A THESIS FOR THE DEGREE OF MASTER SCIENCE

High yield production and separation of furan derivatives from *Quercus mongolica* by oxalic acid pretreatment

신갈나무의 옥살산 전처리를 통한 고수율의 퓨란계 화합물 생산 및 분리

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PROGRAM IN ENVIROMENTAL MATERIALS SCIENCE
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Abstract

High yield production and separation of furan derivatives from *Quercus mongolica* by oxalic acid pretreatment

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In this study, a two-step pretreatment and separation process were carried out for the production of furan derivatives such as 5-hydroxymethlyfurfural (5-HMF), furfural, and 5-methylfurfural (5-MF). Aqueous oxalic acid was used as the solvent in both the 1st and 2nd pretreatments.

Response surface methodology (RSM) was performed to evaluate the effects of variables (X_1: reaction temperature, X_2: acid concentration, and X_3: reaction time) on the pentose yield and to define the optimal conditions for the highest pentose yield during the 1st pretreatment in this study range. The result of RSM analysis shewed that reaction temperature was the most dominant factor, followed by acid concentration and reaction time. The optimal conditions for the maximum pentose yield were a reaction temperature of 147°C, an acid concentration of 2.29% (w/w), and a reaction time of 20 min. Under these conditions, the pentose yield was 14.36% based on the dry weight of the raw material, corresponding to an extraction rate of 81.54% based on the initial weight of pentose in the material.

The liquid hydrolyzate obtained from the 1st pretreatment was used in 2nd pretreatment to produce furan derivatives. The 2nd pretreatment was carried out under various conditions (reaction temperature: 180-230°C, acid concentration: 2-4%, and reaction time: 10 min) to determine the optimal conditions for high yield of furan derivatives and to evaluate the effects of the reaction conditions on the yield of furan derivatives after the 2nd pretreatment. The maximum yield of furan derivatives was 7.66% based on the dry weight of the raw material after pretreatment at 220°C with 2% (w/w) oxalic acid for 10 min. The factor that most influenced the yield of furan derivatives was reaction temperature.

To separate furan derivatives from other compounds in the liquid hydrolyzate after two-step pretreatment under optimal conditions, nanofiltration (NF) and solvent extraction were conducted under various operating conditions (NF: filter type (NE90 and DRM), pH, repetition stage) (Solvent extraction: organic solvent (chloroform, butanol, ethyl acetate, propyl acetate), contact time, the hydrolyzate/solvent volume ratio, the number of extraction stage). Solvent extraction showed better efficiency for the separation of furan derivatives than NF. The best yield was obtained with chloroform-extracted furan derivatives (5.97% based on the dry weight of the raw material, corresponding to the recovery rate of 77.26%).

Key words: *Quercus mongolica*, two-step pretreatment, oxalic acid, furan derivatives, nanofiltration, solvent extraction

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1 Introduction

Since the rapid development through industrial revolution, the energy consumption has been dramatically increased and fossil fuel has become the majority of energy for several years (Ingram & Doran, 1995). However, the use of fossil fuel has produced greenhouse gas such as carbon dioxide, and also caused serious environment problems, for example, global warming, air pollution and acid rain (Saxena et al., 2009). In addition, our excessive overdependence on fossil source could lead to social problems related to price stability of transportation fuel and commodity chemicals because significant portion of materials and chemicals now mostly derived from petroleum. For these reasons, many countries have interested in exploring eco-friendly alternative resource in order to observe various policies and regulations established to reduce CO_2 emission from fossil oil and secure national competitiveness in the international energy market (Cherubini, 2010).

1.1 Lignocellulosic biomass as potential resource

Lignocellulosic biomass, which refers to plant biomass such as grasses, wood and agricultural residue, has currently gained much attention as a promising alternative resource to replace fossil fuel (Kumar et al., 2009). It is renewable, inexpensive and the most abundantly available biopolymer in nature (Behera et al., 2014). According to the research in U.S., the annual available quantities of biomass will be increased from about 119 million dry tons currently to about 129 million dry tons in 2030 (Zhang et al., 2013). Also, it could be regarded as environmental material resulting CO_2 savings due to its property of carbon fixation and dose not compete with food resource. For these reasons, there has been much research to explore and develop new technology using lignocellulose biomass as raw material in order to produce

energy, bio-based chemicals and bio-fuels (Klass, 1998).

Lignocellulosic biomass is mainly composed of three main components, cellulose (35-50%), hemicellulose (25-30%) and lignin (25-30%). Cellulose, the most abundant natural polymer on the earth, is homogenous polysaccharides consisting of the β-1,4 linked linear glucose polymer. It has crystalline structure and higher degree of polymerization than hemicellulose. While Hemicellulose is heterogeneous polysaccharides including hexose (glucose, mannose, and galactose) and pentose (xylose and arabinose). Comparison with cellulose, it is decomposed at lower temperature because of its branched composition (Himmel, 2009; Klass, 1998). Both of two major components can be depolymerized into monosaccharides which are used as source to produce biofuels like bioethanol and sugar degradation products such as furfural, 5-HMF and levulinic acid which can be a promising sustainable intermediate for bio-based feedstock of fine chemical (Yan, 2014; Girisuta, 2006). Lastly, Lignin, complex phenylpropanoid units, is consist of three monomeric precursors, coniferyl alcohol, sinapyl alcohol and coumaryl alcohol, biosynthesized in biomass via the shikimic acid pathway. Although, the exact structure of lignin does not known yet, it is presently regarded as potential aromatic building block in various industries such as fuels, resins and pharmaceuticals (Fan et al., 2014; Smolarski, 2012).

All three main components of lignocellulosic biomass are complexly connected to each other. It could be one of the reasons for biomass recalcitrance, known as natural defending system to protect itself from chemicals and microorganism attacks resulting in its decomposition. (Himmel, 2009; Wayman & Parekh, 1990; Sjostrom, 1993). Therefore, it is essential to take pretreatment process to overcome recalcitrance of lignocellulosic biomass for effective utilization in biorefinery industry.

1.2 Pretreatment to overcome recalcitrance

Pretreatment is one of the essential processes for total utilization of lignocellulosic biomass. Because pretreatment process has a strong influence on final product yield and relevance to efficiency of overall process.

The purpose of pretreatment is: (1) breaking down complex structure of lignocellulosic biomass in order to overcome biomass recalcitrance which is self-defense property against to microorganism and chemicals attacking its structure (2) disrupting crystallinity of cellulose for high sugar yield (3) removing lignin and hemicellulose (4) increasing the porosity of the material, so that microorganism can easily access to the surface of cellulose in the process including the microbial process, for example bioethanol process. (Sun & Cheng, 2002)

Pretreatment can be commonly classified in physical pretreatment, physico-chemical pretreatment, chemical pretreatment and biological pretreatment. First, physical pretreatment is mechanical method such as chipping, grinding and milling for size reduction. The common range of the size after chipping and milling or grinding is 10-30 mm and 0.2-2 mm, respectively (Sun & Cheng, 2002). While Physical pretreatment has high energy consumption, it is the important method to extend surface area which can react with chemical or microorganism and also to reduce cellulose crystallinity (Silverstein, 2005). Second, physico-chemical pretreatment is the combination of physical and chemical pretreatment as its name imparts. The common method is steam explosion which degrades hemicellulose and lignin with high temperature. In this method, the material is chipped firstly and is treated with high pressure for a while, then the pressure reduced quickly so that the material would be exploded by an explosive decompression (McMillan, 1994). Third, chemical pretreatment including acid, alkaline and organosolv process has considered as one of the most promising methods to

adapt in industry. Dilute acid pretreatment is more effective to degrade hemicellulose than lignin, so it can produce high yield of xylose with less severe condition in most lignocellulosic biomass (Himmel et al., 1997). However, it produces toxic products and needs expensive equipment against corrosion (Sun & Cheng, 2002). In addition, a neutralization process is necessary for downstream of fermentation process in bioethanol production. Alkaline pretreatment is believed that lignin is eliminated with the removal of the ester bonds crosslinking between carbohydrate and lignin. Dilute NaOH is used as common chemical in alkaline pretreatment. It makes lignocellulosic material swelling, as its result, a decrease in crystallinity (Tarkow & Feist, 1969; Fang et al., 1987). Organosolv pretreatment is currently evaluated as chemical method and initially developed for pulp production from wood (Pan et al., 2007). In this process, a mixture organic solvent with inorganic acid catalyst hydrolyzes lignin-lignin and lignin-carbohydrate bond (Holtzapple & Humphrey, 1984). Methanol, ethanol, acetone, ethylene glycol, triethylene glycol and tetrahydrofurfuryl alcohol are used as organic solvent and sulfuric acid and hydrochloric acid are commonly used as inorganic acid catalyst (Chum et al., 1988). Lastly, biological pretreatment is the pretreatment using microorganism such as brown rot and white rot for degradation of lignin and hemicellulose (GHOSE, 1978). Brown rots mainly attack cellulose, while white rots attack both cellulose and lignin. Although biological pretreatment can operate in moderate condition, it is difficult to be adopted for industry process because the price of microorganism is expensive and operating cost is high.

To optimize the pretreatment for the target product, it is important to evaluate the effect of pretreatment parameter on the yield of target product. In the chemical pretreatment, reaction time, particle size of material, reaction temperature, solid/liquid ratio and catalyst loading are mainly considered as key factor.

Therefore, there are various pretreatment methods and many parameters, it is necessary to choose suitable pretreatment method and parameters depending on the target products. Also, further research on developing pretreatment process will be need to meet requirements which are high efficient without loss of carbohydrate, cost effective and environment friendly.

1.3 The concept of biorefinery

The concept of biorefinery comes from today's petroleum refinery system. It represents currently an integrated facility that produces energy, transportation fuel, bio–based chemicals and materials from biomass at the same time as shown Fig. 1 (González-Delgado & Kafarov, 2011). By producing multiple products, biorefinery can take advantage of the diversities in biomass components and maximize the value derived from the biomass feedstock while also being able to adapt to changing market conditions.

Biorefinery has progressed through 3 steps. At the first time, it started with phase 1 biorefinery which is a dry-milling plant using corn grain as raw material for bioethanol production. However, phase 1 biorefinery is difficult to meet the changing market condition. So, phase 2 biorefinery developed to overcome the disadvantage of phase 1 and it is a wet-milling plant which can produce various products such as energy, biofuel and bio-based chemicals using corn grain as raw material. Even if phase 2 biorefinery has more flexibility compared to phase 1 biorefinery in final products, it still has the problem of using the corn grain which is food resource. Finally, phase 3 biorefinery has been designed as integrated plant to produce multiple products using lignocellulosic biomass. However, phase 3 biorefinery or more advanced biorefinery is also technically insufficient to produce energy, bio-based chemical and materials. Thus, much effort will be required to develop

the technology which is sustainable, environmentally, resource-friendly and cost effective in biorefinery industry for the future (Kamm et al., 2007; Himmel, 2009).

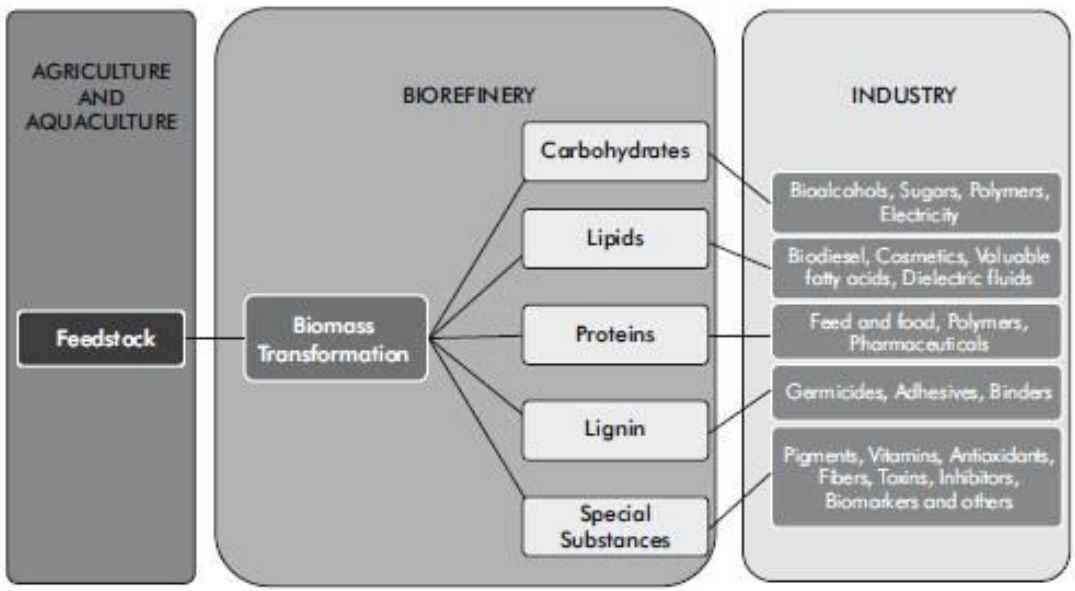


Figure 1. The general scheme of biorefinery concept (González-Delgado & Kafarov, 2011).

1.4 Objectives

The comprehensive purposes of this study are to investigate the optimal condition of furan derivatives production by two-step pretreatment with oxalic acid as catalyst and to evaluate separation methods including nanofiltration and solvent extraction. Each separation process also will be operated at various condition to identify the optimal condition for furan derivatives separation.

Specific purposes of each process are as follows:

Two-step pretreatment

- ✓ Determining the optimal condition of two-step pretreatment with oxalic acid for furan derivatives, especially 5-HMF, furfural, and 5-MF which are major component of furan derivatives from lignocellulosic biomass.

Nanofiltration process

- ✓ Investigating possibility of furan derivatives separation by nanofiltration and solvent extraction with focus on the effects of operating conditions

Standard experiment

- ✓ Conforming the effect of other components on product and separate process

2 Literature reviews

2.1 Studies on pretreatment of lignocellulosic biomass

2.1.1 Oxalic acid pretreatment

Oxalic acid pretreatment, initially designed through the fact that brown-rots secrete oxalic acid to degrade wood fiber and lignin through hydrolysis, was suggested as an alternative pretreatment to sulfuric acid pretreatment. Because it cause less corrosion to reactor than sulfuric acid, and is also more acidic than other organic acids such as formic, acetic, maleic acid due to its dicarboxylic property with two pK_as. Oxalic acid attacks the cell wall structure and leads to the hemicellulose hydrolysis (Kim et al., 2011). Earlier study reported that both of dilute sulfuric acid and oxalic acid achieved about 85% of xylose yield, which means oxalic acid has similar efficiency to sulfuric acid (Zhang et al., 2013). Furthermore this study shows the possibility of using oxalic acid, which is expensive than sulfuric acid on a base weight, as industry catalyst by adding recovery system (Lee et al., 2013).

2.1.2 Two-step acid pretreatment

Two-step pretreatment was suggested for high yield of sugar in the literature several times. Because it can recover higher sugar yield than one-step pretreatment. Nguyen et al reported the research which shows that maximum hydrolysis rate of glucose and mannose is not obtained at the same pretreatment severity. Glucan demands pretreatment of higher severity than mannan to be completely hydrolyzed. This suggests two-step pretreatment, with the first step performed at low severity to hydrolyze the hemicellulose

and the second step, where the solid material from the first step is pretreated again, at higher severity (Nguyen et al., 2000). Also Söderström et al reported the two-step steam pretreatment process with dilute H_2SO_4 impregnation shows attractive advantages, such as high ethanol yield, better utilization of the raw material and lower consumption of enzymes (Söderström et al., 2003).

2.2 Furan derivatives production from lignocellulosic biomass

Lignocellulosic biomass produces furan derivatives such as furfural and 5-HMF as major furan component in the acid pretreatment. They can be a promising component to replace chemicals derived from fossil fuel. The detailed information are as followed.

2.2.1 Furfural production

Hemicellulose, the second most abundant polysaccharide on earth, can produce degradation products such as furfural, 5-HMF, levulinic acid through acid pretreatment (Gallezot, 2012). In the past, the degradation products have been considered as inhibitor which cause the low yield of bioethanol by controlling the growth of the microorganism in fermentation process (Palmqvist et al., 1999; Klinke et al., 2003). However, they were recently identified as feasible alternative resources for bio-based chemicals with adaption of the biorefinery concept. Especially, furfural, selected as one of the most promising chemicals in 21st century proposed by Bozell et al, has regained attention as a biorefinery-based feedstock for future chemicals (Bozell & Petersen, 2010).

Furfural is produced through dehydration of C5 sugars such as xylose and arabinose as shown in Fig. 2. And it can offer a whole new class of chemicals of the furan family through further reaction such as dehydration, hydrogenation, oxidation, condensation, open-ring, and decarbonyl as described in Fig. 3. Therefore, furfural is the key building block for both chemical and fuel industries. It can replace the diminishing fossil-based organics for the production of resins, lubricants, adhesives, and plastics. It is also widely used to produce value-added chemicals, such as furfuryl alcohol, tetrahydrofurfuryl alcohol, and furanoic acid (Gallezot, 2012; Yan et al., 2014).

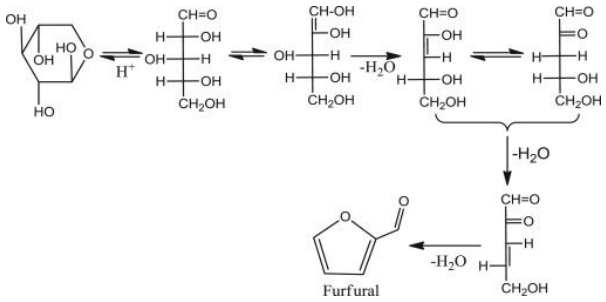


Figure 2. Route of furfural production from C5 sugar (Yan et al., 2014).

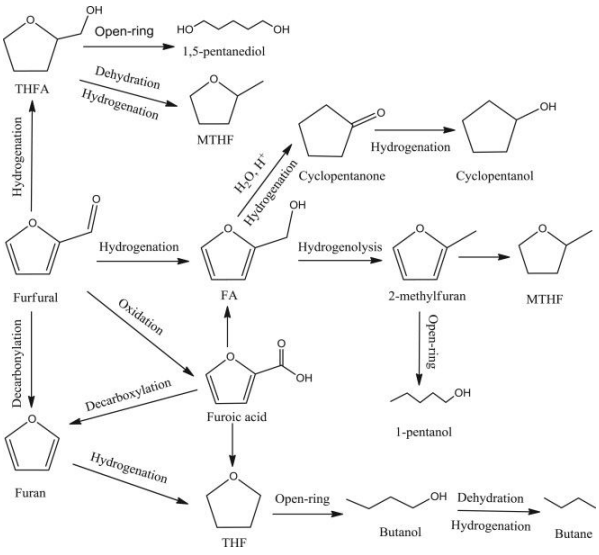


Figure 3. Further reactions from furfural to various value-added chemicals and biofuels (Yan et al., 2014).

2.2.2 5-HMF and other furan derivatives production

5-HMF was obtained by dehydration of fructose in the presence of soluble or solid acid catalysts or from glucose or even polysaccharides by more complex catalytic systems and reaction media shown in Fig. 4.

5-HMF and its derivatives; levulinic acid, 2,5-diformylfuran (2,5-DFF) and 2,5-furandicarboxylic acid (2,5-FDCA), 5-hydroxymethylfuranoic acid, and 2,5-furandicarboxaldehyde were obtained by the catalytic conversion of carbohydrates based on C6 units shown in Fig. 4. And they were identified early as very promising chemical intermediates which could replace other petrochemical-based monomers. For example, 2,5-furandicarboxylic acid is able to replace terephthalic, isophthalic, and adipic acids in the manufacture of polyamides, polyesters, and polyurethanes. Therefore, preparation of 5-HMF with economically acceptable processes will be the key issue in biorefinery industry (Corma et al., 2007, Gallezot, 2012).

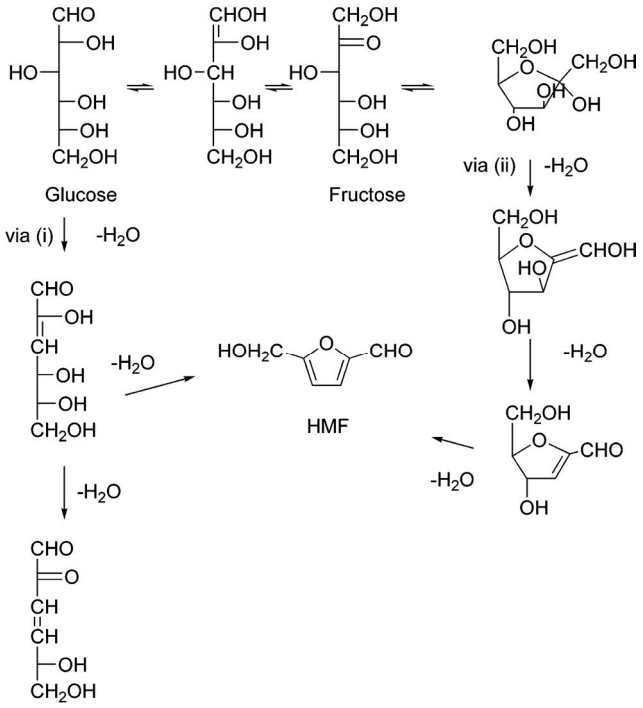


Figure 4. Pathway of 5-HMF formation from C5 and C6 carbohydrate (Corma et al., 2007).

2.3 Separation process of furan derivatives

2.3.1 Nanofiltration

Membrane filtration is one of the physical purification methods to separate by passing liquid which includes the target compound through a special pore sized membrane. It is simply classified into several types by pore size or molecular weight cut off. And filtration was controlled by operation parameters such as pressure, temperature, pH, and concentration of feed.

Nanofiltration is a promising and cost-competitive membrane separation technology. It has a molecular weight cut-off ranging from 150 to 1000 g/mol, enabling high retention of compounds with molecular weight up to 150 to 250 g/mol as well as charged molecules. Thus, nanofiltration has a wide range of applications in fermentation broth separation, sugar fractionation, sugar concentration in biorefinery process (Weng et al., 2009). Liu et al. (2008) applied NF membrane with a molecular weight cut-off of 100 g/mol for concentration and purification of hydrolyzates from hot-water extraction of woody biomass and found that sugars in the hydrolyzates could be cleaned and concentrated by using NF technology. Sjöman et al. (2008) reported purification of xylose in different hemicellulose hydrolyzates with three NF membranes, while recent work by Weng et al. (2010) on the concentration of rice straw hydrolyzates obtained from dilute acid pretreatment by NF also confirmed that NF technology can effectively concentrate sugars in the biomass hydrolyzates.

2.3.2 Solvent extraction

Solvent extraction, named as liquid – liquid extraction, is the one of

the separation methods based on the different distribution of the components to be separated between two liquid phases. It depends on the mass transfer of the component to be extracted from the first liquid phase to the second one (Müller et al., 2000).

As the differing chemical nature of the species, the selection of suitable extractant is the key point for successful separation by solvent extraction. There are several requirements to fulfill to recovery target compounds from aqueous acid hydrolysis stream. First, extractant must have high selectivity to the target compound against to other compounds and need to be chemically stable. Second, it has to be easily regenerate for re-use to increase the efficiency of process. In some cases, extractant which has low boiling point is good to reduce energy consumption in distillation process. Third, it is important to have a large difference in density between extractant and raffinate phase for rapid separation (Vincent Van, 2004).

Several researches have considered the recovery of furfural using solvent extraction. For example, Bruno F. et al. (2012) performed experimental solvent extraction with the standard solution composed water and furfural. Ethyl acetate, propyl acetate, and 1-butanol were prepared as extractant. According to the result of this study, propyl acetate presents better technical characteristics for furfural removal from water (Demesa et al., 2015). In addition, two solvents for the recovery of furfural from aqueous solution was compared. 2-methyltetrahydrofuran (2-MTHF) and tri-n-octylamine in toluene (Alamine 336-toluen), were evaluated. And it turned out that the extraction of furfural was better when using 2-MTHF (Almeida et al., 2012).

Also there are several researches on solvent extraction method related with the recovery of 5-HMF from liquid hydrolyzate. It is not easy to extract from aqueous phase, since the distribution coefficient between the organic and the aqueous phase is not favourable. However, this problem has been overcome by the use of organic solvents such as MIBK (methyl isobutyl

ketone), DCM (dichloromethane), ethyl acetate, THF (tetrahydrofuran), diethyl ether, and acetone, which have been reported to be efficient extraction solvents (Rosatella et al., 2011).

3 Materials and methods

3.1 Materials

Thirty year-old *Quercus mongolica* was supplied by the arboretum of Seoul National University (Anyang, South Korea) and used as raw material in this study. The raw material was milled and reduced to a particle size below 0.5mm (Cutting Mill pulverisette 15, FRITSCH, GERMANY). Then, the samples were air-dried and stored in plastic bags. The moisture content was less than 10% before use. The composition of raw material was determined by NREL method and the results were represented as the yield of components based on dry weight of raw material. Standard materials (glucose, arabinose, and xylose) were purchased from Sigma-Aldrich Co. (Yongin, South Korea).

3.2 Two-step pretreatment

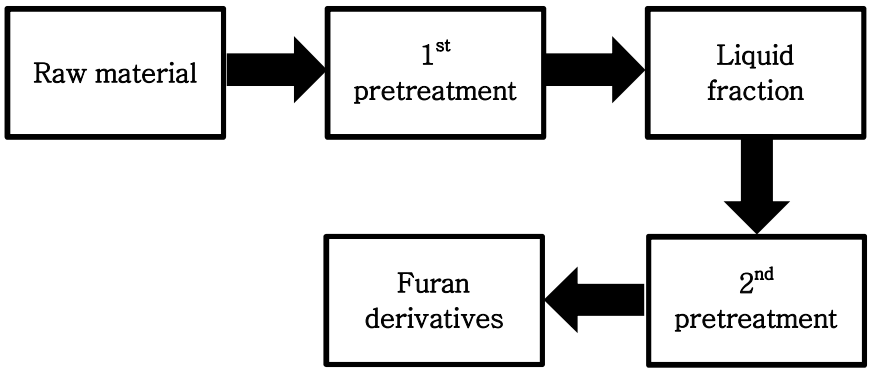


Figure 5. Scheme of two-step pretreatment for furan derivatives production.

Two-step pretreatment was carried out for production of furan derivatives and the whole process was shown in Fig. 5. 1st pretreatment was conducted to release pentose in liquid hydrolyzate and 2nd pretreatment was carried out to produce furan derivatives such as 5-HMF, furfural, and 5-MF.

3.2.1 1st pretreatment

1st pretreatment was performed using a reactor consisted of a 1L volted closure stainless steel reaction vessel (SUS 316), an electric heating mantle, a thermocouple, a pressure gauge, a paddle type impeller, and a control box (HR-8300, Hanwol Engineering Inc.) (Fig. 6). The thermocouple and Teflon impeller were inserted in the reactor to measure the internal temperature and to stir samples evenly, respectively. The temperature of the electric heating mantle and the speed rate of the impeller were controlled by control box.

The milled *Quercus mongolica* and an aqueous oxalic acid were mixed with solid to liquid ratio at 1:7 in the reaction vessel and heated at various reaction conditions following 2^3 factorial design. The pre-heating time was set 50 min to reach the target temperature (not including reaction time). As soon as reaction time was over, the reaction vessel was cooled during 15 min. The pretreated materials were filtered using filter paper (No.2 Adventec, Kyoto, Japan) to divide into solid residue and liquid hydrolyzate fractions. Then liquid hydrolyzate was stored in the glass bottle at 4°C for further use and HPLC (high performance liquid chromatography) analysis.

3.2.1.1 Experimental design and statistical analysis

The statistical approach was adapted to evaluate the effect of pretreatment conditions and to search for the optimal condition of pentose (xylose and arabinose) production during oxalic acid pretreatment as 1st pretreatment. The pretreatment conditions such as reaction temperature (X_1, °C), acid concentration (X_2, %), and reaction time (X_3, min) were selected as the independent variables which can directly influence pentose yield. Each independent variable had different range and was coded in three levels. While, pentose yield based on a dry weight of raw material (%) in liquid fraction after 1st pretreatment was adapted as the dependent variable. To optimize the combination of pretreatment conditions, 17 experimental operations based on 2^3 factorial design, listed in Table 1, were carried out including triplication at the center point (X_1: 140°C, X_2: 2%, X_3: 20min). The statistical approach was performed using Design Expert 8.0.1 software. ANOVA (Analysis of variance) and 3D response surface plots were generated as results for statistical data analysis.

3.2.1.2 Determination of pentose yield

To confirm the optimal condition suggested by RSM (response surface methodology) for the highest pentose yield in the range of this study, 1st pretreatment for determination of pentose yield was conducted as the same way, previously described in 3.2.1 1st pretreatment.

Figure 6. Shape of reactor used for oxalic acid pretreatment.

Table 1. Coding of the condition of experiments based on 2^3 factorial design

Run	Coded level			Variables		
	Reaction temperature X_1	Acid concentration X_2	Reaction time X_3	Reaction temperature (℃)	Acid concentration (%, (w/w))	Reaction time (min)
1	-1	-1	-1	130	1	10
2	1	-1	-1	150	1	10
3	-1	1	-1	130	3	10
4	1	1	-1	150	3	10
5	-1	-1	1	130	1	30
6	1	-1	1	150	1	30
7	-1	1	1	130	3	30
8	1	1	0	150	3	30
9	-1.68	0	0	123.2	2	20
10	1.68	0	0	156.8	2	20
11	0	-1.68	0	140	0.32	20
12	0	1.68	0	140	3.68	20
13	0	0	-1.68	140	2	3
14	0	0	1.68	140	2	37
15	0	0	0	140	2	20
16	0	0	0	140	2	20
17	0	0	0	140	2	20

3.2.2 2nd pretreatment

2nd pretreatment for furan derivatives production was carried out with the same reactor and equipment as 1st step pretreatment. To evaluate the change aspect of composition of liquid hydrolyzate depending on the pretreatment conditions and to investigate the optimal condition of furan derivatives production in the range of pretreatment conditions in this study, the separated liquid hydrolyzate from 1st pretreatment was used and heated at various conditions as shown Table 2. To make 2%, 3%, 4% oxalic acid hydrolyzate, more oxalic acid was added into the liquid hydrolyzate from 1st pretreatment. The pre-heating step and cooling method were also same as 1st pretreatment. Then, liquid hydrolyzate generated from 2nd step pretreatment was stored to use for separation process and HPLC analysis. The results were defined as the component yield based on dry weight of raw material. In addition, the pretreatment with standard materials (glucose, xylose, and arabinose) was conducted at the optimal condition of 2nd pretreatment in order to compare the composition of degradation products and to understand the conversion behavior of furan derivatives.

Table 2. The conditions of 2nd pretreatment

Acid concentration	Reaction temperature	Reaction time
2%	180°C, 190°C, 200°C, 210°C, 220°C, 230°C	10 min
3%		
4%		

3.3 Separation process for furan derivatives

3.3.1 Nanofiltration

Nanofiltration was conducted using Amicon cell (Millipore Amicon stirred cell 8400) with gas pressure, pressure control valve and stirrer located in the cell. Two commercially available NF membranes, NE90 and DRM, were used as filter in this study. They were purchased from Toray and their properties were summarized in Table 3.

To separate furan derivatives from the other compounds, About 40 mL hydrolyzates from two-step pretreatment were injected into the cell and then pumped into the filtration cell at 60 bar with nitrogen gas. After filtration, the collected permeate and corresponding retentate in the cell filter were analyzed for the concentration of sugars and degradation products, especially furan derivatives. The effects of filter type (NE90 and DRM), feed pH (1, 4, 7, and 10), and repetition of filtration on the performance of the NF process were studied to investigate the optimal condition for furan derivatives separation.

3.3.2 Solvent extraction

Solvent extraction was conducted to separate furan derivatives from the other components in liquid hydrolyzate after two-step pretreatment. Various organic solvents including chloroform, butanol, ethyl acetate, and propyl acetate were employed in this study and their properties were sumerized in Table 4.

To screen organic solvents for extraction, the liquid hydrolyzate and organic solvents were mixed together in a 250ml baffled Erlenmeyer flask with shaking (270rpm) at room temperature. Solvent extraction time was set

15, 30, 45, and 60 min. After extraction, the solution was left to be a state of equilibrium (organic and aqueous phases) for 15 min and organic phase which separated from aqueous phase is distilled to remove organic solvent with evaporator (N-1110 series). Then, the sample was diluted with 10 ml acetone for analysis of HPLC. After screening, solvent extraction was conducted with the best solvent to evaluate the effect of the hydrolyzate/solvent ratio and coupling of extraction stage.

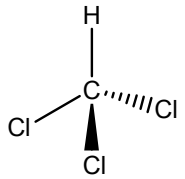
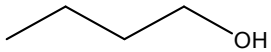
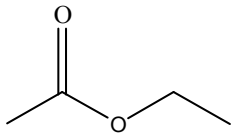
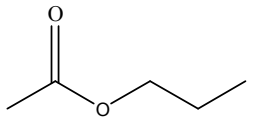
3.3.3 Standard experiment

Standard experiment was conducted at the best separation process selected from the experiments with raw material with nanofiltration or solvent extraction. The model solution was used to identify the effect of other compounds from lignocellulosic biomass on the recovery of furan derivatives in separation process. The model solution was composed of furfural, 5-HMF, and 5-MF and their composition was set as followed by the composition of hydrolyzate after two-step pretreatment under the optimal conditions (1^{st} pretreatment: 147°C, 2.3% oxalic acid, and 20 min and 2^{nd} pretreatment: 220°C, 2% oxalic acid, and 10 min).

Table 3. Characteristic of NF filter used in this study

Filter	**NE90**	**DRM**
Manufacturer	Toray Chemical Korea Inc.	Toray Chemical Korea Inc.
Configuration	Spiral wound	Spiral wound
Filter materials	Polyamide	Polyamide
Molecular weight cutoff (MWCO)	200 Da.	1000 Da.
Max. temperature (°C)	45°C	45°C
Max pressure (bar)	600 psi	600 psi

Table 4. Properties of solvent used in extraction

	Chloroform	Butanol	Ethyl acetate	Propyl acetate
				
Molecular formula	$CHCl_3$	$C_4H_{10}O$	$C_4H_8O_2$	$C_5H_{10}O_2$
Molar mass	119.37 $g \cdot mol^{-1}$	74.12 $g \cdot mol^{-1}$	88.11 $g \cdot mol^{-1}$	102.13 $g \cdot mol^{-1}$
Appearance	Colorless liquid	Colourless, refractive liquid	Colorless liquid	Clear, colorless liquid
Density	1.489 g/cm^3	0.81 g cm^{-3}	0.902 g/cm^3	0.89 g/cm^3
Melting point	−63.5 °C	−89.8 °C	−83.6 °C	−95 °C
Boling point	61.15 °C	117.7 °C	77.1 °C	102 °C
Solubility in water	0.809 g/100 mL (20 °C)	73 g L^{-1}	8.3 g/100 mL	18.9 g/L
Acidity (pKa)	15.7	16.10	25	
Viscosity	0.563 cP	2.573 mPa×s	426 μPa s	
Dielectric constant	4.81	17.5	6.02	

3.4 Analysis of liquid hydrolyzates

3.4.1 Analysis of monomeric sugar content

After 1st step and 2nd step pretreatment, liquid hydrolyzate is filtered by 0.45 μm hydrophilic membrane filter (Adventec Co., Japan) and analyzed their component such as glucose, xylose, galactose, and mannose by Bio-Liquid Chromatography (ICS-2500, Dionex USA) equipped with a CarboPac PA-1 column and Pulsed amprometry (ED40, Gold electrode) as a detector. The mobile phase is potassium hydroxide with 10 μL injection volume with flow1 mL/min flow rate.

3.4.2 Analysis of degradation products

HPLC (Dionex Ultimate 3000, USA) using Aminex 87H column with Refractive index detector (ERC, RefractoMAX520, Japan) is used to determine degradation products such as furan derivatives (furfural, 5-HMF), levulinic acid, formic acid, and acetic acid. Injection volume is 10 μL with 0.01N sulfuric acid and flow rate is 0.5 mL/min at 40°C for 90 min.

4 Results and discussions

4.1 Composition of raw material

The chemical composition of *Quercus mongolica* was determined by NREL method (Sluiter et al., 2008). The raw material was consisted of 58.62% carbohydrates (39.98% glucan, 14.11% xylan, 1.38% arabinan, 1.73% galactan, 1.42% mannan,), 27.62% Klason lignin, 2.3% extract and about 1% ash. The pentose (xylose+arabionose) yield produced in liquid hydrolyzate was 17.61%.

4.2 Pentose production of 1st pretreatment

4.2.1 Analysis of sugar component in liquid hydrolyzate

RSM was performed to see the effect of variables (X_1: reaction temperature, X_2: acid concentration, and X_3: reaction time) on the pentose yield and to search the optimal condition for the highest pentose yield during oxalic acid pretreatment in this study range.

To apply for RSM, 17 experiments based on 2^3 factorial design were carried out with triplication at central point (X_1: 140°C, X_2: 2%, X_3: 20 min). Table 5 shows the composition of sugar contents and pentose in liquid hydrolyzate after 1st oxalic acid pretreatment. Run #16, one of the central point, showed the highest arabinose extraction (1.10%) and run #13 (X_1: 140°C, X_2: 2%, X_3: 3 min) represented the highest xylose extraction (13.44%), simultaneously the highest pentose extraction (14.47%) in this study region. While, run #1 (X1: 130°C, X2: 1%, X3: 10 min) had the least arabinose and xylose solubility (0.73% and 1.57%). This phenomena were considered that

low severity could not fully depolymerize hemicellulose into monomeric sugar. Also, xylose yield was mostly higher than other sugar contents in all samples. And glucose yield was relatively low even glucose composition was more than xylose composition in the raw material. This results indicated that hemicellulose was selectively degraded by acid pretreatment, and it was accorded with previous study (Shin et al., 2015).

Table 5. Analysis of sugar components in liquid hydrolyzate and pentose yield as dependent factor after 1st pretreatment

Run No.	Composition of the components in liquid hydrolyzate					Dependent factor
	Glucose yield (%[a])	Galactose yield (%[a])	Mannose yield (%[a])	Arabinose yield (%[a])	Xylose yield (%[a])	Pentose yield (%[a])
	A	B	C	D	E	Y (D+E)
1	0.52	0.10	0.08	0.73	1.57	2.30
2	1.28	0.65	0.53	0.85	9.60	10.44
3	1.23	0.30	0.28	0.93	7.16	8.09
4	1.44	1.89	0.88	0.96	12.13	13.09
5	1.19	0.31	0.29	0.85	6.58	7.43
6	1.52	1.24	0.75	0.99	13.28	14.27
7	1.43	0.56	0.55	0.91	10.25	11.17
8	1.41	1.40	0.77	0.97	11.59	12.56
9	0.87	0.19	0.17	0.89	4.21	5.10
10	1.64	2.74	0.98	1.02	12.71	13.73
11	0.78	0.16	0.13	0.79	3.02	3.81
12	1.47	1.05	0.76	0.93	12.04	12.98
13	1.55	1.20	0.84	1.04	13.44	14.47
14	1.40	0.45	0.45	0.98	8.91	9.89
15	1.58	0.81	0.80	1.02	12.36	13.38
16	1.69	0.93	0.81	1.10	12.77	13.87
17	1.60	0.85	0.77	1.06	12.80	13.85

[a] based on dry weight of raw material

4.2.2 ANOVA table

Table 6 shows ANOVA (analysis of variance) results representing statistical values. Coefficient estimate was used to establish Eq. (1) shown as below, and Eq. (1) was employed to create the model for the maximum pentose yield by 1st oxalic acid pretreatment. The P-value of the model was lower than 0.05, which indicated that the model was statistically significant within a 95% confidence interval.

$$Y = 13.69 + 2.63 \times X_1 + 1.89 \times X_2 + 0.28 \times X_3 - 1.07 \times X_1 \times X_2 - 0.61 \times X_1 \times X_3 - 0.80 \times X_2 \times X_3 - 1.48 \times X_1^2 - 1.84 \times X_2^2 - 0.51 \times X_3^2 \quad (1)$$

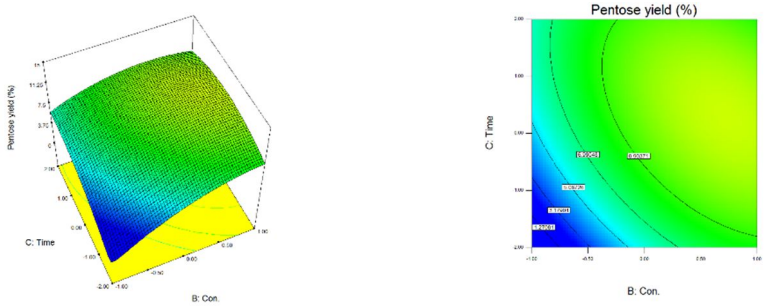
Independent factors (reaction temperature, acid concentration, and reaction time) as shown in ANOVA table were significantly related with pentose yield in pretreatment process. In terms of the influence of single factor on pentose extraction, if the factor has low p-value, it means that the factor has more influence on the pentose yield. Thus, reaction temperature (0.0030) was the most dominant factor, followed by acid concentration (0.0151) and reaction time (0.6538). While, In case of interaction factor, reaction temperature-acid concentration (0.2074) was the most influent factor, followed by acid concentration-reaction time (0.3344), and reaction temperature-reaction time (0.4542). The previous study for optimization of monosaccharides from yellow poplar by oxalic acid using RSM had also similar pattern in xylose extraction (Kim et al., 2011).

Table 6. ANOVA of pentose yield in the liquid hydrolyzate from *Quercus mongolica* after 1st oxalic acid pretreatment

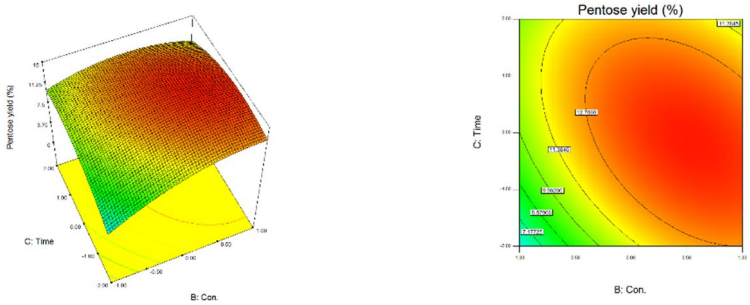
Source	Coefficient estimate	Sum of squares	DF	Mean square	F-value	P-value
Model	13.69	211.79	9	23.53	4.91	0.0238
X_1	2.63	94.28	1	94.28	19.68	0.0030
X_2	1.89	49.01	1	49.01	10.23	0.0151
X_3	0.28	1.05	1	1.05	0.22	0.6538
$X_1 X_2$	-1.07	9.24	1	9.24	1.93	0.2074
$X_1 X_3$	-0.61	3.01	1	3.01	0.63	0.4542
$X_2 X_3$	-0.80	5.15	1	5.15	1.07	0.3344
X_1^2	-1.48	24.81	1	24.81	5.18	0.0570
X_2^2	-1.84	38.31	1	38.31	8.00	0.0255
X_3^2	-0.51	2.88	1	2.88	0.60	0.4638
Residual		33.53	7	4.79		
Lack of Fit		33.37	5	6.67	84.96	0.0117
Pure Error		0.16	2	0.08		
Cor Total		245.32	16			

4.2.3 3D plots and contours representing pentose yield by the change of factors

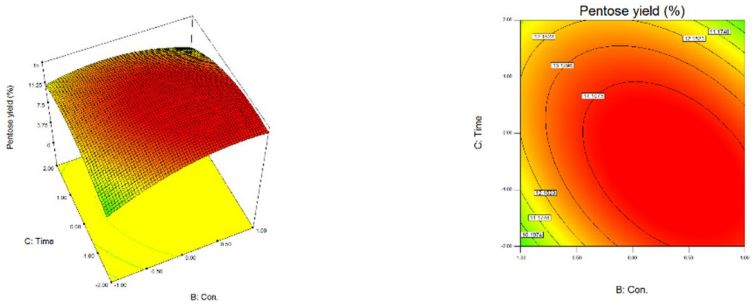
To evaluate the effects on pentose yield in accordance with the change of factors, RSM based on 17 experiments provided 3D plots and contours according to each independent factor (reaction temperature, acid concentration, and reaction time) on pentose yield from *Quercus mongolica* after 1st oxalic acid pretreatment. Fig. 7 shows that the dark region becomes wider, in short, pentose yield rose with an increase of reaction temperature when reaction time and acid concentration was at zero coded level. On the other hand, in case of acid concentration, pentose yield was slightly decreased at 3% acid concentration (Fig. 8). It was considered that pentose was converted into degradation products such as furfural, 5-HMF, formic acid and acetic acid and etc. over the proper severity (Gwak et al., 2012; Shin et al., 2015). Lastly, the reaction time was found to the similar results with in the acid concentration, and the reason was regarded to the same reason as mentioned in the case of acid concentration (Fig. 9).



(A) 130°C

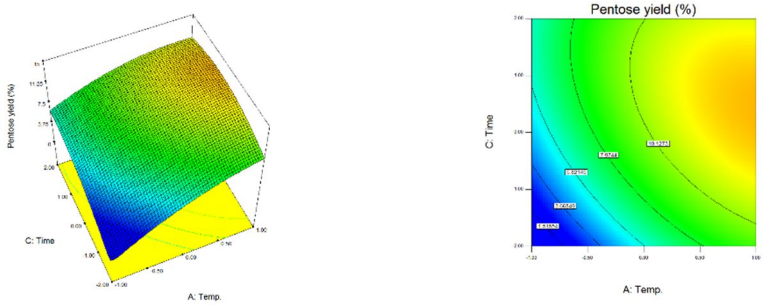


(B) 140°C

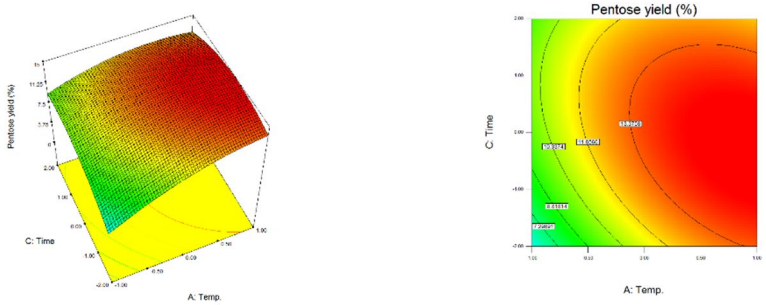


(C) 150°C

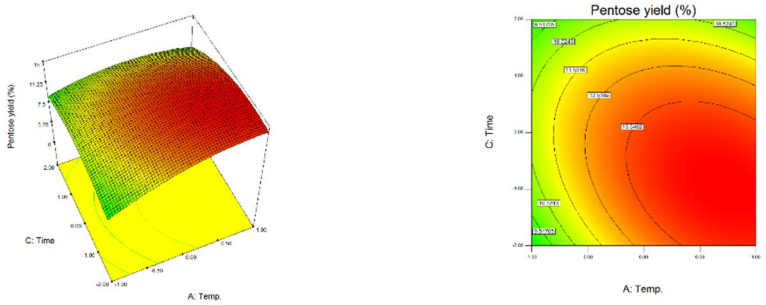
Figure 7. Effect of reaction temperature ranged from 130°C to 150°C on pentose yield from *Quercus mongolica* after 1st pretreatment.



(A) 1%

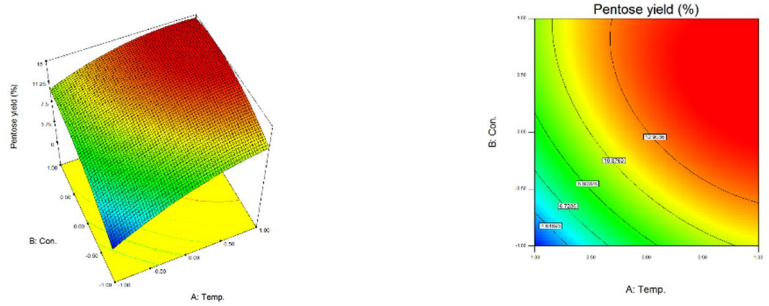


(B) 2%

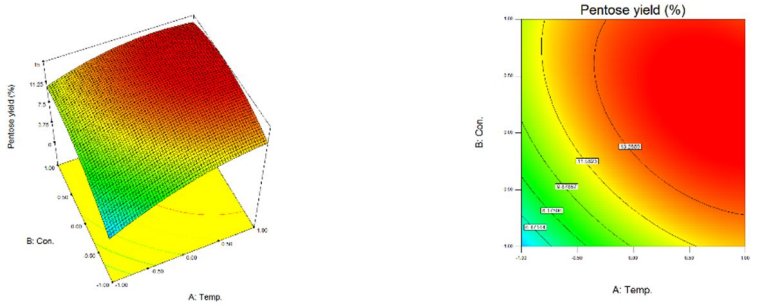


(C) 3%

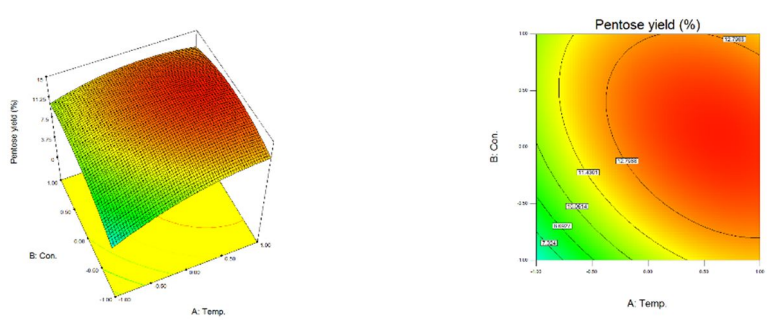
Figure 8. Effect of acid concentration ranged from 1% to 3% on pentose yield from *Quercus mongolica* after 1st pretreatment.



10 min



20 min



30 min

Figure 9. Effect of reaction time ranged from 10 min to 30 min on pentose yield from *Quercus mongolica* after 1st pretreatment.

4.2.4 Confirmation experiment for pentose

As the result of RSM analysis, the optimal condition for the maximum pentose yield was reaction temperature of 147°C, acid concentration of 2.29%, and reaction time of 20 min. In this condition, the predicted maximum pentose yield was 14.98%. To confirm the predicted value, experiments were conducted under the optimal condition, and the results were shown in Table 7. The pentose yield was 14.36%, corresponding to 81.54% pentose extraction, and it indicated that the optimization for pentose yield from *Quercus mongolica* by oxalic acid pretreatment was practicable using RSM. Also, the yield of hexose (glucose, galactose, and mannose) was less than 2%, that is, xylose was effectively isolated as observed in previous researches that oxalic acid intensively makes xylose isolated (Lee & Jeffries, 2011; Lee et al., 2009).

Table 7. The contents of sugars and degradation products in liquid hydrolyzate after the optimal condition of 1st pretreatment (reaction temperature: 147°C, acid concentration: 2.29%, reaction time: 20 min)

Component		Yield[a] (%)
Sugars	Glucose	1.82 (±0.05)
	Mannose	0.87 (±0.01)
	Galactose	1.54 (±0.01)
	Xylose	13.40 (±0.12)
	Arabinose	0.96 (±0.00)
Derivatives products	Formic acid	0.70 (±0.01)
	Acetic acid	4.37 (±0.04)
	Levulinic acid	0.02 (±0.00)
	5-HMF	0.04 (±0.00)
	Furfural	0.70 (±0.01)
	5-MF	0.01 (±0.00)

[a] Based on a dry weight of raw material

4.3 Furan derivatives production of 2nd pretreatment 4

4.3.1 Component yield of liquid hydrolyzate

The liquid hydrolyzate from 1st pretreatment under the optimal condition for high yield of pentose was used for 2nd pretreatment to produce furan derivatives. 2nd pretreatment was conducted at various conditions (reaction temperature: 180-230°C, acid concentration: 2-4%, and reaction time: 10 min) to determine the optimal condition for high yield of furan derivatives, especially furfural, 5-HMF, and 5-MF which are the major furan derivatives from lignocellulosic biomass, and to evaluate effects of reaction conditions on yield of products during 2nd pretreatment. Therefore, Fig. 11, 13, 14, and 15 show that the yield of furan derivatives and its related products in liquid hydrolyzate was described depending on reaction changes of temperature and acid concentration at 10 min reaction time. And all yield was based on dry weight of raw material.

4.3.1.1 Yield of furfural and its related products

Furfural is the most desired chemical among furan derivatives in this study. Because, it was the value-added chemical appointed by US Department of Energy and it could be widely used as biomass feed stock instead of oil based feed in various industry (Bozell & Petersen, 2010). Generally, it is known that furfural is produced from pentose such as xylose and arabinose through dehydration reaction. And at the high severe condition, furfural converts into other degradation products such as formic acid or is used in several other secondary reactions, for example, resinification which is a reaction of furfural itself due to its aldehyde structure which is sensitively

affected to acid, as shown in Fig. 10 (Cho, 2012; Karinen, 2011). Therefore, it is important to know the relationship among pentose (feedstock), furfural (desire product), and formic acid (furfural derivative) for improving furfural production.

To identify their relationship in forming furfural during 2nd pretreatment, each tendency of yield was compared. Furfural yield was increased until 210°C, after that there was hardly changed in amount of furfural ranged from 210°C to 220°C (Fig. 11C), representing the highest furfural yield. Meanwhile, the yields of xylose and arabinose were steadily decreased in that range (Fig. 11A, B). It was considered that furfural was degraded into formic acid or condensed itself (Patil, 2011). It seemed that resinification reaction or other secondary reactions were more active than formation of formic acid over 220°C, because the yield of formic acid was not increased while furfural yield was decreased. Also, it could be assumed that that formic acid was generated from degradation of oxalic acid (Eul et al., 2000).

Lastly, the maximum furfural yield was 6.52%, accordance with theoretical conversion rate of 72.89% which was the similar result with previous studies on production furfural from lignocellulosic biomass using sulfuric acid (Cai, 2014). Therefore, it indicates the feasibility of furfural production using oxalic acid as catalyst through two-step pretreatment process.

In addition to more details about the yield of pentose and formic acid depending on effects of reaction conditions, the yield of xylose and arabinose were steadily decreased when reaction temperature rose, moreover pentose was hardly remained over reaction temperature of 220°C (Fig. 11A, B). In case of formic acid, Fig. 11C indicates that the yield of formic acid was more influenced by acid concentration than reaction temperature. For example, it was obtained the maximum yield of 9.21% with 4% acid concentration at 190°C, but also obtained that of 8.67% with 4% at 230°C while obtained that of 4.61% with 2% at 190°C.

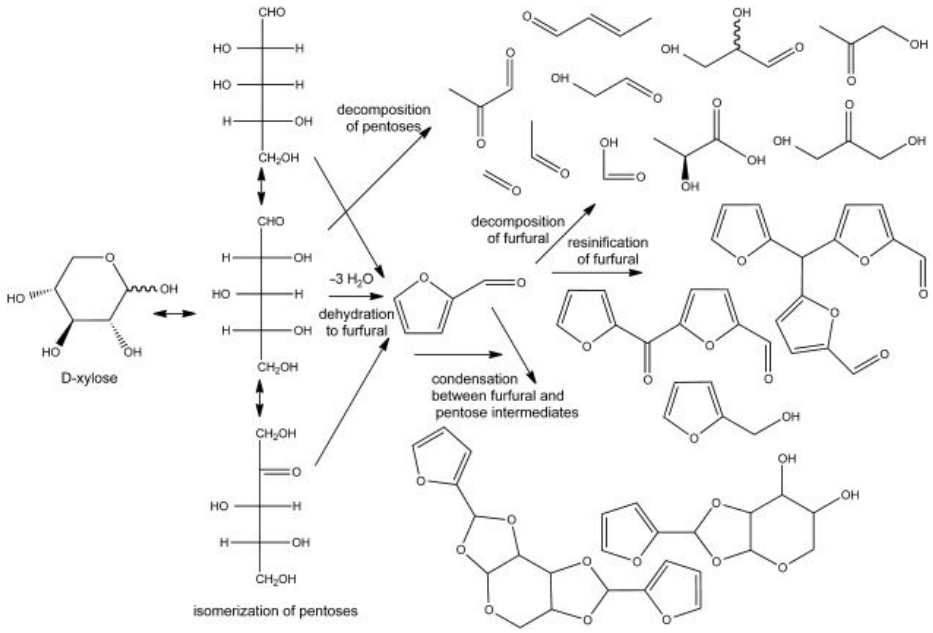
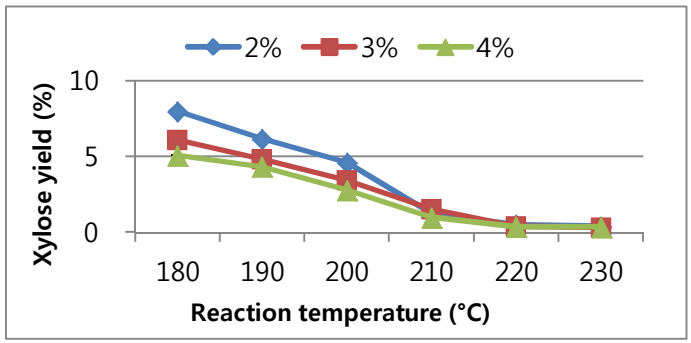
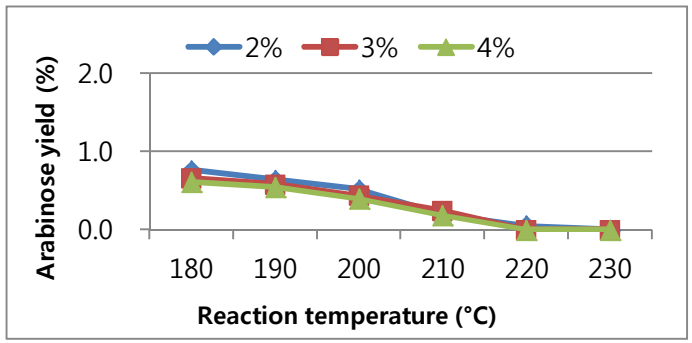


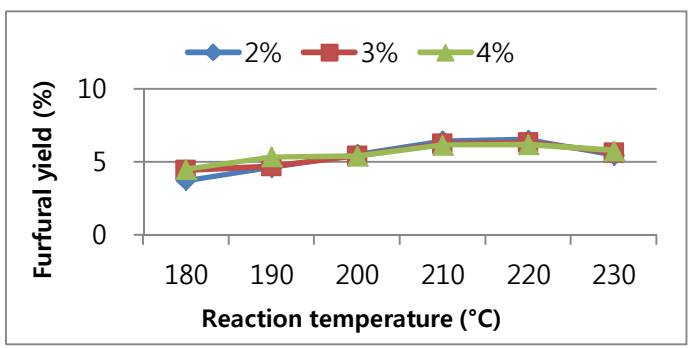
Figure 10. Scheme of reaction pathway of furfural and its secondary reactions.



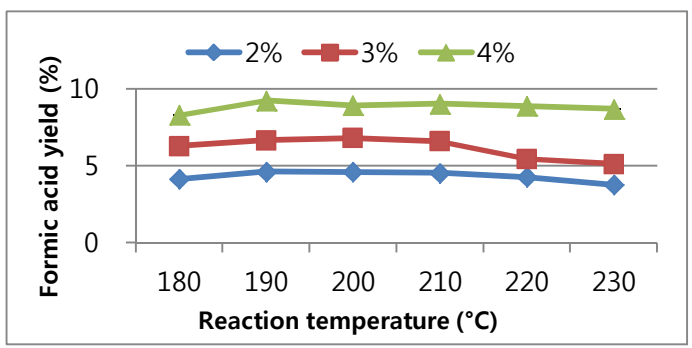
A



B



C



D

Figure 11. Yield of sugars and degradation products in liquid hydrolyzate after 2nd pretreatment depending on reaction temperature and oxalic acid concentration with reaction time fixed at 10 min (A: xylose, B: arabinose, C: furfural, D: formic acid).

4.3.1.2 Yield of 5-HMF and its related products

5-HMF is known as an intermediate products formed from glucose and degraded into levulinic acid and formic acid as described in Fig. 12 (Larsson, 1999). Fig. 13A shows that glucose yield was decreased with rising reaction temperature, especially the slope of graph fell sharply from 210°C to 220°C. Simultaneously, the yield of 5-HMF was increased with rising temperature, and distinctly increased in the same range (Fig. 13B). It was indicated that part of glucose was converted into 5-HMF with a maximum yield of 1% with 2% acid concentration at 220°C for 10 min. Over 220°C, 5-HMF yield was decreased while yield of levulinic acid and formic acid did not change (Fig. 13C, D). This was assumed that 5-HMF converted into not only levulinic acid and formic acid but also other chemicals through secondary reactions and repolymerization.

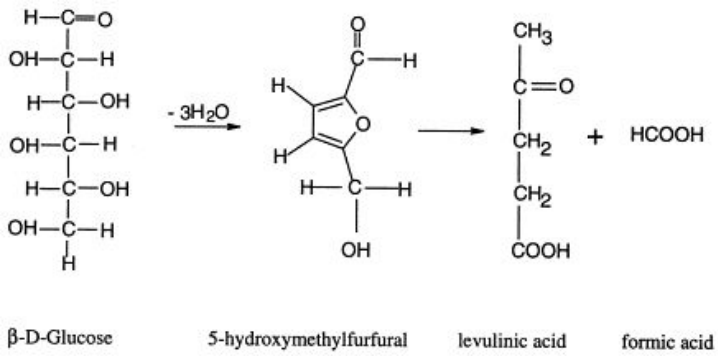
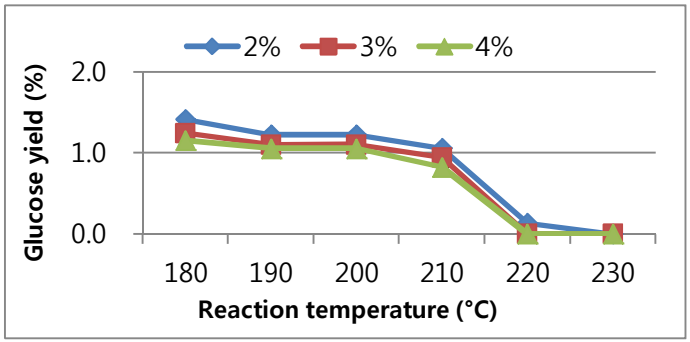
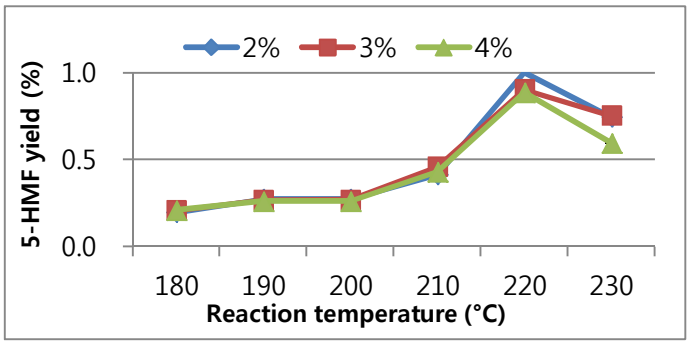


Figure 12. Scheme of conversion pathway of 5-HMF as intermediate.





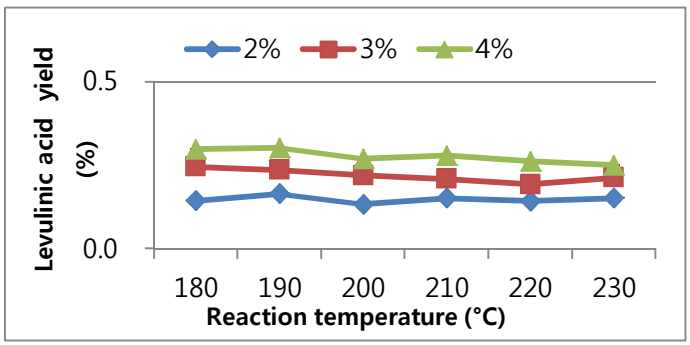


Figure 13. Yield of sugars and degradation products in liquid hydrolyzate after 2nd pretreatment depending on reaction temperature and oxalic acid concentration with reaction time fixed at 10 min (A: glucose, B: 5-HMF, C: levulinic acid).

4.3.1.3 Yield of 5-MF

The graph representing 5-MF yield was gradually risen and it is expected to be further increased (Fig. 14). However, the yield of 5-MF from *Quercus mongolica* was very low, the maximum yield was 0.2% under the condition (reaction temperature: 230°C, acid concentration: 4%).

4.3.1.4 The optimal condition of 2nd pretreatment for furan derivatives

The optimal condition of 2nd pretreatment for furan derivatives (5-HMF, furfural, and 5-MF) was selected at 220°C with 2% acid concentration for 10 min. At that condition, the total yield of furan derivatives was 7.66% and the tendency of its graph was similar to furfural graph because furfural shows the highest proportion, about 85.15%, in furan derivatives in this study (Fig. 15). The composition of hydrolyzate by the optimal condition of 2nd pretreatment shown in Table 8.

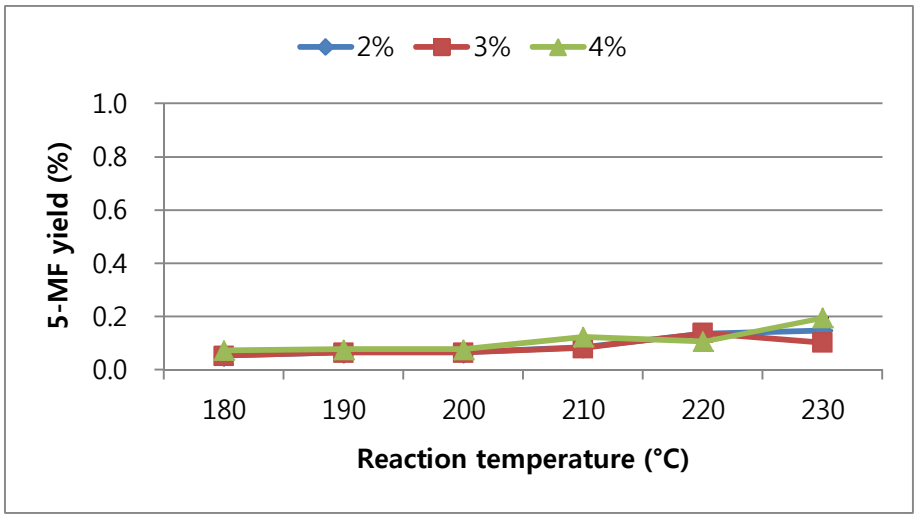


Figure 14. Yield of 5-MF in liquid hydrolyzate after 2nd pretreatment depending on reaction temperature and oxalic acid concentration with reaction time fixed at 10 min.

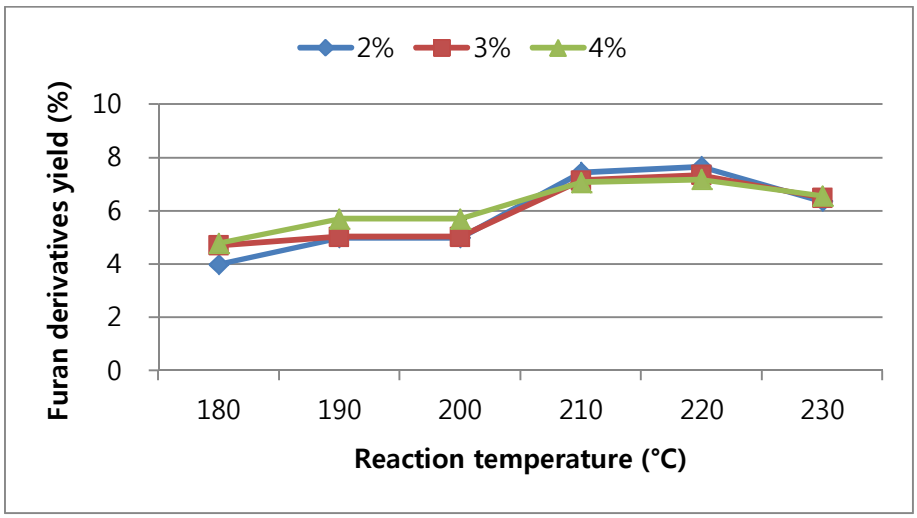


Figure 15. Yield of furan derivatives in liquid hydrolyzate after 2nd pretreatment depending on reaction temperature and oxalic acid concentration with reaction time fixed at 10 min.

Table 8. The contents of sugars and degradation products in liquid hydrolyzate after the optimal 2nd pretreatment (reaction temperature: 220°C, acid concentration: 2%, and reaction time: 10 min)

Component		Yield[a] (%)
Sugars	Glucose	0.02 (±0.05)
	Xylose	0.51 (±0.00)
Degradation products	Formic acid	4.25 (±0.00)
	Acetic acid	4.20 (±0.01)
	Levulinic acid	0.14 (±0.00)
	5-HMF	1.00 (±0.00)
	Furfural	6.52 (±0.01)
	5-MF	0.14 (±0.00)

[a]Based on a dry weight of raw material

4.3.2 Standard experiment at the optimal 2nd pretreatment condition

To understand conversion behavior of furan derivatives and oxalic acid, standard experiments were conducted under the optimal condition of 2nd pretreatment (reaction temperature: 220°C, acid concentration: 2%, and reaction time: 10 min). Glucose, xylose, and arabinose were used as standard materials in this experiments and their composition was set as followed by the composition of hydrolyzate after 1st pretreatment under the optimal conditions.

As the results, glucose generated formic acid, levulinic acid, 5-HMF, furfural, and acetic acid. And there was a little glucose remained. 5-HMF, one of the furan derivatives, was only produced from glucose not pentose as agreed with the previous study (Rosatella, 2011). Meanwhile, furfural, which was generally known as generated from pentose dehydration, was produced from glucose, which is major component of hexose. This was important information of determination for conversion behavior of furfural production, even if the amount of furfural was very low. In case of arabinose and xylose, formic acid and furfural were produced, and small quantity of xylose remained in the xylose standard solution after pretreatment process.

All three standard material generated formic acid. Even glucose and arabinose produced more formic acid than employed amount of glucose and xylose. It was considered that oxalic acid was degraded into formic acid.

As shown in Fig. 16, xylose produced the highest yield of furfural at 5.74%, followed as arabinose at 0.26% and glucose at 0.05%. The total yield of furfural from standard materials was slightly lower than raw material (*Quercus mongolica*). It could be assumed that any other component of lignocellulosic biomass were used in furfural conversion during pretreatment process.

Content		Weight (g)
Glucose (0.32g[a])	Glucose	0.03 (±0.00)
	Formic acid	0.84 (±0.01)
	Acetic acid	0.03 (±0.00)
	Levulinic acid	0.01 (±0.00)
	5-HMF	0.06 (±0.00)
	Furfural	0.01 (±0.00)
Xylose (2.41g[a])	Xylose	0.03 (±0.01)
	Formic acid	0.88 (±0.02)
	Furfural	1.07 (±0.00)
Arabinose (0.17g[a])	Formic acid	0.85 (±0.00)
	Furfural	0.05 (±0.00)

[a] The employed amount of standard material

Table 9. The weight of component produced from standard materials at the optimal condition of 2nd pretreatment (reaction temperature: 220°C, acid concentration: 2%, and reaction time: 10 min)

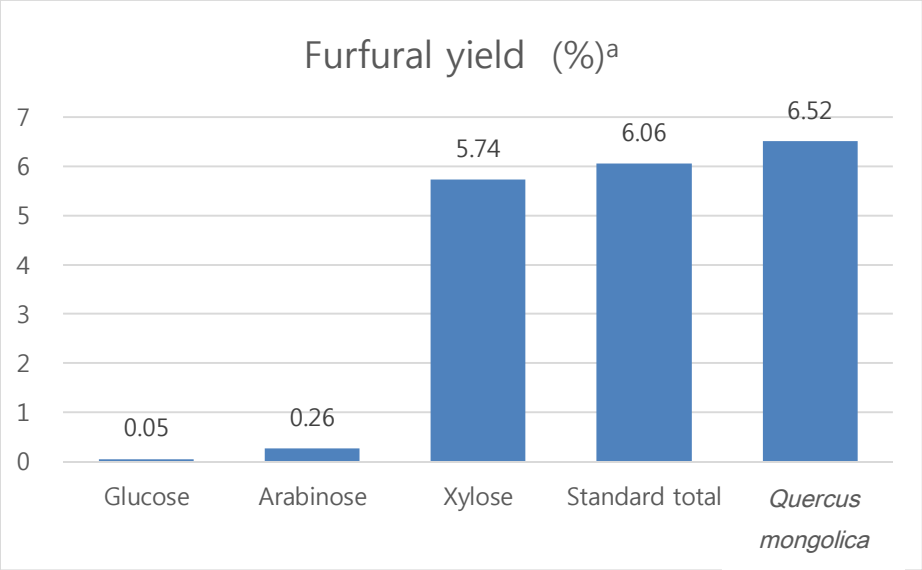


Figure 16. Furfural yield from standard materials and raw material.

4.4 Separation process for furan derivatives

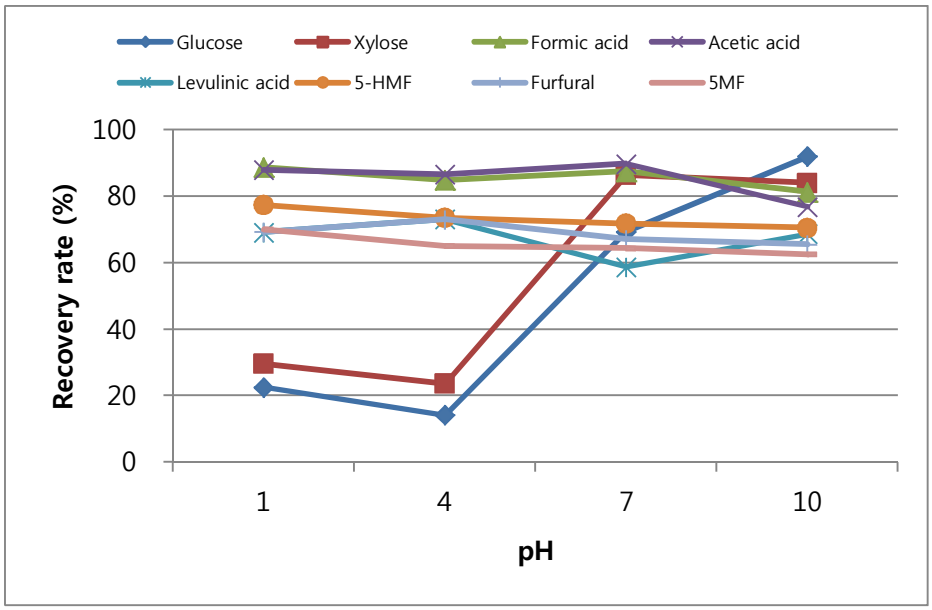
4.4.1 Nanofiltration (NF)

4.4.1.1 Effect of filter, feed pH, and repetition filtration

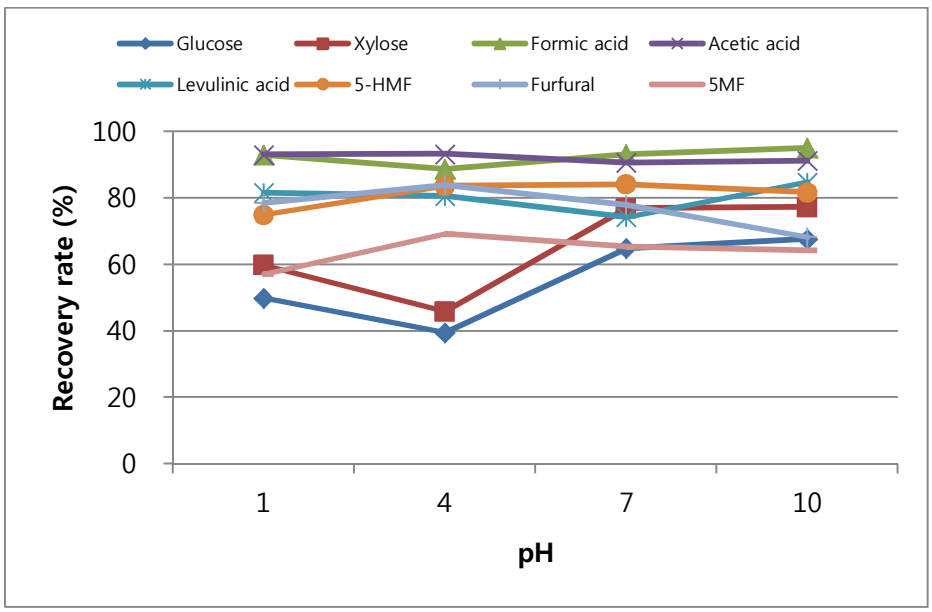
Fig. 18 indicated the recovery rate of components of hydrolyzate at various pH values for NE90 and DRM. The recovery rate of both glucose and xylose by NE90 was low at pH 4 and increased with the increase of the feed pH. The similar tendency was also observed by DRM. This may be linked to the increase of free volume in membrane skin layer. Since polyamide membrane became negative charged at high pH, the membrane repelled each other and resulted in the more open membrane. (Maiti, 2012, Weng et al., 2009)

The recovery rate of acetic acid was almost high at the pH ranged from 1 to 7, while a decrease in recovery rate of acetic acid was observed at pH 10 by NE90 (Fig. 18A). Since the pK value of acetic acid is 4, it almost dissociates and filter negatively charged at pH 11. Therefore, negatively charged acetic acid rejected by the negatively charged filter due to Donnan effect, the phenomenon that the retention of negatively charged ion was high with charged filter compared to un-charged filter. The similar result was reported in the research on separation of inhibitory components such as furfural and acetic acid from pretreated rice straw hydrolyzate using nanofiltration (Qi, 2011). However, the recovery rate of acetic acid did not change by DRM. And it was considered that Donnan effect was not affected because MWCO of DRM was larger than that of NE90. Meanwhile, The recovery rate of formic acid and furan derivatives (5-HMF, furfural, and 5-MF) were almost constant in the pH range examined, with the values higher than approximately 60% for the both filters.

As conclusion, NE90 represented better ability for separation of furan derivatives than DRM. In terms of operating condition, sugars were effectively separated at pH 4. And there was not much different in repetition filtration, but resulted in some loss in both filter.



A



B

Figure 17. Recovery rate of component for NE90 and DRM.

4.4.2 Solvent extraction

4.4.2.1 Effect of solvent and contact time

A preliminary set of experiments was carried out in order to screen the solvent which has high selectivity for furan derivatives and to identify the effect of contact time on the yield of each component. Four solvents including butanol, chloroform, ethyl acetate, and propyl acetate were tested with the hydrolyzate/solvent volume ratio 1:1. Contact time was set 15, 30, 45, and 60 min and the results were shown in Fig. 19.

Butanol extracted all sugars (glucose and xylose), degradation products (formic acid, acetic acid), and furan derivatives (furfural, 5-HMF, 5-MF) from hydrolyzate. Interestingly, butanol has the higher recovery rate of 5-HMF than other solvents with the highest rate of 68%. However, it could not influence on selectivity of furan derivatives because the yield of 5-HMF was low due to the small amount of 5-HMF in hydrolyzate after two-step. The highest total yield of furan derivatives from butanol extraction was 0.81% at the contact time of 15 min. Meanwhile, the yield of each component was very low, almost lower than 1%. And the effect of contact time was not critical in solvent extract process with butanol (Fig. 19A).

Propyl acetate extracted xylose, formic acid, acetic acid, furfural, 5-HMF, and 5-MF and glucose was not extracted. The results were similar to the results with butanol. Both of them did not obtain high selectivity for furfural, less than approximately 2%. The highest total yield of furan derivatives from butanol extraction was 0.94% at the contact time of 60 min (Fig. 19B)

Ethyl acetate also extracted most of components, except glucose like propyl acetate. However, ethyl acetate has the second highest selectivity for furan derivatives. 3.38% of furan derivatives was extracted at the contact time of 60 min. This indicates that ethyl acetate has better selectivity for furan

derivatives than propyl acetate. And it was opposite to the result of research on solvent extraction with standard material (furfural) and solvents (ethyl acetate and propyl acetate) (de Almeida, 2012). Therefore, further research will be needed to identify the different result. Meanwhile, the contact time influenced the yield of component, especially, the other degradation components such as formic acid and acetic acid (Fig. 19C)

Chloroform extracted acetic acid and furan derivatives and showed the highest selectivity for furan derivatives with total yield of 5.80% at the contact time of 15 min. Recovery rate of furfural, 5-MF, and 5-HMF were 83.92%, 70.60%, and 18.56%, respectively. Interestingly, chloroform has relatively higher recovery rate of furfural and lower recovery rate of 5-HMF than the other solvents (Fig. 19D). It was considered that the possibility of hydrogen bonding formation between furfural and chloroform molecules and linked to 'like dissolves like' role (Guo, 2014). And little effect of contact time on furan derivatives were observed in solvent extraction process. In addition, chloroform was considered the best extraction solvents with some reasons in aspect of extraction process. Its boiling point was lower than other solvents, thus it could be easy to regenerate with lower energy consumption. And, it was easier to separate from the feed because its density was different from feed (hydrolyzate) (Richard, 2004)

Therefore, chloroform was selected as the best solute in extracting furan derivatives from hydrolyzate in single stage. And it was used for further experiments for evaluating the effect of the hydrolyzate/solvent volume ratio and the number of extraction stage.

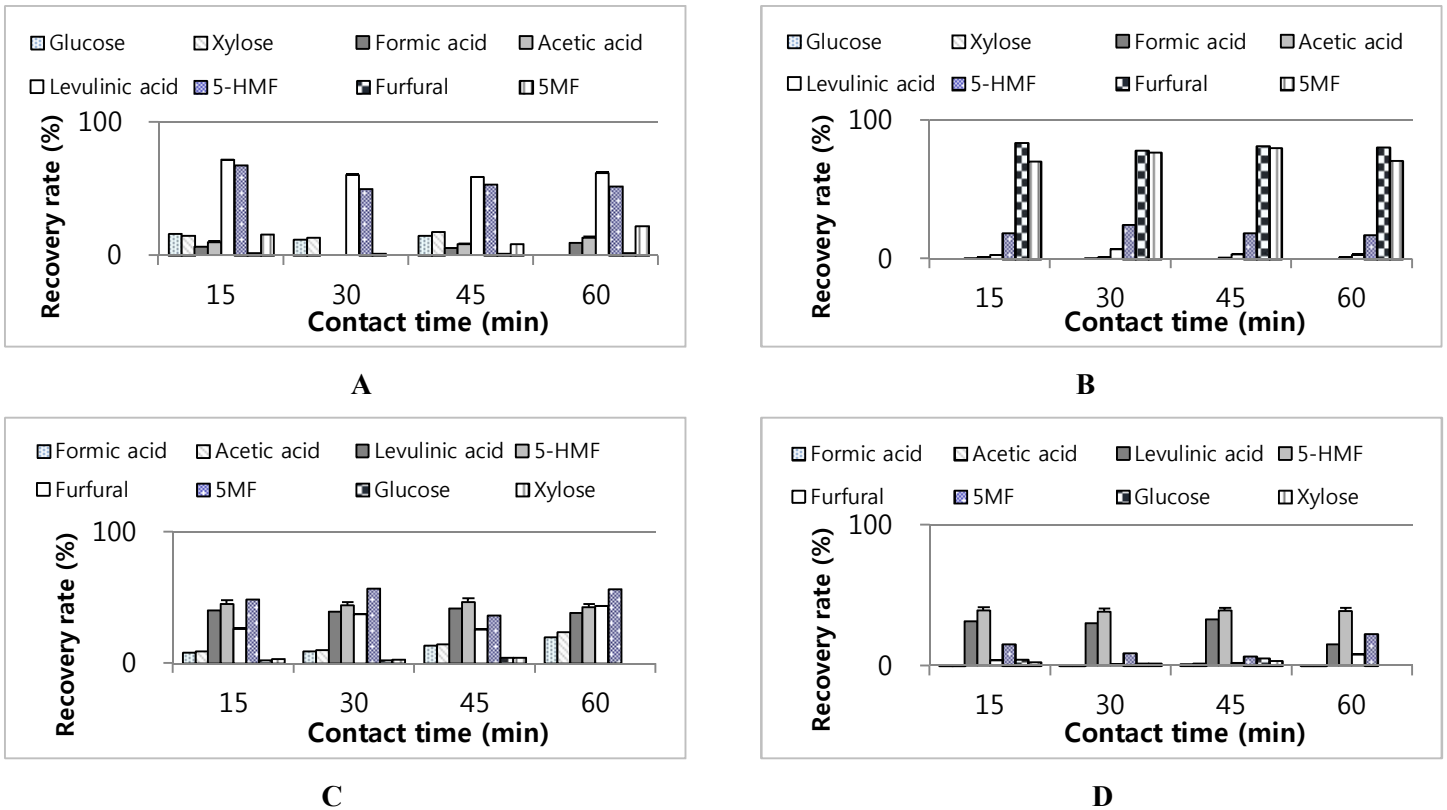


Figure 18. Effect of contact time on component yield and recovery rate of furan derivatives (5-HMF, furfural, and 5-MF) (A: butanol, B: propyl acetate, C: ethyl acetate, D: chloroform)

4.4.2.2 Effect of the hydrolyzate/solvent volume ratio and the number of extraction stage

To evaluate the hydrolyzate/solvent volume ratio and the number of extraction stage on the recovery of furan derivatives and other components, the experiments with chloroform, the best extraction solvent selected in preliminary experiments, were carried out.

Firstly, little effects of the H/S volume ratio on recovery rate of furfural and 5-MF were observed as shown in Fig. 20. The recovery rate of furfural and 5-MF were approximately 80% and 70%, respectively, at all the H/S ratio. On the other hand, the H/S volume ratio influenced the recovery rate of 5-HMF, as followed levulinic acid and acetic acid. Especially, the recovery rate of 5-HMF was increased to double. However, it could not extremely effect on the recovery rate of total furan derivatives because of the small amount of 5-HMF in hydrolyzate. Therefore, the highest recovery rate of furan derivatives was obtained with the value of 75.15% at the H/S volume ratio 1:1.

Also similar tendency were observed from the experiments for evaluating of the number of extraction stage. The recovery of 5-HMF, levulinic acid, and acetic acid were increased when the number of extraction stage increased from 1 to 3 while the recovery rate of furfural and 5-MF did not changed in this study range.

Therefore, it was considered that the H/S ratio and the number of extraction stage were only influenced on the recovery of 5-HMF, levulinic acid, and acetic acid. And the optimal condition of solvent extraction using chloroform was at the H/S volume 1:1 in triplicate extraction, with the highest recovery rate of furan derivatives of 77.26% and its purity rate of 97.81%.

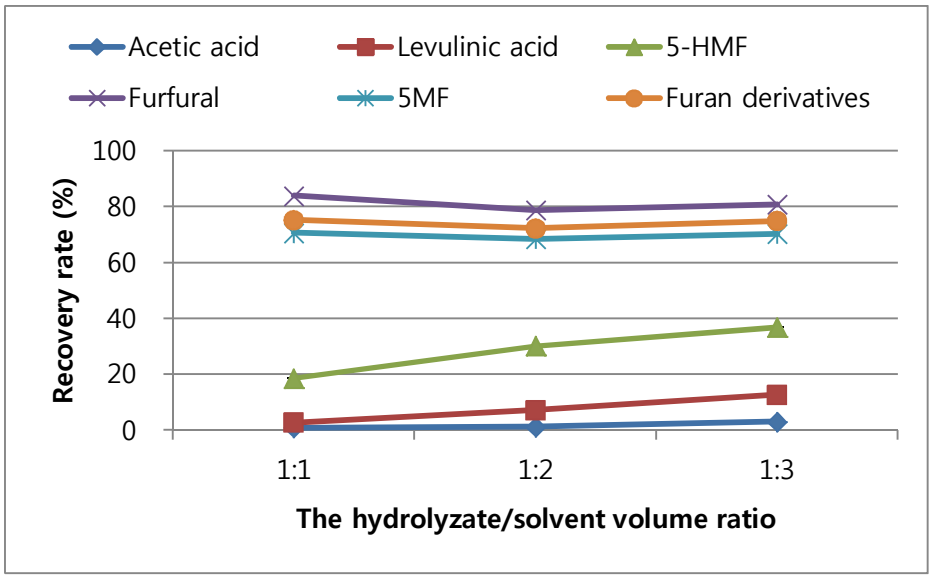


Figure 19. Effect of the hydrolyzate/solvent volume ratio on recovery rate.

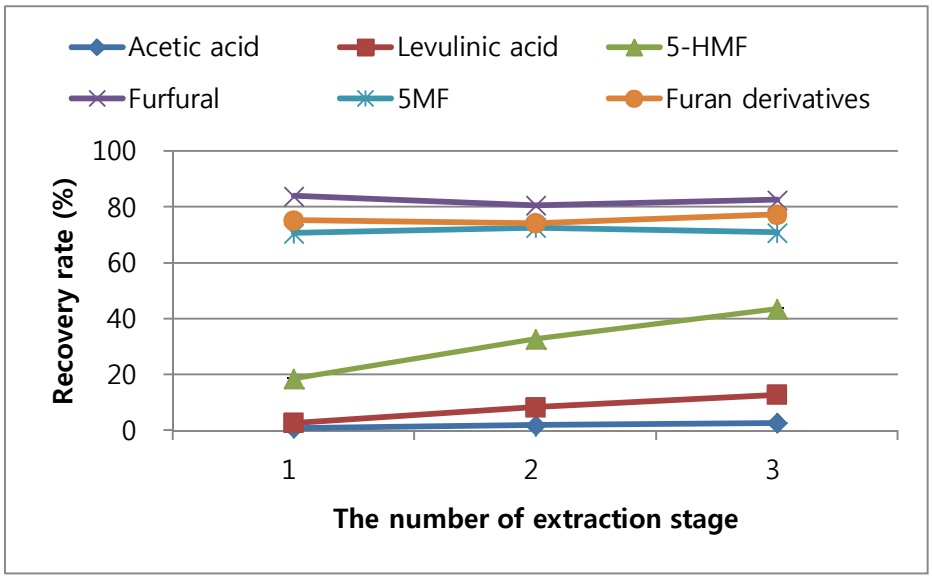


Figure 20. Effect of the number of extraction stage on recovery rate.

4.4.3 Standard experiment at the optimal separation process

Standard experiment was conducted at the best separation process selected from the experiments with raw material above. The model solution was used to identify the effect of other compounds from lignocellulosic biomass in separation process. The model solution was composed of furfural, 5-HMF, and 5-MF and their composition was set as followed by the composition of hydrolyzate after two-step pretreatment under the optimal conditions (1st pretreatment: 147°C, 2.29% oxalic acid, and 20 min and 2nd pretreatment: 220°C, 2% oxalic acid, and 10 min). The best separation process for the highest furan derivatives separation was solvent extraction with chloroform in triple stage.

Fig. 22 indicates the recovery rate of standard material and raw material. All standard material showed higher recovery rate than raw material. Especially, furfural was obtained with the highest recovery rate, over 90%, through standard experiment. It was considered that other components from lignocellulosic biomass influenced recovery of furan derivatives in solvent extraction process.

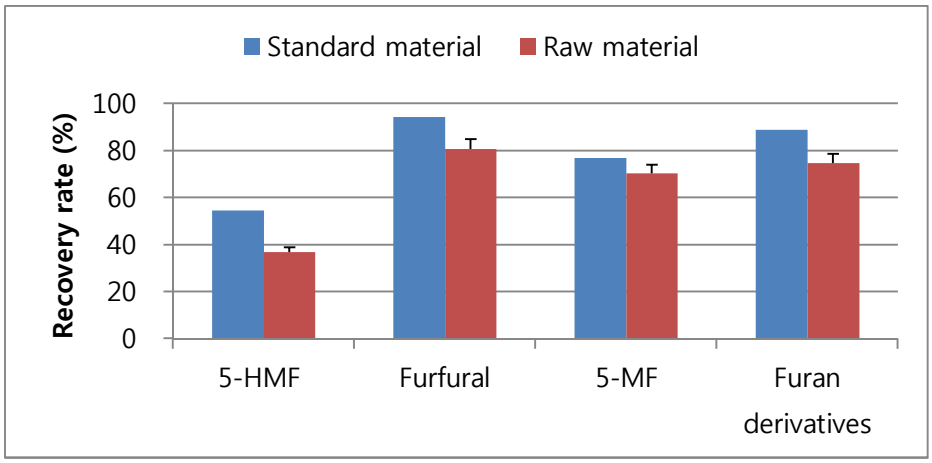


Figure 21. Recovery rate of components from standard materials and raw material by solvent extraction with chloroform.

4.4.4 Mass balance of all process for furan derivatives production

The mass balance of all process at the optimal condition for furan derivatives were shown in Fig. 23. When the basis of 100g raw material were used, 5-HMF of 0.44g, furfural of 5.42g, 5-MF of 0.11g, acetic acid of 0.11g and levulinic acid 0.02g were obtained as final products through two-step pretreatment and solvent extraction with chloroform. The yield of furan derivatives was approximately 6%.

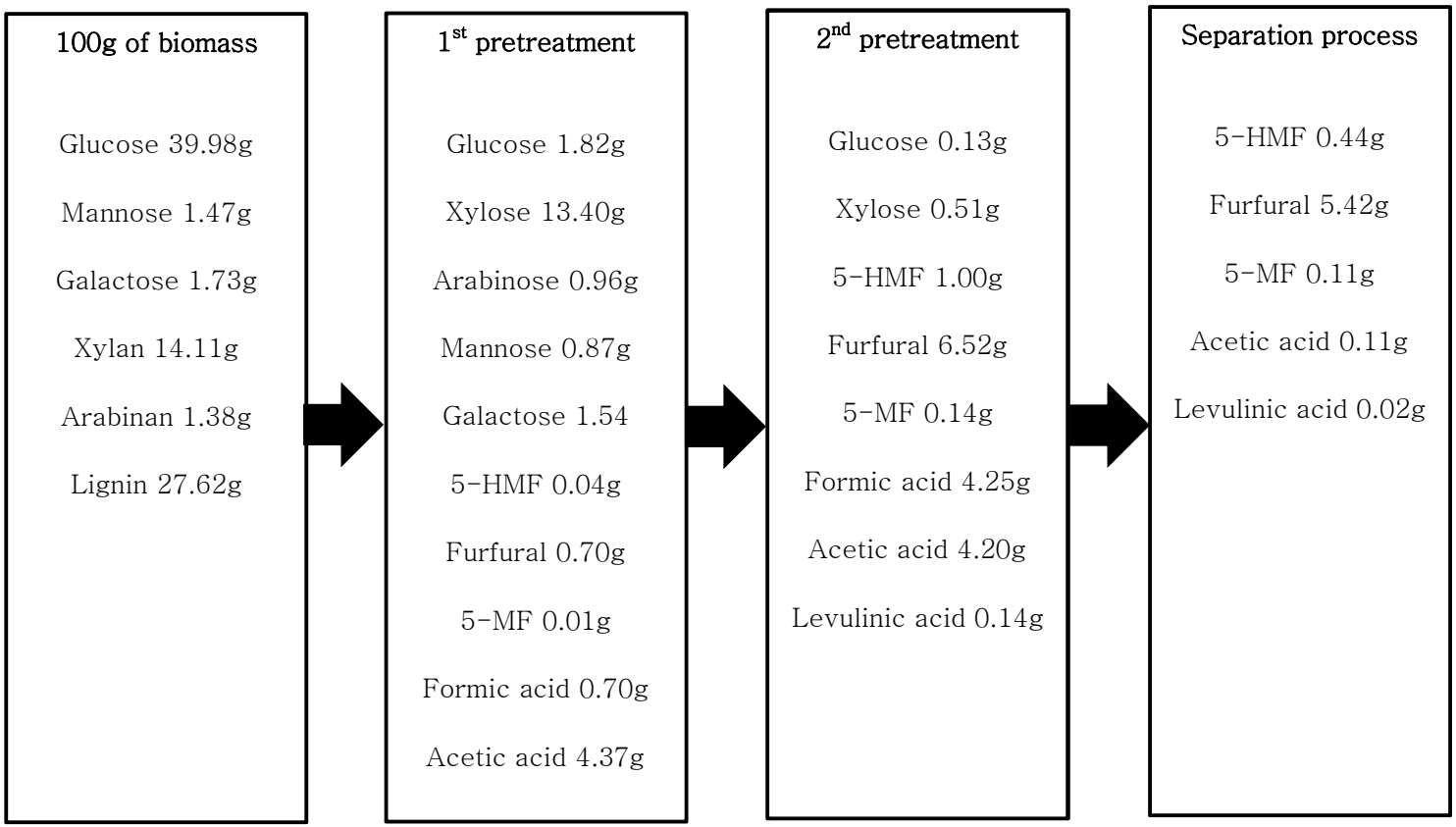


Figure 22. Mass balance of all process for furan derivatives production.

5 Conclusion

A two-step oxalic acid pretreatment of *Quercus mongolica* biomass was conducted to produce furan derivatives such as 5-HMF, furfural, and 5-MF. After production, nanofiltration and solvent extraction were carried out under various operating conditions to identify the optimal conditions for the separation of furan derivatives from other components of the liquid hydrolyzate, including sugars (glucose, xylose) and other degradation products (acetic acid, formic acid, and levulinic acid).

The 1st pretreatment was performed to determine the effects of various parameters (reaction temperature, acid concentration, and reaction time) and to define the optimal conditions for pentose yield by RSM. The results showed that reaction temperature was the most dominant factor affecting pentose yield; the highest yield of pentose was 14.36% under reaction conditions of 2.29% oxalic acid at 147°C for 20 min.

To produce furan derivatives, a 2nd pretreatment was conducted under various conditions (reaction temperature: 180-230°C, acid concentration: 2-4%, reaction time: 10 min). Reaction temperature had a great influence on the production of furan derivatives than acid concentration. The highest yield of furan derivatives was 7.66% under optimal conditions (reaction time: 220°C, acid concentration: 2%, reaction time: 10 min).

Finally, nanofiltration (NF) and solvent extraction were used to separate the reaction products. NE90 filters provided better separation than DRM filters. With both types of filters, glucose and xylose were selectively removed at pH 4 due to the Donnan effect. However, solvent extraction was found to be more selective for furan derivatives than NF. Chloroform was the best extractant producing a yield of furan derivatives of 5.97%, consistent with a recovery rate of 77.26%. It was assumed that chloroform is more able

than the other organic solvents tested (butanol, ethyl acetate, and propyl acetate) to form hydrogen bonds with furan derivatives.

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초 록

신갈나무의 옥살산 전처리를 통한 고수율의 퓨란계 화합물 생산 및 정제

류가희

환경재료과학전공

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본 연구에서는 5-hydroxtmethylfurfural, furfural, 5-methylfurfural과 같은 퓨란계 화합물 생산을 위하여 신갈나무의 2단계 옥살산 전처리를 실시하고 산물들을 분리하였다.

1차 전처리에서는 5탄당 수율에 대한 반응조건들의 영향을 평가하고, 5탄당 생산을 위한 최적 조건을 확인하기 위하여 반응표면분석법을 수행하였다. 그 결과, 반응온도, 산 농도, 반응시간 순으로 5탄당 수율에 영향을 미치는 것으로 구명되었으며, 최적 조건(반응온도 147°C, 산 농도 2.29%, 반응시간 20분)에서 전건 시료 대비 14.36%의 5탄당(초기 시료 5탄당 대비 81.54%)을 얻을 수 있었다.

1차 전처리로부터 생성된 액상 가수분해물을 이용하여 2차 전처리를 실시하였으며, 퓨란계 생산에 대한 처리인자의 영향을 평가하고 최적조건을 탐색하기 위하여 다양한 조건(반응온도:

180~230°C, 산 농도: 2~4%, 반응시간: 10분) 실험을 진행하였다. 최대로 생산된 퓨란계 화합물은 전건시료대비 7.66%로 220°C, 산 농도 2%, 10분 조건에서 얻어졌으며, 반응온도에 의한 영향이 가장 큰 것으로 조사되었다.

마지막으로, 2단계 전처리 후 생성된 액상 가수분해물에 함유된 퓨란계 화합물을 분리하기 위해서, 나노필트레이션과 용매추출이 다양한 조건에서 실시되었다(나노필트레이션: 필터 종류(NE90과 DRM), pH, 반복 여과/ 용매추출: 용매 종류(chloroform, butanol, ethyl acetate, propyl acetate), 추출 시간, 액상 가수분해물과 추출 용매의 부피비, 추출횟수). 그 결과, 용매추출이 퓨란계 화합물을 분리하는 데 있어 나노필트레이션보다 우수한 선택성을 나타냈다. 특히, 클로로폼은 전건시료대비 5.97%의 퓨란계 화합물을 추출하였으며, 이는 회수율 77.26%를 나타냈다.

주요어: 신갈나무, 2단계 전처리, 옥살산, 퓨란계 화합물, 나노필트레이션, 용매추출

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A THESIS FOR THE DEGREE OF MASTER SCIENCE

High yield production and separation of furan derivatives from *Quercus mongolica* by oxalic acid pretreatment

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Advisor Professor : In-Gyu Choi

By Ga-Hee Ryu

PROGRAM IN ENVIROMENTAL MATERIALS SCIENCE
DEPARTMENT OF FOREST SCIENCES
GRADUATE SCHOOL
SEOUL NATIONAL UNIVERSITY

FEBRUARY, 2016

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이 논문을 농학석사학위 논문으로 제출함

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산림과학부 환경재료과학전공
류 가 희

류가희의 석사학위 논문을 인준함

2016 년 1 월

위 원 장 윤 혜 정 (인)
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위 원 여 환 명 (인)

Abstract

High yield production and separation of furan derivatives from *Quercus mongolica* by oxalic acid pretreatment

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In this study, a two-step pretreatment and separation process were carried out for the production of furan derivatives such as 5-hydroxymethlyfurfural (5-HMF), furfural, and 5-methylfurfural (5-MF). Aqueous oxalic acid was used as the solvent in both the 1st and 2nd pretreatments.

Response surface methodology (RSM) was performed to evaluate the effects of variables (X_1: reaction temperature, X_2: acid concentration, and X_3: reaction time) on the pentose yield and to define the optimal conditions for the highest pentose yield during the 1st pretreatment in this study range. The result of RSM analysis shewed that reaction temperature was the most dominant factor, followed by acid concentration and reaction time. The optimal conditions for the maximum pentose yield were a reaction temperature of 147℃, an acid concentration of 2.29% (w/w), and a reaction time of 20 min. Under these conditions, the pentose yield was 14.36% based on the dry weight of the raw material, corresponding to an extraction rate of 81.54% based on the initial weight of pentose in the material.

The liquid hydrolyzate obtained from the 1st pretreatment was used in 2nd pretreatment to produce furan derivatives. The 2nd pretreatment was carried out under various conditions (reaction temperature: 180-230℃, acid concentration: 2-4%, and reaction time: 10 min) to determine the optimal conditions for high yield of furan derivatives and to evaluate the effects of the reaction conditions on the yield of furan derivatives after the 2nd pretreatment. The maximum yield of furan derivatives was 7.66% based on the dry weight of the raw material after pretreatment at 220℃ with 2% (w/w) oxalic acid for 10 min. The factor that most influenced the yield of furan derivatives was reaction temperature.

To separate furan derivatives from other compounds in the liquid hydrolyzate after two-step pretreatment under optimal conditions, nanofiltration (NF) and solvent extraction were conducted under various operating conditions (NF: filter type (NE90 and DRM), pH, repetition stage) (Solvent extraction: organic solvent (chloroform, butanol, ethyl acetate, propyl acetate), contact time, the hydrolyzate/solvent volume ratio, the number of extraction stage). Solvent extraction showed better efficiency for the separation of furan derivatives than NF. The best yield was obtained with chloroform-extracted furan derivatives (5.97% based on the dry weight of the raw material, corresponding to the recovery rate of 77.26%).

Key words: *Quercus mongolica*, two-step pretreatment, oxalic acid, furan derivatives, nanofiltration, solvent extraction

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1 Introduction

Since the rapid development through industrial revolution, the energy consumption has been dramatically increased and fossil fuel has become the majority of energy for several years (Ingram & Doran, 1995). However, the use of fossil fuel has produced greenhouse gas such as carbon dioxide, and also caused serious environment problems, for example, global warming, air pollution and acid rain (Saxena et al., 2009). In addition, our excessive overdependence on fossil source could lead to social problems related to price stability of transportation fuel and commodity chemicals because significant portion of materials and chemicals now mostly derived from petroleum. For these reasons, many countries have interested in exploring eco-friendly alternative resource in order to observe various policies and regulations established to reduce CO_2 emission from fossil oil and secure national competitiveness in the international energy market (Cherubini, 2010).

1.1 Lignocellulosic biomass as potential resource

Lignocellulosic biomass, which refers to plant biomass such as grasses, wood and agricultural residue, has currently gained much attention as a promising alternative resource to replace fossil fuel (Kumar et al., 2009). It is renewable, inexpensive and the most abundantly available biopolymer in nature (Behera et al., 2014). According to the research in U.S., the annual available quantities of biomass will be increased from about 119 million dry tons currently to about 129 million dry tons in 2030 (Zhang et al., 2013). Also, it could be regarded as environmental material resulting CO_2 savings due to its property of carbon fixation and dose not compete with food resource. For these reasons, there has been much research to explore and develop new technology using lignocellulose biomass as raw material in order to produce

energy, bio-based chemicals and bio-fuels (Klass, 1998).

Lignocellulosic biomass is mainly composed of three main components, cellulose (35-50%), hemicellulose (25-30%) and lignin (25-30%). Cellulose, the most abundant natural polymer on the earth, is homogenous polysaccharides consisting of the β-1,4 linked linear glucose polymer. It has crystalline structure and higher degree of polymerization than hemicellulose. While Hemicellulose is heterogeneous polysaccharides including hexose (glucose, mannose, and galactose) and pentose (xylose and arabinose). Comparison with cellulose, it is decomposed at lower temperature because of its branched composition (Himmel, 2009; Klass, 1998). Both of two major components can be depolymerized into monosaccharides which are used as source to produce biofuels like bioethanol and sugar degradation products such as furfural, 5-HMF and levulinic acid which can be a promising sustainable intermediate for bio-based feedstock of fine chemical (Yan, 2014; Girisuta, 2006). Lastly, Lignin, complex phenylpropanoid units, is consist of three monomeric precursors, coniferyl alcohol, sinapyl alcohol and coumaryl alcohol, biosynthesized in biomass via the shikimic acid pathway. Although, the exact structure of lignin does not known yet, it is presently regarded as potential aromatic building block in various industries such as fuels, resins and pharmaceuticals (Fan et al., 2014; Smolarski, 2012).

All three main components of lignocellulosic biomass are complexly connected to each other. It could be one of the reasons for biomass recalcitrance, known as natural defending system to protect itself from chemicals and microorganism attacks resulting in its decomposition. (Himmel, 2009; Wayman & Parekh, 1990; Sjostrom, 1993). Therefore, it is essential to take pretreatment process to overcome recalcitrance of lignocellulosic biomass for effective utilization in biorefinery industry.

1.2 Pretreatment to overcome recalcitrance

Pretreatment is one of the essential processes for total utilization of lignocellulosic biomass. Because pretreatment process has a strong influence on final product yield and relevance to efficiency of overall process.

The purpose of pretreatment is: (1) breaking down complex structure of lignocellulosic biomass in order to overcome biomass recalcitrance which is self-defense property against to microorganism and chemicals attacking its structure (2) disrupting crystallinity of cellulose for high sugar yield (3) removing lignin and hemicellulose (4) increasing the porosity of the material, so that microorganism can easily access to the surface of cellulose in the process including the microbial process, for example bioethanol process. (Sun & Cheng, 2002)

Pretreatment can be commonly classified in physical pretreatment, physico-chemical pretreatment, chemical pretreatment and biological pretreatment. First, physical pretreatment is mechanical method such as chipping, grinding and milling for size reduction. The common range of the size after chipping and milling or grinding is 10-30 mm and 0.2-2 mm, respectively (Sun & Cheng, 2002). While Physical pretreatment has high energy consumption, it is the important method to extend surface area which can react with chemical or microorganism and also to reduce cellulose crystallinity (Silverstein, 2005). Second, physico-chemical pretreatment is the combination of physical and chemical pretreatment as its name imparts. The common method is steam explosion which degrades hemicellulose and lignin with high temperature. In this method, the material is chipped firstly and is treated with high pressure for a while, then the pressure reduced quickly so that the material would be exploded by an explosive decompression (McMillan, 1994). Third, chemical pretreatment including acid, alkaline and organosolv process has considered as one of the most promising methods to

adapt in industry. Dilute acid pretreatment is more effective to degrade hemicellulose than lignin, so it can produce high yield of xylose with less severe condition in most lignocellulosic biomass (Himmel et al., 1997). However, it produces toxic products and needs expensive equipment against corrosion (Sun & Cheng, 2002). In addition, a neutralization process is necessary for downstream of fermentation process in bioethanol production. Alkaline pretreatment is believed that lignin is eliminated with the removal of the ester bonds crosslinking between carbohydrate and lignin. Dilute NaOH is used as common chemical in alkaline pretreatment. It makes lignocellulosic material swelling, as its result, a decrease in crystallinity (Tarkow & Feist, 1969; Fang et al., 1987). Organosolv pretreatment is currently evaluated as chemical method and initially developed for pulp production from wood (Pan et al., 2007). In this process, a mixture organic solvent with inorganic acid catalyst hydrolyzes lignin-lignin and lignin-carbohydrate bond (Holtzapple & Humphrey, 1984). Methanol, ethanol, acetone, ethylene glycol, triethylene glycol and tetrahydrofurfuryl alcohol are used as organic solvent and sulfuric acid and hydrochloric acid are commonly used as inorganic acid catalyst (Chum et al., 1988). Lastly, biological pretreatment is the pretreatment using microorganism such as brown rot and white rot for degradation of lignin and hemicellulose (GHOSE, 1978). Brown rots mainly attack cellulose, while white rots attack both cellulose and lignin. Although biological pretreatment can operate in moderate condition, it is difficult to be adopted for industry process because the price of microorganism is expensive and operating cost is high.

To optimize the pretreatment for the target product, it is important to evaluate the effect of pretreatment parameter on the yield of target product. In the chemical pretreatment, reaction time, particle size of material, reaction temperature, solid/liquid ratio and catalyst loading are mainly considered as key factor.

Therefore, there are various pretreatment methods and many parameters, it is necessary to choose suitable pretreatment method and parameters depending on the target products. Also, further research on developing pretreatment process will be need to meet requirements which are high efficient without loss of carbohydrate, cost effective and environment friendly.

1.3 The concept of biorefinery

The concept of biorefinery comes from today's petroleum refinery system. It represents currently an integrated facility that produces energy, transportation fuel, bio–based chemicals and materials from biomass at the same time as shown Fig. 1 (González-Delgado & Kafarov, 2011). By producing multiple products, biorefinery can take advantage of the diversities in biomass components and maximize the value derived from the biomass feedstock while also being able to adapt to changing market conditions.

Biorefinery has progressed through 3 steps. At the first time, it started with phase 1 biorefinery which is a dry-milling plant using corn grain as raw material for bioethanol production. However, phase 1 biorefinery is difficult to meet the changing market condition. So, phase 2 biorefinery developed to overcome the disadvantage of phase 1 and it is a wet-milling plant which can produce various products such as energy, biofuel and bio-based chemicals using corn grain as raw material. Even if phase 2 biorefinery has more flexibility compared to phase 1 biorefinery in final products, it still has the problem of using the corn grain which is food resource. Finally, phase 3 biorefinery has been designed as integrated plant to produce multiple products using lignocellulosic biomass. However, phase 3 biorefinery or more advanced biorefinery is also technically insufficient to produce energy, bio-based chemical and materials. Thus, much effort will be required to develop

the technology which is sustainable, environmentally, resource-friendly and cost effective in biorefinery industry for the future (Kamm et al., 2007; Himmel, 2009).

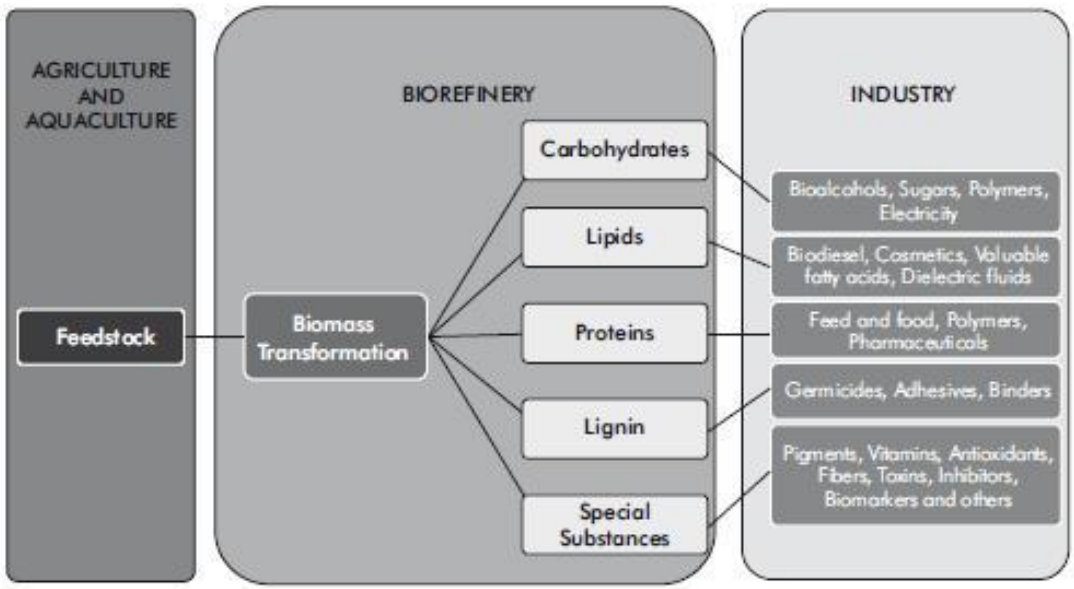


Figure 1. The general scheme of biorefinery concept (González-Delgado & Kafarov, 2011).

1.4 Objectives

The comprehensive purposes of this study are to investigate the optimal condition of furan derivatives production by two-step pretreatment with oxalic acid as catalyst and to evaluate separation methods including nanofiltration and solvent extraction. Each separation process also will be operated at various condition to identify the optimal condition for furan derivatives separation.

Specific purposes of each process are as follows:

Two-step pretreatment

- ✓ Determining the optimal condition of two-step pretreatment with oxalic acid for furan derivatives, especially 5-HMF, furfural, and 5-MF which are major component of furan derivatives from lignocellulosic biomass.

Nanofiltration process

- ✓ Investigating possibility of furan derivatives separation by nanofiltration and solvent extraction with focus on the effects of operating conditions

Standard experiment

- ✓ Conforming the effect of other components on product and separate process

2 Literature reviews

2.1 Studies on pretreatment of lignocellulosic biomass

2.1.1 Oxalic acid pretreatment

Oxalic acid pretreatment, initially designed through the fact that brown-rots secrete oxalic acid to degrade wood fiber and lignin through hydrolysis, was suggested as an alternative pretreatment to sulfuric acid pretreatment. Because it cause less corrosion to reactor than sulfuric acid, and is also more acidic than other organic acids such as formic, acetic, maleic acid due to its dicarboxylic property with two pK_as. Oxalic acid attacks the cell wall structure and leads to the hemicellulose hydrolysis (Kim et al., 2011). Earlier study reported that both of dilute sulfuric acid and oxalic acid achieved about 85% of xylose yield, which means oxalic acid has similar efficiency to sulfuric acid (Zhang et al., 2013). Furthermore this study shows the possibility of using oxalic acid, which is expensive than sulfuric acid on a base weight, as industry catalyst by adding recovery system (Lee et al., 2013).

2.1.2 Two-step acid pretreatment

Two-step pretreatment was suggested for high yield of sugar in the literature several times. Because it can recover higher sugar yield than one-step pretreatment. Nguyen et al reported the research which shows that maximum hydrolysis rate of glucose and mannose is not obtained at the same pretreatment severity. Glucan demands pretreatment of higher severity than mannan to be completely hydrolyzed. This suggests two-step pretreatment, with the first step performed at low severity to hydrolyze the hemicellulose

and the second step, where the solid material from the first step is pretreated again, at higher severity (Nguyen et al., 2000). Also Söderström et al reported the two-step steam pretreatment process with dilute H_2SO_4 impregnation shows attractive advantages, such as high ethanol yield, better utilization of the raw material and lower consumption of enzymes (Söderström et al., 2003).

2.2 Furan derivatives production from lignocellulosic biomass

Lignocellulosic biomass produces furan derivatives such as furfural and 5-HMF as major furan component in the acid pretreatment. They can be a promising component to replace chemicals derived from fossil fuel. The detailed information are as followed.

2.2.1 Furfural production

Hemicellulose, the second most abundant polysaccharide on earth, can produce degradation products such as furfural, 5-HMF, levulinic acid through acid pretreatment (Gallezot, 2012). In the past, the degradation products have been considered as inhibitor which cause the low yield of bioethanol by controlling the growth of the microorganism in fermentation process (Palmqvist et al., 1999; Klinke et al., 2003). However, they were recently identified as feasible alternative resources for bio-based chemicals with adaption of the biorefinery concept. Especially, furfural, selected as one of the most promising chemicals in 21st century proposed by Bozell et al, has regained attention as a biorefinery-based feedstock for future chemicals (Bozell & Petersen, 2010).

Furfural is produced through dehydration of C5 sugars such as xylose and arabinose as shown in Fig. 2. And it can offer a whole new class of chemicals of the furan family through further reaction such as dehydration, hydrogenation, oxidation, condensation, open-ring, and decarbonyl as described in Fig. 3. Therefore, furfural is the key building block for both chemical and fuel industries. It can replace the diminishing fossil-based organics for the production of resins, lubricants, adhesives, and plastics. It is also widely used to produce value-added chemicals, such as furfuryl alcohol, tetrahydrofurfuryl alcohol, and furanoic acid (Gallezot, 2012; Yan et al., 2014).

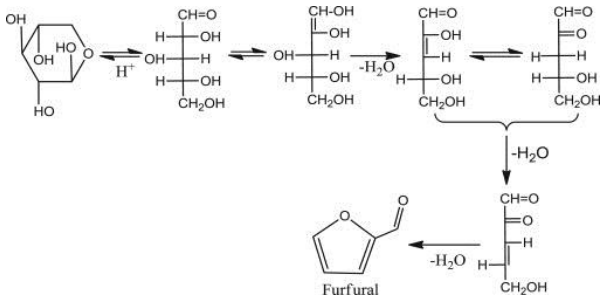


Figure 2. Route of furfural production from C5 sugar (Yan et al., 2014).

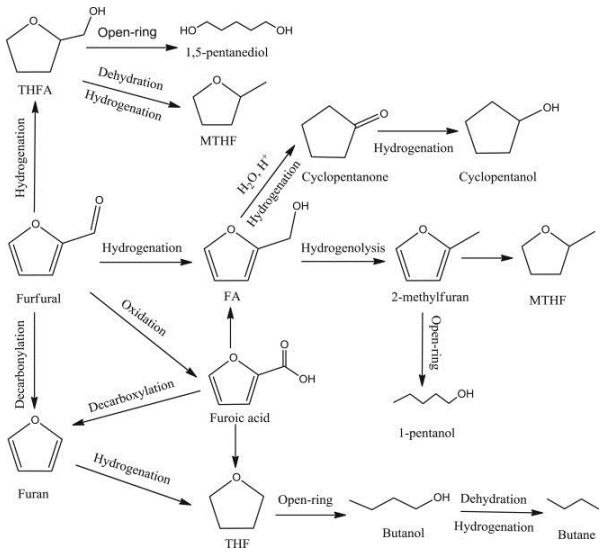


Figure 3. Further reactions from furfural to various value-added chemicals and biofuels (Yan et al., 2014).

2.2.2 5-HMF and other furan derivatives production

5-HMF was obtained by dehydration of fructose in the presence of soluble or solid acid catalysts or from glucose or even polysaccharides by more complex catalytic systems and reaction media shown in Fig. 4.

5-HMF and its derivatives; levulinic acid, 2,5-diformylfuran (2,5-DFF) and 2,5-furandicarboxylic acid (2,5-FDCA), 5-hydroxymethylfuranoic acid, and 2,5-furandicarboxaldehyde were obtained by the catalytic conversion of carbohydrates based on C6 units shown in Fig. 4. And they were identified early as very promising chemical intermediates which could replace other petrochemical-based monomers. For example, 2,5-furandicarboxylic acid is able to replace terephthalic, isophthalic, and adipic acids in the manufacture of polyamides, polyesters, and polyurethanes. Therefore, preparation of 5-HMF with economically acceptable processes will be the key issue in biorefinery industry (Corma et al., 2007, Gallezot, 2012).

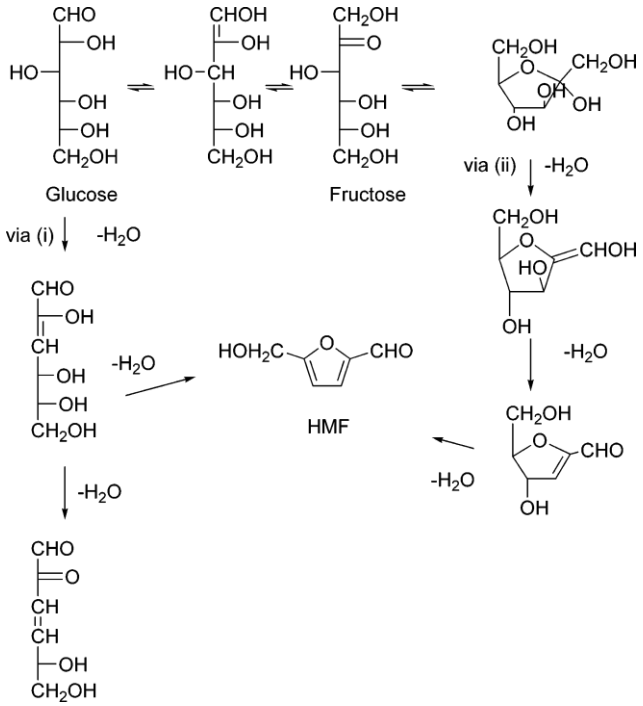


Figure 4. Pathway of 5-HMF formation from C5 and C6 carbohydrate (Corma et al., 2007).

2.3 Separation process of furan derivatives

2.3.1 Nanofiltration

Membrane filtration is one of the physical purification methods to separate by passing liquid which includes the target compound through a special pore sized membrane. It is simply classified into several types by pore size or molecular weight cut off. And filtration was controlled by operation parameters such as pressure, temperature, pH, and concentration of feed.

Nanofiltration is a promising and cost-competitive membrane separation technology. It has a molecular weight cut-off ranging from 150 to 1000 g/mol, enabling high retention of compounds with molecular weight up to 150 to 250 g/mol as well as charged molecules. Thus, nanofiltration has a wide range of applications in fermentation broth separation, sugar fractionation, sugar concentration in biorefinery process (Weng et al., 2009). Liu et al. (2008) applied NF membrane with a molecular weight cut-off of 100 g/mol for concentration and purification of hydrolyzates from hot-water extraction of woody biomass and found that sugars in the hydrolyzates could be cleaned and concentrated by using NF technology. Sjöman et al. (2008) reported purification of xylose in different hemicellulose hydrolyzates with three NF membranes, while recent work by Weng et al. (2010) on the concentration of rice straw hydrolyzates obtained from dilute acid pretreatment by NF also confirmed that NF technology can effectively concentrate sugars in the biomass hydrolyzates.

2.3.2 Solvent extraction

Solvent extraction, named as liquid – liquid extraction, is the one of

the separation methods based on the different distribution of the components to be separated between two liquid phases. It depends on the mass transfer of the component to be extracted from the first liquid phase to the second one (Müller et al., 2000).

As the differing chemical nature of the species, the selection of suitable extractant is the key point for successful separation by solvent extraction. There are several requirements to fulfill to recovery target compounds from aqueous acid hydrolysis stream. First, extractant must have high selectivity to the target compound against to other compounds and need to be chemically stable. Second, it has to be easily regenerate for re-use to increase the efficiency of process. In some cases, extractant which has low boiling point is good to reduce energy consumption in distillation process. Third, it is important to have a large difference in density between extractant and raffinate phase for rapid separation (Vincent Van, 2004).

Several researches have considered the recovery of furfural using solvent extraction. For example, Bruno F. et al. (2012) performed experimental solvent extraction with the standard solution composed water and furfural. Ethyl acetate, propyl acetate, and 1-butanol were prepared as extractant. According to the result of this study, propyl acetate presents better technical characteristics for furfural removal from water (Demesa et al., 2015). In addition, two solvents for the recovery of furfural from aqueous solution was compared. 2-methyltetrahydrofuran (2-MTHF) and tri-n-octylamine in toluene (Alamine 336-toluen), were evaluated. And it turned out that the extraction of furfural was better when using 2-MTHF (Almeida et al., 2012).

Also there are several researches on solvent extraction method related with the recovery of 5-HMF from liquid hydrolyzate. It is not easy to extract from aqueous phase, since the distribution coefficient between the organic and the aqueous phase is not favourable. However, this problem has been overcome by the use of organic solvents such as MIBK (methyl isobutyl

ketone), DCM (dichloromethane), ethyl acetate, THF (tetrahydrofuran), diethyl ether, and acetone, which have been reported to be efficient extraction solvents (Rosatella et al., 2011).

3 Materials and methods

3.1 Materials

Thirty year-old *Quercus mongolica* was supplied by the arboretum of Seoul National University (Anyang, South Korea) and used as raw material in this study. The raw material was milled and reduced to a particle size below 0.5mm (Cutting Mill pulverisette 15, FRITSCH, GERMANY). Then, the samples were air-dried and stored in plastic bags. The moisture content was less than 10% before use. The composition of raw material was determined by NREL method and the results were represented as the yield of components based on dry weight of raw material. Standard materials (glucose, arabinose, and xylose) were purchased from Sigma-Aldrich Co. (Yongin, South Korea).

3.2 Two-step pretreatment

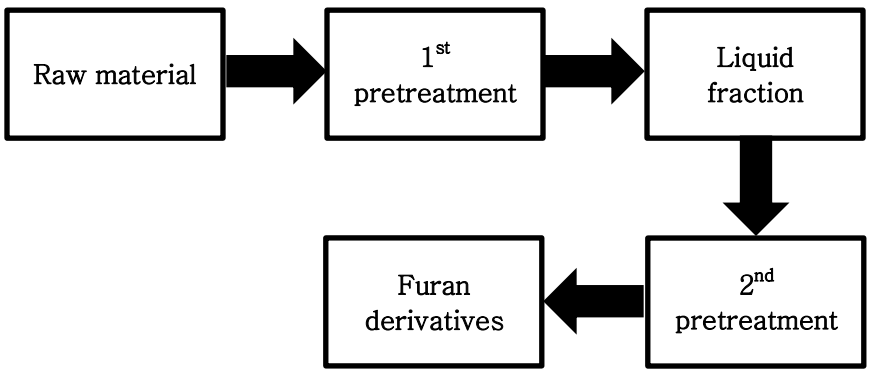


Figure 5. Scheme of two-step pretreatment for furan derivatives production.

Two-step pretreatment was carried out for production of furan derivatives and the whole process was shown in Fig. 5. 1st pretreatment was conducted to release pentose in liquid hydrolyzate and 2nd pretreatment was carried out to produce furan derivatives such as 5-HMF, furfural, and 5-MF.

3.2.1 1st pretreatment

1st pretreatment was performed using a reactor consisted of a 1L volted closure stainless steel reaction vessel (SUS 316), an electric heating mantle, a thermocouple, a pressure gauge, a paddle type impeller, and a control box (HR-8300, Hanwol Engineering Inc.) (Fig. 6). The thermocouple and Teflon impeller were inserted in the reactor to measure the internal temperature and to stir samples evenly, respectively. The temperature of the electric heating mantle and the speed rate of the impeller were controlled by control box.

The milled *Quercus mongolica* and an aqueous oxalic acid were mixed with solid to liquid ratio at 1:7 in the reaction vessel and heated at various reaction conditions following 2^3 factorial design. The pre-heating time was set 50 min to reach the target temperature (not including reaction time). As soon as reaction time was over, the reaction vessel was cooled during 15 min. The pretreated materials were filtered using filter paper (No.2 Adventec, Kyoto, Japan) to divide into solid residue and liquid hydrolyzate fractions. Then liquid hydrolyzate was stored in the glass bottle at 4°C for further use and HPLC (high performance liquid chromatography) analysis.

3.2.1.1 Experimental design and statistical analysis

The statistical approach was adapted to evaluate the effect of pretreatment conditions and to search for the optimal condition of pentose (xylose and arabinose) production during oxalic acid pretreatment as 1st pretreatment. The pretreatment conditions such as reaction temperature (X_1, °C), acid concentration (X_2, %), and reaction time (X_3, min) were selected as the independent variables which can directly influence pentose yield. Each independent variable had different range and was coded in three levels. While, pentose yield based on a dry weight of raw material (%) in liquid fraction after 1st pretreatment was adapted as the dependent variable. To optimize the combination of pretreatment conditions, 17 experimental operations based on 2^3 factorial design, listed in Table 1, were carried out including triplication at the center point (X_1: 140°C, X_2: 2%, X_3: 20min). The statistical approach was performed using Design Expert 8.0.1 software. ANOVA (Analysis of variance) and 3D response surface plots were generated as results for statistical data analysis.

3.2.1.2 Determination of pentose yield

To confirm the optimal condition suggested by RSM (response surface methodology) for the highest pentose yield in the range of this study, 1st pretreatment for determination of pentose yield was conducted as the same way, previously described in 3.2.1 1st pretreatment.

Figure 6. Shape of reactor used for oxalic acid pretreatment.

Table 1. Coding of the condition of experiments based on 2^3 factorial design

Run	Coded level			Variables		
	Reaction temperature X_1	Acid concentration X_2	Reaction time X_3	Reaction temperature (℃)	Acid concentration (%, (w/w))	Reaction time (min)
1	-1	-1	-1	130	1	10
2	1	-1	-1	150	1	10
3	-1	1	-1	130	3	10
4	1	1	-1	150	3	10
5	-1	-1	1	130	1	30
6	1	-1	1	150	1	30
7	-1	1	1	130	3	30
8	1	1	0	150	3	30
9	-1.68	0	0	123.2	2	20
10	1.68	0	0	156.8	2	20
11	0	-1.68	0	140	0.32	20
12	0	1.68	0	140	3.68	20
13	0	0	-1.68	140	2	3
14	0	0	1.68	140	2	37
15	0	0	0	140	2	20
16	0	0	0	140	2	20
17	0	0	0	140	2	20

3.2.2 2nd pretreatment

2nd pretreatment for furan derivatives production was carried out with the same reactor and equipment as 1st step pretreatment. To evaluate the change aspect of composition of liquid hydrolyzate depending on the pretreatment conditions and to investigate the optimal condition of furan derivatives production in the range of pretreatment conditions in this study, the separated liquid hydrolyzate from 1st pretreatment was used and heated at various conditions as shown Table 2. To make 2%, 3%, 4% oxalic acid hydrolyzate, more oxalic acid was added into the liquid hydrolyzate from 1st pretreatment. The pre-heating step and cooling method were also same as 1st pretreatment. Then, liquid hydrolyzate generated from 2nd step pretreatment was stored to use for separation process and HPLC analysis. The results were defined as the component yield based on dry weight of raw material. In addition, the pretreatment with standard materials (glucose, xylose, and arabinose) was conducted at the optimal condition of 2nd pretreatment in order to compare the composition of degradation products and to understand the conversion behavior of furan derivatives.

Table 2. The conditions of 2nd pretreatment

Acid concentration	Reaction temperature	Reaction time
2%	180°C, 190°C, 200°C, 210°C, 220°C, 230°C	10 min
3%		
4%		

3.3 Separation process for furan derivatives

3.3.1 Nanofiltration

Nanofiltration was conducted using Amicon cell (Millipore Amicon stirred cell 8400) with gas pressure, pressure control valve and stirrer located in the cell. Two commercially available NF membranes, NE90 and DRM, were used as filter in this study. They were purchased from Toray and their properties were summarized in Table 3.

To separate furan derivatives from the other compounds, About 40 mL hydrolyzates from two-step pretreatment were injected into the cell and then pumped into the filtration cell at 60 bar with nitrogen gas. After filtration, the collected permeate and corresponding retentate in the cell filter were analyzed for the concentration of sugars and degradation products, especially furan derivatives. The effects of filter type (NE90 and DRM), feed pH (1, 4, 7, and 10), and repetition of filtration on the performance of the NF process were studied to investigate the optimal condition for furan derivatives separation.

3.3.2 Solvent extraction

Solvent extraction was conducted to separate furan derivatives from the other components in liquid hydrolyzate after two-step pretreatment. Various organic solvents including chloroform, butanol, ethyl acetate, and propyl acetate were employed in this study and their properties were sumerized in Table 4.

To screen organic solvents for extraction, the liquid hydrolyzate and organic solvents were mixed together in a 250ml baffled Erlenmeyer flask with shaking (270rpm) at room temperature. Solvent extraction time was set

15, 30, 45, and 60 min. After extraction, the solution was left to be a state of equilibrium (organic and aqueous phases) for 15 min and organic phase which separated from aqueous phase is distilled to remove organic solvent with evaporator (N-1110 series). Then, the sample was diluted with 10 ml acetone for analysis of HPLC. After screening, solvent extraction was conducted with the best solvent to evaluate the effect of the hydrolyzate/solvent ratio and coupling of extraction stage.

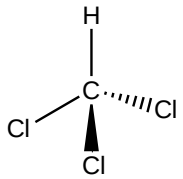
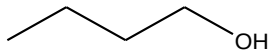
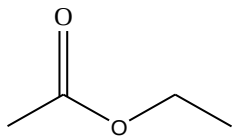
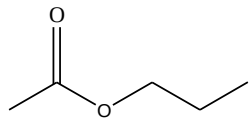
3.3.3 Standard experiment

Standard experiment was conducted at the best separation process selected from the experiments with raw material with nanofiltration or solvent extraction. The model solution was used to identify the effect of other compounds from lignocellulosic biomass on the recovery of furan derivatives in separation process. The model solution was composed of furfural, 5-HMF, and 5-MF and their composition was set as followed by the composition of hydrolyzate after two-step pretreatment under the optimal conditions (1^{st} pretreatment: 147℃, 2.3% oxalic acid, and 20 min and 2^{nd} pretreatment: 220℃, 2% oxalic acid, and 10 min).

Table 3. Characteristic of NF filter used in this study

Filter	**NE90**	**DRM**
Manufacturer	Toray Chemical Korea Inc.	Toray Chemical Korea Inc.
Configuration	Spiral wound	Spiral wound
Filter materials	Polyamide	Polyamide
Molecular weight cutoff (MWCO)	200 Da.	1000 Da.
Max. temperature (℃)	45℃	45℃
Max pressure (bar)	600 psi	600 psi

Table 4. Properties of solvent used in extraction

	Chloroform	Butanol	Ethyl acetate	Propyl acetate
				
Molecular formula	$CHCl_3$	$C_4H_{10}O$	$C_4H_8O_2$	$C_5H_{10}O_2$
Molar mass	119.37 g·mol^{-1}	74.12 g·mol^{-1}	88.11 g·mol^{-1}	102.13 g·mol^{-1}
Appearance	Colorless liquid	Colourless, refractive liquid	Colorless liquid	Clear, colorless liquid
Density	1.489 g/cm^3	0.81 g cm^{-3}	0.902 g/cm³	0.89 g/cm^3
Melting point	−63.5 ℃	−89.8 ℃	−83.6 ℃	−95 ℃
Boling point	61.15 ℃	117.7 ℃	77.1 ℃	102 ℃
Solubility in water	0.809 g/100 mL (20 ℃)	73 g L^{-1}	8.3 g/100 mL	18.9 g/L
Acidity (pKa)	15.7	16.10	25	
Viscosity	0.563 cP	2.573 mPa×s	426 μPa s	
Dielectric constant	4.81	17.5	6.02	

3.4 Analysis of liquid hydrolyzates

3.4.1 Analysis of monomeric sugar content

After 1st step and 2nd step pretreatment, liquid hydrolyzate is filtered by 0.45 μm hydrophilic membrane filter (Adventec Co., Japan) and analyzed their component such as glucose, xylose, galactose, and mannose by Bio-Liquid Chromatography (ICS-2500, Dionex USA) equipped with a CarboPac PA-1 column and Pulsed amprometry (ED40, Gold electrode) as a detector. The mobile phase is potassium hydroxide with 10 μL injection volume with flow1 mL/min flow rate.

3.4.2 Analysis of degradation products

HPLC (Dionex Ultimate 3000, USA) using Aminex 87H column with Refractive index detector (ERC, RefractoMAX520, Japan) is used to determine degradation products such as furan derivatives (furfural, 5-HMF), levulinic acid, formic acid, and acetic acid. Injection volume is 10 μL with 0.01N sulfuric acid and flow rate is 0.5 mL/min at 40°C for 90 min.

4 Results and discussions

4.1 Composition of raw material

The chemical composition of *Quercus mongolica* was determined by NREL method (Sluiter et al., 2008). The raw material was consisted of 58.62% carbohydrates (39.98% glucan, 14.11% xylan, 1.38% arabinan, 1.73% galactan, 1.42% mannan,), 27.62% Klason lignin, 2.3% extract and about 1% ash. The pentose (xylose+arabionose) yield produced in liquid hydrolyzate was 17.61%.

4.2 Pentose production of 1st pretreatment

4.2.1 Analysis of sugar component in liquid hydrolyzate

RSM was performed to see the effect of variables (X_1: reaction temperature, X_2: acid concentration, and X_3: reaction time) on the pentose yield and to search the optimal condition for the highest pentose yield during oxalic acid pretreatment in this study range.

To apply for RSM, 17 experiments based on 2^3 factorial design were carried out with triplication at central point (X_1: 140℃, X_2: 2%, X_3: 20 min). Table 5 shows the composition of sugar contents and pentose in liquid hydrolyzate after 1st oxalic acid pretreatment. Run #16, one of the central point, showed the highest arabinose extraction (1.10%) and run #13 (X_1: 140℃, X_2: 2%, X_3: 3 min) represented the highest xylose extraction (13.44%), simultaneously the highest pentose extraction (14.47%) in this study region. While, run #1 (X1: 130℃, X2: 1%, X3: 10 min) had the least arabinose and xylose solubility (0.73% and 1.57%). This phenomena were considered that

low severity could not fully depolymerize hemicellulose into monomeric sugar. Also, xylose yield was mostly higher than other sugar contents in all samples. And glucose yield was relatively low even glucose composition was more than xylose composition in the raw material. This results indicated that hemicellulose was selectively degraded by acid pretreatment, and it was accorded with previous study (Shin et al., 2015).

Table 5. Analysis of sugar components in liquid hydrolyzate and pentose yield as dependent factor after 1st pretreatment

Run No.	Composition of the components in liquid hydrolyzate					Dependent factor
	Glucose yield (%[a])	Galactose yield (%[a])	Mannose yield (%[a])	Arabinose yield (%[a])	Xylose yield (%[a])	Pentose yield (%[a])
	A	B	C	D	E	Y (D+E)
1	0.52	0.10	0.08	0.73	1.57	2.30
2	1.28	0.65	0.53	0.85	9.60	10.44
3	1.23	0.30	0.28	0.93	7.16	8.09
4	1.44	1.89	0.88	0.96	12.13	13.09
5	1.19	0.31	0.29	0.85	6.58	7.43
6	1.52	1.24	0.75	0.99	13.28	14.27
7	1.43	0.56	0.55	0.91	10.25	11.17
8	1.41	1.40	0.77	0.97	11.59	12.56
9	0.87	0.19	0.17	0.89	4.21	5.10
10	1.64	2.74	0.98	1.02	12.71	13.73
11	0.78	0.16	0.13	0.79	3.02	3.81
12	1.47	1.05	0.76	0.93	12.04	12.98
13	1.55	1.20	0.84	1.04	13.44	14.47
14	1.40	0.45	0.45	0.98	8.91	9.89
15	1.58	0.81	0.80	1.02	12.36	13.38
16	1.69	0.93	0.81	1.10	12.77	13.87
17	1.60	0.85	0.77	1.06	12.80	13.85

[a] based on dry weight of raw material

4.2.2 ANOVA table

Table 6 shows ANOVA (analysis of variance) results representing statistical values. Coefficient estimate was used to establish Eq. (1) shown as below, and Eq. (1) was employed to create the model for the maximum pentose yield by 1st oxalic acid pretreatment. The P-value of the model was lower than 0.05, which indicated that the model was statistically significant within a 95% confidence interval.

$$Y = 13.69 + 2.63 \times X_1 + 1.89 \times X_2 + 0.28 \times X_3 - 1.07 \times X_1 \times X_2 - 0.61 \times X_1 \times X_3 - 0.80 \times X_2 \times X_3 - 1.48 \times X_1^2 - 1.84 \times X_2^2 - 0.51 \times X_3^2 \quad (1)$$

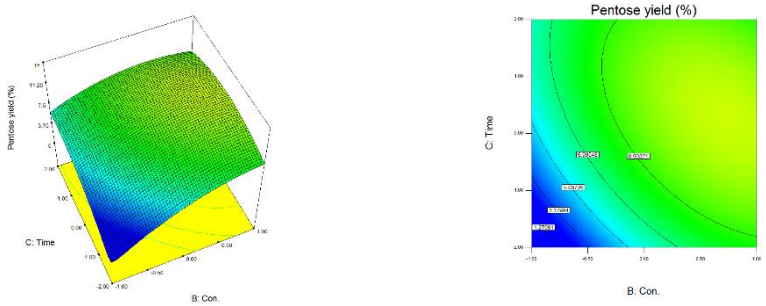
Independent factors (reaction temperature, acid concentration, and reaction time) as shown in ANOVA table were significantly related with pentose yield in pretreatment process. In terms of the influence of single factor on pentose extraction, if the factor has low p-value, it means that the factor has more influence on the pentose yield. Thus, reaction temperature (0.0030) was the most dominant factor, followed by acid concentration (0.0151) and reaction time (0.6538). While, In case of interaction factor, reaction temperature-acid concentration (0.2074) was the most influent factor, followed by acid concentration-reaction time (0.3344), and reaction temperature-reaction time (0.4542). The previous study for optimization of monosaccharides from yellow poplar by oxalic acid using RSM had also similar pattern in xylose extraction (Kim et al., 2011).

Table 6. ANOVA of pentose yield in the liquid hydrolyzate from *Quercus mongolica* after 1st oxalic acid pretreatment

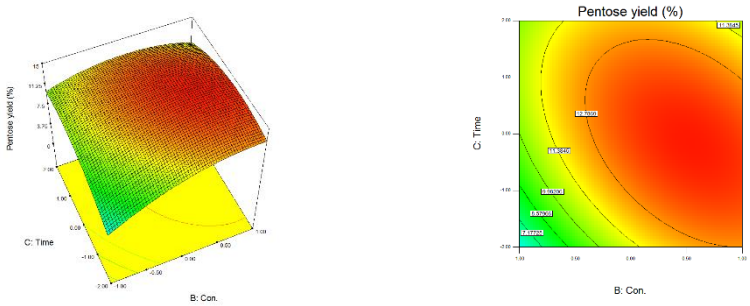
Source	Coefficient estimate	Sum of squares	DF	Mean square	F-value	P-value
Model	13.69	211.79	9	23.53	4.91	0.0238
X_1	2.63	94.28	1	94.28	19.68	0.0030
X_2	1.89	49.01	1	49.01	10.23	0.0151
X_3	0.28	1.05	1	1.05	0.22	0.6538
$X_1 X_2$	-1.07	9.24	1	9.24	1.93	0.2074
$X_1 X_3$	-0.61	3.01	1	3.01	0.63	0.4542
$X_2 X_3$	-0.80	5.15	1	5.15	1.07	0.3344
X_1^2	-1.48	24.81	1	24.81	5.18	0.0570
X_2^2	-1.84	38.31	1	38.31	8.00	0.0255
X_3^2	-0.51	2.88	1	2.88	0.60	0.4638
Residual		33.53	7	4.79		
Lack of Fit		33.37	5	6.67	84.96	0.0117
Pure Error		0.16	2	0.08		
Cor Total		245.32	16			

4.2.3 3D plots and contours representing pentose yield by the change of factors

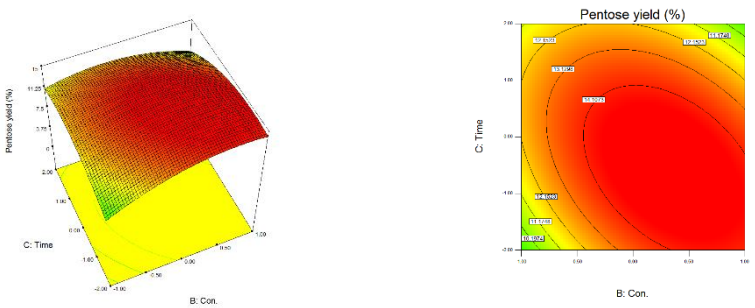
To evaluate the effects on pentose yield in accordance with the change of factors, RSM based on 17 experiments provided 3D plots and contours according to each independent factor (reaction temperature, acid concentration, and reaction time) on pentose yield from *Quercus mongolica* after 1st oxalic acid pretreatment. Fig. 7 shows that the dark region becomes wider, in short, pentose yield rose with an increase of reaction temperature when reaction time and acid concentration was at zero coded level. On the other hand, in case of acid concentration, pentose yield was slightly decreased at 3% acid concentration (Fig. 8). It was considered that pentose was converted into degradation products such as furfural, 5-HMF, formic acid and acetic acid and etc. over the proper severity (Gwak et al., 2012; Shin et al., 2015). Lastly, the reaction time was found to the similar results with in the acid concentration, and the reason was regarded to the same reason as mentioned in the case of acid concentration (Fig. 9).



(A) 130℃

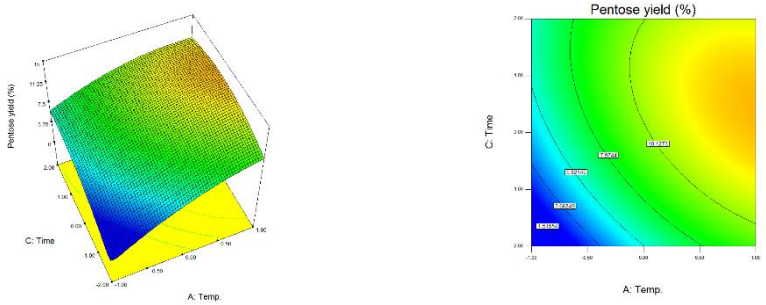


(B) 140℃

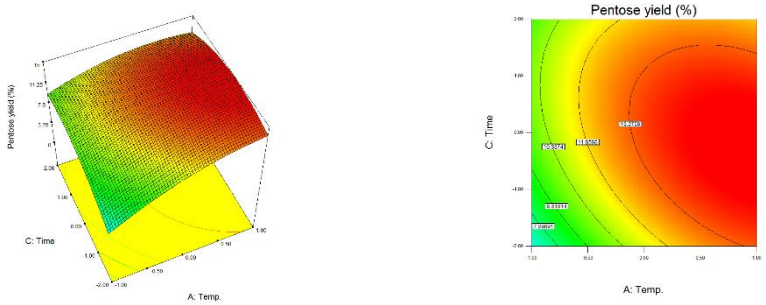


(C) 150℃

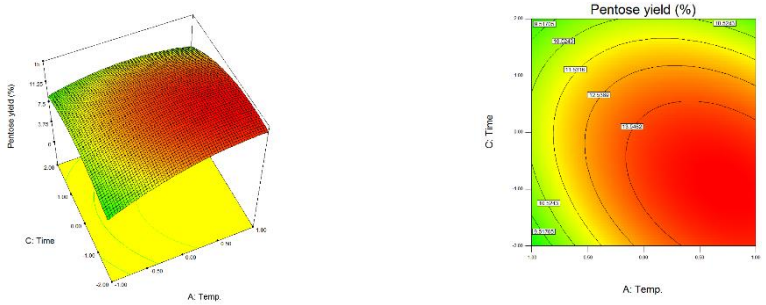
Figure 7. Effect of reaction temperature ranged from 130℃ to 150℃ on pentose yield from *Quercus mongolica* after 1st pretreatment.



(A) 1%

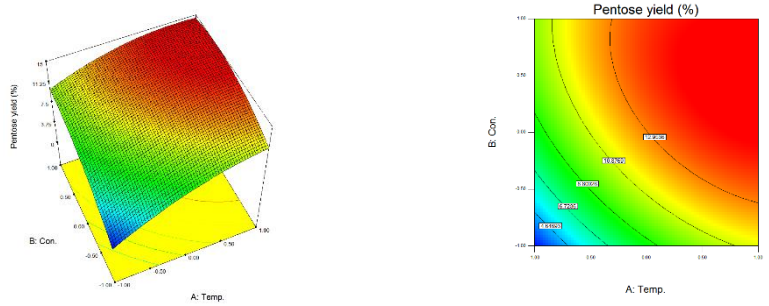


(B) 2%

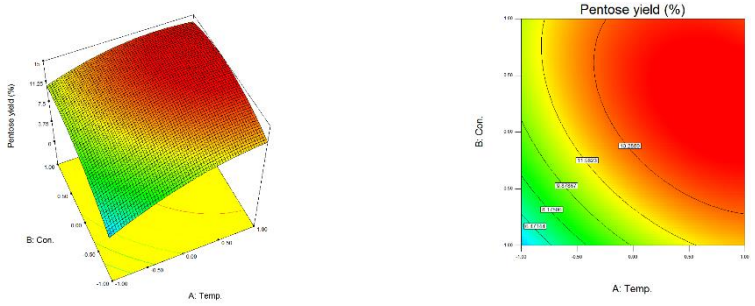


(C) 3%

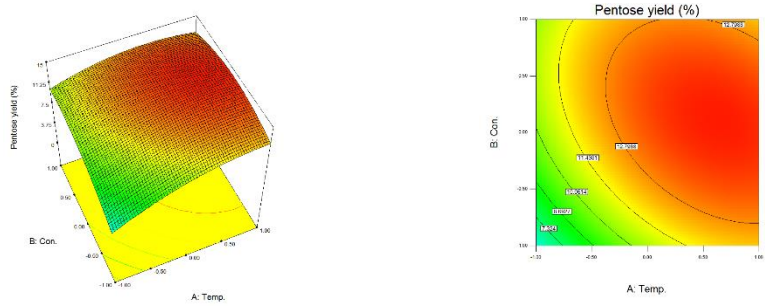
Figure 8. Effect of acid concentration ranged from 1% to 3% on pentose yield from *Quercus mongolica* after 1st pretreatment.



10 min



20 min



30 min

Figure 9. Effect of reaction time ranged from 10 min to 30 min on pentose yield from *Quercus mongolica* after 1st pretreatment.

4.2.4 Confirmation experiment for pentose

As the result of RSM analysis, the optimal condition for the maximum pentose yield was reaction temperature of 147℃, acid concentration of 2.29%, and reaction time of 20 min. In this condition, the predicted maximum pentose yield was 14.98%. To confirm the predicted value, experiments were conducted under the optimal condition, and the results were shown in Table 7. The pentose yield was 14.36%, corresponding to 81.54% pentose extraction, and it indicated that the optimization for pentose yield from *Quercus mongolica* by oxalic acid pretreatment was practicable using RSM. Also, the yield of hexose (glucose, galactose, and mannose) was less than 2%, that is, xylose was effectively isolated as observed in previous researches that oxalic acid intensively makes xylose isolated (Lee & Jeffries, 2011; Lee et al., 2009).

Table 7. The contents of sugars and degradation products in liquid hydrolyzate after the optimal condition of 1st pretreatment (reaction temperature: 147℃, acid concentration: 2.29%, reaction time: 20 min)

Component		Yield[a] (%)
Sugars	Glucose	1.82 (±0.05)
	Mannose	0.87 (±0.01)
	Galactose	1.54 (±0.01)
	Xylose	13.40 (±0.12)
	Arabinose	0.96 (±0.00)
Derivatives products	Formic acid	0.70 (±0.01)
	Acetic acid	4.37 (±0.04)
	Levulinic acid	0.02 (±0.00)
	5-HMF	0.04 (±0.00)
	Furfural	0.70 (±0.01)
	5-MF	0.01 (±0.00)

[a] Based on a dry weight of raw material

4.3 Furan derivatives production of 2nd pretreatment 4

4.3.1 Component yield of liquid hydrolyzate

The liquid hydrolyzate from 1st pretreatment under the optimal condition for high yield of pentose was used for 2nd pretreatment to produce furan derivatives. 2nd pretreatment was conducted at various conditions (reaction temperature: 180-230℃, acid concentration: 2-4%, and reaction time: 10 min) to determine the optimal condition for high yield of furan derivatives, especially furfural, 5-HMF, and 5-MF which are the major furan derivatives from lignocellulosic biomass, and to evaluate effects of reaction conditions on yield of products during 2nd pretreatment. Therefore, Fig. 11, 13, 14, and 15 show that the yield of furan derivatives and its related products in liquid hydrolyzate was described depending on reaction changes of temperature and acid concentration at 10 min reaction time. And all yield was based on dry weight of raw material.

4.3.1.1 Yield of furfural and its related products

Furfural is the most desired chemical among furan derivatives in this study. Because, it was the value-added chemical appointed by US Department of Energy and it could be widely used as biomass feed stock instead of oil based feed in various industry (Bozell & Petersen, 2010). Generally, it is known that furfural is produced from pentose such as xylose and arabinose through dehydration reaction. And at the high severe condition, furfural converts into other degradation products such as formic acid or is used in several other secondary reactions, for example, resinification which is a reaction of furfural itself due to its aldehyde structure which is sensitively

affected to acid, as shown in Fig. 10 (Cho, 2012; Karinen, 2011). Therefore, it is important to know the relationship among pentose (feedstock), furfural (desire product), and formic acid (furfural derivative) for improving furfural production.

To identify their relationship in forming furfural during 2nd pretreatment, each tendency of yield was compared. Furfural yield was increased until 210℃, after that there was hardly changed in amount of furfural ranged from 210℃ to 220℃ (Fig. 11C), representing the highest furfural yield. Meanwhile, the yields of xylose and arabinose were steadily decreased in that range (Fig. 11A, B). It was considered that furfural was degraded into formic acid or condensed itself (Patil, 2011). It seemed that resinification reaction or other secondary reactions were more active than formation of formic acid over 220℃, because the yield of formic acid was not increased while furfural yield was decreased. Also, it could be assumed that that formic acid was generated from degradation of oxalic acid (Eul et al., 2000).

Lastly, the maximum furfural yield was 6.52%, accordance with theoretical conversion rate of 72.89% which was the similar result with previous studies on production furfural from lignocellulosic biomass using sulfuric acid (Cai, 2014). Therefore, it indicates the feasibility of furfural production using oxalic acid as catalyst through two-step pretreatment process.

In addition to more details about the yield of pentose and formic acid depending on effects of reaction conditions, the yield of xylose and arabinose were steadily decreased when reaction temperature rose, moreover pentose was hardly remained over reaction temperature of 220℃ (Fig. 11A, B). In case of formic acid, Fig. 11C indicates that the yield of formic acid was more influenced by acid concentration than reaction temperature. For example, it was obtained the maximum yield of 9.21% with 4% acid concentration at 190℃, but also obtained that of 8.67% with 4% at 230℃ while obtained that of 4.61% with 2% at 190℃.

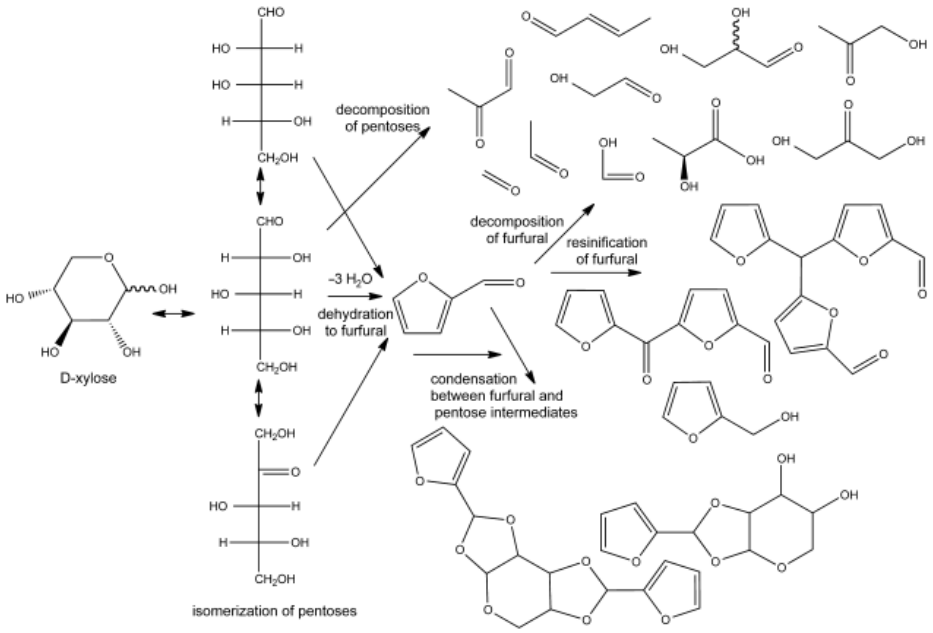
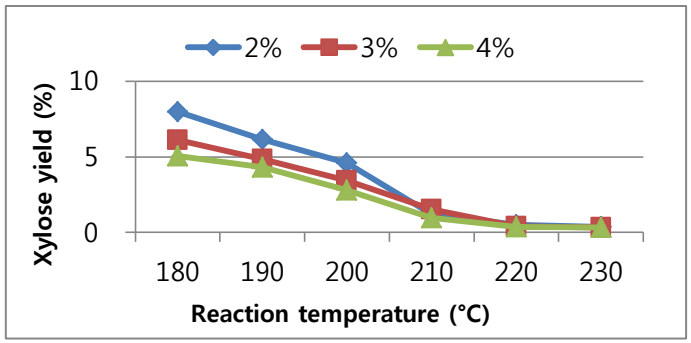
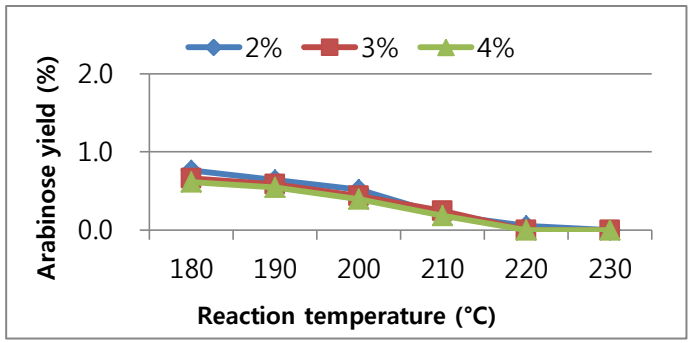


Figure 10. Scheme of reaction pathway of furfural and its secondary reactions.



A



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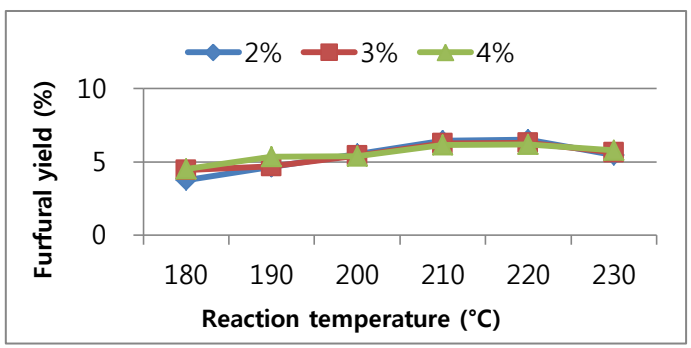

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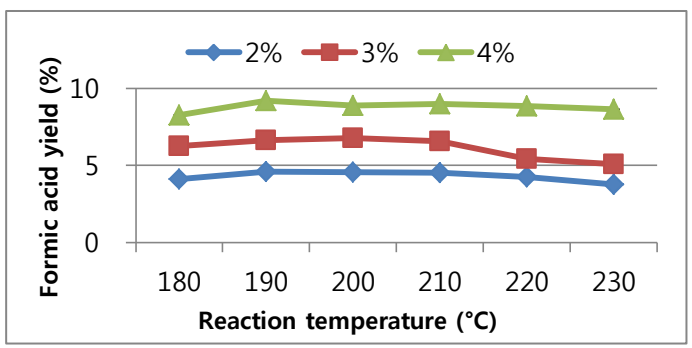


D

Figure 11. Yield of sugars and degradation products in liquid hydrolyzate after 2nd pretreatment depending on reaction temperature and oxalic acid concentration with reaction time fixed at 10 min (A: xylose, B: arabinose, C: furfural, D: formic acid).

4.3.1.2 Yield of 5-HMF and its related products

5-HMF is known as an intermediate products formed from glucose and degraded into levulinic acid and formic acid as described in Fig. 12 (Larsson, 1999). Fig. 13A shows that glucose yield was decreased with rising reaction temperature, especially the slope of graph fell sharply from 210℃ to 220℃. Simultaneously, the yield of 5-HMF was increased with rising temperature, and distinctly increased in the same range (Fig. 13B). It was indicated that part of glucose was converted into 5-HMF with a maximum yield of 1% with 2% acid concentration at 220℃ for 10 min. Over 220℃, 5-HMF yield was decreased while yield of levulinic acid and formic acid did not change (Fig. 13C, D). This was assumed that 5-HMF converted into not only levulinic acid and formic acid but also other chemicals through secondary reactions and repolymerization.

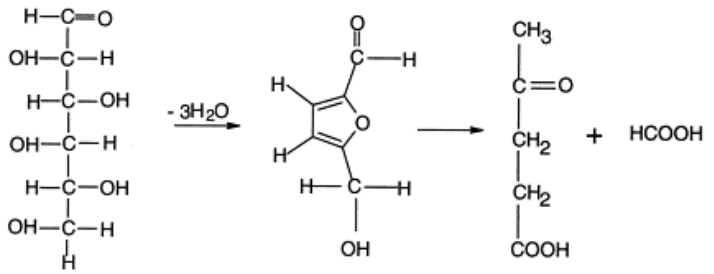
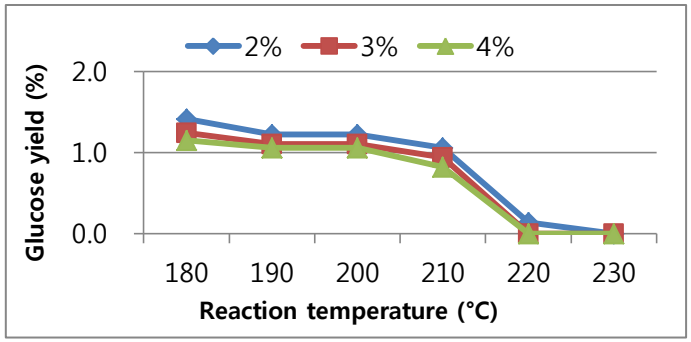
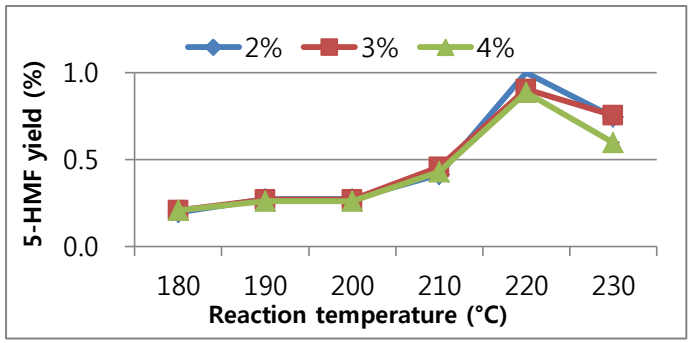


Figure 12. Scheme of conversion pathway of 5-HMF as intermediate.





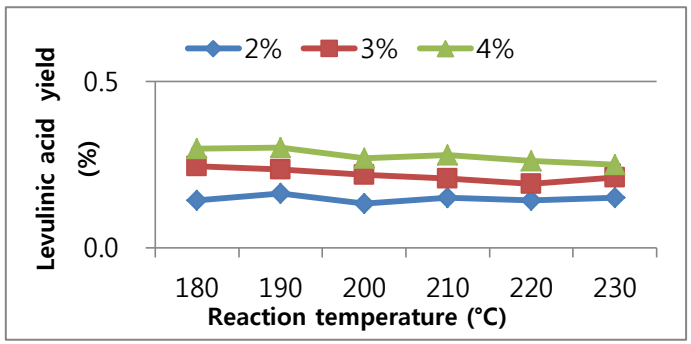


Figure 13. Yield of sugars and degradation products in liquid hydrolyzate after 2nd pretreatment depending on reaction temperature and oxalic acid concentration with reaction time fixed at 10 min (A: glucose, B: 5-HMF, C: levulinic acid).

4.3.1.3 Yield of 5-MF

The graph representing 5-MF yield was gradually risen and it is expected to be further increased (Fig. 14). However, the yield of 5-MF from *Quercus mongolica* was very low, the maximum yield was 0.2% under the condition (reaction temperature: 230℃, acid concentration: 4%).

4.3.1.4 The optimal condition of 2nd pretreatment for furan derivatives

The optimal condition of 2nd pretreatment for furan derivatives (5-HMF, furfural, and 5-MF) was selected at 220℃ with 2% acid concentration for 10 min. At that condition, the total yield of furan derivatives was 7.66% and the tendency of its graph was similar to furfural graph because furfural shows the highest proportion, about 85.15%, in furan derivatives in this study (Fig. 15). The composition of hydrolyzate by the optimal condition of 2nd pretreatment shown in Table 8.

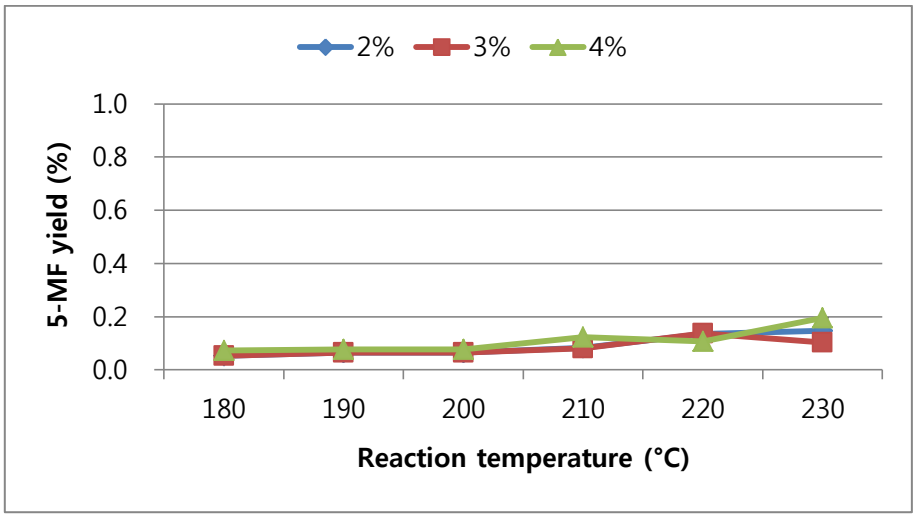


Figure 14. Yield of 5-MF in liquid hydrolyzate after 2nd pretreatment depending on reaction temperature and oxalic acid concentration with reaction time fixed at 10 min.

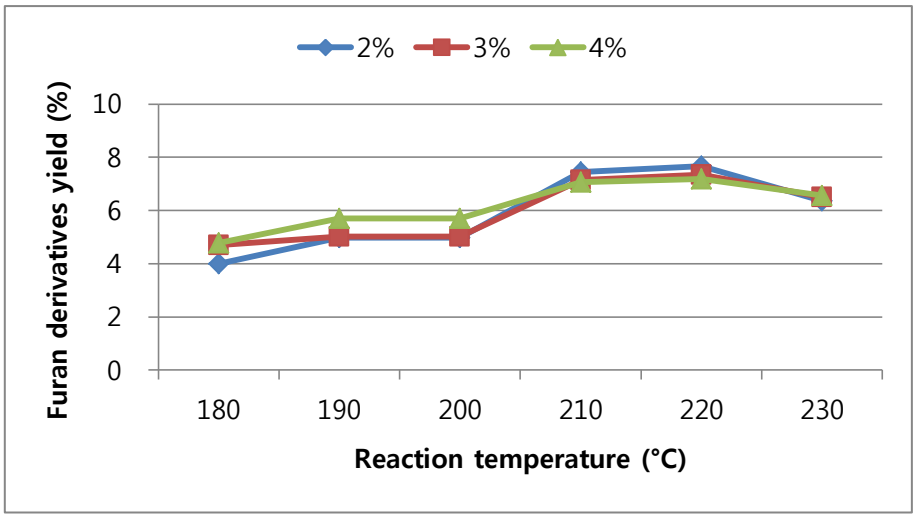


Figure 15. Yield of furan derivatives in liquid hydrolyzate after 2nd pretreatment depending on reaction temperature and oxalic acid concentration with reaction time fixed at 10 min.

Table 8. The contents of sugars and degradation products in liquid hydrolyzate after the optimal 2nd pretreatment (reaction temperature: 220℃, acid concentration: 2%, and reaction time: 10 min)

Component		Yield[a] (%)
Sugars	Glucose	0.02 (±0.05)
	Xylose	0.51 (±0.00)
Degradation products	Formic acid	4.25 (±0.00)
	Acetic acid	4.20 (±0.01)
	Levulinic acid	0.14 (±0.00)
	5-HMF	1.00 (±0.00)
	Furfural	6.52 (±0.01)
	5-MF	0.14 (±0.00)

[a]Based on a dry weight of raw material

4.3.2 Standard experiment at the optimal 2nd pretreatment condition

To understand conversion behavior of furan derivatives and oxalic acid, standard experiments were conducted under the optimal condition of 2nd pretreatment (reaction temperature: 220℃, acid concentration: 2%, and reaction time: 10 min). Glucose, xylose, and arabinose were used as standard materials in this experiments and their composition was set as followed by the composition of hydrolyzate after 1st pretreatment under the optimal conditions.

As the results, glucose generated formic acid, levulinic acid, 5-HMF, furfural, and acetic acid. And there was a little glucose remained. 5-HMF, one of the furan derivatives, was only produced from glucose not pentose as agreed with the previous study (Rosatella, 2011). Meanwhile, furfural, which was generally known as generated from pentose dehydration, was produced from glucose, which is major component of hexose. This was important information of determination for conversion behavior of furfural production, even if the amount of furfural was very low. In case of arabinose and xylose, formic acid and furfural were produced, and small quantity of xylose remained in the xylose standard solution after pretreatment process.

All three standard material generated formic acid. Even glucose and arabinose produced more formic acid than employed amount of glucose and xylose. It was considered that oxalic acid was degraded into formic acid.

As shown in Fig. 16, xylose produced the highest yield of furfural at 5.74%, followed as arabinose at 0.26% and glucose at 0.05%. The total yield of furfural from standard materials was slightly lower than raw material (*Quercus mongolica*). It could be assumed that any other component of lignocellulosic biomass were used in furfural conversion during pretreatment process.

Content		Weight (g)
Glucose (0.32g[a])	Glucose	0.03 (±0.00)
	Formic acid	0.84 (±0.01)
	Acetic acid	0.03 (±0.00)
	Levulinic acid	0.01 (±0.00)
	5-HMF	0.06 (±0.00)
	Furfural	0.01 (±0.00)
Xylose (2.41g[a])	Xylose	0.03 (±0.01)
	Formic acid	0.88 (±0.02)
	Furfural	1.07 (±0.00)
Arabinose (0.17g[a])	Formic acid	0.85 (±0.00)
	Furfural	0.05 (±0.00)

[a] The employed amount of standard material

Table 9. The weight of component produced from standard materials at the optimal condition of 2nd pretreatment (reaction temperature: 220℃, acid concentration: 2%, and reaction time: 10 min)

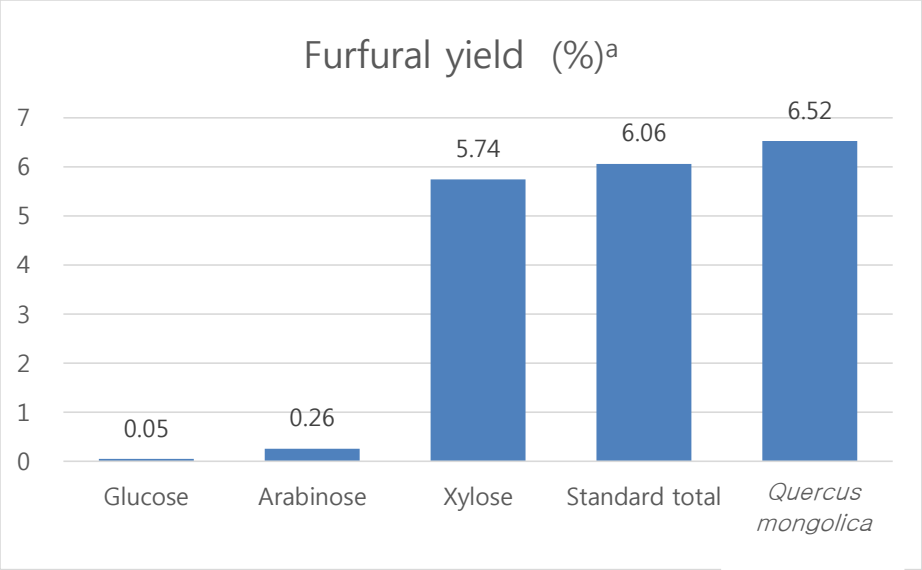


Figure 16. Furfural yield from standard materials and raw material.

4.4 Separation process for furan derivatives

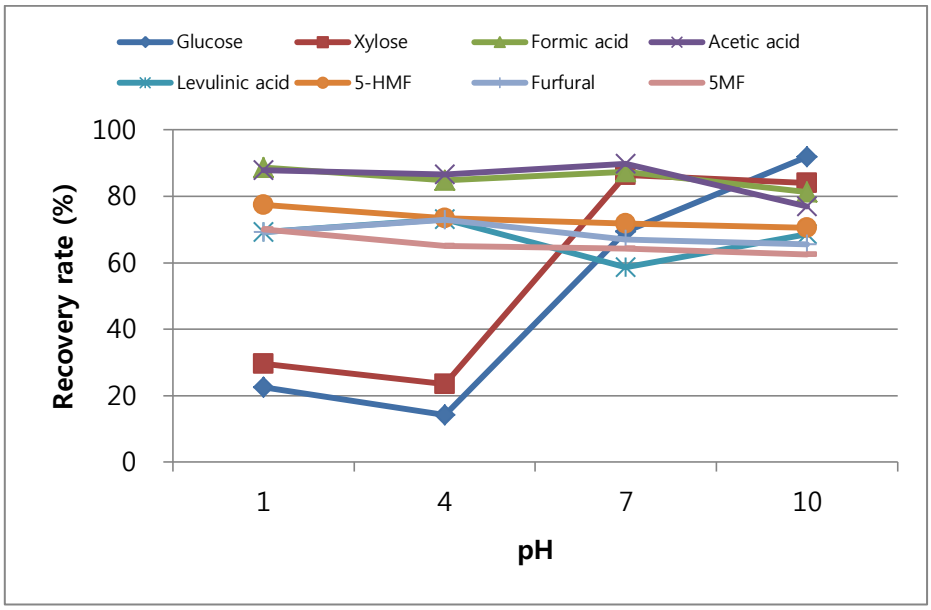
4.4.1 Nanofiltration (NF)

4.4.1.1 Effect of filter, feed pH, and repetition filtration

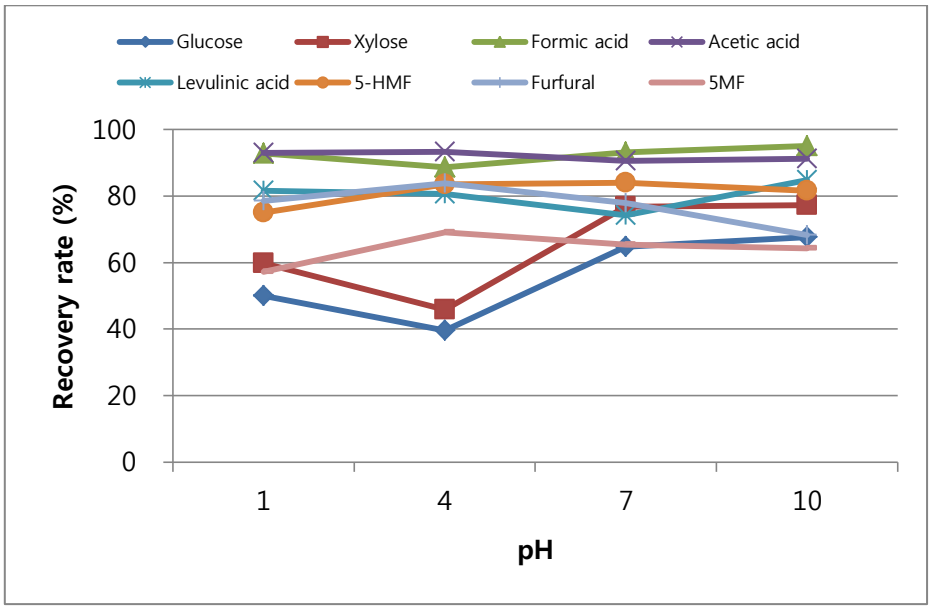
Fig. 18 indicated the recovery rate of components of hydrolyzate at various pH values for NE90 and DRM. The recovery rate of both glucose and xylose by NE90 was low at pH 4 and increased with the increase of the feed pH. The similar tendency was also observed by DRM. This may be linked to the increase of free volume in membrane skin layer. Since polyamide membrane became negative charged at high pH, the membrane repelled each other and resulted in the more open membrane. (Maiti, 2012, Weng et al., 2009)

The recovery rate of acetic acid was almost high at the pH ranged from 1 to 7, while a decrease in recovery rate of acetic acid was observed at pH 10 by NE90 (Fig. 18A). Since the pK value of acetic acid is 4, it almost dissociates and filter negatively charged at pH 11. Therefore, negatively charged acetic acid rejected by the negatively charged filter due to Donnan effect, the phenomenon that the retention of negatively charged ion was high with charged filter compared to un-charged filter. The similar result was reported in the research on separation of inhibitory components such as furfural and acetic acid from pretreated rice straw hydrolyzate using nanofiltration (Qi, 2011). However, the recovery rate of acetic acid did not change by DRM. And it was considered that Donnan effect was not affected because MWCO of DRM was larger than that of NE90. Meanwhile, The recovery rate of formic acid and furan derivatives (5-HMF, furfural, and 5-MF) were almost constant in the pH range examined, with the values higher than approximately 60% for the both filters.

As conclusion, NE90 represented better ability for separation of furan derivatives than DRM. In terms of operating condition, sugars were effectively separated at pH 4. And there was not much different in repetition filtration, but resulted in some loss in both filter.



A



B

Figure 17. Recovery rate of component for NE90 and DRM.

4.4.2 Solvent extraction

4.4.2.1 Effect of solvent and contact time

A preliminary set of experiments was carried out in order to screen the solvent which has high selectivity for furan derivatives and to identify the effect of contact time on the yield of each component. Four solvents including butanol, chloroform, ethyl acetate, and propyl acetate were tested with the hydrolyzate/solvent volume ratio 1:1. Contact time was set 15, 30, 45, and 60 min and the results were shown in Fig. 19.

Butanol extracted all sugars (glucose and xylose), degradation products (formic acid, acetic acid), and furan derivatives (furfural, 5-HMF, 5-MF) from hydrolyzate. Interestingly, butanol has the higher recovery rate of 5-HMF than other solvents with the highest rate of 68%. However, it could not influence on selectivity of furan derivatives because the yield of 5-HMF was low due to the small amount of 5-HMF in hydrolyzate after two-step. The highest total yield of furan derivatives from butanol extraction was 0.81% at the contact time of 15 min. Meanwhile, the yield of each component was very low, almost lower than 1%. And the effect of contact time was not critical in solvent extract process with butanol (Fig. 19A).

Propyl acetate extracted xylose, formic acid, acetic acid, furfural, 5-HMF, and 5-MF and glucose was not extracted. The results were similar to the results with butanol. Both of them did not obtain high selectivity for furfural, less than approximately 2%. The highest total yield of furan derivatives from butanol extraction was 0.94% at the contact time of 60 min (Fig. 19B)

Ethyl acetate also extracted most of components, except glucose like propyl acetate. However, ethyl acetate has the second highest selectivity for furan derivatives. 3.38% of furan derivatives was extracted at the contact time of 60 min. This indicates that ethyl acetate has better selectivity for furan

derivatives than propyl acetate. And it was opposite to the result of research on solvent extraction with standard material (furfural) and solvents (ethyl acetate and propyl acetate) (de Almeida, 2012). Therefore, further research will be needed to identify the different result. Meanwhile, the contact time influenced the yield of component, especially, the other degradation components such as formic acid and acetic acid (Fig. 19C)

Chloroform extracted acetic acid and furan derivatives and showed the highest selectivity for furan derivatives with total yield of 5.80% at the contact time of 15 min. Recovery rate of furfural, 5-MF, and 5-HMF were 83.92%, 70.60%, and 18.56%, respectively. Interestingly, chloroform has relatively higher recovery rate of furfural and lower recovery rate of 5-HMF than the other solvents (Fig. 19D). It was considered that the possibility of hydrogen bonding formation between furfural and chloroform molecules and linked to 'like dissolves like' role (Guo, 2014). And little effect of contact time on furan derivatives were observed in solvent extraction process. In addition, chloroform was considered the best extraction solvents with some reasons in aspect of extraction process. Its boiling point was lower than other solvents, thus it could be easy to regenerate with lower energy consumption. And, it was easier to separate from the feed because its density was different from feed (hydrolyzate) (Richard, 2004)

Therefore, chloroform was selected as the best solute in extracting furan derivatives from hydrolyzate in single stage. And it was used for further experiments for evaluating the effect of the hydrolyzate/solvent volume ratio and the number of extraction stage.

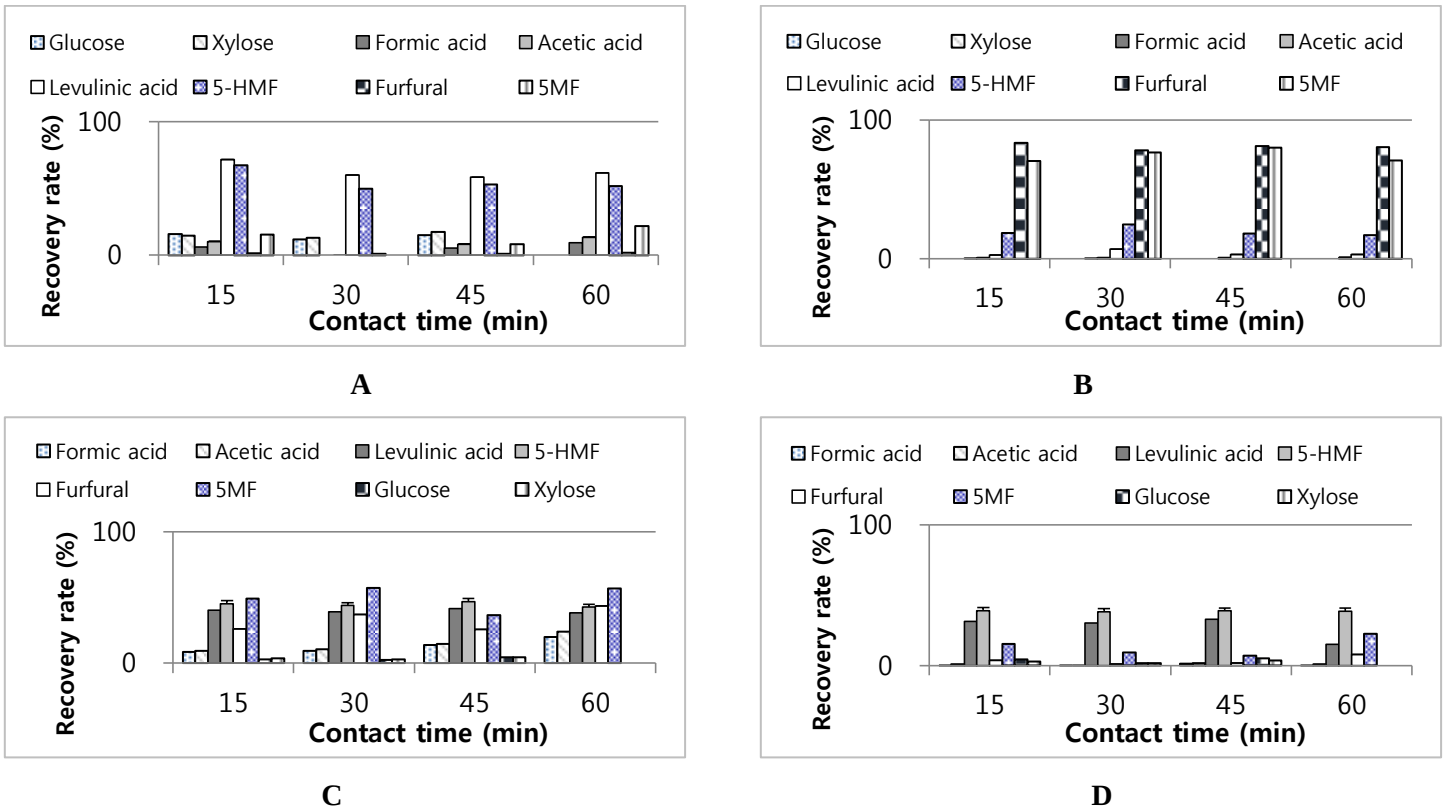


Figure 18. Effect of contact time on component yield and recovery rate of furan derivatives (5-HMF, furfural, and 5-MF) (A: butanol, B: propyl acetate, C: ethyl acetate, D: chloroform)

4.4.2.2 Effect of the hydrolyzate/solvent volume ratio and the number of extraction stage

To evaluate the hydrolyzate/solvent volume ratio and the number of extraction stage on the recovery of furan derivatives and other components, the experiments with chloroform, the best extraction solvent selected in preliminary experiments, were carried out.

Firstly, little effects of the H/S volume ratio on recovery rate of furfural and 5-MF were observed as shown in Fig. 20. The recovery rate of furfural and 5-MF were approximately 80% and 70%, respectively, at all the H/S ratio. On the other hand, the H/S volume ratio influenced the recovery rate of 5-HMF, as followed levulinic acid and acetic acid. Especially, the recovery rate of 5-HMF was increased to double. However, it could not extremely effect on the recovery rate of total furan derivatives because of the small amount of 5-HMF in hydrolyzate. Therefore, the highest recovery rate of furan derivatives was obtained with the value of 75.15% at the H/S volume ratio 1:1.

Also similar tendency were observed from the experiments for evaluating of the number of extraction stage. The recovery of 5-HMF, levulinic acid, and acetic acid were increased when the number of extraction stage increased from 1 to 3 while the recovery rate of furfural and 5-MF did not changed in this study range.

Therefore, it was considered that the H/S ratio and the number of extraction stage were only influenced on the recovery of 5-HMF, levulinic acid, and acetic acid. And the optimal condition of solvent extraction using chloroform was at the H/S volume 1:1 in triplicate extraction, with the highest recovery rate of furan derivatives of 77.26% and its purity rate of 97.81%.

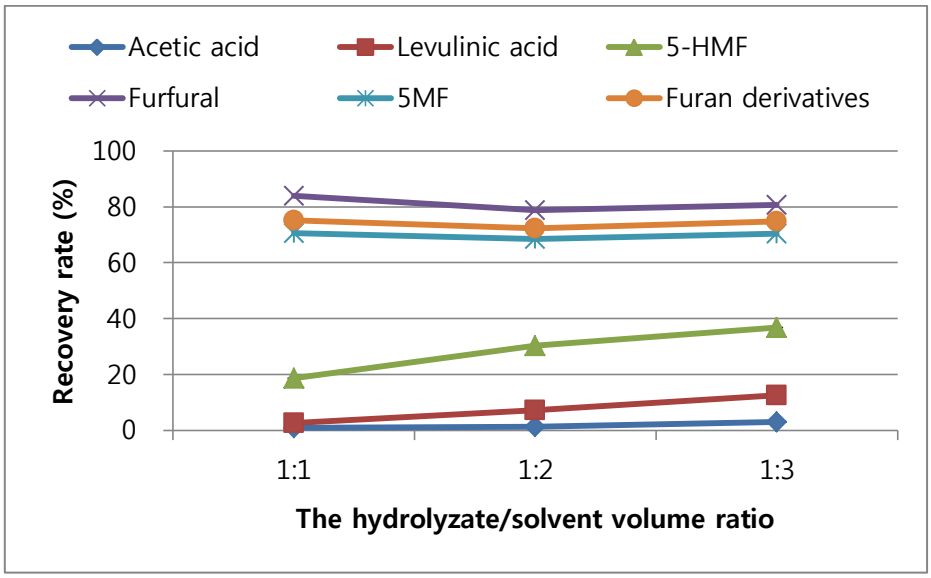


Figure 19. Effect of the hydrolyzate/solvent volume ratio on recovery rate.

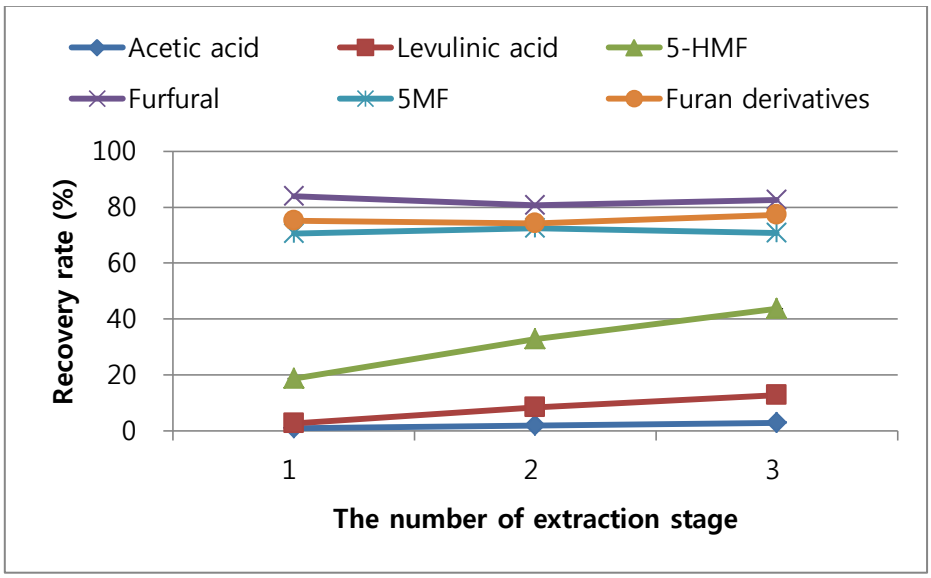


Figure 20. Effect of the number of extraction stage on recovery rate.

4.4.3 Standard experiment at the optimal separation process

Standard experiment was conducted at the best separation process selected from the experiments with raw material above. The model solution was used to identify the effect of other compounds from lignocellulosic biomass in separation process. The model solution was composed of furfural, 5-HMF, and 5-MF and their composition was set as followed by the composition of hydrolyzate after two-step pretreatment under the optimal conditions (1^{st} pretreatment: 147℃, 2.29% oxalic acid, and 20 min and 2^{nd} pretreatment: 220℃, 2% oxalic acid, and 10 min). The best separation process for the highest furan derivatives separation was solvent extraction with chloroform in triple stage.

Fig. 22 indicates the recovery rate of standard material and raw material. All standard material showed higher recovery rate than raw material. Especially, furfural was obtained with the highest recovery rate, over 90%, through standard experiment. It was considered that other components from lignocellulosic biomass influenced recovery of furan derivatives in solvent extraction process.

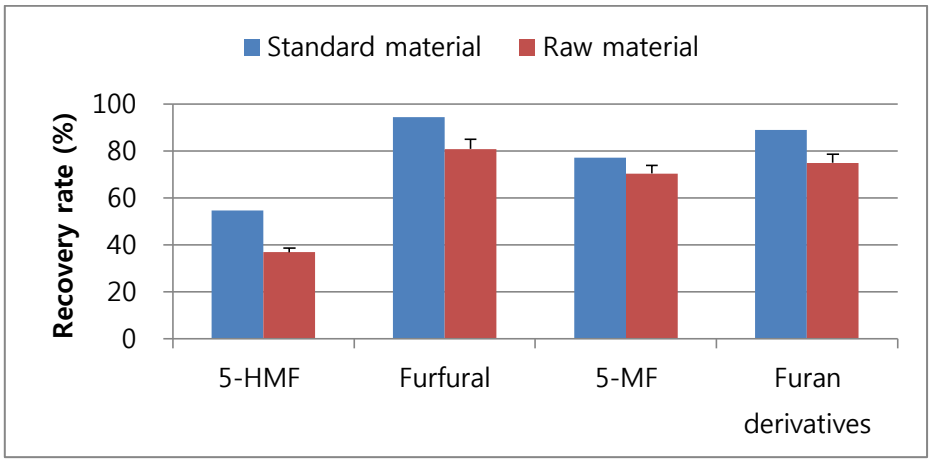


Figure 21. Recovery rate of components from standard materials and raw material by solvent extraction with chloroform.

4.4.4 Mass balance of all process for furan derivatives production

The mass balance of all process at the optimal condition for furan derivatives were shown in Fig. 23. When the basis of 100g raw material were used, 5-HMF of 0.44g, furfural of 5.42g, 5-MF of 0.11g, acetic acid of 0.11g and levulinic acid 0.02g were obtained as final products through two-step pretreatment and solvent extraction with chloroform. The yield of furan derivatives was approximately 6%.

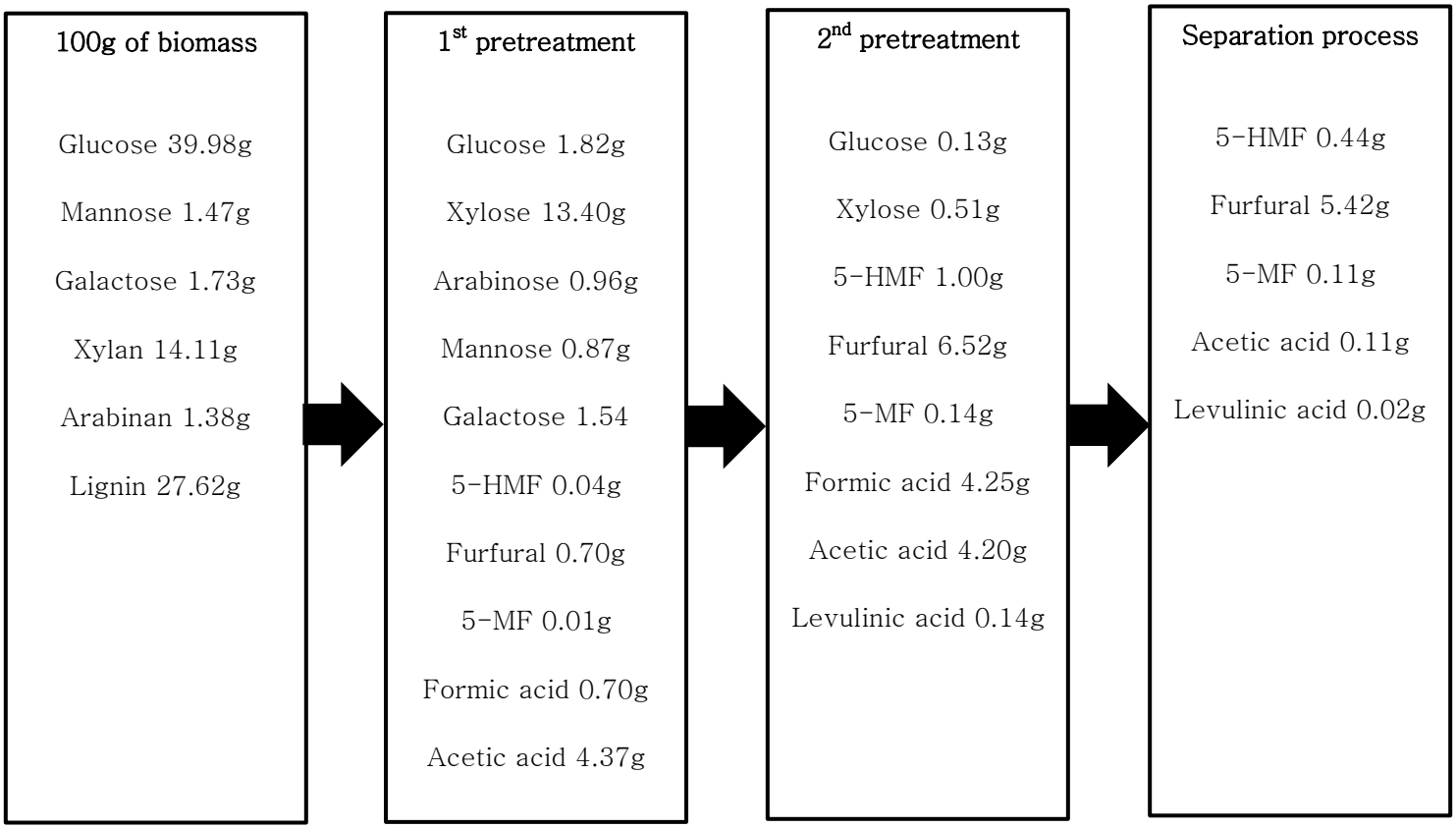


Figure 22. Mass balance of all process for furan derivatives production.

5 Conclusion

A two-step oxalic acid pretreatment of *Quercus mongolica* biomass was conducted to produce furan derivatives such as 5-HMF, furfural, and 5-MF. After production, nanofiltration and solvent extraction were carried out under various operating conditions to identify the optimal conditions for the separation of furan derivatives from other components of the liquid hydrolyzate, including sugars (glucose, xylose) and other degradation products (acetic acid, formic acid, and levulinic acid).

The 1st pretreatment was performed to determine the effects of various parameters (reaction temperature, acid concentration, and reaction time) and to define the optimal conditions for pentose yield by RSM. The results showed that reaction temperature was the most dominant factor affecting pentose yield; the highest yield of pentose was 14.36% under reaction conditions of 2.29% oxalic acid at 147°C for 20 min.

To produce furan derivatives, a 2nd pretreatment was conducted under various conditions (reaction temperature: 180-230℃, acid concentration: 2-4%, reaction time: 10 min). Reaction temperature had a great influence on the production of furan derivatives than acid concentration. The highest yield of furan derivatives was 7.66% under optimal conditions (reaction time: 220°C, acid concentration: 2%, reaction time: 10 min).

Finally, nanofiltration (NF) and solvent extraction were used to separate the reaction products. NE90 filters provided better separation than DRM filters. With both types of filters, glucose and xylose were selectively removed at pH 4 due to the Donnan effect. However, solvent extraction was found to be more selective for furan derivatives than NF. Chloroform was the best extractant producing a yield of furan derivatives of 5.97%, consistent with a recovery rate of 77.26%. It was assumed that chloroform is more able

than the other organic solvents tested (butanol, ethyl acetate, and propyl acetate) to form hydrogen bonds with furan derivatives.

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초 록

신갈나무의 옥살산 전처리를 통한 고수율의 퓨란계 화합물 생산 및 정제

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본 연구에서는 5-hydroxtmethylfurfural, furfural, 5-methylfurfural과 같은 퓨란계 화합물 생산을 위하여 신갈나무의 2단계 옥살산 전처리를 실시하고 산물들을 분리하였다.

1차 전처리에서는 5탄당 수율에 대한 반응조건들의 영향을 평가하고, 5탄당 생산을 위한 최적 조건을 확인하기 위하여 반응표면분석법을 수행하였다. 그 결과, 반응온도, 산 농도, 반응시간 순으로 5탄당 수율에 영향을 미치는 것으로 구명되었으며, 최적 조건(반응온도 147°C, 산 농도 2.29%, 반응시간 20분)에서 전건 시료 대비 14.36%의 5탄당(초기 시료 5탄당 대비 81.54%)을 얻을 수 있었다.

1차 전처리로부터 생성된 액상 가수분해물을 이용하여 2차 전처리를 실시하였으며, 퓨란계 생산에 대한 처리인자의 영향을 평가하고 최적조건을 탐색하기 위하여 다양한 조건(반응온도:

180~230°C, 산 농도: 2~4%, 반응시간: 10분) 실험을 진행하였다. 최대로 생산된 퓨란계 화합물은 전건시료대비 7.66%로 220°C, 산 농도 2%, 10분 조건에서 얻어졌으며, 반응온도에 의한 영향이 가장 큰 것으로 조사되었다.

마지막으로, 2단계 전처리 후 생성된 액상 가수분해물에 함유된 퓨란계 화합물을 분리하기 위해서, 나노필트레이션과 용매추출이 다양한 조건에서 실시되었다(나노필트레이션: 필터 종류(NE90과 DRM), pH, 반복 여과/ 용매추출: 용매 종류(chloroform, butanol, ethyl acetate, propyl acetate), 추출 시간, 액상 가수분해물과 추출 용매의 부피비, 추출횟수). 그 결과, 용매추출이 퓨란계 화합물을 분리하는 데 있어 나노필트레이션보다 우수한 선택성을 나타냈다. 특히, 클로로폼은 전건시료대비 5.97%의 퓨란계 화합물을 추출하였으며, 이는 회수율 77.26%를 나타냈다.

주요어: 신갈나무, 2단계 전처리, 옥살산, 퓨란계 화합물, 나노필트레이션, 용매추출

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