glucose and other monomer sugars take place to form several products such as fructose, 5- HMF, furfural, pyruvaldehyde, glyceraldehyde and glycolaldehyde. Moreover, hemicellulose is hydrolyzed into high molecular weight oligomers, low molecular weight oligomers, xylose, furfural, glycolaldehyde and glyceraldehydes. Re-polymerization, isomerization and fragmentation of these small products occur into liquid, gaseous and solid products at higher reaction temperatures (Cardenas-Toro et al. 2014). In addition to these products, organic acids, such as levulinic acid, formic acid, acetic acid, etc. are also formed. Among them, levulinic acid is the desired product in this study. Percentage values of cellulose conversion and product yields at different temperatures are given in Figure 4.



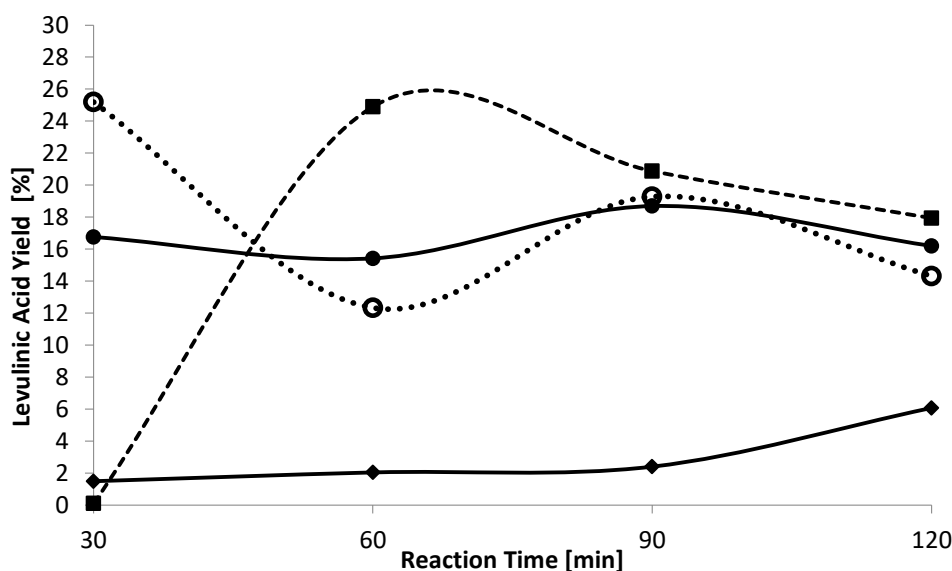
**Figure 4:**  
Conversion of cellulose and distribution of the liquid products after 60 min with 50 mM  $H_2SO_4$  at different reaction temperatures (pressure range: 5-64 bar)

As seen in Figure 4, 70% of cellulose was successfully degraded after 60 min at 280 °C. At 150 °C temperature and 5 bar pressure, the amount of remaining cellulose was weighted as 3.61 g from the initial mass of 4 g. This temperature and pressure was not sufficient for the disruption of glycosidic bonds of cellulose. When temperature was increased to 200 °C, there was a sudden raise in both the yield of levulinic acid and degradation of cellulose while the amount of 5-HMF in liquid product decreased to 1.7%. This result indicated that liquid products were formed from cellulose degradation under hydrothermal conditions through two steps and at higher reaction temperatures glucose isomerized to fructose, which by-turn converted to 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 (Cardenas-Toro et. al. 2014; Rosatella et. al. 2011; Zeng et. al. 2010). As it can be seen from the product distribution in Figure 5, while levulinic acid and formic acid yields were increasing, the yield of 5-HMF was decreasing meaning that 5-HMF was further converted to levulinic and



formic acids. 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 quite low (Kruse et. al. 2003).

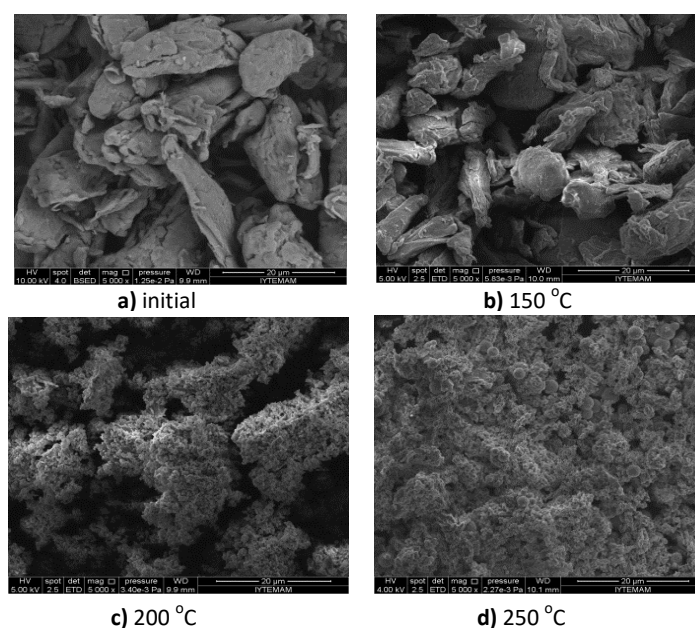
In the case of levulinic acid yield, as the desired product, effect of temperature at different reaction times is given in Figure 5. When reaction was carried out at 200 °C, for the first 30 mins, no levulinic acid was detected in the liquid product solution. However, at the end of 60 mins levulinic acid yield reached to 25%. At higher temperatures (250 °C), levulinic acid formation was enhanced (25%) within the first 30 mins. However, longer reaction times until 120 mins caused a decrease in the yield of levulinic acid to 14%. The opposite case was observed for lower reaction temperature such as 150 °C. As the reaction time was longer, the yield of levulinic acid increased from 1.5% to 6% which was still too low compared to the values at 200 °C. Since the optimum conditions were determined by the help of HPLC analysis results, such information is given by drawing figures showing yield of the compounds (in %) in the liquid product solution.



**Figure 5:**

*Levulinic acid yield with 50 mM H<sub>2</sub>SO<sub>4</sub> from 30 mins to 120 mins at various reaction temperatures of 150, 200, 250 and 280 °C (Symbols: □ 150 °C; ● 200 °C; ○ 250 °C; ◇ 280 °C)*

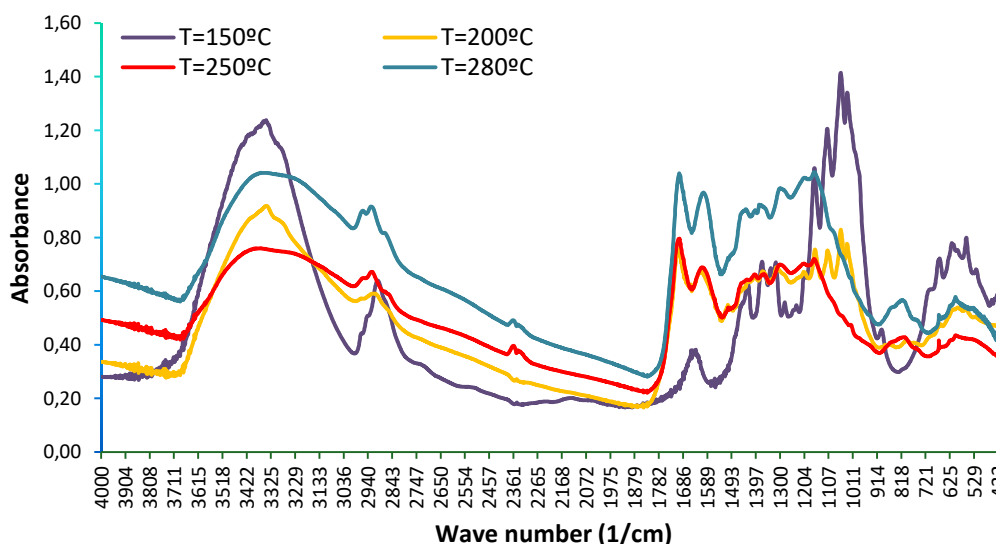
The SEM images taken after the treatment of cellulose at 150, 200 and 250 °C temperatures are shown in Figure 6. For comparison, the crystal structure of initial cellulose is also given in Figure 6 (a). As temperature increased (250 °C), it was seen that in the solid product together with some unreacted cellulose, round shaped, agglomerated nano and micro-sized particles were also present. These black, insoluble particles are known as ‘humins’ having with particle sizes of 5–15 μm.



**Figure 6:**

*SEM images (same scale-1000X) of a) initial cellulose and solid products after treatment with 50 mM H<sub>2</sub>SO<sub>4</sub> at b) 150 °C; c) 200 °C; d) 250 °C*

In Figure 7, the spectrum of FTIR analysis after hydrothermal treatment of cellulose in the presence of 50 mM H<sub>2</sub>SO<sub>4</sub> is shown. In ranges from 3364 cm<sup>-1</sup> broad O-H stretching vibrations observed which is highest at 150 °C, indicates presence of the functional alcohols, phenols groups. Peak around 2750-2950 cm<sup>-1</sup> of shown C-H stretching vibrations, which indicates presence aromatic hydrocarbons. The peak around 1620-1707 cm<sup>-1</sup> which is higher at 280 °C, belongs to C=O stretching vibrations which indicates the presence of aliphatic groups. Peaks at 1215-1275 cm<sup>-1</sup> correspond to stretching of aliphatic C-H groups. Moreover, 1030 cm<sup>-1</sup> represents stretching of C-O alcohol esters.



**Figure 7:**

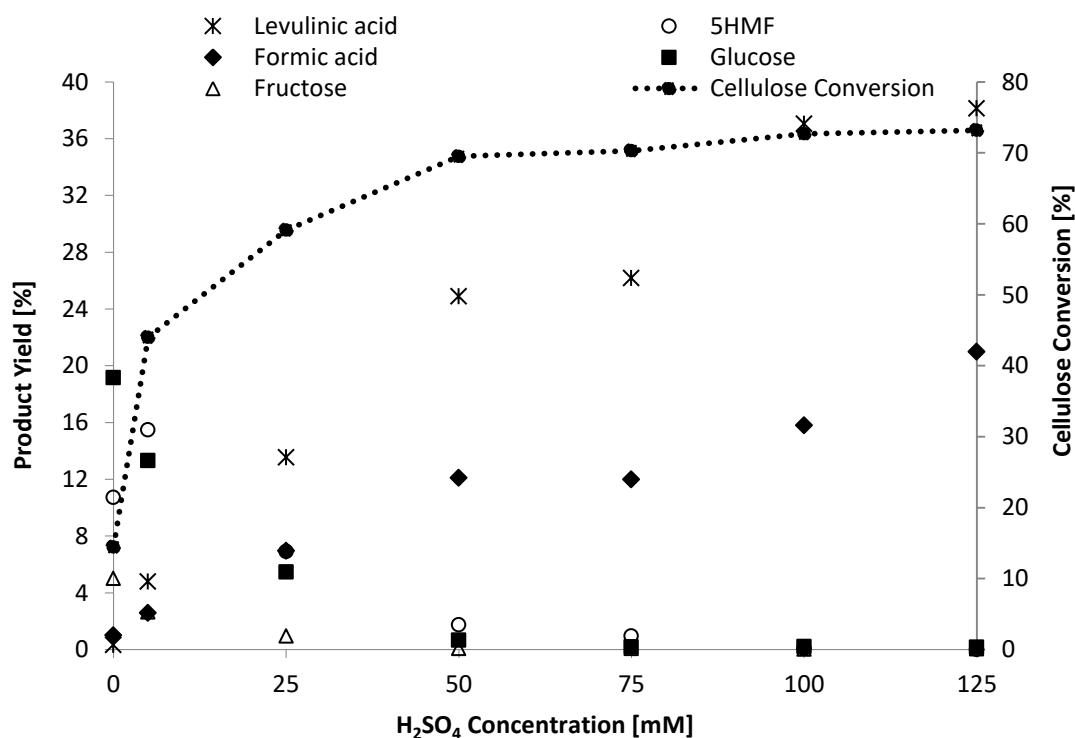
*Comparison of FTIR spectrum of solid residue after treatment with 50 mM H<sub>2</sub>SO<sub>4</sub> at different reaction temperatures for 60 min*

### **3.2 Effect of reaction pressure on cellulose degradation and product yields**

Mostly, oxidation under hydrothermal conditions involves the liquid phase oxidation of chemicals with a gaseous source of oxygen (usually air). In subcritical water reaction medium, since the operation is carried out at high-temperature and high-pressure aqueous conditions, the generation of oxygen can be controlled. In order to investigate the effect of initial pressure loading on cellulose conversion and product distribution, separate experiments were carried out by initially loading 5, 10 and 15 bars nitrogen gas into the reactor vessel. When the set conditions were reached without any pressure loading, the reaction pressure ranged from 5 to 64 bars depending on the reaction temperature. When the results were analyzed, it was realized that there were no significant changes in cellulose conversion with initial pressure loading. At subcritical water reaction runs in liquid phase and there is low dependence of water properties on the pressure so that the rate constant of cellulose decomposition does not increase even if the reaction pressure is increased (Sasaki et. al. 2007). Oppositely, at supercritical water treatment, the rate constant gradually increase with an increase in pressure since the thermodynamic properties of water in supercritical state are strongly depended on pressure. In supercritical water, decomposition of substrate may run in two reaction pathways such as ionic and free radical mechanisms. Ionic pathway dominates if there is high density and low temperature, whereas free radical reaction occurs if there is high temperature and low density. Additionally, in supercritical state, water density, dielectric constant and ion product increase with increasing pressure (Kruse et. al. 2003; Akiya and Savage 2002). However, since this study was carried out at temperatures below the critical point of water, it was seen that initial pressure had almost no effect on conversion of cellulose.

### **3.3 Effect of external oxidizer addition on cellulose degradation and product yields**

One of the aims of this study is to compare the oxidizer-free and oxidizer-added experimental results on the conversion of cellulose and levulinic acid yield. The effect of external oxidizer was examined by adding 0-125 mM sulfuric acid ( $H_2SO_4$ ) to the feeding solution of cellulose. The degradation percentage of cellulose (on secondary y-axis) and the product yields are illustrated in Figure 8. Addition of an acid greatly enhanced the cellulose conversion. Without  $H_2SO_4$ , 15% of cellulose was degraded, whereas with 50 mM  $H_2SO_4$ , this value increased to about 70%. Further increase in the acid concentration did not affect the degradation efficiency, which might be explained by the saturation of the solution with  $H_2SO_4$ .

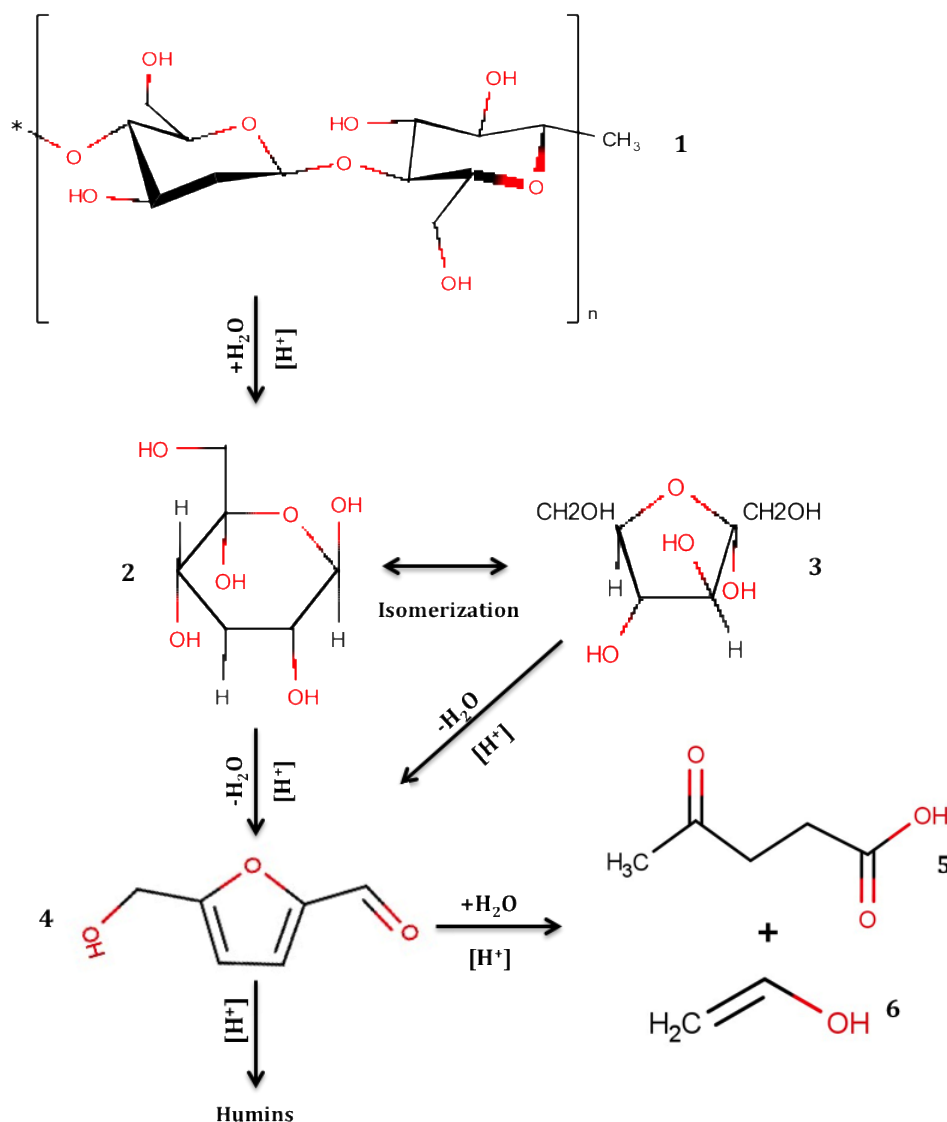


**Figure 8:**

*Effect of H<sub>2</sub>SO<sub>4</sub> concentration on the conversion of cellulose and product yields (reaction time: 60 min; reaction temperature: 200 °C)*

In this study, 50 mM H<sub>2</sub>SO<sub>4</sub> was mainly used as an oxidizer, however, to compare the product distribution with addition of different amounts of acid, 5, 25, 75, 100 and 125 mM of H<sub>2</sub>SO<sub>4</sub> were also used. In Figure 8, yield of levulinic acid increased with increasing H<sub>2</sub>SO<sub>4</sub> concentration and reached from 4.8 % (with 5 mM H<sub>2</sub>SO<sub>4</sub>) to 38% with the addition of 125 mM H<sub>2</sub>SO<sub>4</sub>. 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 H<sub>2</sub>SO<sub>4</sub>, respectively. However, the yields of other products such as glucose, fructose, and 5-HMF were started to decrease with increasing acid concentration in the feed. 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 (Cardenas-Toro et. al. 2014; Rosatella et. al. 2011; Zeng et. al. 2010).

The formation pathway of levulinic acid from cellulose degradation in hot-pressurized water is schematically given in Figure 9. In the first step, the glycosidic bonds of cellulose (1) are broken down into low molecular weight fragments and finally to glucose (2). This reaction was enhanced in the presence of an acid (H<sub>2</sub>SO<sub>4</sub> or H<sub>2</sub>O<sub>2</sub>). When H<sub>2</sub>O<sub>2</sub> was used, this reaction was promoted resulting in high yield of glucose in the liquid product solution. Glucose is decomposed to 5-HMF (4), which is further converted to levulinic acid (5) and formic acid (6). 5-HMF can also be formed from the decomposition of fructose (3), which is a structural isomer of glucose. High concentration of H<sub>2</sub>SO<sub>4</sub> greatly affected the yield of levulinic acid as previously shown in Figure 8.



**Figure 9:**  
*Decomposition pathway of cellulose to levulinic acid*

In some experiments carried out at high temperatures ( $T > 250$  °C), by-products such as insoluble dark-brown substances, known as humins, and gas products due to thermal degradation of reactants/products were generated. The elemental analysis confirmed the presence of humins. The composition (in wt%) of a typical humin sample contained 60.4 wt% of carbon and 4.9 wt% of hydrogen, which are close to the elemental composition given in the literature (Grisuta et. al. 2006) (C, 63.1; H, 4.2). Further evidence for the formation of substantial amounts of humins was obtained from SEM images of solid product at 250 °C (Figure 6). These SEM images showed that in the solid product together with some unreacted cellulose, round shaped, agglomerated nano and micro-sized particles were also present. These black, insoluble particles humins have particle sizes of between 5–15  $\mu\text{m}$ . The formation of these humin particles resulted in a decrease in the yield of levulinic and formic acids at 250 °C for longer reaction times ( $t > 90$  mins).

In this study, other possible by-products of the hydrolysis of cellulose are gas phase components from thermal degradation of reactants and/or products. To gain insights, the gas products were identified with gas chromatography with a thermal conductivity detector (GC-

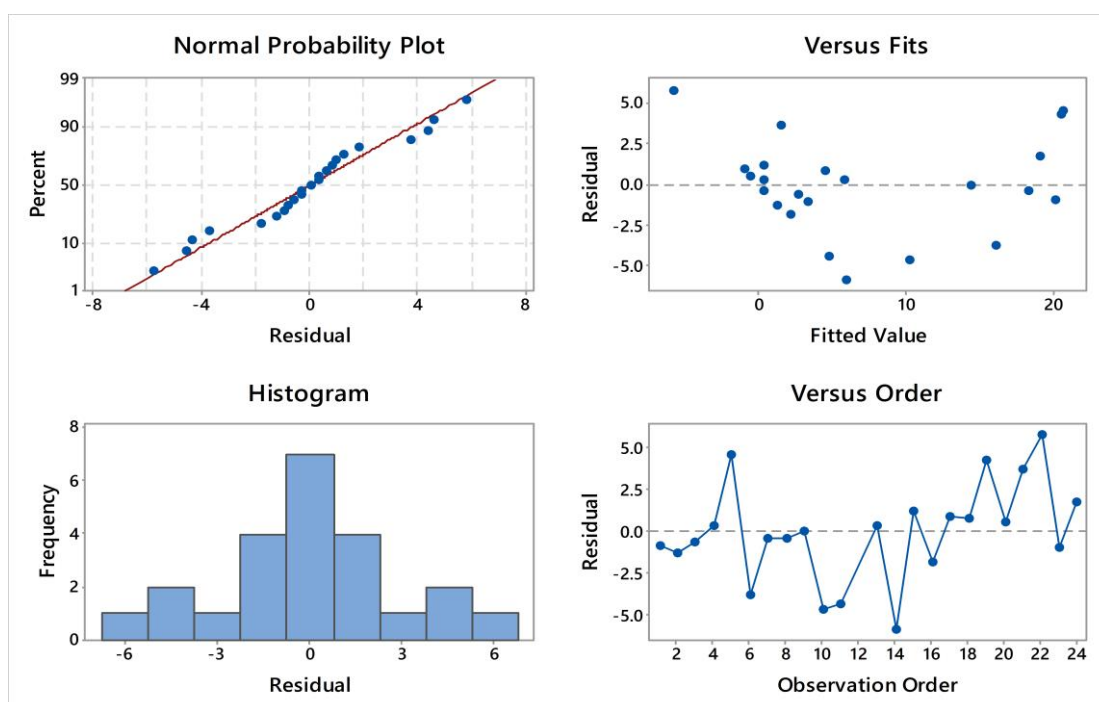
TCD) at higher reaction temperatures (250 °C and 280 °C). The main gas products were carbon monoxide and carbon dioxide whereas hydrogen and methane were also formed in trace amounts. When the reaction temperature increased from 250 °C to 280 °C, the concentration of carbon monoxide was found to be 172.5 µg/ml; whereas under same conditions, carbon dioxide amount decreased from 586.3 to 427.1 µg/ml. This points out that water gas shift reaction shifts through left-hand side towards carbon monoxide when the reaction temperature increased (Yakaboğlu et al. 2015). Comparatively, small amount of hydrogen gas was obtained and it decreased to 1.89 µg/ml when temperature was increased from 250 °C to 280 °C. This implies that the formation of gas-phase compounds is only a minor reaction pathway under the reaction conditions applied in this study.

Table 4 indicates summary of ANOVA test for cellulose conversion values. On this table, p-values (less than  $p < 0.1$ ) of each affecting parameters are given individually to consider the interactions between the parameters. The  $R^2$  with a value of 0.99 indicates that model is well fit to the observed response. The model accuracy is also confirmed by histograms including normal probability and residual plots (Figure 10).

As seen in Table 4, in the model, some of the interaction terms were removed due to the lack of fit ( $p > 0.1$ ) and considered as disturbance in the system causing error in the measured responses. As an individual parameter, reaction time has the p value of 0.148, which is higher than the confidence interval indicating that time has no significant effect on the conversion of cellulose. However, interaction parameter (temp\*time) showed that coupled effect of temperature and time was significant and thus, model did not avoid time as insignificant parameter. Similar result was obtained for the interaction parameter of  $H_2SO_4$ \*time, which has a p value of 0.028. In order to visualize the effect of parameters, response surface plots (Figure 10) of cellulose conversion was plotted by constant concentration of 50 mM  $H_2SO_4$  at reaction temperatures of 150 and 250 °C. Temperature had the highest impact on cellulose degradation since temperature increases up to 220 °C as ionic product concentration increases in subcritical water region (Dinjus and Kruse 2004).

**Table 4. Statistical analysis results for converted cellulose**

Source	DF	Sum of squares	Mean of squares	F value	P value
Model	17	19941.3	1173.02	445.93	0.000
Linear	6	16053.4	2675.57	1017.12	0.000
Temperature	2	11796.6	5898.30	2242.25	0.000
$H_2SO_4$	1	1992.3	1992.29	757.37	0.000
Time	3	22.1	7.36	2.80	0.148
2-way interactions	11	3699.9	336.36	127.87	0.000
Temp* $H_2SO_4$	2	3386.7	1693.33	643.72	0.000
Temp*Time	6	67.4	11.23	4.27	0.066
$H_2SO_4$ *Time	3	57.8	19.28	7.33	0.028
Error	5	13.2	2.63		
Total	22	19954.5			



**Figure 10:**

*Histogram plots of converted cellulose, a) normal probability plot of residuals; b) residuals versus predicted values; c) frequency histogram of residuals; d) observation orders of residuals*

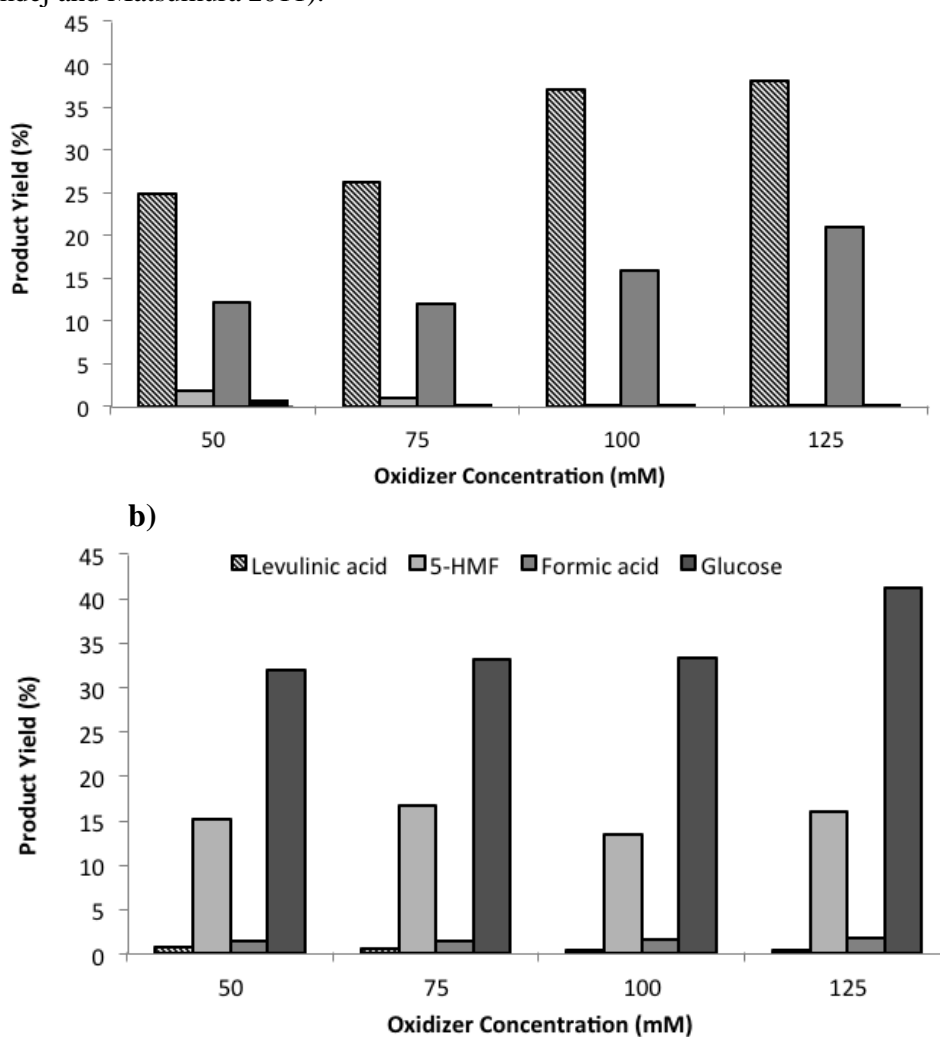
### 3.4 Effect of external oxidizer kind on cellulose degradation and product yields

The main idea of using oxidizing agent is to improve conversion of cellulose and increase the product yields-especially levulinic acid yield- by reducing the reaction temperature and time leading to energy efficient process. After the investigation of  $\text{H}_2\text{SO}_4$  effect, separate experiments were carried out by adding  $\text{H}_2\text{O}_2$  to the reaction solution with amounts of 50, 75, 100 and 125 mM at 200 °C for 60 min reaction time. When the conversion of cellulose was evaluated, it was observed that conversion of cellulose in the presence of  $\text{H}_2\text{O}_2$  was relatively lower than that of  $\text{H}_2\text{SO}_4$ . Under same conditions, cellulose conversion with 125 mM of  $\text{H}_2\text{SO}_4$  was 73.2 %, whereas it was only 17.6% with the same amount of  $\text{H}_2\text{O}_2$ .

pH of the aqueous solution was recorded as 3.2 without acid addition treatment. After hydrothermal conversion in the presence of 125 mM  $\text{H}_2\text{SO}_4$  and 125 mM  $\text{H}_2\text{O}_2$  treatments, pH values were found as 1.2 and 1.8, respectively. The difference of pH is attributed to the organic acid formation amount from the decomposition of sugars and organic acid diversity. Identified organic acids were acetic, formic, lactic, pyruvic, levulinic and glycolic acids from the degradation of cellulose in the subcritical water treatment. It was expected that the product distribution in the presence of  $\text{H}_2\text{O}_2$  and  $\text{H}_2\text{SO}_4$  would also be different and was confirmed by both HPLC (Figure 11) and GC-MS analysis (Figure 12).

According to HPLC and GC-MS analyses (Figures 11 and 12), it can be concluded that acid type affected diversely to the product distribution. In the case of  $\text{H}_2\text{O}_2$ , the yield of glucose was higher than that of  $\text{H}_2\text{SO}_4$  since the dehydration of glucose occurs easily in the presence of low acid concentration (Takeuchi et al. 2008). On the other hand, the catalytic effect of  $\text{H}_2\text{SO}_4$  was significantly higher than that of  $\text{H}_2\text{O}_2$  in the formation of levulinic acid by accelerating the rate of rehydration of 5-HMF to levulinic acid. With adding more  $\text{H}_2\text{SO}_4$  (125 mM), the yield of levulinic acid severely increased and reached to 38.1%, however; it was considerably low with a value of 0.5% in 125 mM  $\text{H}_2\text{O}_2$ . On the contrary, the use of  $\text{H}_2\text{O}_2$  was quite favorable in the

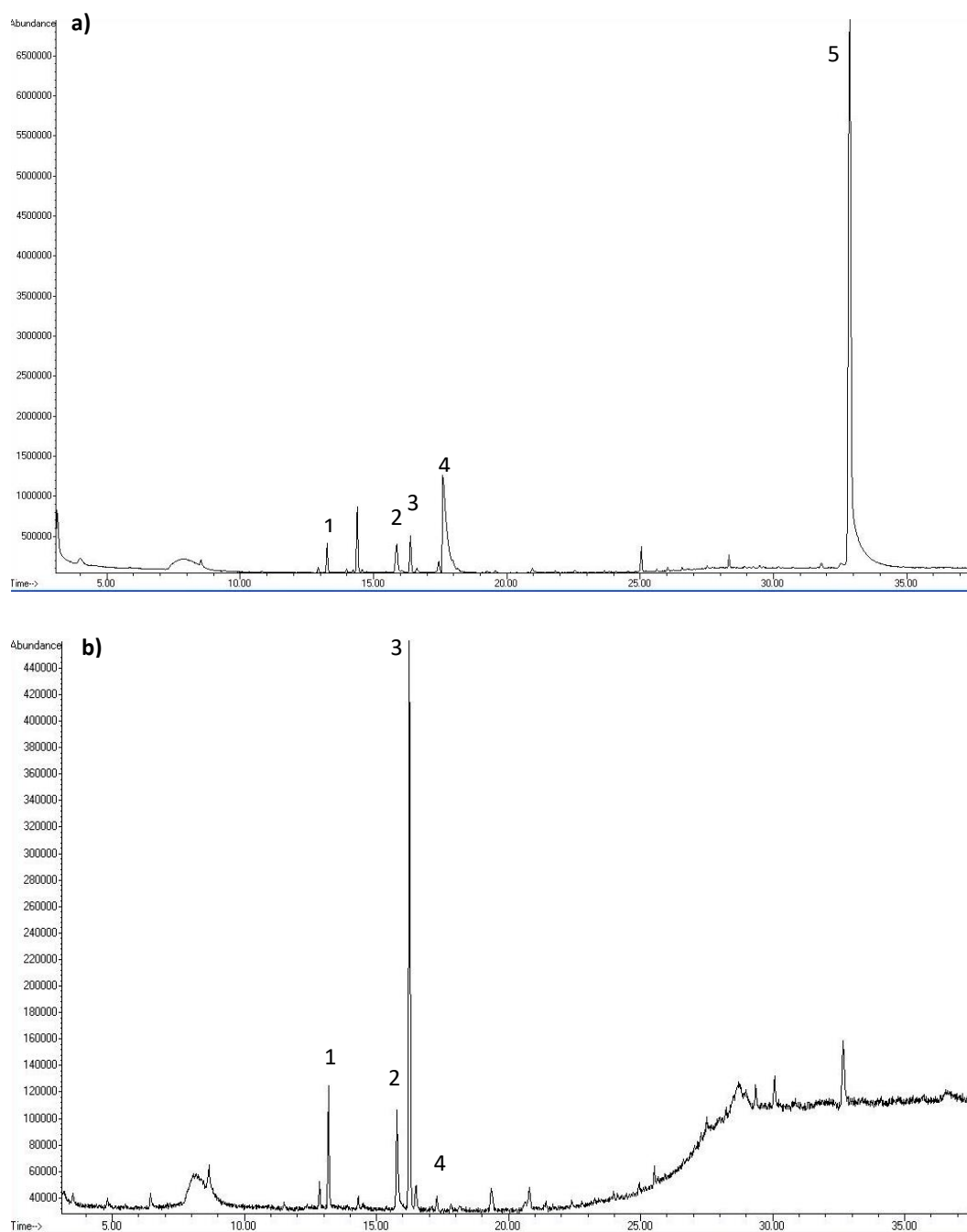
formation of 5-HMF since the yield of 5-HMF reached the highest value (16.1%) in the case of adding 100 mM H<sub>2</sub>O<sub>2</sub>. Almost no 5-HMF remained in the product solution after the treatment with 125 mM of H<sub>2</sub>SO<sub>4</sub>. This different behavior might be explained by two cases: The first case may be the degradation of HMF to furfuryl alcohol, which was further converted to levulinic acid with acid-catalyzed treatment (Rackemann and Doherty 2011). The other case might be related to the polymerization of HMF that may cause char formation in hot compressed water (Promdej and Matsumura 2011).



**Figure 11:** Effect of **a)** sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and **b)** hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) addition on the yields of levulinic acid, 5-HMF, formic acid and glucose at 200 °C after 60 mins

The HPLC and GC-MS analyses showed that between these two oxidizing agents, H<sub>2</sub>SO<sub>4</sub> was favorable in terms of levulinic acid production resulting to 38% of levulinic acid yield with the addition of 125 mM of H<sub>2</sub>SO<sub>4</sub>. Totally different product profiles of two oxidizing agents showed that the external oxidizer type should be selected depending on the desired products.





**Figure 12:**  
*GC-MS spectrum of the liquid products after the treatment with a) 125 mM H<sub>2</sub>SO<sub>4</sub> and b) 125 mM H<sub>2</sub>O<sub>2</sub> for 60 min at a reaction temperature of 200 °C (symbols: 1: hydroxyacetone; 2: acetic acid; 3: furfural; 4: formic acid; 5: levulinic acid)*

#### 4. CONCLUSION

Degradation of microcrystalline cellulose as a model compound of biomass in hot-pressurized water was investigated to obtain value-added chemicals with a special interest to levulinic acid. In order to clarify the effects of reaction parameters on the degradation of cellulose and the yield of levulinic acid, series experiments were carried out at different reaction temperatures, pressures, acid concentration, acid types and reaction times. After the treatment of cellulose in hot-pressurized water with the addition of H<sub>2</sub>SO<sub>4</sub>, levulinic acid, 5-HMF and formic acid were produced as the main liquid products. Additionally, small amounts of other organic acids (glycolic acid, lactic acid, etc.) aldehydes, monomers and oligomers were generated as minor products. Addition of 125 mM of H<sub>2</sub>SO<sub>4</sub> as an external oxidant greatly enhanced the production of levulinic acid from cellulose under hydrothermal conditions by yielding 38% of levulinic acid at 250 °C at the end of 60 min. Under same conditions, 73% of cellulose was successfully degraded. When separate experiments were conducted with the addition of H<sub>2</sub>O<sub>2</sub> to compare the effects of external oxidize types, it was observed that level of cellulose conversion with 125 mM of H<sub>2</sub>O<sub>2</sub> was 18%, although addition of same amount of H<sub>2</sub>SO<sub>4</sub> increased cellulose conversion to 73%. The product distribution was also very different, as expected. Although 125 mM of H<sub>2</sub>O<sub>2</sub> was added to the feed solution, the yield of levulinic acid was still less than 3%.

#### ACKNOWLEDGEMENT

This research is financially supported by Marie Curie Career Integration Grants (FP7-PEOPLE-2012-CIG) with a project number of PCIG11-GA-2012-321741. I would like to thank to my graduate student Saken Dadenov for his help in the experiments. I also appreciate to “Environmental Reference Research and Development Center” at Izmir Institute of Technology for their support in the product analyses.

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