LIQUID HOT WATER PRETREATMENT OF AGRICULTURAL RESIDUES AND EVALUATION OF A SOLVOTHERMAL FRACTIONATION PROCESS

MR. SAKSIT IMMAN
ID: 52920008

A THESIS SUBMITTED AS A PART OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
IN ENERGY TECHNOLOGY

THE JOINT GRADUATE SCHOOL OF ENERGY AND ENVIRONMENT
AT KING MONGKUT'S UNIVERSITY OF TECHNOLOGY THONBURI

2ND SEMESTER 2013

Liquid Hot Water Pretreatment of Agricultural Residues and Evaluation of a Solvothermal Fractionation Process

Mr. Saksit Imman
ID: 52920008

A Thesis Submitted as a Part of the Requirements
for the Degree of Doctor of Philosophy
in Energy Technology

The Joint Graduate School of Energy and Environment
at King Mongkut's University of Technology Thonburi

2nd Semester 2013

Thesis Committee

……………………………………………………
(Assoc. Prof. Dr. Navadol Laosiripojana) Advisor

……………………………………………………
(Dr. Verawat Champreda) Co-Advisor

……………………………………………………
(Asst. Prof. Dr. Worapon Kiatkittipong) Member

……………………………………………………
(Dr.Vorakan Burapatana) Member

……………………………………………………
(Dr. Nawin Viriya-empikul) Member

……………………………………………………
(Prof. Dr. William Doherty) External Examiner

Thesis Title: Liquid Hot Water Pretreatment of Agricultural Residues and Evaluation of a Solvothermal Fractionation Process

Student's name, organization and telephone/fax numbers/email

Mr. Saksit Imman
The Joint Graduate School of Energy and Environment (JGSEE)
King Mongkut's University of Technology Thonburi (KMUTT)
126 Pracha Uthit Rd., Bangmod, Tungkru, Bangkok 10140 Thailand
Telephone: 0-8615-84845
Email: imman_s@hotmail.com; saksit016@hotmail.com

Supervisor's name, organization and telephone/fax numbers/email

Assoc. Prof. Dr. Navadol Laosiripojana
The Joint Graduate School of Energy and Environment (JGSEE)
King Mongkut's University of Technology Thonburi (KMUTT)
126 Pracha Uthit Rd., Bangmod, Tungkru, Bangkok 10140 Thailand
Telephone: 02-872-6736 ext 4146
Email: navadol_l@jgsee.kmutt.ac.th

Co-supervisor's name, organization and telephone/fax numbers/email

Dr. Verawat Champreda
Enzyme Technology Laboratory
Bioresources Technology Unit
National Center for Genetic Engineering and Biotechnology
113 Thailand Science Park
Paholyothin Rd., Klong 1, Klong Luang, Pathumthani 12120, Thailand
Tel. (66) 2 564 6700 ext 3473 Fax (66) 2 564 6707
E-mail: verawat@biotec.or.th

Topic: Liquid Hot Water (LHW) Pretreatment of Agricultural Residues and Evaluation of A Solvothermal Fractionation Process

Name of student: Mr. Saksit Imman **Student ID**: 52920008
Name of Supervisor: Assoc. Prof. Dr. Navadol Laosiripojana

ABSTRACT

Several liquid biofuels and chemicals can be produced from biomass via the fermentation of sugars derived through the saccharification cellulose and hemicellulose components of the biomass. Pretreatment/fractionation of the biomass is an essential process that improves carbohydrate digestibility by enzymes. This project investigated the use of compressed liquid hot water (LHW) for the pretreatment of rice straw (RS), bagasse (BG), corn stover (CS) and empty palm fruit bunch (EPFB). The prime aim was to ascertain whether the pretreatment technology could process a feedstock that constitutes these residues. The study extended the investigation using homogeneous acids (H_2SO_4, HCl, H_3PO_4 and oxalic acid) and NaOH catalyst on the pretreatment of rice straw.

The pretreatment of compressed LHW on the agricultural residues was found to be effective in hemicellulose solubilization and the digestibility of the cellulose-enriched residues by enzymatic hydrolysis. Optimum processing conditions were 200°C with reaction times between 5 and 20 min. The maximum amounts of glucose and pentose present in the substrates were 408.8-482.7 mg/g and 81.1-174.0 mg/g respectively. Comparative analysis on the basis of severity factor showed susceptibility to LHW treatment was in the order BG > RS > CS > EPFB. Saccharification of the substrates gave maximum glucose yields of 75.7-82.3% and pentose yields of 27.4-42.4%. The inhibitory compounds furfural (0.77-2.35 mg/mL) and 5-hydroxy furfural (HMF, 0.32-0.67 mg/mL) were under the threshold for ethanolgens. Surface area and structural changes, and the removal of hemicellulose were parameters that impacted on the effectives of the biomass to enzymatic hydrolysis.

The presence of homogeneous acid catalysts (0.25 % w/v) or NaOH (0.25-1.0 w/v) effectively enhanced the enzymatic digestibility of the rice straw substrate at temperatures lower than LHW in the absence of a catalyst. Oxalic acid (0.25 %w/v) was found to be the best promoter at the optimum operating temperature of 160°C for 10 min, as it resulted in the highest glucose yield of 84.2% and the lowest formation of inhibitory furan compounds. This glucose yield is slightly higher than a value of 82.3% obtained from a substrate that was derived by treatment with the alkali, NaOH (0.25, w/v) at 140°C for 10 min gave a glucose yield of 79.7% after enzymatic hydrolysis. The formation of HMF (0.02-0.26 mg/mL) and furfural (0.13-0.42 mg/mL) was lower than the concentration obtained with the acid-catalysed process. Sodium hydroxide was found to be a higher effective promoter for LHW at lower temperature. This could have been due to the higher acidity of water at increasing temperatures, which neutralized the alkali promoter in the reaction.

The clean fractionation (CF) process (i.e. a single-step fractionation) was studied as an alternative to obtain substrates for enzymatic hydrolysis for subsequent processing. This was conducted with a ternary mixture of water/ethanol/organic solvent that allowed the effective separation of cellulose, hemicellulose and lignin. The process allowed lignin to be recovered with high purity, thus providing the potential to produce high value products from lignin instead of burning it for it energy value. The organic solvents evaluated were methyl isobutyl ketone (MIBK), ethyl acetate (EA) and toluene (TOL), while H_2SO_4, HCL, and H_3PO_4 were the promoters examined. Methyl isobutyl ketone is the current choice of organic solvent used in the CF process. However, ethyl acetate was found low costs and toxicity.

Ethyl acetate was found to be a superior composite solvent to the conventional solvent MIBK by providing higher glucan and lignin yields. Glucan and xylan could be further solubilized and lignin recovery increased at higher temperature and acid concentration. The optimal fractionation conditions were found to be a water/ethanol/ethyl acetate (62.5%:25%:12.5%) mixture using 0.05 M H_2SO_4 at 160°C for 1 h, which gave the separation of a cellulose-enriched solid with 71.4 wt% glucan yield. The maximum of hemicellulose of 71.3 wt% was obtained as sugars, and dehydration products in the aqueous/alcohol phase were minimal. 84.9 wt% of lignin was recovered in the organic solvent phase with no cross-contamination of sugars.

Finally, microwave-assisted heating at 300 W for 1 h resulted in comparable glucan and lignin yields in the respective phases to conventional heating with the advantage of increased pentose yield and lower furan formation in the aqueous phase. The study provided a basis for efficient pretreatment/fractionation strategies for implementation in biorefineries using local agricultural feedstocks.

Keywords: Biorefinery, Enzymatic hydrolysis, Fractionation, Homogeneous catalyst, Lignocellulosic

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation and sincere gratitude to my advisor, Assoc. Prof. Dr. Navadol Laosiripojana (Joint Graduate School of Energy and Environment, JGSEE) and my co-advisor, Dr. Verawat Champreda (National Center for Genetic Engineering and Biotechnology, BIOTEC) for giving me an opportunity to be under their supervision. Without their encouragement, my graduate study and thesis would not have materialized. I would like to thank them for their kindness, motivation, understanding, patience and most importantly, their continuous support during my study. I also would like to thank them for teaching me numerous lessons, insightful guidance and suggestions. Their invaluable lessons are really inspired, practical and helpful. I can use their guidance throughout my study, life, and further in my career.

I would like to thank my examining committee, Asst. Prof. Dr. Worapon Kiatkittipong of Department of Chemical Engineering, Faculty of Engineering, Silpakorn University, Dr. Vorakan Burapatana of PTT Research and Technology Institute, PTT Public Company Limited, Dr. Nawin Viriya-empikul of National Nanotechnology Center (NANOTEC), National Science and Technology Development Agency (NSTDA), and Professor William Doherty, Principal Research Fellow, Centre for Tropical Crops and Biocommodities, Sugar Research and Innovation and Cooperatives for their kindness, helpful discussions, comments and suggestions.

I am also deeply thankful to my friends and especially, all members of the Enzyme Technology Laboratory Bioresource Unit, National Center for Genetic Engineering and Biotechnology (BIOTEC) for their assistance, guidance, and most importantly, their friendship during my research there. It is the one of the best memories in my life.

My deepest appreciation and sincere gratitude go to my parents, who have always loved, encouraged, and also shared the hard times with me.

I thankfully acknowledge the Enzyme Technology Bioresource Unit, National Center for Genetic Engineering and Biotechnology (BIOTEC) for providing laboratory equipment and facilities.

At last, I would like to thank the Thailand Graduate Institute of Science and Technology (TGIST), Grant No. TGIST 01-53-020, for all financial support during my study and research. I gratefully acknowledge the financial support provided by the Joint Graduate School of Energy and Environment (JGSEE), King Mongkut's University of Technology Thonburi under the JGSEE Research Scholar ship, Contract No. JGSEE 434.

CONTENTS

CONTENTS (Cont')

CONTENTS (Cont')

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Cont’)

LIST OF FIGURES (Cont')

LIST OF FIGURES (Cont')

LIST OF FIGURES (Cont')

LIST OF FIGURES (Cont’)

CHAPTER 1
INTRODUCTION

1.1 Rationale

Today, the world is facing anenergy crisis because it is mainly reliant on fossil-based oil (i.e., conventional oil) which is continuously rising in price. There is also the environmental issue associated with CO_2 and toxic gas emission. In Thailand, demand for fuel is rising because of population growth and, like the rest of the world, increasing demand industry and the transportation sector. Imported oil supplies have risen substantially to fulfill demand as only 10% of the required fuel [Koide *et al.* 1982] resulting in trade deficit for the nation.

There are several procedures to minimize energy and environmental problems. One of the promising current approaches is to develop clean alternative fuels to replace conventional oil, for example, hydrogen, gas-to-liquid (GTL), biomass-to-liquid (BTL), dimethyl-ether (DME), biodiesel, and bioethanol. At present, bioethanol is considered a potential clean alternative that can be produced from lignocellulosic biomass. Lignocellulosic agricultural by-products are considered potent feedstocks for bioethanol production in Thailand. There are many types and various amount of lignocellulosic biomass in the country as shown in Table 1.1. Consequently, the uses of these biomasses as feedstock for bioethanol productions would be a great choice for Thailand. If we can develop the efficient techniques to convert these feedstocks to bioethanol, it will absolutely help the nation to reduce the fuel import rate as well as decrease CO_2 emission rate from the combustion of fuel.

Table 1.1 : Information of biomass in Thailand [National Statistics Office., 2007]

Crop	Production 2003(ktons)	Residues	RPR	Surplus Availability Factor	Calorific Value (MJ/kg)	Potential Energy (TJ)
Rice	26,057	Husk	0.230	0.493	14.27	42,162
		Straw	0.447	0.684	10.24	81,581
Sugarcane	64,973	Bagasse	0.291	0.207	14.40	56,358
		Top &Trash	0.302	0.986	17.39	336,447
Maize	4,230	Corn cob	0.273	0.670	18.04	13,958
Cassava	16,868	Stalk	0.088	0.407	18.42	11,128
		Husk	0.362	0.595	16.23	4,957
		Shell	0.160	0.378	17.93	1,538
Coconut	1,418	Empty Buncheses	0.049	0.843	15.40	902
		Frond	0.255	0.809	16.00	4,130

1.2 Biomass potential in Thailand

According to the Thailand Energy Situation (1994) published by the Department of Energy Development and Promotion, Thailand's total primary energy supply in 1994 amounted to about 2700 PJ (65.82 Mtoe; million tons oil equivalent, 10,000 kcal/l equivalent in oil), an increase of 11% over the previous year, of which 58.4% came from indigenous sources and 41.6% from imported sources, as shown in Table 1.2. The total production of renewable energy or biomass energy in 1994 was 810 PJ (19.4 Mtoe), an increase of 9.3% over the previous year, accounted for 51.6% of the total indigenous energy production. It is noteworthy that hydro-power, geothermal, solar, and wind energies are excluded from renewable energy in the data. As seen in Table 2.1, biomass energy including fuel wood, rice husk, and bagasse were a major contribution to the primary energy supply, making up more than half as regards the indigenous energy source. The total energy of biomass was 810 PJ (19.348 Mtoe).

Table 1.2 Energy balance in Thailand (1994) [a]

	Total biomass	Fossil energy	
		Domestic	Import
Total primary energy supply	810 (19,378)	761 (18,197)	1,181 (28,244)
Total final consumption	480 (11,477)	1,853 (44,335)	

[a]Reference: Thailand Energy station 1994 [unit: PJ(M toe)]

Table 1.3 shows the information on biomass energy in Thailand. Fuel wood was the dominant source (83.7%), followed by bagasse (13.8%) and rice husk (2.5%). Fuel wood includes both wood used directly and as a raw material for the production of charcoal. The energy content of biomass residue was estimated from the data of crop-to-residue ratio, moisture content, and heating value of the residue of selected agricultural products.

Table 1.3 Biomass energy in Thailand (1994) [a]

	Fuel wood	**Charcoal**	**Rice husk**	**Bagasse**	**Total biomass energy**
Total primary energy supply PJ(k toe)	16,221(678)	7(0.3)	483(20.2)	6,667(111.5)	19,378(810)
Total final consumption PJ(k toe)	3,902(163)	4,458(186.3)	450(18.5)	2,667(111.5)	11,477(480)

[a] Reference: Thailand Energy Situation 1994 [unit: PJ(k toe)]

Table 1.4 shows the energy content of the biomass residue. The production of rice was 18.447 Mt in 1994. Rice husk and rice straw were generated from paddy. Bhattacharya *et al*. (1989) reported that the rice-to-husk ratio was 0.267 and rice-to-straw ratio was 1.695 on an air-dried basis. The heating values of these two types of residue were assumed to be 16.3 GJ/t on a dry basis, being the same value as reported by Hall *et al*. (1993). The energy content of rice husk and rice straw was 70.3 and 445 PJ, respectively. For sugar cane, the production in 1994 was 37.569 Mt. The sugar cane-to-bagasse ratio was assumed to be 0.29 on the basis of a 5-year study by the National Energy Administration (1991). The moisture content of bagasse was taken to be 49% and the heating value was 17.33 GJ/t (Bhattacharya et al., 1989). The energy content is thus estimated to be 96.3 PJ. Similar procedures were taken for corn cob and cassava stalk to calculate their energy content [Bhattacharya *et al*. 1989; Hall *et al.* 1993]. Although biomass residue other than those listed in Table 1.3 has to be taken into consideration, the coconut shell and husk, palm frond, old fruit trees, cotton stalk, cassava stalk, groundnut shell were neglected since their production is relatively small.

Table 1.4 Energy of biomass residue

	Production (kton)	Residue	Residue coefficient	Production (kton)	Moisture content	Dry weight (kton)	Energy content (PJ)
Rice straw	18,447	Rice husk	0.267	4,925	0.124	4,314	70.3
		Rice straw	1.695	31,268	0.127	27,297	445
Sugarcane	37,569	Bagasse	0.29	10,895	0.490	5,556	96.3
Corn	3,800	Corn cob	0.273	1037	0.0753	959	17.0
Cassava	19,091	Cassava stalk	0.088	1,380	0.30	1,176	20.8
Wood	1,548	Saw dust	0.1	155	0.20	124	2.0
Total	80,455			49,960		39,426	649.4

By comparing Table 1.3 with Table 1.4, we can see that bagasse is completely utilized as an energy source, while rice straw seems to have a greater potential. However, rice straw is already used as an industrial raw material for fibers in pulp production. It is also extensively used as animal feed, compost, and soil conditioner on agricultural land. Rice husk is also utilized as filler in the brick-manufacturing industry and as bedding material for animals. Corn cobs are used as wood charcoal additives and animal feed.

For sawdust or wood dust, which is by-products of cutting, grinding, drilling, sanding, or otherwise pulverizing wood with a saw or other tool, only a small portion is used as a dumping material for landfill in sawdust mounds in the making of charcoal. Taking this into consideration, there is not a great potential for these agricultural wastes as energy sources, but all of the types of residue are not fully utilized. It is reasonable to assume that 10% of total residue is effectively used as energy or an extra 65 PJ can be produced.

Biomass is a renewable resource produced from organic matter, including agriculture residues, and is the main renewable energy resource available, especially in agricultural countries, such as Thailand. Thailand has many kinds of biomass, for example, rice husk, rice straw, corncob, coconut shell, palm shell, cassava pulp and sugarcane bagasse. With advances in chemical engineering and biotechnology, many processes have been established for energy production from biomass. These materials can be used as fuel to heat water to produce steam or processed into liquids and gases, which can be converted to energy and fuel via thermochemical and biochemical conversions. The products at the end of the conversion routes are diverse, but can be divided into four main groups: biodiesel mainly fatty acid methyl ester (FAME), alcohols (ethanol/methanol), synthetic fuel (BTL) and hydrogen. The main biomass energy conversion routes are shown in Figure 1.1.

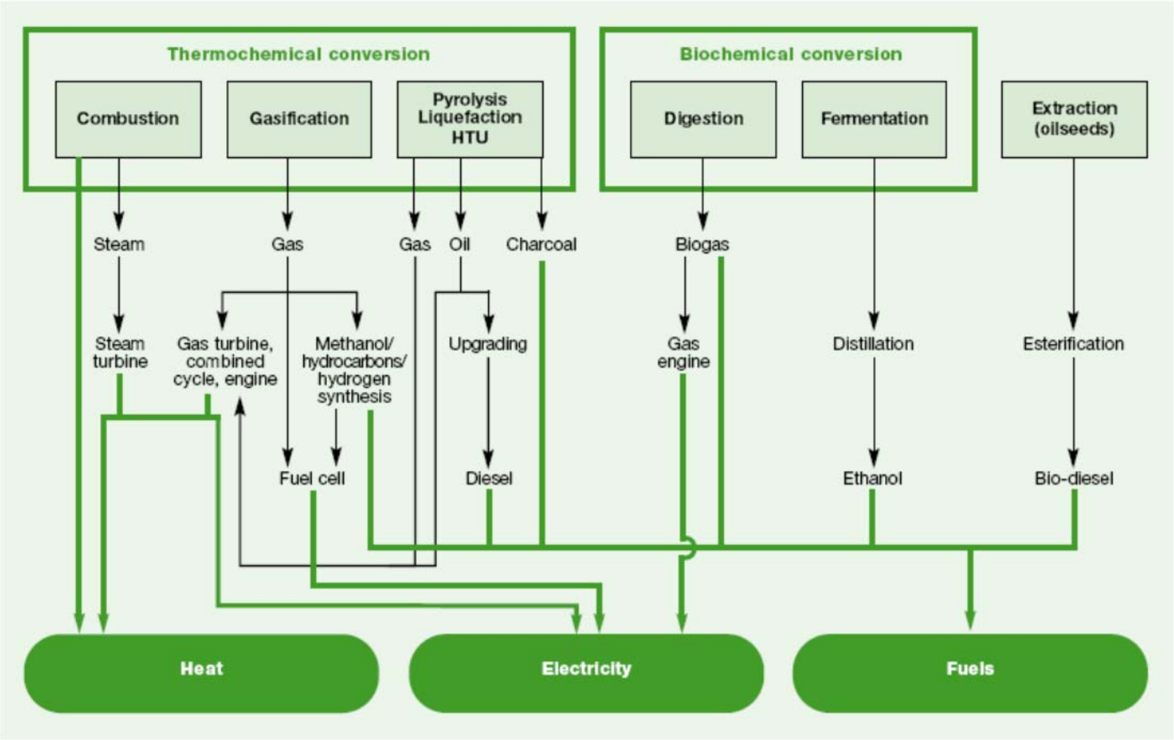


Figure 1.1 Main biomass energy conversion pathways [Vessia *et al*. 2005]

Ethanol production from lignocellulosic biomass is one of the choice fuels for the future worldwide and one of the most dependable processes available in the fuel sector. The virtues of using ethanol as fuel are high ability to reduce CO_2 emissions more than 90% and high quality, which is not depend on any limitation of use in either today's engine or foreknowledge next-generation engines. Ethanol fuels have great potential for securing supply, mitigating climate change and providing added value in rural areas. Ethanol is a clear, colorless, flammable, oxygenated hydrocarbon with the chemical formula C_2H_5OH. Ethanol can be used in a substitute for gasoline. Ethanol can be mixed in gasoline up to 20 percent without engine modification [Koide *et al.* 1982]. Ethanol is normally blended at 10% ethanol with 90% gasoline (a mixture called gasohol). In addition, ethanol can be used as a feedstock for the product of Ethyl-Tertiary- Butyl-Ether or ETBE by mixing ethanol and isobutylene and reacting them with heat over a catalyst. ETBE is an oxygenated fuel that can be blended with gasoline. ETBE will be used to replace Methyl-Tertiary- Butyl-Ether or MTBE, which is widely used as an oxygenate and octane booster to replace lead in the gasoline pool. The role of MTBE has recently been questioned because of its problems with groundwater pollution [Wheals *et al.* 1999]. It has to be imported, and costs Thailand about 4 billion baht per year [Thepkanjana. 2002]

1.3 Research Objectives

The project studied the effects of thermochemical processes on the modification of lignocellulosic biomass compositions and structures, focusing on pretreatment and fractionation processes. It involved the following areas of research activities:

1. The use of liquid hot water (LHW) for the pretreatment of selected agricultural residues. The process efficiency was evaluated based on enzymatic digestibility and the proportion of inhibitor products formed.
2. The effects of catalyst type and loading on the pretreatment of rice straw using LHW. The parameters examined for this part of the study are: sugar recovery, hemicellulose content and digestibility.
3. The use of a ternary mixture of water/ethanol/organic solvent to study the fractionation of rice straw into its main components.

1.4 Hypothesis

1. The LHW process can improve the enzymatic digestibility of pretreated agricultural residues.

2. The addition of homogeneous catalyst can improve LHW efficiency.

3. The Clean fractionation process (CF) can separate lignocellulosic biomass components with a high yield by each fraction.

4. Pretreatment and fractionation of lignocellulosic can improve the utilization of lignocellulosic biomass for biorefinery applications.

1.5 Scope of this work

This research focuses on the studies of lignocellulosic biomass pretreatment and fractionation by thermochemical processes (i.e. hydrothermal and solvothermal) with and without the presence of homogeneous catalysts. The project is divided into three sections.

1. Study the efficiency of LHW on improving the digestibility of five selected lignocellulosic biomasses, as well as its impact on biomass structures and physicochemical properties based on liquid water processes in the absence of liquid-state acid and alkali on pretreatment in batch systems under various conditions.
2. Explore the use of external catalysts on the LHW process by a comparative study of different homogeneous acid and alkalines on catalytic LHW pretreatment of rice straw. Effects of catalyst types on sugar recovery from hemicellulose hydrolysis and enzymatic digestibility of the pretreated biomass were investigated under different LHW conditions.

3. The effects of organic solvents and acid promoters on the fractionation of rice straw using a modified clean fractionation process (CF) were studied. In addition, the use of microwave-assisted heating to replace the conventional external heating was reported. The work provides an efficient alternative approach for efficient separation of primary lignocellulosic components with high recoverability and selectivity for further valorization in integrated biorefineries.

CHAPTER 2
LITERATURE REVIEW

The main objective of the research is to study thermochemical processes for the pretreatment/fractionation of lignocellulosic biomass. It aims to evaluate liquid hot water (LHW) for the pretreatment of agricultural residues for subsequent enzymatic hydrolysis to simple sugars. The study was complemented with the fractionation of biomass into its components using organic solvents.

2.1 Biomass structure

Biomass is the renewable carbon source for the production of energy and materials. As carbon in biomass is from the result of photosynthesis of carbon dioxide from the atmosphere, it is considered carbon neutral and would be considered a truly a potential green feedstock for industry.

The conversion of biomass to value-added products depends on the physical properties and chemical compositions of the feedstocks. Biomass contains varying composition of cellulose, hemicellulose lignin, and ash, etc. In general, lignocellulosic biomass comprised of cellulose (40-60%), hemicelluloses (20-40%), and lignin (10-25%). Examples of lignocellulosic biomass with potential for biomass conversion industries are trees, agricultural residues, crops, grasses [U.S. Department of energy, 2006].

Cellulose is a polymer consisting of glucose molecules approximately in the range of 10,000 units. The direction of the linkages and additional hydrogen bonding make the polymer rigid and difficult to break. Hydrolysis of cellulose can be occurred by acids catalyzed reactions, enzymes and thermal processes. This process is also called saccharification, which yields glucose which is a six-carbon hexose as the final product.

Hemicelluloses are heterogeneous polymers of pentoses (xylose, arabinose), hexoses (mannose, glucose, galactose), and sugar acids. Unlike cellulose, hemicelluloses are not chemically homogeneous. In softwood, hemicelluloses mainly consist of glucomannans, whereas in hardwood hemicelluloses contain mostly xylan. [Saha *et al.* 2003]. Xylan is the major component of many plant materials. It is a heteropolysaccharide with homopolymeric backbone chains of 1,4-linked β-D-xylopyranose units. In addition to xylose, xylans may contain arabinose, glucuronic acid or its 4-*O*-methyl ether. The frequency and composition of branches are dependent on the source of xylan [Saha *et al*. 2003]. The component of backbone are *O*-acetyl, α-L-arabinofuranosyl, α-1,2-linked glucuronic or 4-*O*-methylglucuronic acid substituents. Nevertheless, unsubstituted linear xylans have also been isolated from guar seed husk, esparto grass, and tobacco stalks [Saha *et al*. 2003]. Xylans can thus be categorized as linear homoxylan, arabinoxylan, glucuronoxylan, and glucuronoarabinoxylan.

Lignin in the cell walls of the plant cells are highly branched and mononuclear aromatic polymers. Lignin is present in the complex of cellulose as a lignocellulosic complex. It is a large complex polymer of phenenyl propane and methoxy group, a non-carbohydrate polyphenolic substance that encrusts the cell wall and cements the cells together. It is degradable by only a few organisms, into several higher value products such as organic acids, phenols and vanillin. Valuable fuel additives may be produced from lignin via chemical processes.

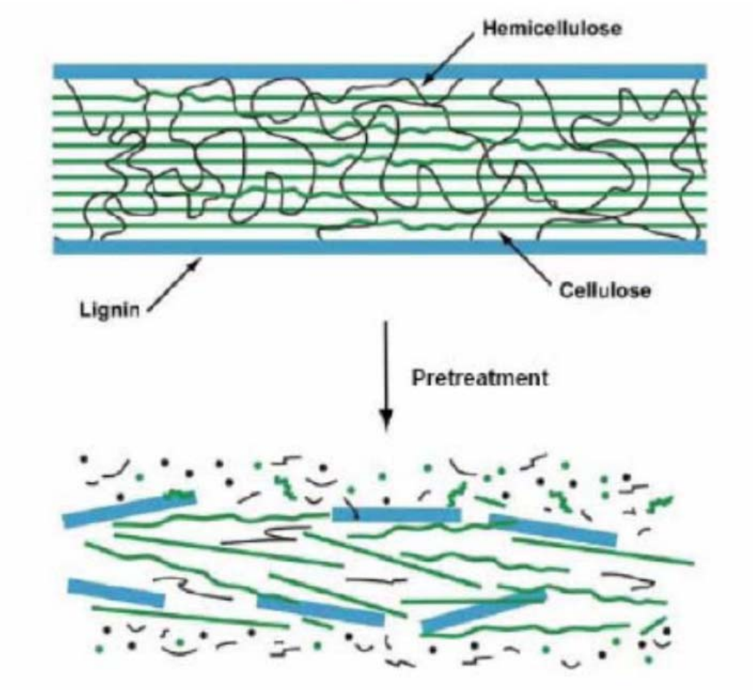


Figure 2.1 Physical deconstruction and fractionation

Hemicelluloses and lignin must be removed before the hydrolysis of cellulose can efficiently occur as they provide a protective cover around cellulose. The highly insoluble and recalcitrant crystalline structure of cellulose should also be transformed to amorphous form. Consequently, efficient pretreatment step are necessary for efficient degradation of cellulose. After the cellulose and hemicelluloses have been saccharified, the sugar generated can be further converted to the desirable value-added products using fermentation, biochemical, thermochemical or catalytic processes.

2.2 Pretreatment technologies

A worldwide pretreatment process is difficult to envision because of the diverse nature of different biomass feedstocks. A multitude of different pretreatment technologies have been suggested during the last decades. They can be classified into biological, physical, chemical and thermo-chemical pretreatments, according to the different forces or energy consumed in the pretreatment process. Combination of these methods has been also studied.

2.2.1 Biological pretreatments

Fungal pretreatment has been explored to upgrade lignocellulosic materials for feed and paper applications. Recently, this environmentally friendly approach has received renewed attention as a pretreatment method for enhancing the enzymatic saccharification of lignocellulosic biomass in ethanol production processes. Biological pretreatments employ microorganisms mainly brown, white and soft-rot fungi which degrade lignin and hemicelluloses and with little degradation on cellulose (Sanchez. 2009). Lignin degradation by white-rot fungi, the most effective for biological pretreatment of lignocellulosic materials, occurs through the action of lignin-degrading enzymes such as peroxidases and laccases (Kumar *et al.* 2009a).

Several white-rot fungi, such as *Phanerochaete chrysosporium, Ceriporia lacerata, Cyathus stercolerus, Ceriporiopsis subvermispora, Pycnoporus cinnarbarinus* and *Pleurotus ostreaus*, have been examined on different lignocellulosic biomass showing high delignification efficiency (Kumar *et al.*, 2009a; Shi *et al.*, 2008). Results from other recent studies have shown that fungal pretreatment of wheat straw for 10 days with a high lignin-degrading and low cellulose-degrading fungus (fungal isolate RCK-1) resulted in a reduction in acid loading for hydrolysis, an increase in the release of fermentable sugars and a reduction in the concentration of fermentation inhibitors (Hoyer *et al.* 2013) .

Ethanol yield and volumetric productivity with *Pichia stipitis* from subsequent fermentation were 0.48 g/ g and 0.54 g/L h, respectively (Kuhar *et al*. 2008).

2.2.2 Physical pretreatments

Physical pretreatments can be divided into two methods i.e. mechanical comminution and extrusion. The objective of the mechanical comminution is the reduction of particle size and the crystallinity of lignocellulose in order to increase the specific surface and reduce the degree of polymerization. This can be produced by a combination of chipping, grinding or milling depending on the final particle size of the material (10–30 mm after chipping and 0.2–2 mm after milling or grinding) (Sun *et al*. 2002). Different milling processes (ball milling, two-roll milling, hammer milling, colloid milling and vibro energy milling) can be used to improve the enzymatic hydrolysis of lignocellulosic materials (Taherzadeh *et al*. 2008). The power requirement of this pretreatment is relatively high depending on the final particle size and the biomass characteristics. Taking into account the high energy requirements of milling and the continuous rise of energy prices, it is likely that this process is not economically feasible (Hendriks *et al*. 2009).

Extrusion process is a novel and promising physical pretreatment method for biomass conversion to ethanol production. In extrusion, the materials are subjected to heating, mixing and shearing, resulting in physical and chemical modifications during the passage through the extruder. Screw speed and barrel temperature are believed to disrupt the lignocellulose structure causing defibrillation, fibrillation and shortening of the fibers, and finally, increasing accessibility of carbohydrates to enzymatic attack (Karunanithy *et al*. 2008). The different reactor parameters must be taken into account to achieve the highest efficiency in the process. In recent studies, the application of enzymes during the extrusion process is being considered as a promising technology for ethanol production.

2.2.3 Chemical pretreatments

2.2.3.1 Alkali pretreatments

Alkali pretreatment increases cellulose digestibility, solubilize lignin, and causes minor cellulose and hemicellulose solubilization than acid or hydrothermal processes (Carvalheiro *et al*. 2008). Alkali pretreatment can be performed at room temperature and times ranging from seconds to days. It is described to cause less sugar degradation than acid pretreatment and it was shown to be more effective on agricultural residues than on wood materials (Kumar *et al*. 2009a). Nevertheless, possible loss of fermentable sugars and production of inhibitory compounds must be taken into consideration to optimize the pretreatment conditions. Sodium, potassium, calcium and ammonium hydroxides are suitable alkaline pretreatments. NaOH causes swelling, increasing the internal surface of cellulose and decreasing the degree of polymerization and crystallinity, which provokes lignin structure disruption (Taherzadeh *et al*. 2008). NaOH has been reported to increase hardwood digestibility from 14% to 55% by reducing lignin content from 24–55% to 20% (Kumar *et al*. 2009a).

In the AFEX process, biomass is treated with liquid anhydrous ammonia at pretreatment temperatures in the range of 60-100°C for several times. Pressure is released rapidly resulting in a expansion of the ammonia gas that causes swelling and disruption of biomass structure and decrystallization of cellulose. AFEX has been reported to decrease cellulose crystallinity and disrupt lignin-carbohydrates linkages (Laureano-Perez *et al*. 2005). Small amount of the solid is solubilized during the pretreatment (Wyman *et al*. 2005a). Digestibility of biomass is increased after AFEX pretreatment due to deacetylation of hemicellulose and removes the acetyl groups from lignocellulosic materials (Kumar *et al*. 2009b; Galbe and Zacchi *et al*, 2007) and therefore the enzymatic hydrolysis results in greater yields. In the hydrolysis of pretreated biomass, cellulase and hemicellulases will be required due to the considerable remaining hemicellulose in the pretreated solid. The AFEX pretreatment is more effective on agricultural residues with limited effectiveness demonstrated on woody biomass and other high lignin feedstocks (Wyman *et al*. 2005a). More than 90% conversion of cellulose and hemicellulose to simple sugars achieved for different of lignocellulosic biomass (Teymouri *et al*. 2005).

Calcium hydroxide, also known as lime, has been widely studied. Lime pretreatment delignifies the biomass, although it increases its crystallinity. Addition of enzyme performance by enhancing lignin removal results in reducing non-productive adsorption sites for enzymes and increasing cellulose accessibility (Kim *et al*. 2006). Lime also removes acetyl groups from hemicelluloses reducing steric hindrance of enzymes and enhancing cellulose digestibility (Mosier *et al*. 2005b). Lime pretreatment has been proven successfully at temperatures from 85–150 ˚C and for 3–13 h with corn stover (Kim *et al.* 2006) or poplar wood (Chang *et al.* 2001). Pretreatment with lime has lower cost and less safety requirements compared with NaOH or KOH pretreatments and can be easily recovered from hydrolysate by reaction with CO_2 (Mosier *et al.* 2005b).

The addition of an oxidant agent (oxygen/H_2O_2) to alkaline pretreatment (NaOH/$Ca(OH)_2$) can improve the performance by favoring lignin removal (Carvalheiro *et al.* 2008). Ethanol yields of 0.33 g/g have been obtained in simultaneous saccharification and cofermentation (SSCF) processes with *Escherichia coli* FBR5 from wheat straw pretreated with alkali peroxide (Saha *et al.* 2006). Furthemore, no furfural or Hydroxymethylfurfural (HMF) were detected in hydrolysates obtained with alkaline peroxide pretreatment which favours the fermentation step in an ethanol production process (Taherzadeh *et al.* 2008).

2.2.3.2 Acid pretreatment

The main objective of the acid pretreatments is to solubilize the hemicellulosic fraction of the biomass and to make the cellulose more accessible to enzymes. This type of pretreatment can be performed with concentrated or diluted acid, but concentrated acid is less attractive for ethanol production due to equipment corrosion problems and acid recovery. The high operational and maintenance costs reduce the interest of applying the concentrated acid pretreatment at commercial scale (Wyman. 1996).

Diluted acid pretreatment appears to be a more feasible method for industrial applications and has been studied for pretreating a wide range of lignocellulosic biomass. Different types of reactors such as percolation, plug flow, shrinking-bed, batch and countercurrent reactors have been applied for the pretreatment of lignocellulosic materials (Taherzadeh *et al.* 2008). It can be performed at high temperatures (e.g. 180 ^{o}C) during a short period of time, or at lower temperatures (e.g. 120 ^{o}C) for longer retention time (30–90 min). The solubilized hemicellulose mainly consists of xylan, is also converted to fermentable sugars. Nevertheless, depending on the process temperature, some sugar degradation compounds such as furfural and HMF and aromatic lignin degradation compounds are detected, and affect the microorganism metabolism in the fermentation step (Saha *et al.* 2005). Nevertheless, this pretreatment generates lower degradation products than concentrated acid pretreatments. High yields from hydrolysis have been reported when pretreating lignocellulosic materials with diluted H_2SO_4 which is the most studied acid. Hydrochloric acid, phosphoric acid and nitric acid have also been tested (Mosier *et al.* 2005a). Saccharification yield as high as 74% was shown when wheat straw was subjected to 0.75% v/v of H_2SO_4 at 121 ^{o}C for 1 h (Saha *et al.* 2005). Olive tree biomass was pretreated with 1.4% H_2SO_4 at 210˚C resulting in 76.5% of hydrolysis yields (Cara *et al.* 2008). Recently, ethanol yield as high as 0.47 g/g glucose was achieved in fermentation tests with cashew apple bagasse pretreated with diluted H_2SO_4 at 121˚C for 15 min (Rocha *et al.* 2009).

Organic acids, such as fumaric or maleic acids, are appearing as alternatives to enhance cellulose hydrolysis for ethanol production. In this context, both acids were compared with sulfuric acid in terms of hydrolysis yields from wheat straw and formation of sugar degradation compounds during pretreatment. Results showed that organic acids can pretreat wheat straw with high efficiency although fumaric acid was less effective than maleic acid. Furthermore, less amount of furfural was formed in the maleic and fumaric acid pretreatments than with sulfuric acid (Kootstra *et al.* 2009).

2.2.3.3 Ozonolysis

Ozone is a powerful oxidant that shows high delignification efficiency (Sun *et al.*, 2002). This lignin removal increases the yields of the simple sugars as a result of enzymatic hydrolysis. The pretreatment is usually performed at room temperature and normal pressure and does not lead to the formation of inhibitory compounds that can affect the subsequent hydrolysis and fermentation. Ozonolysis has been applied on several agricultural residues, such as wheat straw and rye straw, increasing in both cases the enzymatic hydrolysis yield after ozonolysis pretreatment (Garcia-Cubero *et al.,* 2009). Despite some interesting results, further research has to be performed regarding ethanol production from lignocellulosic materials pretreated with ozone. An important drawback to consider is the large amounts of ozone needed, which can make the process economically unfeasible (Sun *et al* 2002).

2.2.3.4 Organosolv

Various organic or aqueous solvent mixtures can be utilized, including methanol, ethanol, acetone, ethylene glycol and tetrahydrofurfuryl alcohol, in order to solubilize lignin and provide treated cellulose suitable for enzymatic hydrolysis (Zhao *et al.* 2009a). Compared to other chemical pretreatments, the main advantage of the organosolv process is the recovery of relatively pure lignin as a by-product (Zhao *et al.* 2009a). In some studies, these mixtures are combined with acid catalysts (HCl, H_2SO_4, oxalic or salicylic) to break hemicellulose bonds. A high yield of xylose can usually be obtained with the addition of acid. However, this acid addition can be avoided for a satisfactory delignification by increasing process temperature (above 185 ^{o}C). High lignin removal (70%) and minimum cellulose loss (less than 2%) are achieved, as it has been demonstrated as a potential method for lignocellulosic (Papatheofanous *et al.* 1995). Removal of solvents from the system is necessary using appropriate extraction and separation techniques, e.g., evaporation and condensation, and they should be recycled to reduce operational costs. Solvents need to be separated because they might be inhibitory to enzymatic hydrolysis and fermentative microorganisms (Sun *et al.* 2002). The high commercial price of solvents is another important factor to consider for industrial applications. For economic reasons, among all possible solvents, the low-molecular weight alcohols with lower boiling points such as ethanol and methanol are favored.

2.2.3.5 Ionic liquids (ILs) pretreatment

The use of ILs as solvents for the pretreatment of cellulosic biomass has recently received much attention. ILs are salts, typically composed of large organic cations and small inorganic anions, which exist as liquids at relatively low temperatures, often at room temperature. Their solvent properties can be varied by adjusting the anion and the alkyl constituents of the cation. These interesting properties include chemical and thermal stability, non-flammability, low vapour pressures and a tendency to remain liquid in a wide range of temperatures (Hayes. 2009). ILs are called ‘‘green” solvents. Carbohydrates and can be dissolved in ILs via breaking the hydrogen bonding network of the polysaccharide by anion of the ILs. As a result, the intricate network of non-covalent interactions among biomass polymers of cellulose, hemicellulose, and lignin is effectively disrupted while minimizing formation of degradation products. However, most data showing the effectiveness of ILs has been developed using pure crystalline cellulose, and its applicability to a more complex combination of constituents in Lignocellulosic biomass requires further studies.

For the large-scale application of ILs, thedevelopment of energy efficient recycling methods for ILs is a prerequisite and should be investigated in detail. Toxicity to enzymes and fermentative microorganisms must be also studied before ILs can be considered a real option for biomass pretreatment (Yang *et al*. 2008; Zhao *et al*. 2009b). Depending on the amount of ILs residues remaining, significative negative effect on cellulase activity may be observed (Alvira *et al*. 2010). Thus, ILs residues removal would be required to prevent decrease of final sugars concentrations. In a pretreatment study using 1-ethyl-3-methyl imidazolium diethyl phosphate, the yield of reducing sugars from wheat straw pretreated with this ionic liquid at 130 ˚C for 30 min was 54.8% after being enzymatically hydrolyzed for 12 h (Li et al. 2009). The fermentability of the hydrolysates obtained after enzymatic saccharifications of the regenerated wheat straw was also evaluated. Results obtained using *Saccharomyces cerevisiae* indicated that wheat straw pretreated by this IL did not bring any negative effect on the growth of *S. cerevisiae* (Li *et al*. 2009). Further research is needed to improve the economics of ILs pretreatment before they can be applied at industrial scale. Technology is still expensive and commercial IL recovery methods have not been fully developed. In addition, techniques need to be developed to recover hemicellulose and lignin from solutions after extraction of cellulose

(Hayes. 2009). Despite of these current limitations, advanced research e.g. as potential synthesis of ILs from carbohydrates, may play a role in reducing their cost. Development of ILs pretreatment offers a great potential for future lignocellulose biorefinering processes.

2.2.4 Thermochemical pretreatments

2.2.4.1 Steam explosion: SO_2 steam explosion

Steam explosion is the most widely employed physico-chemical pretreatment for lignocellulosic biomass. It is a hydrothermal pretreatment in which the biomass is subjected to pressurized steam for a period of time ranging from seconds to several minutes, and then suddenly depressurized. This pretreatment combines mechanical forces and chemical effects due to the hydrolysis (autohydrolysis) of acetyl groups present in hemicellulose. Autohydrolysis takes place when high temperatures promote the formation of acetic acid from acetyl groups; furthermore, water can also act as an acid at high temperatures. The mechanical effects are caused because the pressure is suddenly reduced and fibers are separated owing to the explosive decompression. In combination with the partial hemicellulose hydrolysis and solubilization, the lignin is redistributed and to some extent removed from the material [Pan *et al.* 2005]. Removal of hemicelluloses exposes the cellulose surface and increases enzyme accessibility to the cellulose microfibrils. The most important factors affecting the effectiveness of steam explosion are particle size, temperature, residence time and the combined effect of both temperature (T) and time (t), which is described by the severity factor (R_o) [$R_o = t \times e^{[T-100/14.75]}$] being the optimal conditions for maximum sugar yield a severity factor between 3.0 and 4.5 [Alfani *et al.* 2000]. Higher temperatures result in an increased removal of hemicelluloses from the solid fraction and an enhanced cellulose digestibility, thought they also promote higher sugar degradation.

Steam explosion process offers several attractive features when compared to other pretreatment technologies. These include the potential for significantly lower environmental impact, lower capital investment, more potential for energy efficiency, less hazardous process chemicals and conditions and complete sugar recovery [Avellar *et al.* 1998]. Among the main advantages, it is an attractive process because it makes limited use of chemicals and low energy input with no recycling or environmental cost. It is remarkable that energy use for obtaining small chips size before pretreatment can make up one third of the power requirements of the entire process [Hamelinck *et al.* 2005]. Although low cost of the chemical is also advantages for steam explosion, however , the addition of an acid catalyst has been also used to increase cellulose digestibility and improve hemicelluloses hydrolysis [Sun *et al.* 2002]. Cost reduction and low energy consumption are required for effective pretreatment, for example, no particle size reduction and non-acid addition would be desirable to optimize the effectiveness on the process [Hamelinck *et al.* 2005]. Although acid addition (e.g. H_2SO_4) in steam explosion has been introduced for the solubilization of hemicellulose increasing and the optimal pretreatment temperature decreasing, however, a partial hydrolysis of cellulose has been observed [Brownell *et al.* 1986; Tengborg *et al.* 1998]. On the other hand, the main drawbacks when using acids are related to equipment requirements and higher formation of degradation compounds [Mosier *et al.* 2005b; Palmqvist *et al.* 2000]. In general, SO_2-catalyzed steam explosion is regarded as one of the most effective pretreatment method for softwood material [Tengborg *et al.* 1998].

Steam explosion technology has been proven for ethanol production from a wide range of raw materials as poplar [Oliva *et al.* 2003], olive residues [Cara *et al.* 2006], herbaceous residues as corn stover [Varga *et al.* 2004], and wheat straw [Ballesteros *et al.* 2006]. It has successfully performed with hardwoods and agricultural residues or herbaceous biomass but it is not very effective for softwoods due to its low content of acetyl groups in the hemicellulosic portion [Sun *et al.* 2002]. With the aim of maximizing sugar recoveries, some authors have suggested a two-step pretreatment [Tengborg *et al.* 1998]. In the first step, pretreatment is performed at low temperature to solubilize the hemicellulosic fraction, and the cellulose fraction is subjected to a second pretreatment step at temperatures higher than 210°C. It offers some additional advantages such as higher ethanol yields, better use of the raw material and lower enzyme dosages during enzymatic hydrolysis [Soderstrom *et al.* 2002]. Nevertheless, an economic evaluation is needed to determine the effectiveness of an additional steam explosion [Galbe *et al.* 2007].

The main drawbacks of steam explosion pretreatment are the partially hemicellulose degradation and the generation of some toxic compounds that could affect the following hydrolysis and fermentation steps [Oliva *et al.* 2003]. The toxic compounds generated and their amounts depend on the raw material and the harshness of the pretreatment. Hence, the necessity of using a robust strain in the subsequent fermentation step. The major inhibitors are furan derivatives, weak acids and phenolic compounds. The main furan derivatives are furfural and 5-hydroxymethyl furfural derived from pentoses and hexoses degradation, respectively. Both have been reported as inhibitors by the prolongation of the lag phase during batch fermentation [Palmqvist *et al.* 2000].

Weak acids generated during steam explosion are mostly acetic acid, formed from the acetic groups present in the hemicellulosic fraction, and formic and levulinic acids derived from further degradation of furfural and HMF. Wide range of phenolic compounds is generated due to the lignin breakdown varying widely between different raw materials. Several detoxification methods have been studied in order to reduce the inhibitory effect caused by these compounds on enzymes and yeasts.

2.2.4.2 Liquid hot water

Liquid hot water is a hydrothermal treatment that does not require rapid decompression and does not employ any catalyst or chemicals. Pressure is applied to maintain water in the liquid state at elevated temperatures (160–240°C) and toprovoke alterations in the structure of the lignocellulose. The objective of the liquid hot water is to solubilize mainly the hemicellulose, to make the cellulose more accessible and to avoid the formation of inhibitors. The slurry generated after pretreatment can be filtered to obtain two fractions: one solid cellulose-enriched fraction and a liquid fraction rich in hemicellulose derived sugars. To avoid the formation of inhibitors, the pH should be kept between 4 and 7 during the pretreatment because at this pH hemicellulosic sugars are retained in oligomeric form and monomers formation is minimized. Therefore LHW has the advantages of producing less by-products inhibitory to ethanologens and lower energy requirement [Mosier *et al.* 2005a]. Liquid hot water has been shown to remove up to 80% of the hemicellulose and to enhance the enzymatic digestibility of pretreated material in herbaceous feedstocks, such as corn stover [Mosier *et al.* 2005a], sugarcane bagasse [Laser *et al.* 2002] and wheat straw [Perez et al. 2008].

Two-step pretreatment has been studied to optimize hemicellulosic sugars recovery and to enhance enzymatic hydrolysis yields. Lignin is partially depolymerized and solubilized as well during hot water pretreatment but complete delignification is not possible using hot water alone, because of the recondensation of soluble components originating from lignin. The properties of water will be discussed from the macroscopic and microscopic point of view. It has been to be indicated that LHW at high temperature has macroscopic properties like a non-polar solvent, however, the single molecules are still polar. This seems to be a contradiction and has consequences for chemical reactions (Kruse *et al*, 2007)

The treatment system can be broadly categorized into batch-type and flow-type systems. Batch-type systems are not flexible enough to control treatment time, temperature, and pressure, and excessive decomposition of hydrolyzed products from cellulose inevitably takes place. On the other hand, a flow-type system can flexibly regulate these treatment conditions, and can hydrolyze cellulose without excessive decomposition. However, this system is not enough to regulate the decomposition pathway of cellulose in supercritical and subcritical water. Since the properties of supercritical water and subcritical water can be regulated by their temperature, pressure, and density, it is important to select the pressure and temperature for controlling the decomposition pathway of cellulose.

Flowthrough systems have been reported to remove more hemicellulose and lignin than batch systems to from some materials. Addition of external acid during the flow through process has been also studied but it is discussed if hemicellulose and lignin removal is increased with the acid addition [Wyman *et al.* 2005a]. In general, liquid hot water pretreatments are attractive from a cost-savings potential: no catalyst requirement and low-cost reactor construction due to low-corrosion from a cost- saving perspective. It has also the major advantage that the solubilized hemicellulose and lignin products are present in lower concentration, due to higher water input, and subsequently concentration of degradation products is reduced. In comparison to steam explosion, higher pentosan recovery and lower formation of inhibitors are obtained, however, water demand and energy requirement are higher, and the technology has not yet been developed to commercial scale.

Different pretreatment methods for lignocellulosic materials have been described and widely studied to improve ethanol production processes. All these methods should make the lignocellulose accessible to enzymatic reactions, where the crystallinity of cellulose, its accessible surface area, and lignin and hemicelluloses disposal are the main substrate-related factors affecting the enzymatic hydrolysis. Figure 1.2 shows the main advantages and disadvantages of the most promising pretreatment methods.

Liu *et al.* (2005) studied the pretreatment of corn stover to enhance hemicellulose sugar recovery and the enzymatic digestibility of cellulose using partial flow of liquid hot water. Performance of batch and flowthrough operations was compared for corn stover pretreated with liquid hot water at 200 °C. The results showed that flowthrough operation was achieved higher xylose sugar yields in the range of 84–89% and 46.6 % for batch pretreatment. Corn stover was pretreated by partial flow had higher enzymatic digestibility (88–90%) than batch operations (∼85%) at otherwise identical conditions, apparently due to much higher lignin removal for the former (40–45% for flowthrough operations and 10–12% for batch operations).

Yu *et al*. (2008) studied the pretreatment of rice straw by a liquid hot water process for enzymatic hydrolysis. The purpose of this study was to investigate the efficiency of HCW pretreatment on rice straw based on sugar production and inhibitor formation. HCW pretreatment was carried out at a temperature ranging from 140 to 240 °C for 10 or 30 min. The results showed that maximal production of total glucose was obtained at 180 °C and below accounted for 4.4–4.9% of glucan in raw material. Total xylose production peaked at 180 °C, accounting for 43.3% of xylan in raw material for 10-min pretreatment and 29.8% for 30-min pretreatment. The production of acetic acid increased at higher temperatures and longer treatment time and furfural production achieved the maximum (2.8 mg/ml) at 200 °C for both 10-min and 30-min processes. The glucose yield by enzymatic hydrolysis of pretreated rice straw was no less than 85% at 180 °C and above for 30-min pretreatment and at 200 °C and above for 10-min pretreatment.

Liu *et al*. (2003) investigated the effects of flow on the fate of hemicellulose, lignin and total mass using liquid hot water pretreatment of corn stover in a small tubular flowthrough system at 180, 200 and 220 ˚C. They found that hemicelluloses degradation increased with flow, especially at high temperatures. The dissolved xylan in the hydrolysate was mostly oligomers over this temperature range, and the fraction as oligomers increased with flow rate. Also of importance, lignin removal increased from less than about 30% for batch reactors to about 75% at high flow rates and was nearly linearly related to hemicellulose release for the flowthrough reactor.

Laser *et al*. (2002) studied the pretreatment of sugar cane bagasse with either liquid hot water (LHW) or steam using the same reactor. Solid concentration was varied from 1% to 8% for LHW pretreatment and was more than 50% for steam pretreatment. The reaction temperature and time ranged from 170 to 230 °C and 1 to 46 min, respectively. Key performance metrics, included fiber reactivity, xylan recovery, and the extent to which pretreatment hydrolysate inhibited glucose fermentation. The results showed that LHW pretreatment achieved ⩾80% conversion by simultaneous saccharification and fermentation (SSF), ⩾80% xylan recovery, and no hydrolysate inhibition of glucose fermentation yield. Combined effectiveness was not as good for steam pretreatment due to low xylan recovery. SSF conversion increased and xylan recovery decreased as xylan dissolution increased for both modes. SSF conversion, xylan dissolution, hydrolysate furfural concentration, and hydrolysate inhibition increased, while xylan recovery and hydrolysate pH decreased, as a function of increasing LHW pretreatment solids concentration (1–8%).

Hideno *et al.* (2009) used rice straw as feedstock in liquid hot water wet disk milling pretreatment without sulfuric acid for enzymatic hydrolysis. Glucose and xylose yields by wet disk milling, ball milling, and liquid hot water treatment were 78.5% and 41.5%, 89.4% and 54.3%, and 70.3% and 88.6%, respectively. Wet disk milling and liquid hot water treatment increased sugar yields without decreasing their crystallinity.

2.2.4.3 Liquid hot water reaction on cellulose

Liu and Wyman (2005) studied the effect of dilute sulfuric acid on the efficiency of flowthrough pretreatment processes of corn stover. The experiment was carried out with a flow rate of 10 mL/min and operating temperature 200 ˚C with enough volume (25.4 mm OD ×10.7 mm length, internal volume of 37.8 mL) to allow the use of 6.5 g of corn stover as the feedstock. Cellulose digestibility was about 85% for batch, 88–90% for partial flow, and 95% for flowthrough operation. The enhanced cellulose digestibility obtained with the flowthrough or partial flow operation is perhaps due to increased availability of cellulose to enzymatic attack, as higher proportions of hemicellulose, and particularly, lignin were removed.

Kabyemela *et al.* (1998) investigated the reaction pathways to evaluate the reaction kinetics of cellobiose in sub- and supercritical water conditions. The decomposition of cellobiose was via hydrolysis to glucose and pyrolysis to glycosylerythrose and glycosylglycolaldehyde, which further hydrolyzed to erythrose plus glucose, and glycolaldehyde plus glucose, respectively. The hydrolysis and pyrolysis rates for the general decomposition pathway of cellobiose were evaluated. The cellobiose hydrolysis rate was similar to the glycosylerythrose and glycosylglycolaldehyde hydrolysis rates. The pyrolysis rate of cellobiose decreased with pressure in the supercritical water conditions giving an increase in hydrolysis selectivity. This may be a result of the cage effect of the solvent around the transition species that suppresses the decomposition.

Sakaki *et al.* (2002) studied the saccharification of cellulose with liquid hot water at temperatures in the range of 300 to 400 °C in batch experiments. Cellulose was hydrolyzed with liquid hot water (HCW; ~310 °C, 9.8 MPa) using an HCW flow reactor. HCW was continuously delivered into a reactor charged with cellulose. The effluent from the reactor was cooled and separated into a water-soluble fraction (WS) and a water insoluble fraction which deposited after cooling (DP). Cellulose started to decompose into a water-soluble fraction (WS) and water in the soluble fraction, which was deposited after cooling (DP) when temperatures in HCW were delivered above 230 °C. The results suggested that cellulose was hardly solubilized when lignocellulosic biomass was treated with HCW at 190-230 °C. Therefore, they first examined the decomposition temperature of cellulose in HCW. HCW was delivered at 180 °C for 20 min, and then the HCW temperature was raised to 285 °C. The results are shown in Figure 3. Cellulose hardly decomposed at 180 °C and started to decompose to WS and DP when the temperature of the HCW reached around 230 °C. The integral decomposition yield of cellulose amounting to 79% and 21wt % of the unreacted cellulose remained in the reactor when the HCW temperature reached 285 °C.

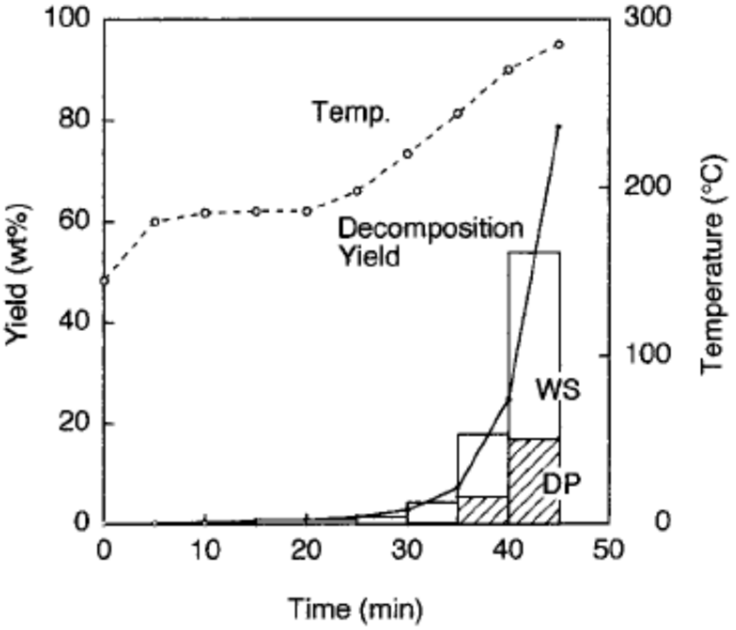


Figure 2.2 Relation between decomposition of cellulose and temperature of HCW. [Sakaki *et al*., 2002]

Ehara *et al* (2002) studied microcrystalline cellulose (avicel) between the batch-type and flow-type systems to elucidate the decomposition mechanisms of cellulose into supercritical water, and to explore the process to achieve a high yield of sugars. As a result, the flow-type system was able to liquefy avicel without producing any supercritical water-insoluble residue. Although hydrolyzed products such as glucose and fructose, and pyrolyzed products such as levoglucosan, 5-hydroxymethyl furfural, erythrose, methylglyoxal, glycolaldehyde and dihydroxyacetone were found in common from the water-soluble portion treated by both systems, the flow-type system gave a water soluble portion with more hydrolyzed and less pyrolyzed products, together with water-soluble oligosaccharides consisting of cellobiose to cellododecaose and their decomposed products at their reducing end of glucose, such as [β-D-glucopyranosyl]$_{1–11}$, β -D-levoglucosan, [β -D-glucopyranosyl]$_{1–11}$, β-D-erythrose and [β-D-glucopyranosyl]$_{1–11}$, β-D-glycolaldehyde. In addition, the precipitates of polysaccharides were recovered after 12 h setting of the water-soluble portion. These results indicated that the flow-type system can hydrolyze cellulose with minimizing pyrolyzed products. On the other hand, the batch-type system resulted in a higher yield of the pyrolyzed products due to the longer treatment, but a higher yield of glucose due possibly to the higher pressure and concomitantly higher ionic product of water. Based on these lines of evidence, the process to increase the yield of the sugar is discussed under supercritical water treatment.

Ehara *et al.* (2005) compared the decomposition of cellulose between supercritical water (400°C, 40MPa) and subcritical water (280°C, 40MPa) treatments to elucidate the difference in their decomposition behavior. The supercritical water treatment was found to be more suitable for obtaining high yields of hydrolyzed products. However, cellulose was found to be more liable to fragment under supercritical water treatment, resulting in a decrease in the yield of hydrolyzed products. On the contrary, cellulose was found to be liable to more dehydration in the subcritical water treatment. Based on these results, they have proposed the combined process of short supercritical water treatment followed by subcritical water treatment so as to inhibit fragmentation.

Saka *et al.* (1999) developed and designed the supercritical water biomass conversion system in a laboratory. The reaction vessel with cellulose sample was treated with this system at supercritical state of water for a designated period (3–105 s) under the conditions of a tin bath temperature of 500˚C and pressure of 35 MPa. The recovered products of hydrolysates were then analyzed by high performance liquid chromatography (HPLC). The obtained results indicated that a high amount of glucose and levoglucosan can be achieved from both celluloses I and II for 5–10s supercritical treatment, while that from starch for 3–5 s treatment. Although this difference could be due to a difference in the molecular structure between cellulose and starch, a difference between celluloses I and II was not significant. Instead, an accessibility of the water towards cellulose molecules seemed to be significant for their chemical conversion. With the longer treatment, amounts of these compounds observed were decreased due to decomposition. Therefore, it may be concluded that, compared with acid hydrolysis or enzymatic saccharification, cellulose may be hydrolyzed to glucose and its derivatives more or less to the same degree as in corn starch under supercritical state. This finding suggests that the supercritical treatment can overcome the difficulties in hydrolyzing cellulose to glucose, found in the acid hydrolysis or enzymatic saccharification techniques

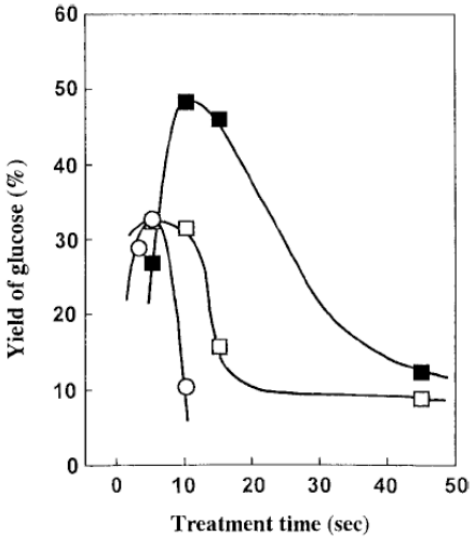


Figure 2.3 The change of glucose yield from Avicel (cellulose I), Lyocell (cellulose II) and starch after supercritical water treatment: □, cellulose I; ■, cellulose II; ○, starch. [Saka and Ueno, 1999]

Ishikawa *et al* (2001) studied the chemical conversion of cellulose as treated in supercritical methanol using a batch-type reaction vessel at temperatures from 220 to 450°C and pressures from 14 to 72MPa. Supercritical methanol treatment at 350°C and 43MPa for 7 min was sufficient to convert microcrystalline cellulose (avicel) to the methanol-soluble. To study the kinetics of the decomposition of cellulose, the decomposition rate constants were obtained, and rapid increase was observed at about 270°C which was about 30°C higher than the critical temperature of methanol. The main products from cellulose decomposition were methylated cellotriose, methylated cellobiose, methyl α-D-glucosides and β-D-glucosides, levoglucosan and 5-hydroxymethylfurfural. Monomeric compounds such as methyl α-D-glucosides and β-D-glucosides were stable in supercritical methanol, allowing high yields of monomeric products by supercritical methanol treatment. Based on these results, a pathway of cellulose decomposition treated in supercritical methanol was proposed. These findings suggest that the supercritical methanol treatment of various cellulosic materials may be suitable to obtain useful chemicals and liquid fuels without using fossil resources.

2.2.4.4 Liquid Hot Water Reaction on Hemicellulose

Jacobsen *et al.* (2002) reported on the pretreatment by liquid flow past solid increases hemicellulose removal yields particularly at higher flow rates the effect of varying sugarcane bagasse concentrations on xylose monomer and oligomer yields, which were experimentally measured in a batch reactor without adding acids or other chemicals at 200 °C. A greater drop in pH was observed at higher solids concentrations, as anticipated. Furthermore, only about 7-13% of the total xylose recovered in solution was as monomers at the maximum total xylose yield point, with the rest being oligomers, and although monomer yields could be increased at longer hold times, overall yields declined. These results and the general yield versus time profiles are consistent with the predictions of first-order models.

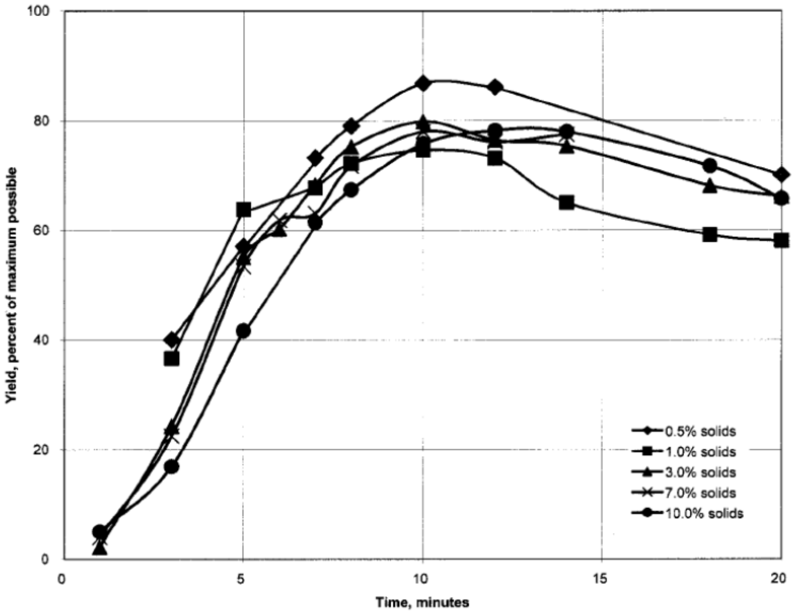


Figure 2.4 Total soluble xylose yield as monomers and oligomers versus reaction time for batch hydrolysis of sugarcane bagasse with varying solids concentrations without acid addition at 200 °C. [Jacobsen *et al.* 2002].

Liu *et al.* (2003) studied the effects of flow on the fate of hemicellulose, lignin, and total mass for hot-water pretreatment of corn stover in a small tubular flowthrough reactor at 180, 200, and 220 °C. Solubilization of hemicellulose increased with flow, especially at high temperatures; a result that is inconsistent with traditional first-order kinetic models. The dissolved xylan in the hydrolyzate was mostly oligomers over this temperature range, and the fraction as oligomers increased with the flow rate. Also of importance, lignin removal increased from less than about 30% for batch reactors to about 75% at high flow rates and was nearly linearly related to hemicellulose release for the flowthrough reactor. These observations suggest that mass transfer or other physical factors, and not strictly first-order homogeneous chemical kinetics, impact hemicellulose hydrolysis. In addition, lignin appears to be released throughout hydrolysis, but its fate may be governed by subsequent precipitation reactions unless it is removed first.

Perez *et al*. (2008) studied the optimization of process variables (temperature and residence time) in liquid hot water (LHW) pretreatment of wheat straw for ethanol production was addressed by means of the design of experiments. They studied the recovery of hemicellulose-derived sugars (HDS) in the liquid fraction and the enzymatic susceptibility of the solid residue obtained after filtration of pretreated material were considered as response variables to different processes conditions. Results show that when optimization was performed considering response variables jointly, optimal conditions were 188 ˚C and 40 min, leading to HDS recovery yield of 43.6% of HDS content in raw material and enzymatic hydrolysis (EH) yield of 79.8% of theoretical. Nonetheless, after response variables were analyzed separately, 71.2% of HDS recovery was obtained at 184 ˚C and 24 min whereas conditions of 214 ˚C and 2.7 min led to a maximum EH yield of 90.6% of theoretical ethanol yield. So, a two-step pretreatment would be the most adequate process configuration to get a maximum recovery of fermentable sugars although economic and energetic aspects of such approach should be considered.

2.2.4.5 Decomposition Behavior of Plant Biomass in Liquid Hot Water

For the conversion of lignocellulosics into energy and chemicals, the saccharification of cellulose and hemicelluloses followed by fermentation is one of the methods to obtain ethanol, which can be used not only as a useful chemical, but also as a liquid fuel. The two major methods for hydrolysis of lignocellulosics are by the use of acid and enzyme. Lignocellulosics can be separated to carbohydrate-derived and lignin-derived products by supercritical water treatment. The carbohydrate fraction mainly consists of polysaccharides, oligosaccharides, monosaccharides, and their decomposed products, are an appropriate source of ethanol fermentation which mainly consists of monomeric and oligomeric lignin derived products, may be useful as alternatives to aromatic chemicals from fossil fuel resources. To realize this process, the treatment conditions must be improved and optimized to produce a high yield of fermentable sugars. Thus, optimization of liquid hot water reactions has been attempted by developing various treatment systems with appropriate treatment time, temperature, and pressure.

Ando *et al.* (2000) studied the decomposition behaviors of bamboo, chinquapin (hardwood), and Japan cedar (softwood) in liquid hot water, which were examined using a HCW flow type reactor to achieve the maximum utilization of plant biomass. HCW was passed through the reactor (3.6 mL) loaded with the biomass sample powder (177-250 μm) at a flow rate of 10 mL/min under 9.8 MPa. The temperature of HCW was maintained at 180 °C for 20 min, after which it was raised to about 285 °C at a rate of 5 °C /min and maintained at 285 °C for about 7 min. By these operations, free sugars, some of lignin and most of hemicellulose, were first solubilized in water and flowed out during the initial 20 min flow of HCW. Cellulose started to decompose when the temperature of HCW was over 230 °C. The orders of elution of these components were common for three biomass samples, although the amount of products obtained during the heat-up period up to 180 °C and that of residue which finally remained in the reactor differed with the samples.

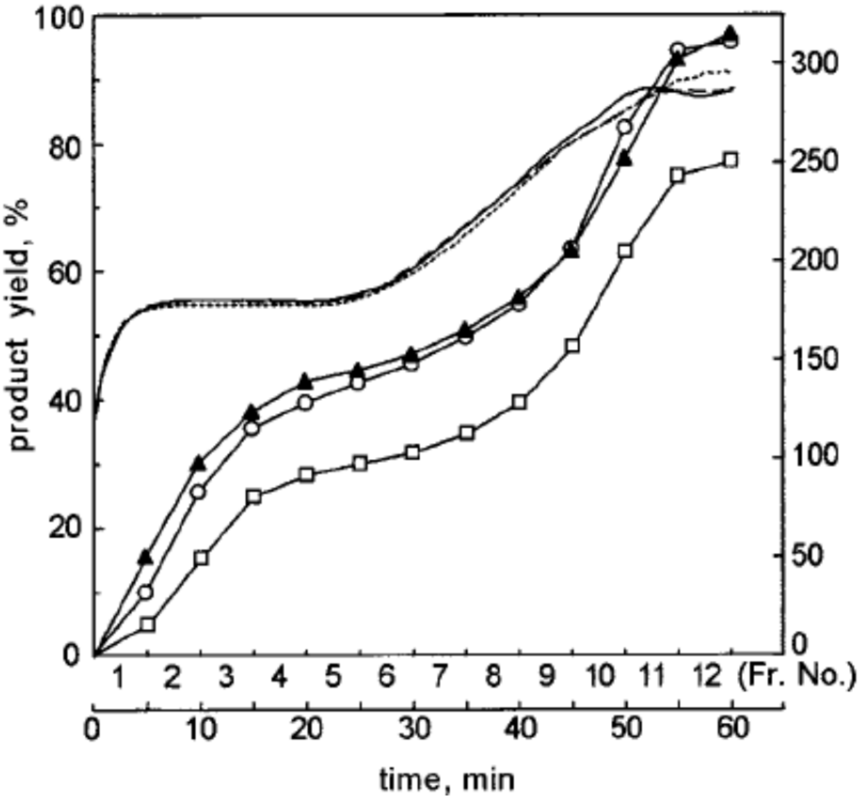


Figure 2.5 Relationship between the accumulated yield of the product (○, bamboo; ▲, chinquapin; □, Japan cedar) and the temperature of HCW (−, bamboo; - - -, chinquapin; Japan cedar) [Ando *et al.* 2000].

Recently, there has been an increased interest in using biomass as a source of hydrogen fuel. Several authors [Yu et al. 1993; Xu et al. 1996; Cortright et al. 2002) have investigated the hydrothermal gasification of biomass. Most of these studies have used glucose as a model compound, chiefly because it is the main building block of biomass and because it is water soluble. Cortright et al. have reported that hydrogen can be produced from sugars at temperatures near 227 °C in an aqueous-phase reforming process using a platinum-based catalyst. Several biomass-derived materials that are soluble in water have been used for the aqueous hydrogen generation process. Since hydrothermal gasification of biomass to hydrogen necessitates using catalytic materials, it is important to produce water–soluble products from biomass so that these products will be compatible with the heterogeneous catalytic gasification.

Table 2.1 Summary of the advantages and disadvantages with different methods for pretreating lignocellulosic biomass.

Pretreatment method	Advantages	Disadvantages

Biological	- Degrades lignin and hemicellulose - Low energy consumption	- Low rate of hydrolysis
Milling	- Reduces cellulose crystallinity	- High power and energy consumption
Steam explosion	- Causes lignin transformation and hemicellulose solubilization - Cost-effective - Higher yield of glucose and hemicellulose	- Generation of toxic compounds - Partial hemicellulose degradation
AFEX	- Increases accessible surface area - Low formation of inhibitors	- Not efficient for raw materials with high lignin content - High cost of large amount of ammonia
CO_2 explosion	- Increases accessible surface area - Cost-effective - Do not imply generation of toxic compounds	- Does not affect lignin and hemicelluloses - Very high pressure requirements
Wet oxidation	- Efficient removal of lignin - Low formation of inhibitors - Minimizes the energy demand (exothermic)	- High cost of oxygen and alkaline catalyst
Ozonolysis	- Reduces lignin content - Does not imply generation of toxic compounds	- High cost of large amount of ozone needed
Organosolv	- Causes lignin and hemicellulose hydrolysis	- High cost - Solvents need to be drained and recycled
Concentrated acid	- High glucose yield - Ambient temperatures	- High cost of acid and need to be recovered - Reactor corrosion problems - Formation of inhibitors
Diluted acid	- Less corrosion problems than concentrated acid - Less formation of inhibitors	- Generation of degradation products
NaOH	- Low pretreatment temperature - Generated lower amount of inhibitory - Easily to handle	- Less effective on biomass which higher lignin content - Utilizes a huge amount of water for washing salts of alkali
$Ca(OH)_2$	- Inexpensive reagent - Easily handled - Low pretreatment temperature	- Very limited solubility - Longer pretreatment times - Higher quantities of water for pretreatment
LHW	- Low temperature - Less formation of inhibitors	- Amount of solubilized product is higher - Large volume of water involved
Aqueous ammonia	- High selectivity for reaction with lignin - Short retention time	- Ineffective of high lignin containing biomass - Negative impact on environment for industrial scale application

Among the different methods, chemical and thermochemical are currently the most effective and include the most promising technologies for industrial applications. The combination of different pretreatments has been also considered and might be interesting for obtaining the optimal fractionation of the different components and reach very high yields.

2.3 Biomass hydrolysis

Biomass hydrolysis is required in order to change the structure of cellulosic materials and to make cellulose more accessible to the enzymes, which convert the carbohydrate polymers into fermentable sugars. During the hydrolysis of lignocellulosic materials, many by-products can be formed that act as inhibitors for enzymatic hydrolysis and cellular growth. In addition, biomass hydrolysis is one of the most expensive processing steps for biofuel production. Therefore, selection of the best method for a certain raw material is very important. There are a lot of hydrolysis methods for lignocellulosic materials, but each method has advantages and disadvantages depending on feedstock. The main differences in the hydrolysis can be performed by acid hydrolysis, enzymatic hydrolysis and thermochemical hydrolysis.

2.3.1 Acid hydrolysis

Regardless of the process, biomass feedstocks must be broken or shredded into particles small enough to permit diffusion of heat, acid, and/or enzymes into their structures. For dilute and concentrated acid hydrolysis, fractionation to particle sizes on the order of chips used in commercial pulping for papermaking is satisfactory. In dilute acid hydrolysis, dilute acid, e.g. 1.5% sulfuric acid, is added and the mixture heated to moderate temperatures (140–160˚C (280–320˚F)), thereby releasing most hemicellulosic (C_5 and C_6) sugars. Cellulosic sugar (glucose, C_6) is then isolated by raising reactor temperatures to 200–240˚C (390–460˚F). The elevated temperature readily frees glucose, but degrades some freed sugars. Substantial reductions in overall sugar yields can occur, and large proportions of C_5 sugars are degraded and rendered unavailable for fermentation to ethanol or use as co-products. In addition, degradation products inhibit organisms/enzymes used for fermentation. Recent research suggests that a more desirable alternative involves eliminating the high temperature step, and coupling the first low temperature step with enzymatic hydrolysis [Yu *et al.* 2008].

Concentrate-acid processes enable the hydrolysis of both hemicelluloses and celluloses. The solubilisation of polysaccharides is reached using different acid concentrations, like 72% H_2SO_4, 41% HCl or 100% TFA [Fengel *et al.* 1983]. HCl and TFA have the advantage to be easier recovered. Concentrate-acid based processes have the advantage to allow operating at low/medium temperatures leading to the reduction in the operational costs. The formation of degradation products is low, although its formation rate can be severely affected by slight changes in temperature [Camacho *et al.* 1996]. One of the key steps of these pre-treatments is the acid recovery, which is mandatory for their economical viability [Goldstein. 1983] Moreover, the equipment corrosion is an additional disadvantage. Nevertheless, there seems to be a renewable interest in these processes [Zhang *et al.* 2007] owing to the moderate operation temperatures and because no enzymes are required.

2.3.2 Enzymatic Hydrolysis

Enzymatic hydrolysis is based on the same principles of biomass microbial decomposition as an integral part of the global carbon cycle. The research on the mechanism of the microbial degradation of cellulose has provided the conceptual framework for an enzymatic conversion plant. Some researcher first suggested that an enzyme known as $C1$ decrystallizes the cellulose, followed by a consortium of hydrolytic enzymes, known as Cx, which breaks down the cellulose to sugar. Further research revealed that enzymes work in a more sophisticated way. There are three major classes of cellulase enzymes: (1) endoglucanases, which act randomly on soluble and insoluble glucose chains, (2) exoglucanases, which include glucanhydrolases that preferentially liberate glucose monomers from the end of the cellulose chain and cellobihydrolases that preferentially liberate cellobiose from the end of the cellulose chain, and (3) *β*-glucosidases, which liberate D-glucose from cellobiose dimmers and soluble cellodextrins. These enzymes work together synergistically in a complex interplay for efficient decrystallization and hydrolysis of native cellulose [Yu *et al.* 2008].

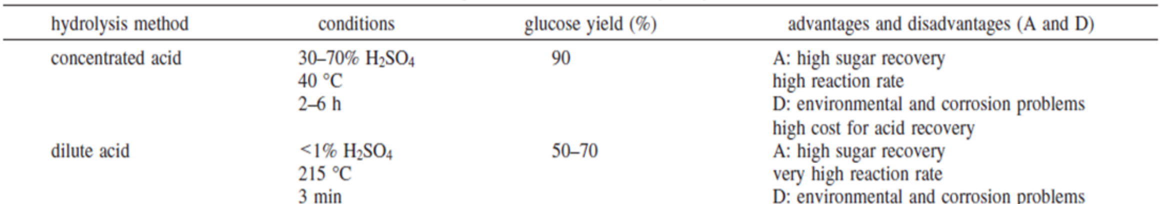
At present, an economically viable enzymatic hydrolysis process for lignocellulosic materials is hindered by several technical problems: (1) Although a high yield (75–95%) of glucose can be potentially achieved, enzymatic hydrolysis reactions are much slower than acid hydrolysis, requiring days rather than hours or minutes for completion. (2) Lignocellulose is difficult to convert into sugars by enzymes because of its high crystallinity, low surface area, and heterogeneous nature, as well as cellulose protection by lignin and sheathing by hemicellulose. An effective pretreatment step seems to be necessary to break the lignin seal and reduce cellulose crystallinity. (3) Cellulase enzymes are expensive to produce and have a very low specific cellulase activity. (4) The hydrolysis reactions also suffer from end-product inhibition by sugar and time-dependent loss of cellulase activity. (5) Thermal inactivation limits the efficiency of cellulase recycling. Despite these disadvantages, enzymatic hydrolysis is promising for ethanol production from lignocellulosic biomass because of its ability to produce a high yield of relatively pure glucose without the generation of glucose degradation products and mild reaction conditions [Yu *et al*. 2008].

2.3.3 Thermochemical hydrolysis

The thermo-chemical conversion processes have two basic approaches. The first is the gasification of biomass and its conversion to hydrocarbons. The second approach is to liquefy biomass directly by high-temperature pyrolysis, high-pressure liquefaction, ultrapyrolysis, or supercritical extraction. These processes convert the biomass into energy-rich useful products. The choice of conversion process depends upon the type and quantity of biomass feedstock, the desired form of the energy, i.e. end use requirements, environmental standards, economic conditions and project specific factors. Different thermo-chemical conversion processes include combustion, gasification, liquefaction, hydrogenation and pyrolysis. Although pyrolysis is still under developing stage but during current energy scenario, pyrolysis has received special attention as it can convert biomass directly into solid, liquid and gaseous products by thermal decomposition of biomass in the absence of oxygen. Pyrolysis offers efficient utilization of particular importance for agriculture countries with vastly available biomass by-products.

Table 2.2 shows a comparison of the process conditions and performance of various hydrolysis methods. Dilute acid hydrolysis achieves a sugar yield of 50–70%. At present, enzymatic hydrolysis can obtain a sugar yield of 75–85% and a projected yield of 85–95% with further research. Although acid processes are matured technologies, enzymatic processes have comparable costs and the potential of future cost reductions as technology improved. HCW technology is considered to be a green technology. However, the current sugar yield is still too low for biomass hydrolysis in HCW; hence, further research is needed. Each method has its own advantages and disadvantages. It is of great importance to choose a suitable method for biomass of difference types. For example, the concentrated acid may be favored when there is significant variability in feedstock characteristics, such as municipal solid waste (MSW) [Yu *et al*. 2008].

Table 2.2 Comparisons of different hydrolysis methods [Yu *et al*. 2008].



hydrolysis method	conditions	glucose yield (%)	advantages and disadvantages (A and D)
concentrated acid	30–70% H_2SO_4 40 °C 2–6 h	90	A: high sugar recovery high reaction rate D: environmental and corrosion problems high cost for acid recovery
dilute acid	<1% H_2SO_4 215 °C 3 min	50–70	A: high sugar recovery very high reaction rate D: environmental and corrosion problems

2.4 Biomass fractionation

Efficient separation of constitutive biomass components and the cost barrier is one of the major obstacles to the efficient utilization of renewable resources. However, such separation is mandatory for using sustainable derived plant (renewable) resources as feedstocks for chemicals and materials, which are currently obtained from fossil carbon resources. The extraction of lignin and noncrystalline carbohydrates (hemicellulose or polyoses) from wood is commercially practiced by the pulp and paper industry. However, while the paper industry has managed to define conditions under which cellulose-rich pulp fibers could be isolated and purified efficiently, the process has failed to achieve efficient fractionation performance by wasting (through underutilizing the noncellulosic biomass as process fuel) half of the plant resource. Most fractionation processes are based on the use of physicochemical pretreatment that increased the reactivity of the lignocellulosic structure, followed by one or more separation and purification stages to isolate products.

Currently, there have been very limited reports on single or multi-step thermochemical biomass polymer fractionation aiming for the separation of intact biomass polymers. Most research has focused on the effect of thermochemical processes on biomass pretreatment to improved digestibility, which resulted in the degradation of some biomass components. Most studies focused on wood while fractionation of wheat straw has been paid less attention despite the magnitude of the annual production of straw. Research on wheat straw fractionation has been centered on the pretreatment by dilute acid hydrolysis or autohydrolysis. Complete fractionation sequences of wheat straw components combined steam pretreatment at low temperature to extracted hemicellulose followed by ethanol at high temperature with delignification. [Hongzhang *et al.* 2007].

Rowland *et al.* (1994) studied delignification on an acid-detergent fiber pre-extraction using grass, straw, heather and spruce litter. The results showed that sulphuric acid hydrolysed cellulose component with 72% yield while dissolve most of the hemicellulose and offered a method to separate the residual alpha-cellulose fraction.

Sasaki *et al.* (2002) pretreated bagasse by hydrothermal treatment using a semi-batch reactor to develop a new biomass fractionation method that has a low impact on the environment. It was found that lignin and hemicellulose could be mostly extracted as a water-soluble fraction at 200-230˚C. Cellulose fraction was hydrolyzed in the temperatures range of 230–280 °C or recovered as solid residue from this treatment. Crystal structure and the chemical composition of the residue were in good accordance with those of untreated crystalline cellulose.

Allen *et al.* (1996) investigated the fractionation of sugar-cane bagasse (10-15 g oven dry basis) without size reduction using liquid hot water (190-230˚) for 45 s – 45 min. They found that more than 50% of the lignocellulosic biomass was solubilized. Hemicellulose was solubilized more than 60%, while cellulose could be solubilized less than 10%. After a mild posthydrolysis, recovery of the hemicellulose as monomeric sugars exceeded 80%. Less than 5% of the hemicellulose was converted to furfural.

Black *et al.* (1988) developed a method on separating lignocellulosic material into lignin, cellulose and dissolved sugars. Herbaceous biomass was digested at elevated temperatures in a single-phase mixture of alcohol, water and a water-immiscible organic solvent e.g. a ketone. The amount of water or organic solvent was adjusted after digestion leading to phase separation. The lignin was present in the organic solvent while the cellulose was present in a solid phase and the aqueous phase contained hemicellulose and dissolved sugars. The results showed that the kason lignin from poplar wood chip, had 88% purity. Dissolved sugars mainly from hemicellulose were contained in the combined alcohol-aqueous fraction at18% yield based on the wood charged. For the sugarcane bagasse the yield of lignin was 32% and yield of hemicellulose was 19%.

Hasegawa *et al.* (2004) developed a separation process for hemicellulose, cellulose, and lignin from oil palm shell, Japanese apricot tree using a thermochemical approach. Biomass treated in hot water at 180 °C was extracted in a flowing stream of water/acetone mixture under 10 MPa at 230 °C. Through the hot water treatment, hemicellulose in biomass was successfully recovered as saccharides, leaving lignin and cellulose as a solid.

Through the sequential extraction by the water/acetone solvent, lignin was depolymerized into the water/acetone-soluble compounds and the residual cellulose was partly dehydrated. The other method is a one-step process, in which biomass was directly extracted in 50% water/acetone solution at 200 °C using a batch reactor, and the residue was pure cellulose.

Bozell *et al.* (2011) developed a biomass fractionation process (Clean fractionation, CF) for the separation of lignocellulosic raw material into cellulose, hemicellulose, and lignin. The lignocellulosic material is separated with a ternary mixture of methyl isobutyl ketone, ethanol and water in the presence of an acid promoter, which selectively dissolves lignin and hemicellulose, leaving cellulose as an undissolved solid. The resulting single phase liquor is treated with water giving an organic phase containing lignin and an aqueous phase containing hemicellulose. For woody feedstocks, the yield of the cellulose fraction across all separations averaged 47.7 wt% (±1.1). Representative separations gave cellulose fractions with average Klason lignin contents of 2.0% at acid concentrations of 0.1 M H_2SO_4 or greater. Little or no galactose, mannose or arabinose is observed in the cellulose, and at an acid concentration of 0.2 M, average xylose contents as low as 0.22% were observed. The average glucan contents for representative cellulose samples of 92.7% were observed, and rose as high as 98.2% for separations using 0.2 M H_2SO_4. Glucan contents as high as 97% were also observed if the cellulose was bleached using either a QPD (Bleaching sequence; Q= chelation, P = peroxide, D = ClO_2) or QPDE (Bleaching sequence; Q= chelation, P = peroxide, D = ClO_2, E = hot extraction) sequence. The average yield of the lignin fraction was 18.3 wt%. Representative lignin samples gave an average Klason lignin value of 91% with selected lignin samples exhibiting residual sugar levels of <0.5%. The aqueous hemicellulose fraction contains a higher level of non-sugar components, but can be purified by ion exchange chromatography.

Cybulska *et al.* (2012) modified a clean fractionation process using ethyl acetate-ethanol-water mixture to separate lignin, hemicellulose, and cellulose fractions. Different proportions of constituents in the solvent mixture were tested. In order to improve lignin recovery, the process was catalyzed with sulfuric acid. Optimization was performed for fractionation processing conditions using lignin recovery and glucose yields as primary response variables. Optimal conditions resulted in a 51.33% lignin recovery, 53.82% hydrolysis glucose yield and 33.73% xylose yield in the aqueous fraction. To improve cellulose digestibility, solid fraction was subjected to hydrothermal post-treatment, for which optimal processing conditions were found, resulting in 78.93% hydrolysis glucose yield.

Monteil-Rivera *et al*. (2012) explored the potential applicability of microwaves to isolate lignin from triticale straw. A central composite design (CCD) was used to optimize the processing conditions for the microwave (MW)-assisted extraction of lignin from triticale straw. Maximal lignin yield (91%) was found when using 92% EtOH, 0.64 N H_2SO_4, and 148°C. The yield and chemical structure of MW-extracted lignin were compared to those of lignin extracted with conventional heating. Under similar conditions, MW irradiation led to higher lignin yields, lignins of lower sugar content, and lignins of smaller molecular weights. Except for these differences the lignins resulting from both types of heating exhibited comparable chemical structures.

CHAPTER 3
METHODOLOGY

3.1 Raw materials

All agricultural residues were obtained locally in Thailand. Sugarcane bagasse (BG) was from Phu Kiaew Bioenergy, Chaiyapoom province. Rice straw (RS) was collected from a local field in Pathumthani province. Corn stover (CS) was from Suwan farm, Nakorn Ratchasrima. Empty palm fruit bunch (EPFB) was from Suksomboon Palm Oil, Co. Ltd., Cholburi. The biomass was physically processed using a cutting mill (Retsch SM2000) and sieved to isolate particles of diameter of 250–420 μm. The processed biomass was then used as starting material for experiments. Chemical compositions (percent lignin, cellulose, hemicelluloses, and ash) were analyzed using the standard NREL method [Sluiter *et al*. 2008].

3.2 Autohydrolysis pretreatment

Pretreatment was performed in a multireactor system consisting of a 6×50 mL reactor in a temperature-controlled jacket with vertical shaking system to provide optimal mixing. The individual stainless steel reactor vessel was installed with a thermocouple to record the temperature inside the reactor. The biomass was pretreated under a set of conditions with varying temperatures (160–200 °C) and residence times (5, 10, and 20 min), while substrate loading was fixed at 10 % (w/v) and the initial pressure was at 25 bars under nitrogen. The reactor was quenched in a water bath after heating at the desired conditions. The pretreated solid biomass was separated from the liquid fraction by filtration and then thoroughly washed with distilled water on a Buchner funnel. The sample was dried at 60 °C to constant weight before being subjected to enzymatic hydrolysis to evaluate the improvement on biomass digestibility.

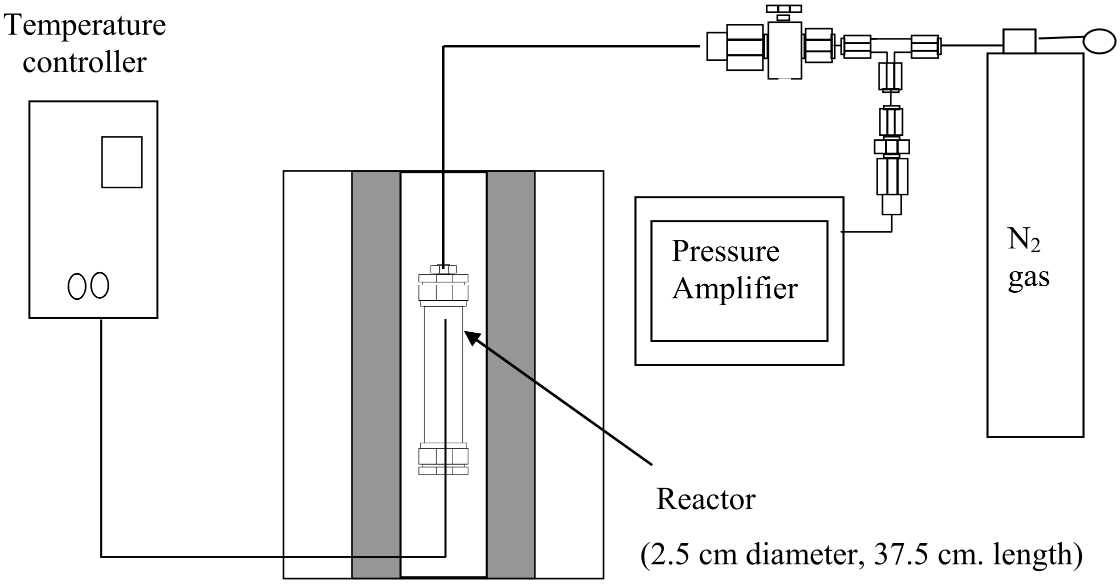


Figure 3.1 The experimental set-up for LHW system

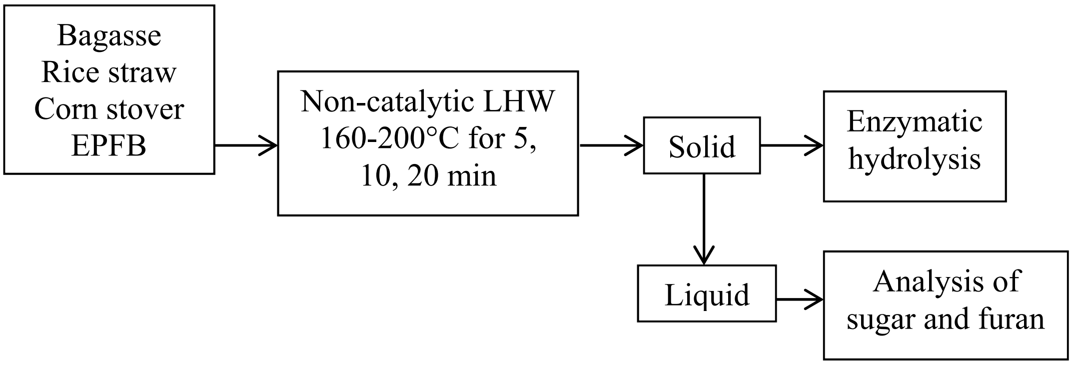


Figure 3.2 Process flow for non-catalytic LHW pretreatment of agricultural residues

3.3 Catalytic liquid hot water pretreatment

The pretreatment process was studied in a stainless steel reactor (2.5 cm diameter and 37.5 cm in length with the wall thickness of 2 mm and the total volume of 50 mL). The individual reactor vessel was installed with a thermocouple to measure the actual temperature inside the reactor. The standard reaction contained 2 g of rice straw and 20 mL of distilled water with 0.25% (v/v) acid (H_2SO_4, H_3PO_4, HCl, and oxalic acid) or 0.25% (w/v) alkaline (NaOH) as a catalyst. Nitrogen was flowed into the reactor for purging and adjusting the initial pressure to 20 bars. The reactors were placed in furnace slots in a reactor system consisting 6 x 50 mL-reactor in a temperature-controlled jacket with vertical shaking system to provide optimal mixing. The reaction was co-heated to 140-180°C for 5-20 min. Uniformity in heating could be achieved due to the relatively small reactor size. There was no significant difference between the temperature measured inside the reactor and the furnace temperature. The reaction was quenched in a water bath after heating under the desired conditions. The solid cellulose fraction was separated by filtration on filter paper using a Bushner funnel and washed with distilled water and dried at 60°C to constant weight before being subjected to enzymatic hydrolysis. The liquid fraction was collected for the analysis of sugar and inhibitory by-products by HPLC.

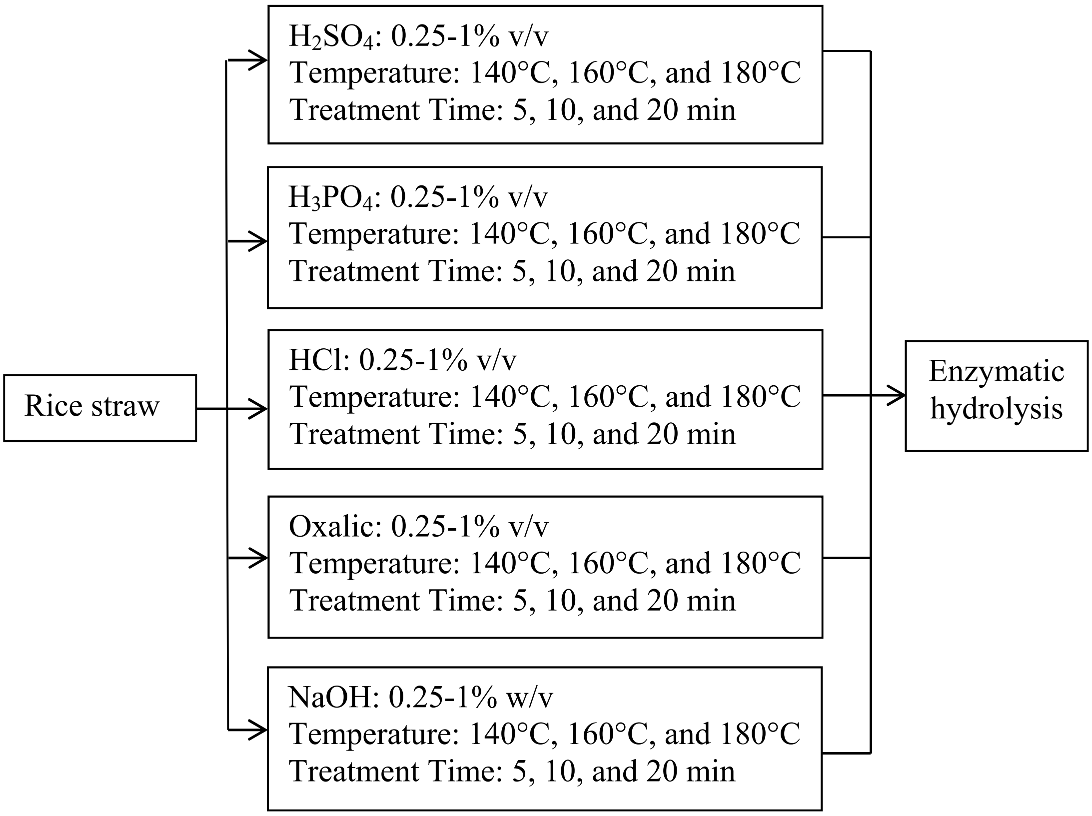


Figure3.3 Process conditions for LHW pretreatment of rice straw

Figure 3.4 The experimental set up apparatus: (A) Furnace and (B) Reactor

3.4 Enzymatic hydrolysis

The pretreatment efficiencies were assessed based on the digestibility of the pretreated solid residues using a commercial enzyme mixture. The hydrolysis reactions of 1-mL total volume contained 5% (w/v) pretreated substrate with 10 filter paper unit (FPU)/g Trichoderma reesei cellulase (CelluclastTM 1.5 L, Novozymes AS, Bagvaerd, Denmark) supplemented with 330 IU/g Aspergillus niger β-glucosidase (Novozym 188, Novozymes AS) and 120 IU/g based on endo-xylanase activity of Humicola inosolens hemicellulase (Optimash® BG, Danisco AS, Copenhagen, Denmark) in 50 mM sodium acetate buffer, pH 5.0. The reactions were incubated at 50 °C for 72 h with vertical mixing at 30 rpm. The experiments were done in triplicate. Polysaccharide degrading activities were analyzed based on the amount of liberated reducing sugars using the 3,5-dinitrosalisylic acid method [Miller *et al* 1995]. FPU for cellulase was analyzed according to a standard method [Adney *et al.* 1996]. β-Glucosidase activity was determined using pnitrophenyl-β-D-glucopyranoside as the substrate [Harnpicharnchai *et al.* 2009]. One international unit (IU) was defined as the amount of enzyme which produced 1 µmol of reducing sugar or p-nitrophenolate in 1 min.

3.5 Conventional Heating Process

The CF process in this study is a modification of the method described by Bozell *et al*. (2011). The fractionation process was studied in a stainless steel reactor (2.5 cm diameter and 37.5 cm in length with the wall thickness of 2 mm and the total volume of 50 mL). The individual reactor vessel was installed with a thermocouple to follow the actual inside temperature. The solvent ratio in the ternary mixture (water/ethanol/ and methyl isolbutylketone (MIBK), ethyl acetate (EA), toluene (TOL), or diethyl ether (DEE) was set up according to the phase diagram to obtain a single phase mixture [Bozell *et al.* 2011; Trofimova *et al.* 2012; Gomis *et al.* 2008; Merzougui *et al.* 2011].

The standard reaction contained 1.5 g of rice straw and 15 mL of the single-phase solvent mixture containing water/ethanol/methyl isolbutylketone (MIBK) (24%:32%:44%) or water/ethanol/ethyl acetate (EA) (62.5%:25%:12.5%) or other solvents as indicated. Nitrogen was flowed into the reactor for purging and adjusting the initial pressure to 20 bars. The reactors were placed in furnace slots in a multi-reactor system consisting 6 x 50 mL-reactor in a temperature-controlled jacket with vertical shaking system to provide optimal mixing. The reaction was co-heated to 140-180^{o}C for 1 h in the absence or presence of different mineral acids (H_2SO_4, HCl, and H_3PO_4) at varying concentration (0.05 M - 0.2 M). The reaction was quenched in a water bath after heating under the desired conditions. The solid cellulose-enriched fraction was separated by filtration on filter paper using a Bushner funnel and washed with 40 mL MIBK or EA and then with 100 mL water, and dried at 60^{o}C. The liquid fraction was combined with the rinsate and placed into a separatory funnel. Water was added to the aqueous-organic fraction until phase separation was obtained. The mixture was stirred and then placed at room temperature for 20 min for complete phase separation. The aqueous phase containing hemicelluloses and soluble products was recovered. The separated organic phase was dried at 105^{o}C to obtain lignin.

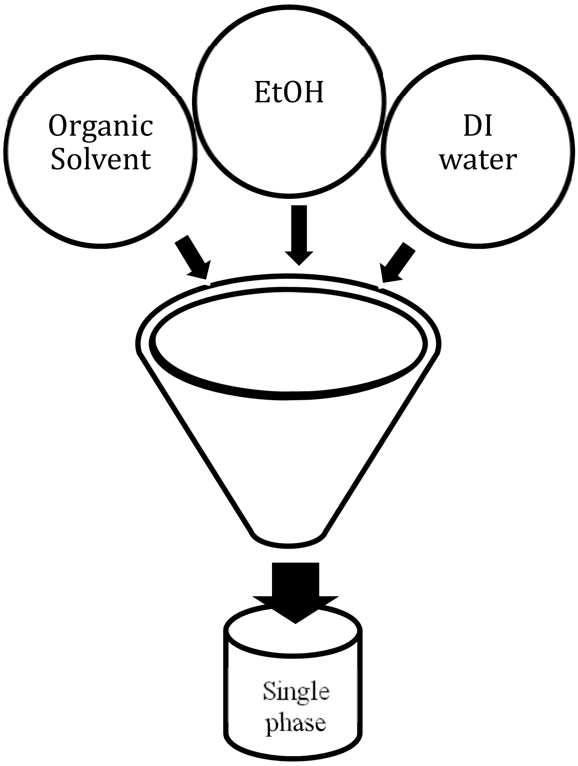
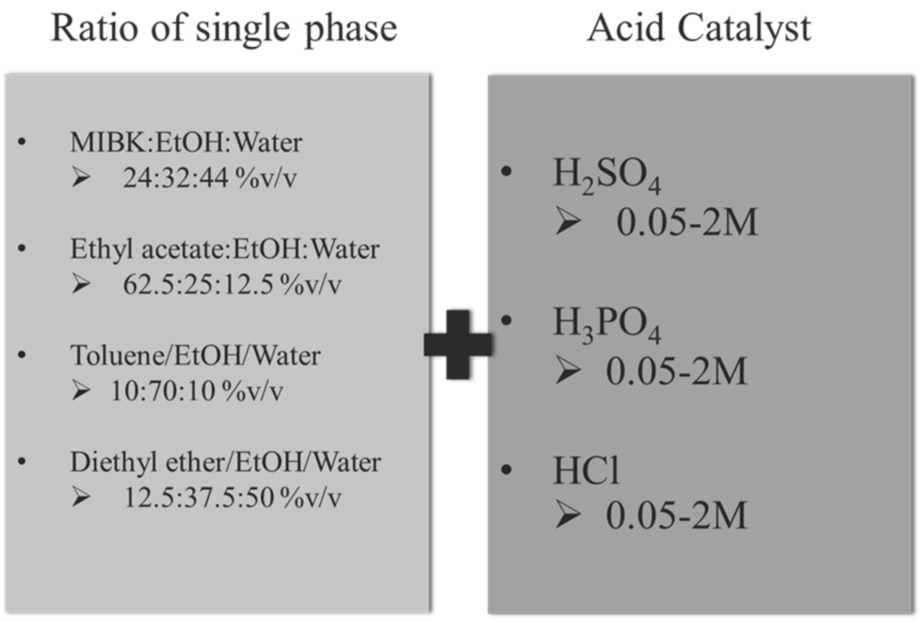


Figure3.5 Preparation of ternary mixture solvents


Ratio of single phase

- MIBK:EtOH:Water
  - 24:32:44 %v/v
- Ethyl acetate:EtOH:Water
  - 62.5:25:12.5 %v/v
- Toluene/EtOH/Water
  - 10:70:10 %v/v
- Diethyl ether/EtOH/Water
  - 12.5:37.5:50 %v/v

Acid Catalyst

- H_2SO_4
  - 0.05-2M
- H_3PO_4
  - 0.05-2M
- HCl
  - 0.05-2M

Figure3.6 Ternary mixture ratio with different concentrations of acid catalysts

3.6 Microwave-Assisted Process

Microwave assisted reactions were conducted in a 500-mL round bottom flask connected to a glass condenser to recover the evaporated solvent. The standard reaction contained 1.5 g of rice straw and 15 mL of the single-phase solvent mixture (water/ethanol/EA). The reaction was heated in a modified home microwave oven (MW71B, Samsung, Korea) set at 200-450 W for 1 h in the absence or presence of different mineral acids (H_2SO_4, HCl, and H_3PO_4) at varying concentrations (0.05 M - 0.2 M). The reaction was then stopped by quenching in a water bath. Phase separation of the reaction mixture was carried out as described above. Temperature in the reactors was estimated using a thermogun (Welch-Allyn Thermoscan PRO 4000 Thermometer, Welch Allyn, New York, NY).

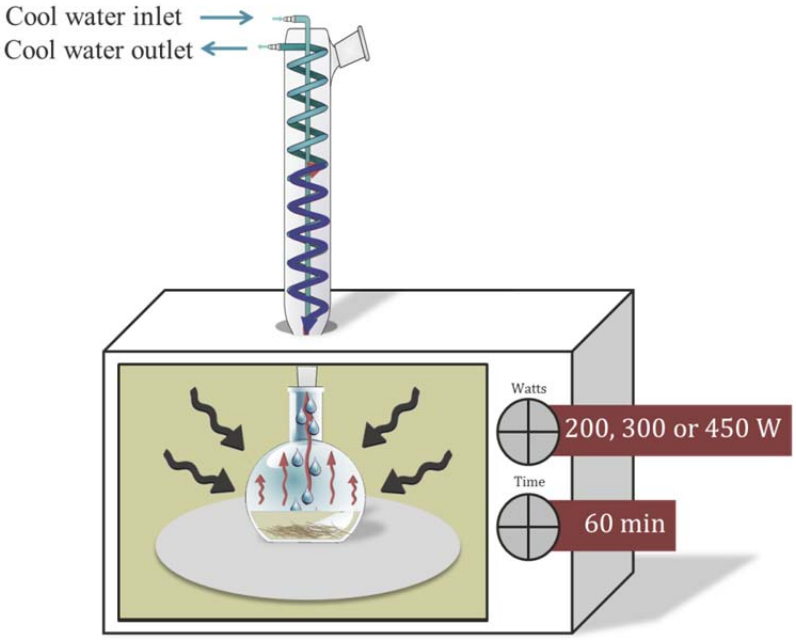


Figure 3.7 Microwave heating process

3.7 Analytical methods of lignocellulose components and products

Chemical compositions (% cellulose, hemicelluloses, lignin, and ash) in the solid/ aqueous/organic phases were analyzed using the standard NREL method [Sluiter *et al.* 2008]. Fermentable sugar profiles and inhibitory by-products (5-hydroxymethyl furfural and furfural) were analyzed on a high performance liquid chromatograph (SPD-M10A DAD, Shimadzu, Kyoto, Japan) equipped with a refractive index detector using an Aminex

HPX-87H column (Bio-Rad, Hercules, CA, USA) operating at 65˚C with 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.5 mL/min. The product yield is reported as the percentage of the product obtained based on its content in the native rice straw on a dried weight basis. The reaction selectivity for glucan (G), hemicellulose (H), and lignin (L) is reported as its relative content in the final fractionated products.

$$\text{Product yield} = \left(\frac{\text{Grams of the product obtained}}{\text{Grams of the product biomass}}\right) \times 100 \tag{2.1}$$

$$\text{Relative content} = \left(\frac{\text{Grams of product}}{\text{Grams of G + H + L + ash in the sample}}\right) \times 100 \tag{2.2}$$

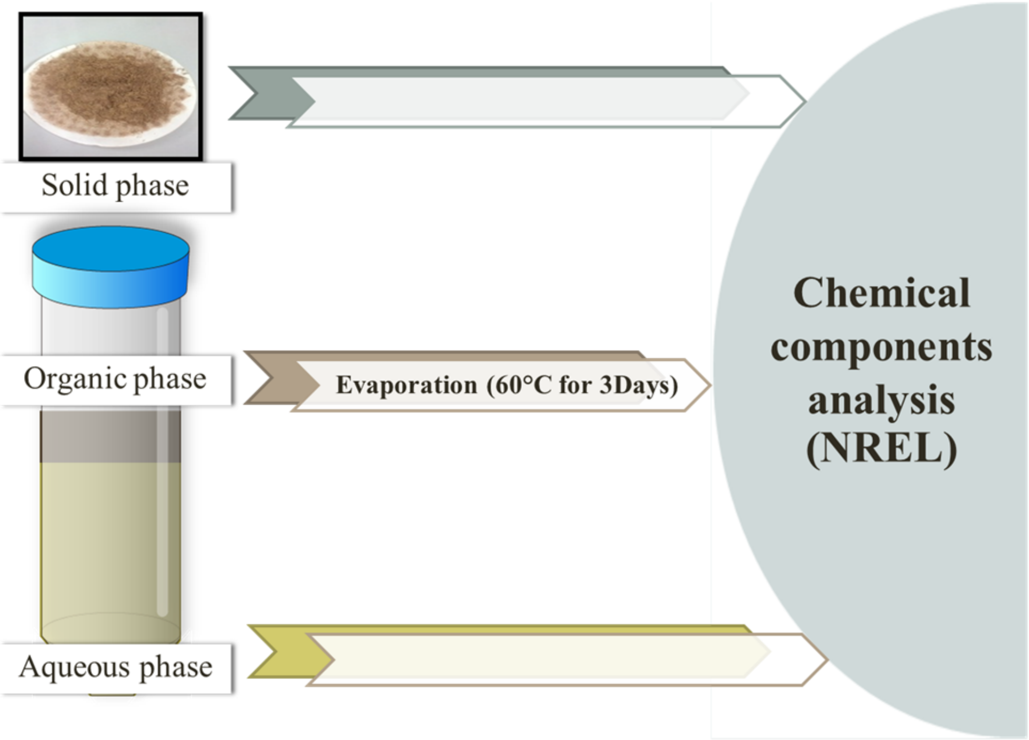


Figure 3.8 Chemical components analysis

3.8 Physical analysis of solid residue

3.8.1 Scanning electron microscopy

The microstructures of the native rice straw and the solid residues obtained from the fractionation process were analyzed by scanning electron microscopy (SEM) using a JSM-6301F Scanning Electron Microscope (JEOL, Tokyo, Japan). The samples were dried and coated with gold for analysis. An electron beam energy of 5 kV was used for analysis.

3.8.2 X-ray diffraction

Crystallinities of the native and separated solid fractions were determined by X-ray diffraction (XRD) using an X'Pert PRO diffractometer (PANalytical, Almelo, The Netherlands). The samples were scanned in a range of 2θ=10°–30° with a step size of 0.02° at 500 kV, 30 mA and radiation at Cu Kα (λ=1.54 Å). Crystallinity was calculated according to the following equation [Li *et al.* 2010].

$$CrI(\%) = \left[\frac{I_{002} - I_{amorphous}}{I_{002}}\right] \times 100$$

in which I_{002} is the intensity for the crystalline portion of biomass (i.e. cellulose) at $2\Theta = 22.4$ and $I_{amorphous}$ is the peak for the amorphous portion (i.e. cellulose, hemicellulose, and lignin) at $2\Theta = 18.0$.

3.8.3 BET surface area measurement

The total surface area of the raw material and solid residues from fractionation was determined using the method of Brunauer, Emmett, and Teller (BET) on a Belsorp-max TPDpro (BEL Japan, Tokyo, Japan) equipped with a thermal conductivity detector (Semi-diffusion type, 4-element W-Re filament) at the National Nanotechnology Center, Thailand.

3.8.4 Fourier-Tranformed Infrared spectroscopy analysis

FT-IR measurements were performed in a Perkin–Elmer 16PC instrument by direct transmittance using a KBr pellet technique. Each spectrum was recorded over 20 scans in the range from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}. Background spectra were collected before every sampling. KBr was previously oven-dried to avoid interferences due to the presence of water. The commercial lignin from SIGMA-ALDRICH was used as the reference.

CHAPTER 4

AUTOHYDROLYSIS OF TROPICAL AGRICULTURAL RESIDUES BY COMPRESSED LIQUID HOT WATER

Chapter Summary

Pretreatment is an essential step in biorefineries for improving the digestibility of recalcitrant agricultural feedstocks prior to enzymatic hydrolysis to composite sugars, which can be further converted to fuels and chemicals. LHW has been used for pretreatment of several lignocellulosic feedstocks including various herbaceous agricultural residues and energy plant. However, there has been no report on comparative analysis of LHW efficiency on different plant biomass differing in their physical and chemical characteristics using the same system and operational conditions, which will be important for implementation of LHW as a common pretreatment process for biorefineries operating with various agricultural biomass. In this chapter, the effect of LHW treatment on improve the digestibility of sugarcane bagasse (BG), rice straw (RS), corn stover (CS), and empty palm fruit bunch (EPFB) were investigated. The impact of the process on biomass structures and physico-chemical properties were determined. The study evaluated the potential of LHW as a pretreatment technology for a feedstock made of various agricultural reesidues.

4.1 Composition of biomass

The lignocellulosic substrates used in this study are basically different in their physical properties and chemical characteristics. BG represents a biomass with rigid and compact morphology, while RS has a packed and complex structure with high silica content. CS is a heterogeneous substrate containing stalks and leaves. EPFB is highly rigid and has rough, uneven, and undulating structure with high lignin content. All these biomass represents potent lignocellulosic feedstocks in tropical countries. The constituents of raw materials used in this study are presented in Table 4.1. The native biomass contained various ratios of holocellulose (59.5–66.7 %) and lignin (23.8–30.9 %). Among all feedstocks, BG contained the highest cellulose content (39.7 %), whereas RS contained the lowest fraction of hemicellulose (21.4 %) but the highest ash content (14.2 % w/w).

The highest lignin content was found in EPFB, which was reflected by its observed rigid structure. The biomass compositions in this study were in the same range as those previously reported [Hsu *et al*. 2010; Qin *et al*. 2012]. The observed differences in physical structures and compositions, thus make these feedstocks useful for the comparison of the effects of pretreatment on enzymatic susceptibility.

Table 4.1 Chemical composition of native lignocellulosic biomass

Biomass	Cellulose (%w/w)	Hemicellulose (%w/w)	Lignin (%w/w)	Ash (%w/w)
RS.	38.1	21.4	26.7	14.2
BG.	39.7	28.0	24.6	7.2
CS.	36.5	30.4	23.8	8.4
EPFB.	35.8	27.8	30.9	8.5

4.2 Effects of LHW on Lignocellulosic Biomass

4.2.1 Autohydrolysis of Rice Straw in Liquid Fraction

The effects of LHW on autohydrolysis of biomass were studied by the analysis of the released sugars and degraded products in the liquid fraction after pretreatment. LHW led to extensive solubilization and hydrolysis of the hemicelluloses in the native biomass. A trend was observed of increasing pretreatment temperature and time with increasing sugar concentration, particularly pentoses (xylose and arabinose) in the liquid phase. The highest pentose concentrations were found at 200 °C with the residence time of 5 min for CS and 10 min for BG, RS, and EPFB. These pretreatments yielded 67.8, 94.1, 54.7, and 85.9 mg/g of dried biomass (Fig. 4.1A-B and Fig. 4.2A-B), respectively, corresponding to 22.6, 29.7, 19.7, and 27.3 % of pentose yield from the native substrates, respectively, under the conditions for maximal released pentose. In contrast, low amounts of glucose (3.9–13.2 mg/g) were released into the liquid phase, indicating limited hydrolysis of the crystalline cellulose fraction under the experimental pretreatment conditions. Increasing concentrations of dehydration by-products in the liquid phase were found with increasing pretreatment temperature and time, with a marked increase at 200 °C. Furfural from the dehydration of the released pentoses from hemicelluloses was found as the major degraded product, while hydroxymethylfurfural (HMF) from degradation of hexoses, particularly glucose (released from hemicelluloses or partial hydrolysis of cellulose) was detected as the minor dehydration product. The maximum concentrations of furfural and HMF were observed at 200 °C for 10 min in the range of 1.7–2.4 and 0.3–0.7 mg/mL, respectively, depending on the specific feedstocks. Dehydration product concentrations decreased with increasing pretreatment time at 200 °C, which could be due to further degradation of furans to aldehydes and organic acids, such formic and acetic acids [Yu *et al.* 2010].

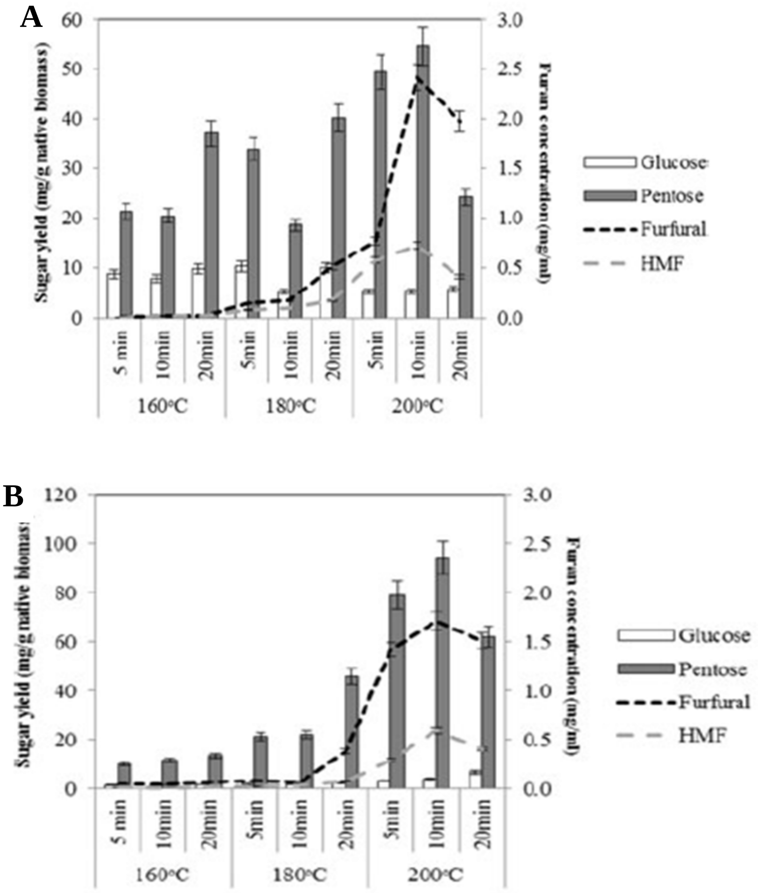


Figure 4.1 Effects of compressed liquid hot water pretreatment on autohydrolysis of the sugar and by-product into the liquid fraction. The biomass was pretreated at 10% (w/v) solid loading at different temperatures and residence times with the initial pressure at 25 bars: (A) Rice straw and (B) Sugarcane bagasse.

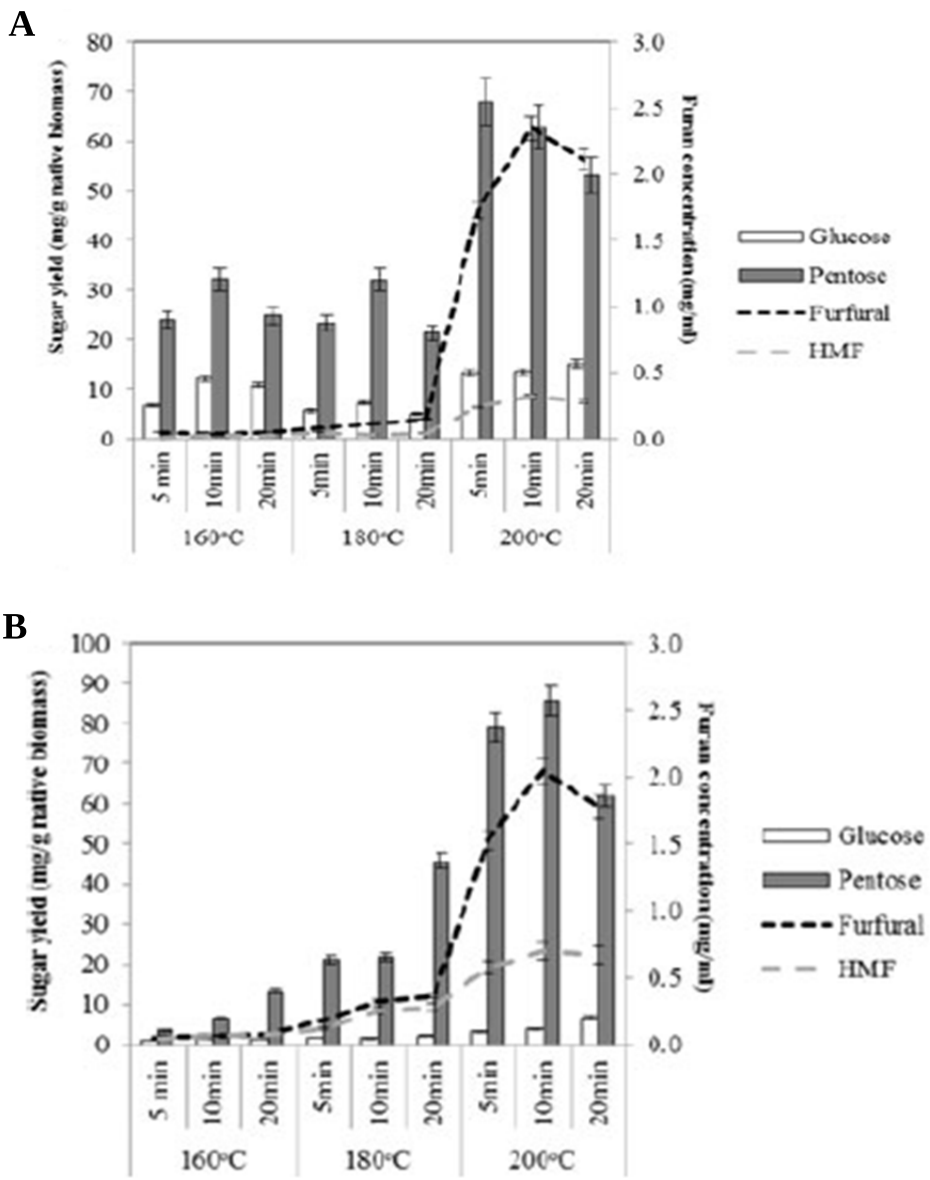


Figure 4.2 Effects of compressed liquid hot water pretreatment on autohydrolysis of the sugar and by-product into the liquid fraction. The biomass was pretreated at 10% (w/v) solid loading at different temperatures and residence times with the initial pressure at 25 bars: (A) Corn stover and (B) Empty palm fruit bunch.

4.2.2 Enzymatic Hydrolysis of the Solid Residues

The effect of LHW pretreatment on the digestibility of the pretreated biomass was determined based on sugars released after enzymatic hydrolysis. The results showed that increasing pretreatment temperature and time led to increased digestibility of the solid residues. The maximal glucose yields were achieved at 200 °C with the residence time of 5 min for RS, 10 min for BG and CS, and 20 min for EPFB, which led to the glucose yields of 425.4, 482.7, 437.7, and 409.8 mg/g of pretreated biomass, respectively, after 72 h of enzymatic hydrolysis, corresponding to 82.3, 75.7, 76.1, and 79.0 % glucose yield from the native biomass, respectively (Fig. 4.3A-B and Fig. 4.4A-B). These yields were 7.7–11.7-fold greater than the glucose yields obtained from non-pretreated biomass. Higher pentose yields were observed under milder conditions (160–180 °C), reflecting the higher hydrolyzable characteristic of hemicelluloses. The highest pentose yield from hydrolysis of the residual hemicellulose was obtained with pretreatment conditions at 180 °C with the residence time of 5 min for RS, CS, and EPFB and 10 min for BG, resulting in the pentose yields of 139.1, 234.3, 235.5, and 247.5 mg/g of pretreated substrate, respectively, equivalent to 47.1, 51.2, 48.3, and 60.3 % pentose yield from native biomass, respectively. These yields were 2.3–3.7-fold greater than pentose yield from the non-pretreated biomass. Higher reaction temperatures and times of LHW pretreatment thus tended to improve the digestibility of biomass.

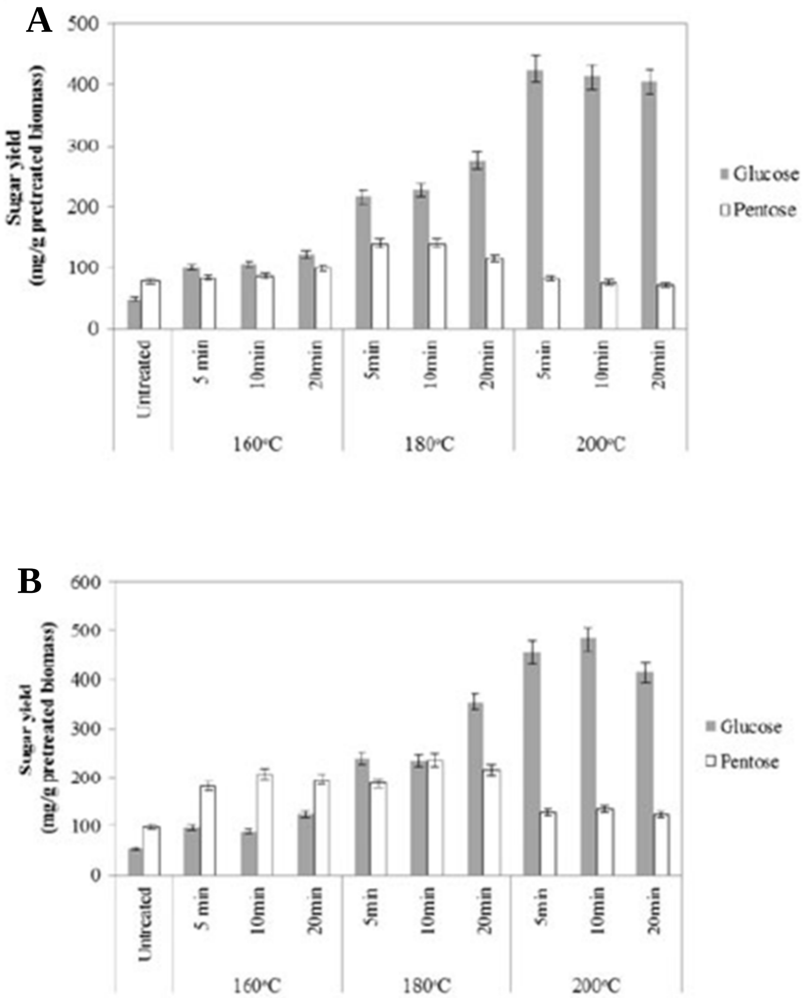


Figure 4.3 Effects of compressed liquid hot water pretreatment on enzymatic digestibility of the solid residues. The biomass was pretreated at 10% (w/v) solid loading at different temperatures and residence times with the initial pressure at 25 bars: (A) Rice straw and (B) Sugarcane bagasse.

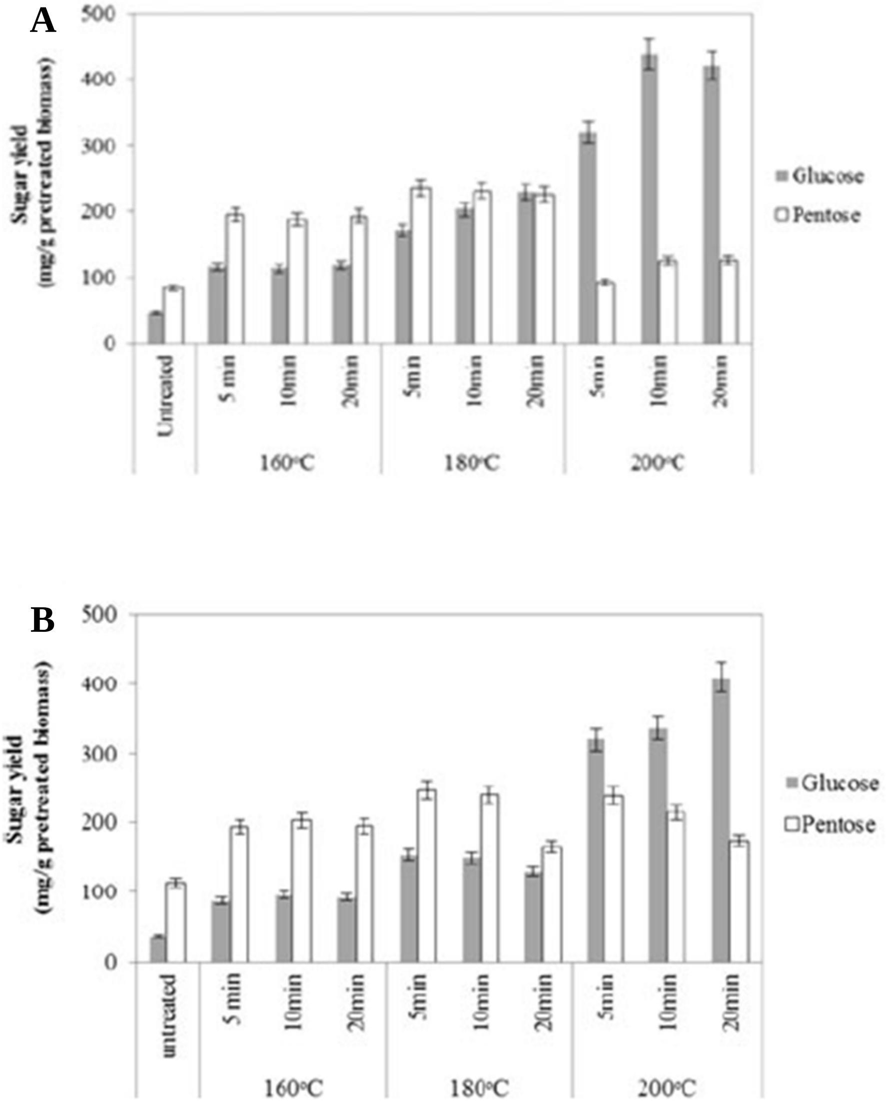


Figure 4.4 Effects of compressed liquid hot water pretreatment on enzymatic digestibility of the solid residues. The biomass was pretreated at 10% (w/v) solid loading at different temperatures and residence times with the initial pressure at 25 bars: (A) Corn stover and (B) Empty palm fruit bunch.

4.2.3 Optimal LHW Pretreatment Conditions

According to our result, the pretreatment temperature was the most important factor for sugar production, i.e. enzymatic digestibility of the solid residues and autohydrolysis of the biomass into the liquid phase for an LHW process, whereas pretreatment time was of lesser influence. The optimal pretreatment conditions based on the maximized overall sugar yields were at 200 °C for all substrates with the residence time of 5 min for RS, 10 min for BG and CS, and 20 min for EPFB, which led to the glucose and pentose yields of 0.308–0.348 and 0.081–0.174 g/g dried native biomass from hydrolysis of the solid residues, respectively, and 0.050–0.094 g/g of pentose recovery in the liquid phase (Table 4.2). The inhibitory furan by-products were in the range of 0.77–2.35 mg/mL for furfural and 0.32–0.67 mg/mL for HMF. These levels of byproducts are not inhibitory to Saccharomyces cerevisiae or Candida guilliermondii, which tolerate HMF and furfural up to 2 mg/mL [Sanchez *et al.* 1998].

Table 4.2 Summary of components in solid and liquid fractions from LHW pretreatment under the optimal conditions

Biomass	**Temperature (°C)**	**Time (min)**	**Liquid fraction**				**Solid fraction**	
			Glucose[a] (mg/g)	**Pentose [a] (mg/g)**	**HMF[a] (mg/g)**	**Furfural [a] (mg/g)**	**Glucose[b] (mg/g)**	**Pent.[b] (mg/g)**
RS.	200	5	5.3	49.6	5.8	7.7	425.4	81.0
BG.	200	10	4.0	94.1	5.9	17.1	482.7	136.0
CS.	200	10	13.5	62.7	3.2	23.5	437.7	124.4
EPFB.	200	20	6.6	62.1	6.7	17.8	409.8	174.0

[a] Based on native biomass

[b] Based on pretreated biomass

The amounts of obtained sugars from the LHW pretreatment process to enzymatic hydrolysis is shown in Table 4.3. In brief, all biomass at a solid loading of 10 % (w/v) was pretreated under the optimized conditions and the remaining insoluble fraction was separated from the pretreatment hydrolysate prior to enzymatic saccharification. Minority of the glucose was released into the liquid fraction (0.9–3.3 %), while a substantial fraction of hemicelluloses was present as pentoses in the liquid fraction (20.5–29.7 %). Overall, a high glucose yield of 75.7–82.3 % (w/w) from the starting materials was obtained after enzymatic hydrolysis of the solid fractions.

Table 4.3 Amount of obtained sugars from LHW pretreatment and enzymatic hydrolysis

Biomass	Composition				Solid fraction			Enzymatic hydrolysis fraction	
	Cel (g)	**Hemi (g)**	**Lig (g)**	**Ash (g)**	**Glc (g)**	**Pent (g)**	**Furan (g)**	**Glc (g)**	**Pent (g)**
RS.	0.191	0.107	0.133	0.072	0.003	0.025	0.014	0.174	0.033
BG.	0.199	0.140	0.121	0.041	0.002	0.047	0.023	0.167	0.047
CS.	0.183	0.152	0.124	0.044	0.007	0.031	0.034	0.154	0.044
EPFB.	0.179	0.139	0.153	0.043	0.003	0.031	0.031	0.157	0.067

The decomposition of lignocellulosic components under pressurized compressed hot water has been reported to occur at different temperatures. Hydrolysis of hemicellulose starts at 160 °C, while lignin start melting at 170–180 °C and partially reprecipitated on the biomass surface after the pretreatment [Dien *et al.* 2009; Ritchie *et al.* 1990]. Degradation of the crystalline cellulose was found at temperatures >200 °C [Levan *et al.* 1989]. This thus suggests that improvement in biomass digestibility is mainly related to removal of hemicellulose under the experimental pretreatment conditions.

The optimal conditions for LHW in this study are in the same range as those previously reported for various agricultural residues and softwood, e.g., poplar, which are in the range of 180–220 °C under non-catalytic conditions [Kim *et al.* 2009], while lower temperatures in the range of 120–195 °C are used under acid-catalyzed process using dilute H_2SO_4 for corn stover and rice hull pretreatment [Schell *et al.* 2003; Saha *et al.* 2005]. Variation in sugar recovery has been reported for pretreatment of lignocellulosic biomass with different physical structures and chemical composition with different pretreatment technologies [Galbe *et al.* 2012]. Among various thermal and chemical processes, LHW provides high recovery yields (>70 %) irrespective of the biomass. High glucose recovery yield ≥90 % was achieved for pretreatment of corn stover with partial flow hot water [Liu *et al.* 2005] or from batch LHW pretreatment of alfalfa fiber [Sreenath *et al.* 1999]. The highest cellulose recovery yields of 83.0–88.5 % were also obtained by LHW pretreatment of switchgrass and oil palm frond [Garlock *et al.* 2012; Goh *et al.* 2012]. Relatively lower recovered glucose yields (70.0–86.0 %) have been reported for other widely used pretreatment methods, e.g., alkaline pretreatment [Zhao *et al*. 2008], supercritical carbon dioxide [Srinivasan *et al*. 2012], and AFEX pretreatment [Kim *et al*. 2008].

LHW is also superior to many other pretreatment technologies in that it generates lower amounts of sugar degradation by-products due to the bulk water volume and obviation of external acid catalysts [Hendricks A.T.W.M. *et al*. 2009]. This advantage of LHW over other pretreatment methods is particularly important since the principal sugar degradation products, viz., furans, are growth inhibitory to ethanologens by various mechanisms. For instance, furfural inhibits glycolytic enzymes in vitro and has an additional inhibitory effect on aldehyde dehydrogenase activity, resulting in an accumulation of acetaldehyde, which prolongs the lag phase of S. cerevisiae. HMF is less inhibitory than furfural, but it remains in the medium longer owing to its low conversion rate [Zaldivar *et al*. 2005]. Our study thus suggested potential of LHW for effective one-step partial separation of hemicellulose-enriched fraction with high recovery of hydrolyzed cellulosic sugars in the solid fraction for further processing.

4.2.4 Effects of LHW Pretreatment on Structural Properties of Biomass

Changes in biomass microstructures pretreated under the conditions for maximal sugar yields were analyzed using SEM (Fig. 4.5). Comparison of the SEM images of the native and pretreated biomass shows that microstructures of the agricultural residues are disrupted by pretreatment. Cavities and cracks in the plant cell wall were observed in the pretreated biomass, which reflect the removal of hemicellulose and modification of the surface lignin [Pan *et al*. 2005]. These structural modifications led to an increase in available surface area as revealed by BET analysis (Table 4.4). Substantial increases in accessible surface area in the range of 49.3–69.4 % were observed for all biomass samples after pretreatment. The accessible surface area is regarded as one of the most important factors affecting the effectiveness of enzymatic digestibility [Kumar *et al*. 2011].

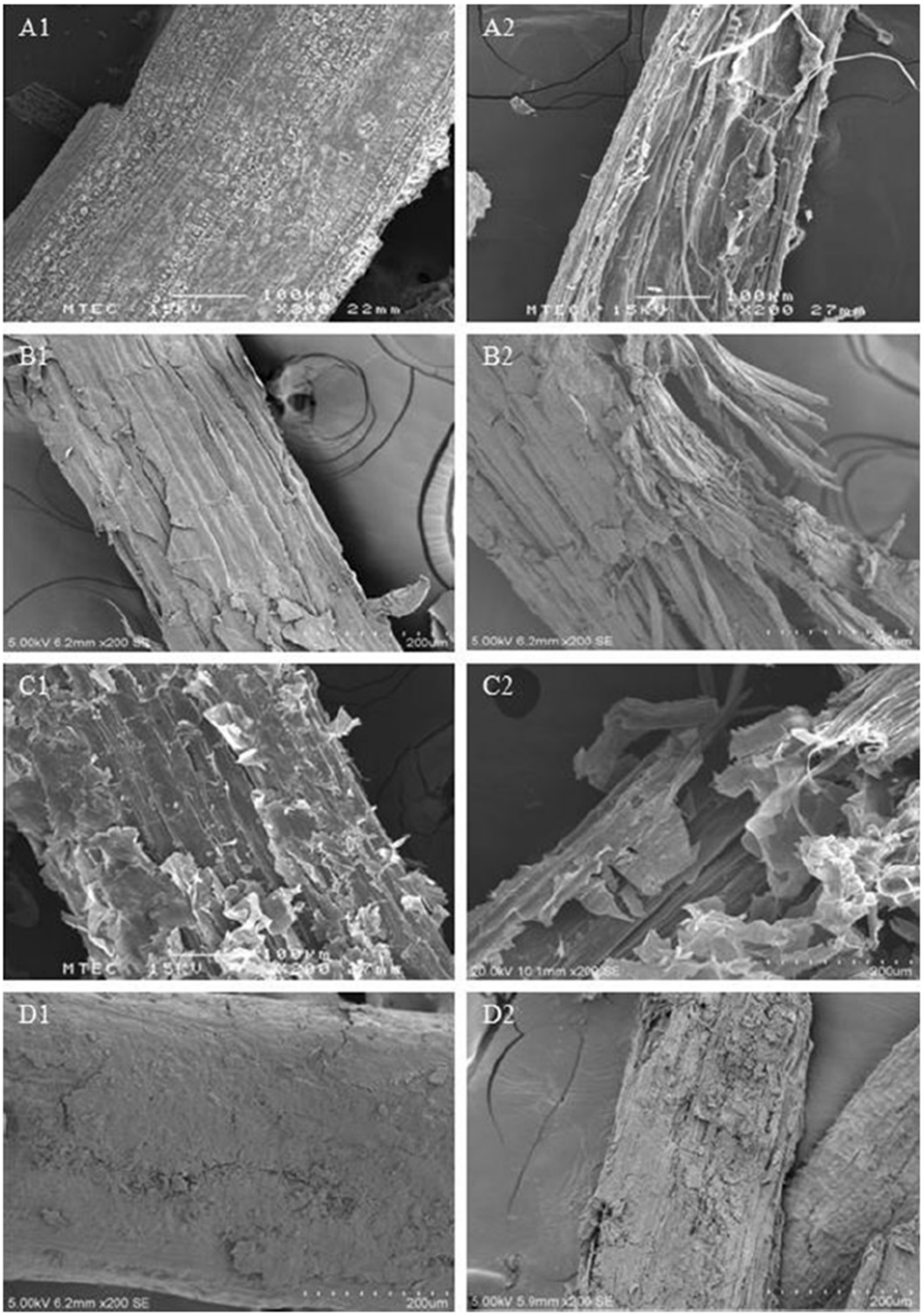


Figure 4.5 Scanning electron micrographs of native and pretreated biomass under the optimal conditions: native (A1) and pretreated (A2) rice straw; native (B1) and pretreated (B2) bagasse; native (C1) and pretreated (C2) corn stover; native (D1) and pretreated (D2) empty palm fruit bunch (Magnification of ×200).

Crystallinity of biomass is also considered an important factor influencing the efficiency of enzymatic hydrolysis. According to an analysis of the XRD patterns, higher CrI (72.66– 78.92%) was found for all biomass samples pretreated under the conditions for maximal sugar production compared with the native biomass (62.03–68.51%), which is equivalent to 9.4– 13.4% increases in the crystallinity indices (CrI) (Table 4.4). The highest increase in CrI was observed for CS followed by EPFB, RS, and BG, which showed the lowest CrI increase. The increases in CrI were related to effects of LHW on removal of the amorphous xylan and lignin fractions, while it showed less effect on the disruption of the highly crystalline cellulose. Increases in biomass crystallinity were previously reported in various biomasses pretreated by LHW, e.g., *Tamarix ramosissima* pretreated using LHW [Xiao *et al.* 2013] and also other pretreatment methods, e.g., microwave-assisted acid pretreatment [Binod *et al.* 2012] and steam explosion [Pang *et al.* 2013]. On the other hand, complete destruction of crystalline cellulose has been reported for some pretreatment techniques, e.g. ionic liquid [Zhu *et al.*, 2006].

Table 4.4 BET surface area and crystallinity index of untreated and pretreated at optimal conditions

Biomass	Surface area (m^2/g)		Degree of crystallinity (%)	
	Native	Pretreated	Native	Pretreated
RS.	2.2	7.8	68.5	72.7
BG.	3.9	9.8	63.3	78.9
CS.	2.7	6.7	62.0	75.4
EPFB.	3.9	7.7	64.2	77.0

Different structural changes due to modifications of cellulose crystallinity, available surface area, and lignin and hemicellulose removal have been reported for various kinds of lignocellulosic biomass pretreated by LHW, e.g. corn stover, wheat straw, and soybean straw [Wan *et al.*, 2011]. The main effect of LHW on modification of biomass microstructures and physicochemical properties by degradation of hemicelluloses is similar to some other thermochemical pretreatments, e.g., stream, acid, and CO_2 explosion, of which also show relatively weak effects on disruption of the crystalline cellulose structures [Hendricks A.T.W.M. *et al.*, 2009]. The increased enzymatic digestibility of the LHW-pretreated biomass is thus caused primarily by solubilisation of hemicellulose with much less effect on lignin.

4.3 Chapter conclusion

In this chapter, autohydrolysis by compressed liquid hot water (LHW) pretreatment of various tropical agricultural residues, including sugarcane bagasse (BG), rice straw (RS), corn stover (CS), and empty palm fruit bunch (EPFB), was investigated. It was found that LHW pretreatment at 200°C for 5–20 min resulted in high levels of hemicellulose solubilization into the liquid phase and marked improvement on enzymatic digestibility of the solid cellulose-enriched residues. The maximal yields of glucose and pentose were 409.8–482.7 mg/g and 81.1–174.0 mg/g of pretreated substrates, respectively. Comparative analysis based on severity factor showed varying susceptibility of biomass to LHW in the order of BG> RS> CS> EPFB. Structural analysis revealed surface modification of the pretreated biomass along with an increase in crystallinity index. Overall, 75.7–82.3% yield of glucose and 27.4–42.4% yield of pentose from the dried native biomass was recovered in the pretreated solid residues, while 18.3–29.7 % of pentoses were recovered in the liquid phase with dehydration by-product concentration under the threshold for ethanologens. The results suggest the potential of LHW as an efficient pretreatment strategy for implementation in biorefineries using various seasonal agricultural feedstocks.

CHAPTER 5

EFFECTS OF ACID AND ALKALINE PROMOTERS ON COMPRESSED LIQUID HOT WATER PRETREATMENT

Chapter Summary

In this chapter, the effects of homogeneous acid and alkaline promoters on the efficiency and selectivity of LHW pretreatment of rice straw were investigated. In order to explore the use of external promoters on LHW, this study reports a comparison of different homogeneous acids and alkalis on catalytic LHW pretreatment of rice straw under pressurized water conditions aiming to enhancing hemicellulose hydrolysis and improving cellulose digestibility which reflected in greater sugar recoveries in the liquid and saccharified solid phases under different LHW conditions employing different promoters. The works shows potential of external promoters for promoting efficiency of LHW pretreatment of lignocelluloses for further conversion to valorized products in sugar platform biorefinery.

5.1 Catalytic liquid hot water

According to compositional analysis, the native rice straw used in this study contained cellulose (35.8%), hemicellulose (21.5%), and lignin (24.4%) as the major constituents with a high ash content (15.0%) composed mainly of silica (Sun *et al*. 2000). The high silica nature makes RS a unique characteristic for processing compared to other lignocellulosic agricultural residues.

5.1.1 Influences of temperature and time on LHW pretreatment

In the first step, rice straw was pretreated under non-catalytic LHW conditions at varying temperatures (140-180°C) and times (5-20 min) in the absence of catalysts. Hemicellulose was partially solubilized and hydrolyzed to monomeric sugars under the experimental conditions. An increasing trend of pentoses (xylose and arabinose) in the liquid fractions was found with increasing temperature and time at low severity conditions (Fig. 5.1A). The maximal pentose yield of 44.67% was obtained at 180°C for 5 min and decreased afterwards. Lower solubilization of glucan to glucose (< 11%) was observed under the operating conditions. High temperature conditions also promoted the degradation of sugars to furans as observed in this study. These were further hydrolyzed to organic acids e.g. formic acid, acetic acid and levulinic acid [Jonsson *et al*. 2013]. Trend of sugar dehydration products i.e. furfural and HMF was observed; however, with relatively low concentration. The highest furfural and HMF concentrations of 0.09 and 0.24 mg/mL were found at 180°C for 5 min. Further increase in operating time led to decreasing furfural and HMF presumably due to their further decomposition to small organic acids. Increasing temperature in LHW pretreatment up to 160°C led to increasing enzymatic digestibility of the biomass (Fig 5.1B).

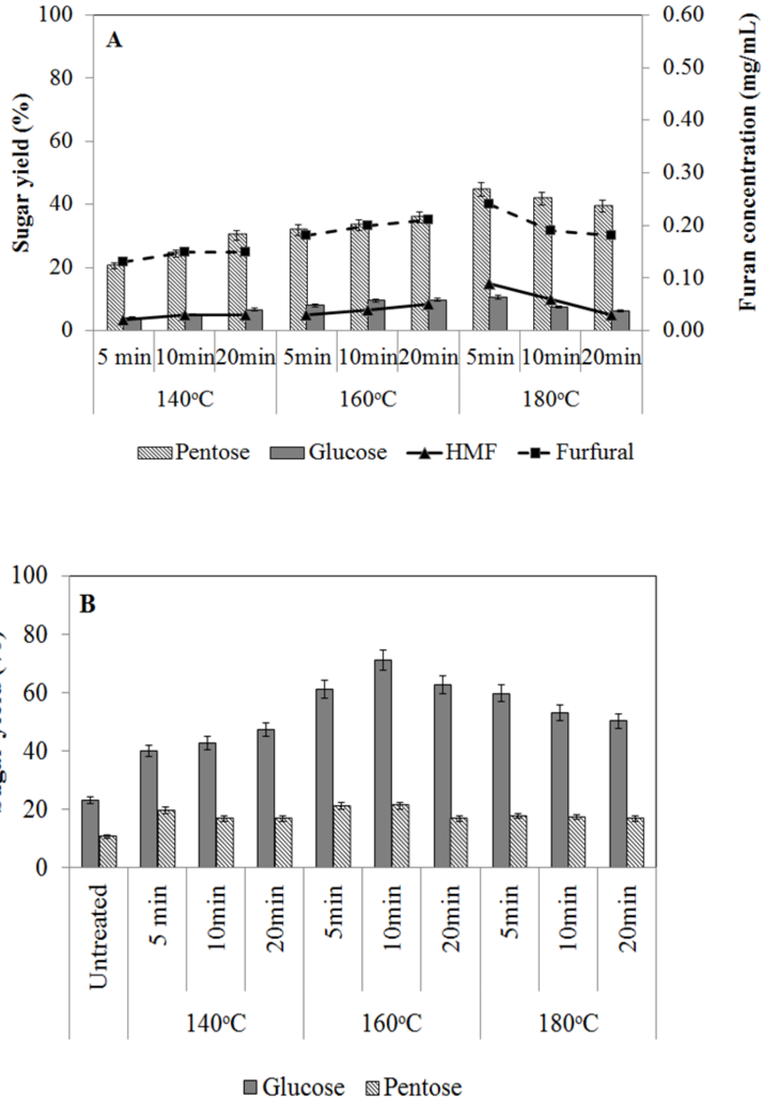


Figure 5.1 Effects of temperature and time on LHW pretreatment of rice straw under non-catalytic conditions. The biomass was pretreated at 10% (w/v) solid loading at different temperatures and residence times with the initial pressure at 25 bars: (A) soluble products in liquid phase and (B) fermentable sugars from enzymatic hydrolysis of the solid phase

Glucose constitutes the majority of monomeric sugars from enzymatic hydrolysis. The highest glucose yield of 71.22% from the native rice straw was recovered after enzymatic hydrolysis of the solid residue pretreated at 160°C for 10 min while 21.4% pentose was obtained as a minor product under this experimental condition. Further increase in pretreatment severity led to lower sugar recovery from hydrolysis of the solid fractions due to higher polysaccharide solubilization as shown by increasing the weight loss from the pretreatment process.

5.1.2 Effects of acid and alkaline catalysts

5.1.2.1 Homogeneous acid catalysts

Mineral acids are basically used for enhancing efficiency in the hydrothermal pretreatment of different biomass e.g. hardwood, softwood, and agricultural residues [Mosier *et al*. 2005; Saha *et al*. 2005]. The influences of homogeneous strong acid (H_2SO_4, HCl, H_3PO_4) catalysts at concentration of 0.25-1% on efficiency and selectivity of LHW pretreatment of rice straw were firstly studied at 140-180^{o}C with the operating time of 5-20 min. The addition of all catalysts tested led to increasing hemicellulose solubilization in the order of H_2SO_4 > HCl> H_3PO_4 which led to higher pentose recovery equivalent to 0.91-2.46 folds compared to those obtained with the non-catalytic process under respective conditions (Fig. 5.2, Fig. 5.3, and Fig 5.4). The highest pentose yield of 81.5% was obtained under LHW conditions at 180^{o}C for 5 min in the presence of 0.5% v/v H_2SO_4 as the catalyst. Increasing trend of inhibitory sugar degradation by-products was found with increasing temperature and time while the highest accumulation of HMF and furfural were found with H_2SO_4 and HCl while H_3PO_4 led to the lowest inhibitor formation. Among the acids tested, H_2SO_4 was found to be the superior catalyst in terms of product yield and selectivity. This led to the pentose yield of 41.4-81.5% equivalent to 1.21-2.46 folds in pentose yield to those obtained using non-catalytic control reactions while the accumulation of HMF (0.10-0.40 mg/mL) and furfural (0.22-0.66 mg/mL) (Fig. 5.2) was lower than HCl (Fig. 5.4). However, the accumulated inhibitory by-product levels were lower than the maximal tolerance threshold for ethanolgens (2 mg/mL for furfural and HMF) [Sanchez *et al*. 1988].

The use of acid-catalyzed reaction also led to markedly increase in sugar recovery after enzymatic hydrolysis of the pretreated solid residues (Figs. 5.5, 5.6, 5.7). The highest glucose recovery was 82.3% using H_3PO_4 as the catalyst with the operating temperature of 160^{o}C for 20 min (Fig. 5.6B), which equivalent to 1.31 folds compared to that obtained under the non-catalytic condition. The use of acid catalysts also led to increasing reaction selectivity which resulted in lower pentose obtained from enzymatic hydrolysis of the solid fractions compared to those obtained under the non-catalytic conditions. The sugar yield achieved was comparable to that obtained using the non-catalytic LHW pretreatment, which thus indicated energy savings in the pretreatment process by the use of acid catalysts.

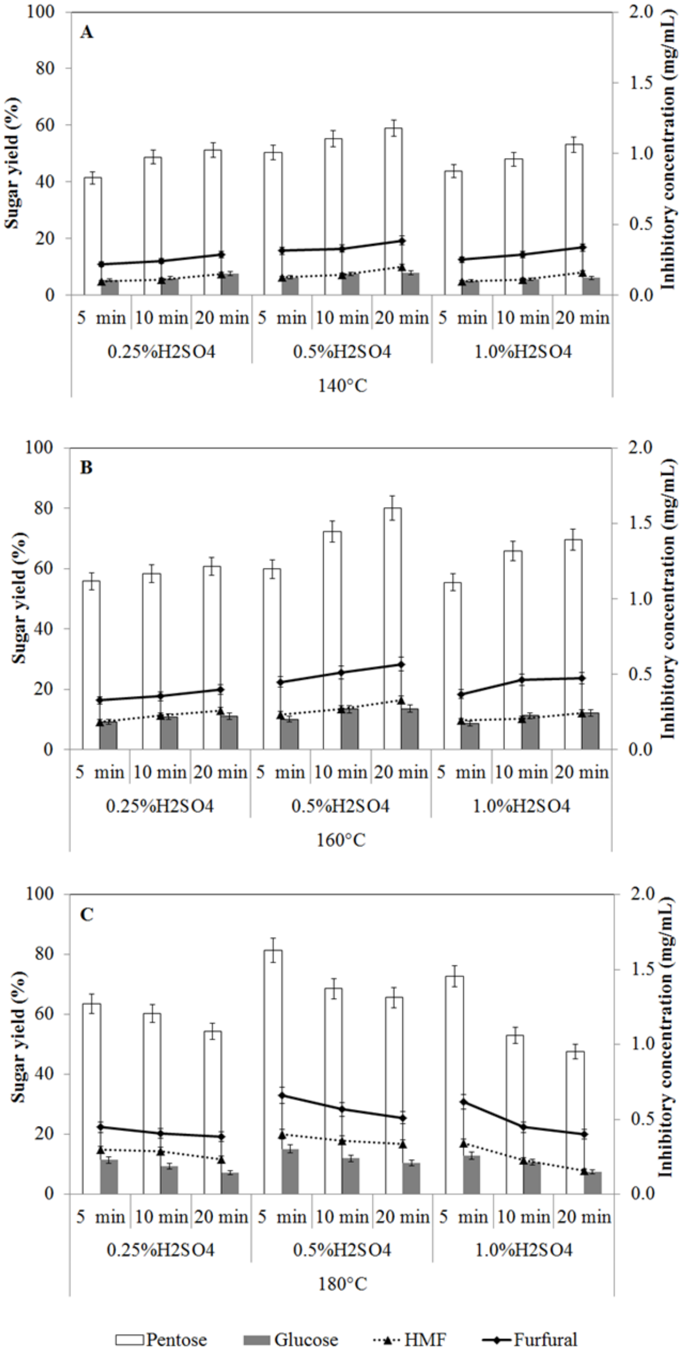


Figure 5.2 Effects of acid catalysts on LHW pretreatment of rice straw. The biomass was pretreated at 10% (w/v) solid loading at 140-180°C at varying residence times in the presence of 0.25-1% (v/v) H_2SO_4 acid as the catalyst with the initial pressure at 25 bars: (A) 140°C, (B) 160°C and (C) 180°C

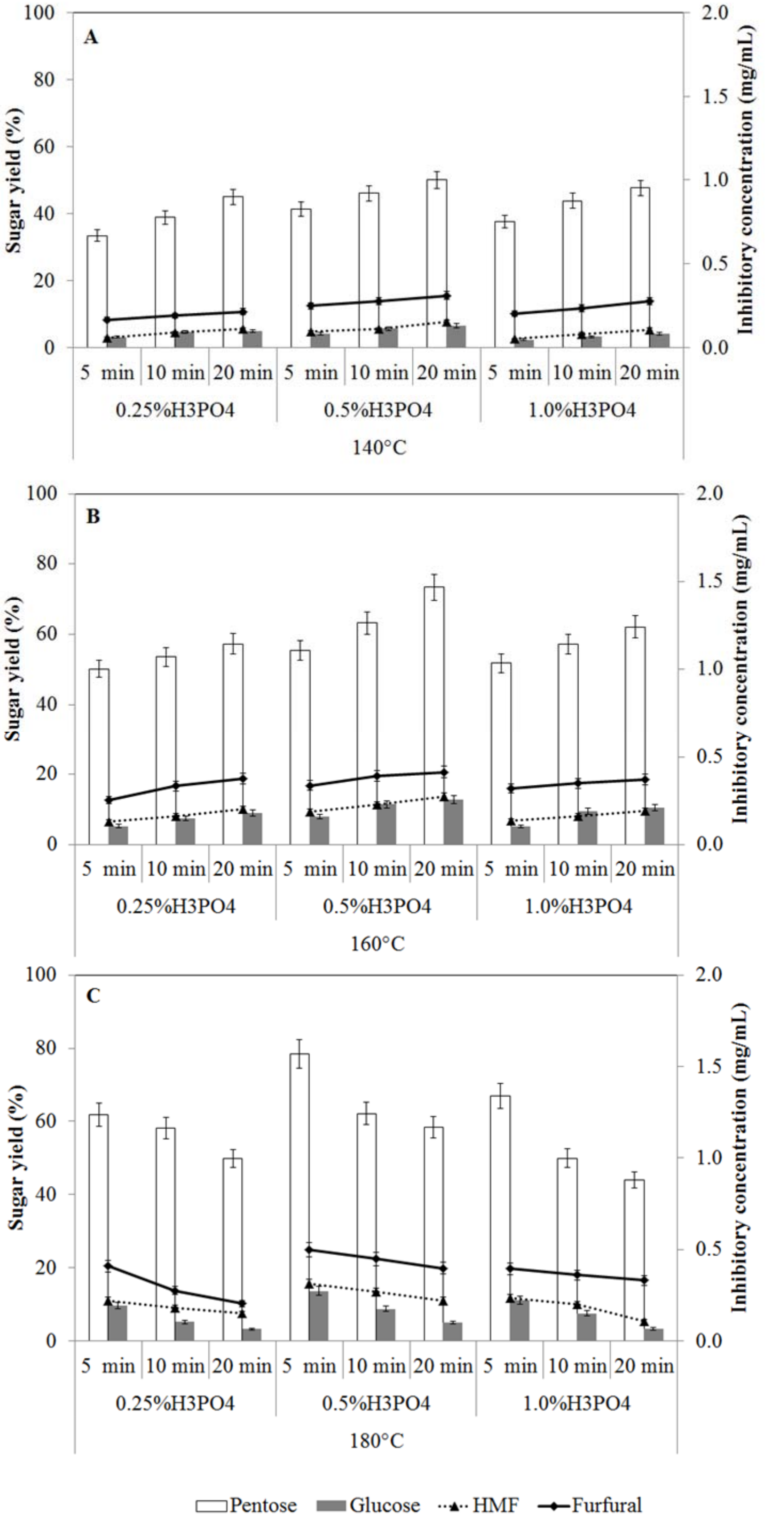


Figure 5.3 Effects of acid catalysts on LHW pretreatment of rice straw. The biomass was pretreated at 10% (w/v) solid loading at 140-180°C at varying residence times in the presence of 0.25-1% (v/v) H_3PO_4 acid as the catalyst with the initial pressure at 25 bars: (A) 140°C, (B) 160°C and (C) 180°C

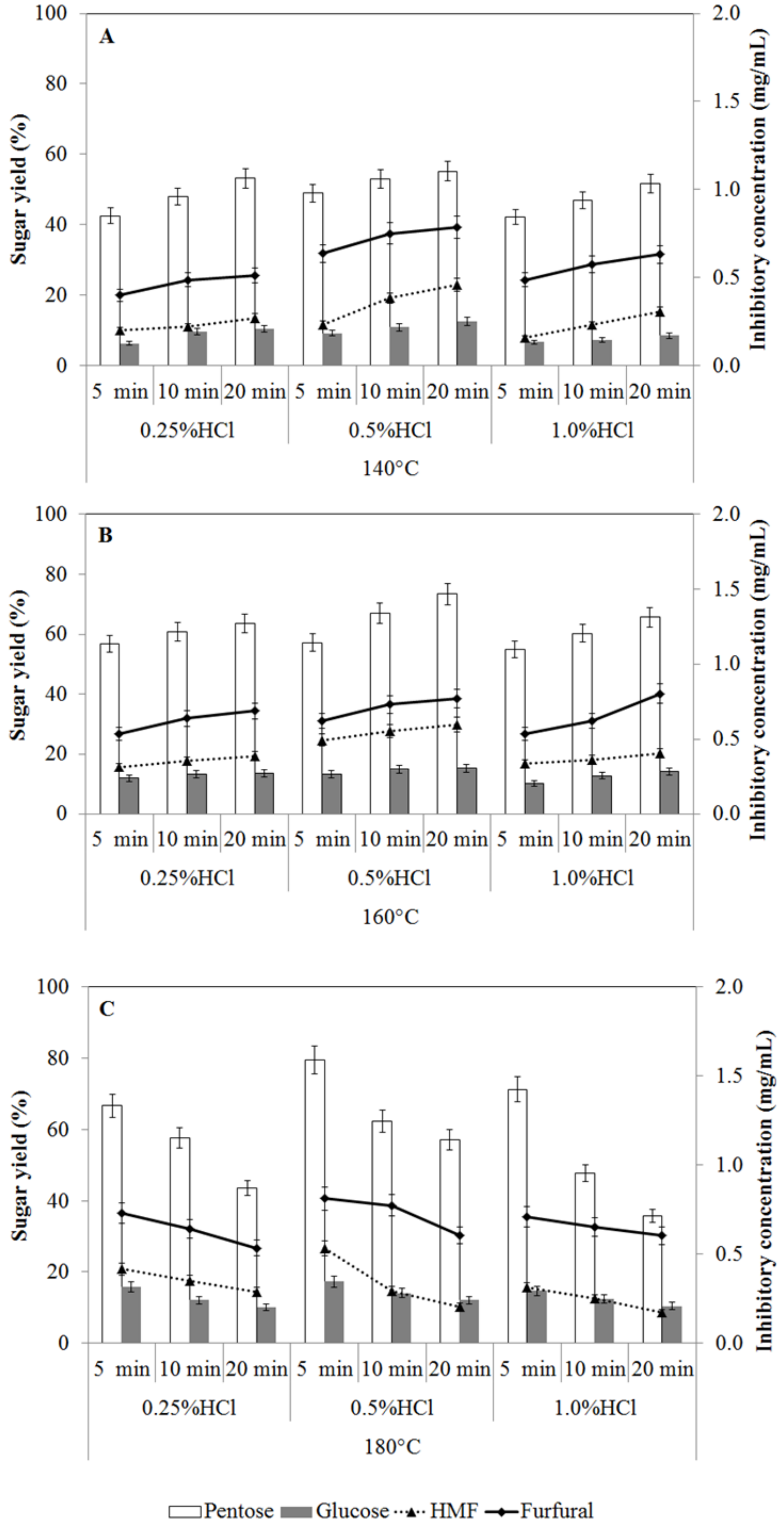


Figure 5.4 Effects of acid catalysts on LHW pretreatment of rice straw. The biomass was pretreated at 10% (w/v) solid loading at 140-180°C at varying residence times in the presence of 0.25-1% (v/v) HCl acid as the catalyst with the initial pressure at 25 bars: (A) 140°C, (B) 160°C and (C) 180°C

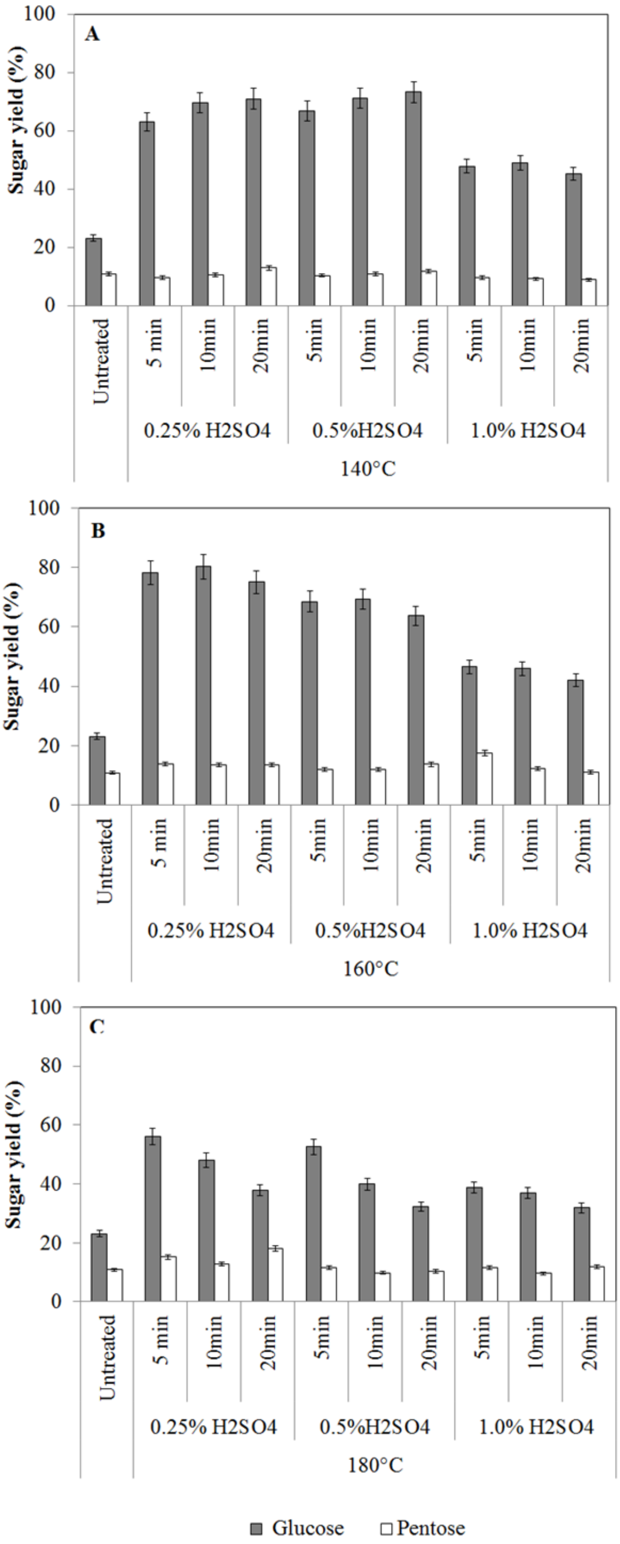


Figure 5.5 Effects of enzymatic hydrolysis on catalysts LHW pretreatment of rice straw. The biomass was pretreated at 10% (w/v) solid loading at 140-180°C at varying residence times in the presence of 0.25-1% (v/v) H_2SO_4 acid as the catalyst with the initial pressure at 25 bars: (A) 140°C, (B) 160°C and (C) 180°C.

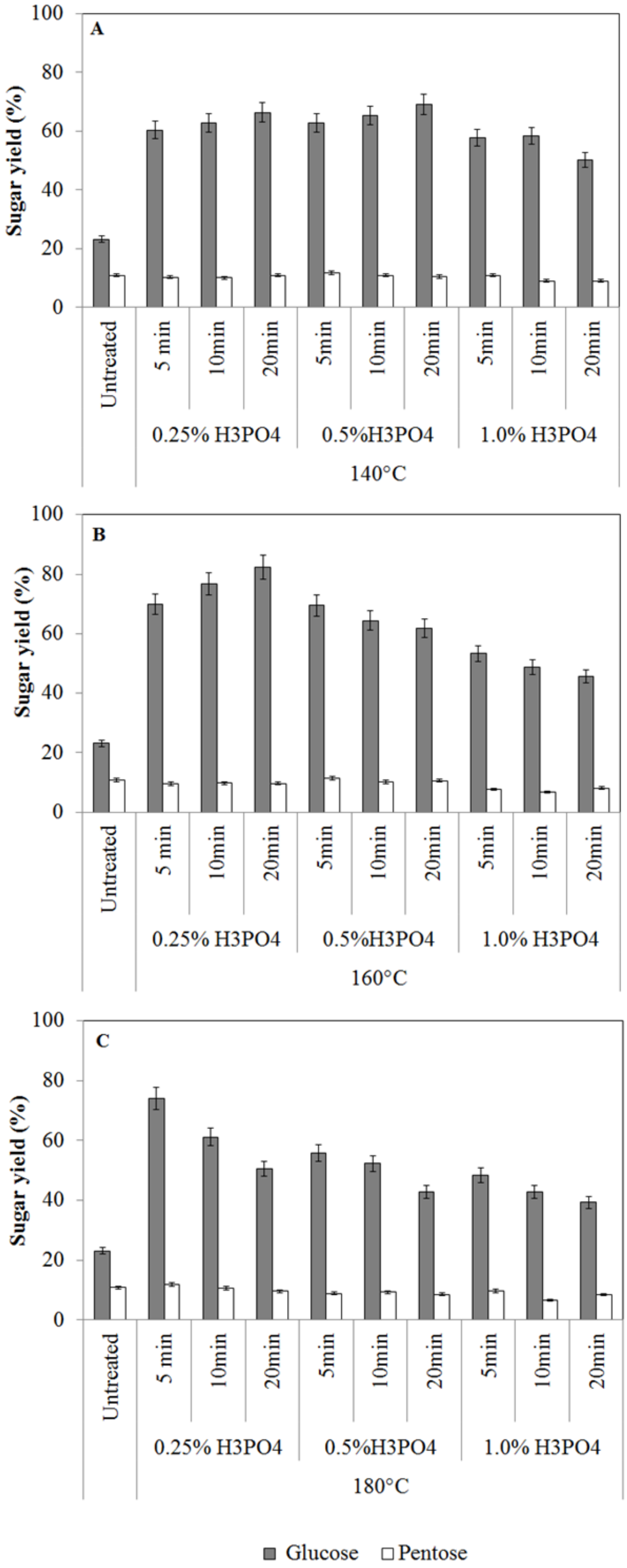


Figure 5.6 Effects of enzymatic hydrolysis on catalysts LHW pretreatment of rice straw. The biomass was pretreated at 10% (w/v) solid loading at 140-180°C at varying residence times in the presence of 0.25-1% (v/v) H_3PO_4 acid as the catalyst with the initial pressure at 25 bars: (A) 140°C, (B) 160°C and (C) 180°C

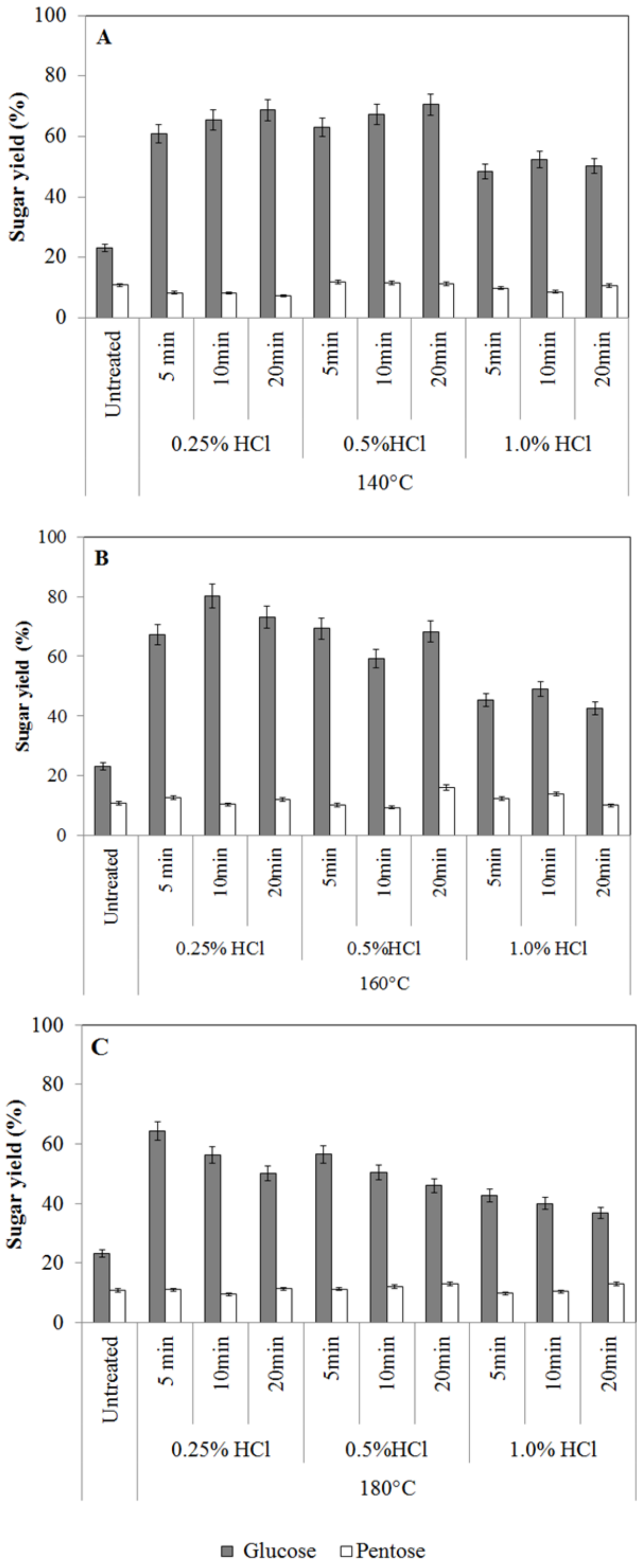


Figure 5.7 Effects of enzymatic hydrolysis on catalysts LHW pretreatment of rice straw. The biomass was pretreated at 10% (w/v) solid loading at 140-180°C at varying residence times in the presence of 0.25-1% (v/v) HCl acid as the catalyst with the initial pressure at 25 bars: (A) 140°C, (B) 160°C and (C) 180°C.

The use of weak acids as catalysts in the LHW process also showed a similar trend to those observed with strong acids on improving hemicellulose solubilization and biomass digestibility. The use of weak dicarboxylic acids as catalysts has also received increasing attention due to their less toxicity and compatibility to subsequent processing of the solubilized fractions by fermentation or catalytic routes [Lee *et al*. 2011; Kootsta *et al*. 2009].

The advantage of dicarboxylic acid catalysts on promoting less furan formation in hydrothermal biomass pretreatments as compared to strong acids has been previously reported [Lu *et al*. 2007]. Addition of 0.5% oxalic acid at 180°C for 5 min (Fig. 5.8C) led the highest pentose yield of 79.2% in the liquid phase and 59.9% glucose yield from hydrolysis of the solid fraction (Fig. 5.9C). However, the use of weak acid showed higher selectivity for products in the liquid fraction as observed by substantially lower solubilization of glucan yield into the liquid phase (4.2-18.8%) (Fig. 5.8). This was also associated with lower formation of HMF (0.06-0.35 mg/mL) and furfural (0.18-0.56 mg/mL) compared to the use of H_2SO_4 and higher pentose yield from enzymatic hydrolysis solid fraction. The solid fraction obtained from pretreatment was further to enzymatic hydrolysis. From the results showed that oxalic acid as catalysts, glucose was the major products obtained in hydrolyzate. Glucose yield obtained from oxalic acid pretreatment was 43.3-84.2% with increasing pretreatment temperature (Fig. 5.9). Previous studied, dicarboxylic organic acids like maleic acid and oxalic acid have been shown to possess higher activity on enhancing cellulose digestibility compared to H_2SO_4 in hydrothermal pretreatment of corncob [Lee *et al*. 2011]. The use of weak dicarboxylic acids as catalysts has also received increasing attention due to their less toxicity and compatibility to subsequent processing of the solubilized fractions by fermentation or catalytic routes [Lee et al. 2011; Kootsta *et al*. 2009].

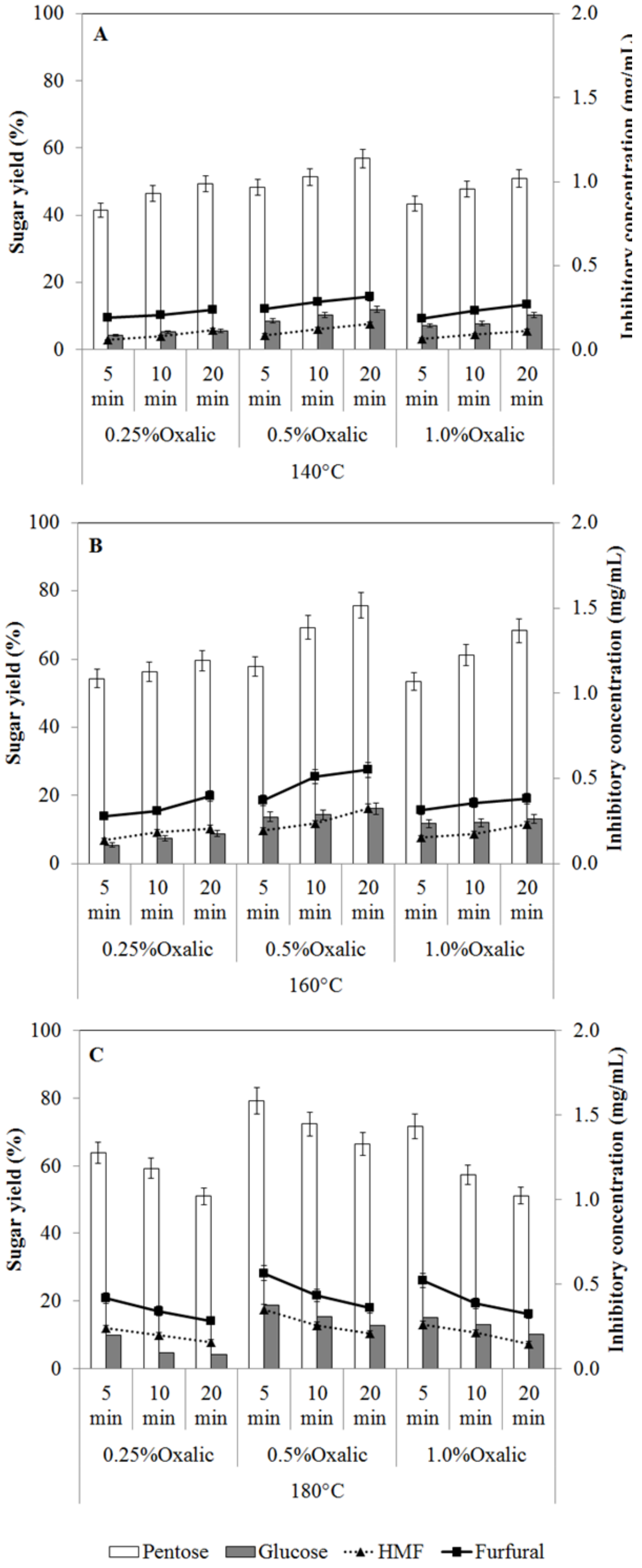


Figure 5.8. Effects of oxalic acid catalysts on LHW pretreatment of rice straw. The biomass was pretreated at 10% (w/v) solid loading at 140-180°C at varying residence times in the presence of 0.25-1% (v/v) acid as the catalyst with the initial pressure at 25 bars: (A) 140°C, (B) 160°C and (C) 180°C.

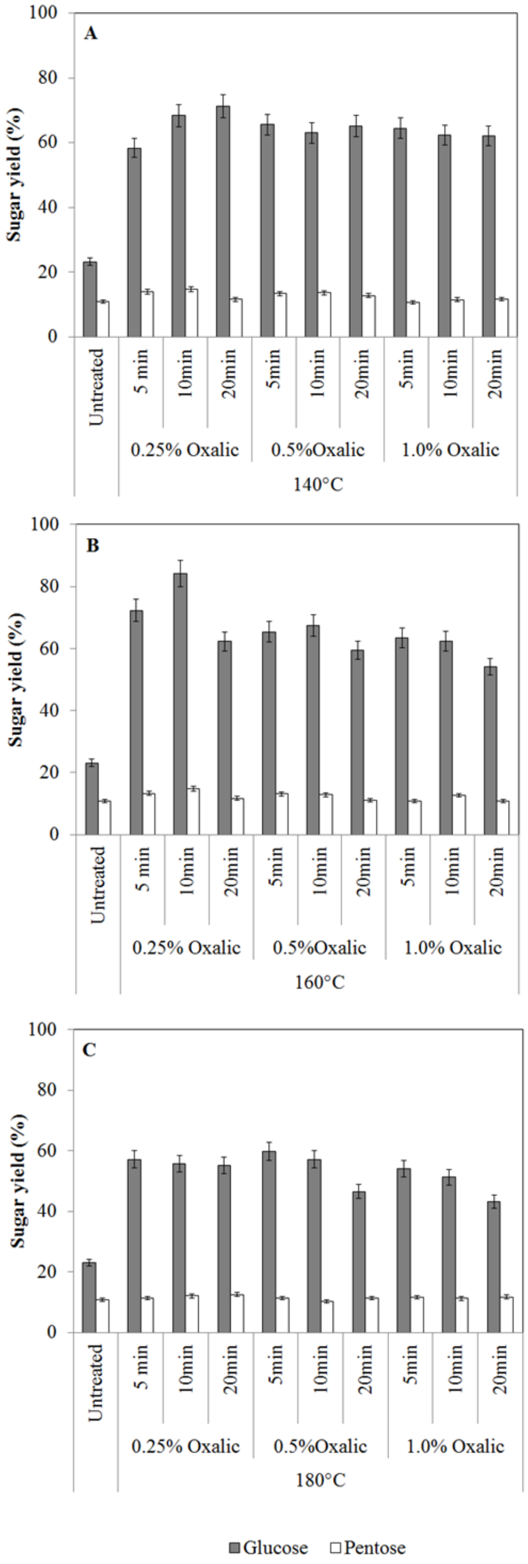


Figure 5.9. Effects of enzymatic hydrolysis on oxalic acid catalysts LHW pretreatment of rice straw. The biomass was pretreated at 10% (w/v) solid loading at 140-180°C at varying residence times in the presence of 0.25-1% (v/v) acid as the catalyst with the initial pressure at 25 bars: (A) 140°C, (B) 160°C and (C) 180°C.

5.1.2.2 Homogeneous alkaline catalysts

The addition of NaOH at 0.25-1% (w/v) to the LHW process at 140-180°C led to increasing hemicellulose solubilization as compared to the non-catalyzed reactions under the experimental conditions. The use of 0.5 % w/v NaOH with the operating at 180°C for 5 min resulted in the highest soluble pentose yield of 79.4% equivalent to 1.78 fold compared to that obtained using the non-catalytic LHW reaction (Fig. 5.10C). This was associated with substantially lower glucan degradation which led to decreased glucose hydrolysis into the liquid fraction as observed by a lower solubilized glucose yield (14.7%) compared to that obtained using the non-catalytic reaction (10.6%). The formation of sugar degradation by-products was also lower (0.02-0.26 mg/ml and 0.13-0.42 mg/mL for HMF and furfural, respectively) compared to the acid-catalyzed process. The use of alkaline in the LHW process led to achieved glucose yield of 79.7% from enzymatic hydrolysis of the solid fraction (Fig. 5.11A); however, with slightly higher pentose associated with the solid phase. The results thus suggested higher performance of alkaline catalyst on increasing LHW pretreatment efficiency under the experimental conditions.

Alkalis show strong effects on the delignification of biomass in hydrothermal pretreatment processes. Different alkalines, e.g. sodium hydroxide, ammonia, and lime, have been used as a basic strategy on the delignification of agricultural residues, e.g. corn stover, rice straw, and napier grass [Xu *et al.* 2011; Xu *et al.* 2012; Chen *et al.* 2012]. However, most alkaline-catalyzed study was performed under relatively lower temperatures (25-121°C) with long pretreatment time (30 min to 24 h) while the study under high-temperature LHW conditions has been very limited [Chiesa *et al.* 2014]. In our study, the use of low dosages of NaOH at 140-180°C led to increasing pentose solubilization into the liquid fraction to the comparable extent to those obtained using the acid catalysts while more pronounced effects on improving cellulose digestibility was observed, which resulted in the higher glucose yield from saccharification of the solid residues compared to the use of acids under LHW conditions at the same temperature. Mechanistically, alkalines cleave hydrolysable linkages in lignin and glycosidic bonds of polysaccharides, which result in reduction in the degree of polymerization and crystallinity, swelling of the fibers, as well as disruption of the lignin structure [Gupta. 2010]. This suggested the major effects of alkaline-catalyzed LHW on delignification and partial hydrolysis of the hemicelluloses, which in overall led to high susceptibility of the pretreated biomass to enzymatic hydrolysis.

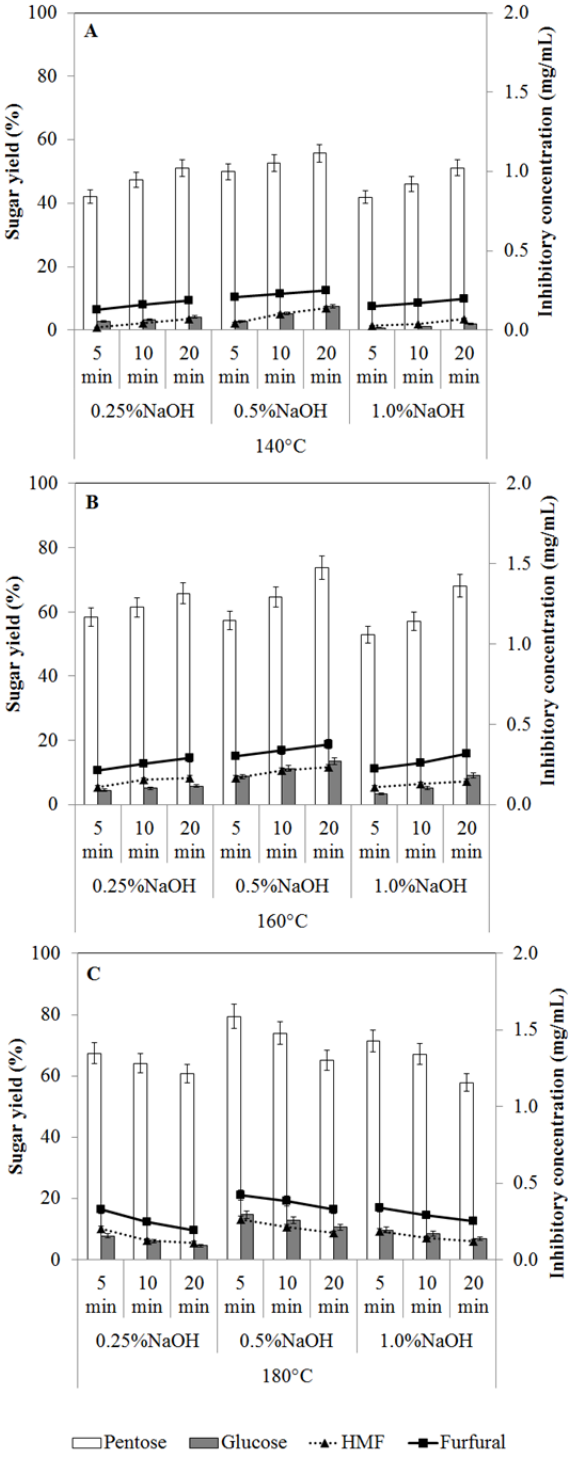


Figure 5.10 Effects of NaOH catalysts on LHW pretreatment of rice straw. The biomass was pretreated at 10% (w/v) solid loading at 140-180°C at varying residence times in the presence of 0.25-1% (w/v) NaOH as the catalyst with the initial pressure at 25 bars: (A) 140°C, (B) 160°C and (C) 180°C.

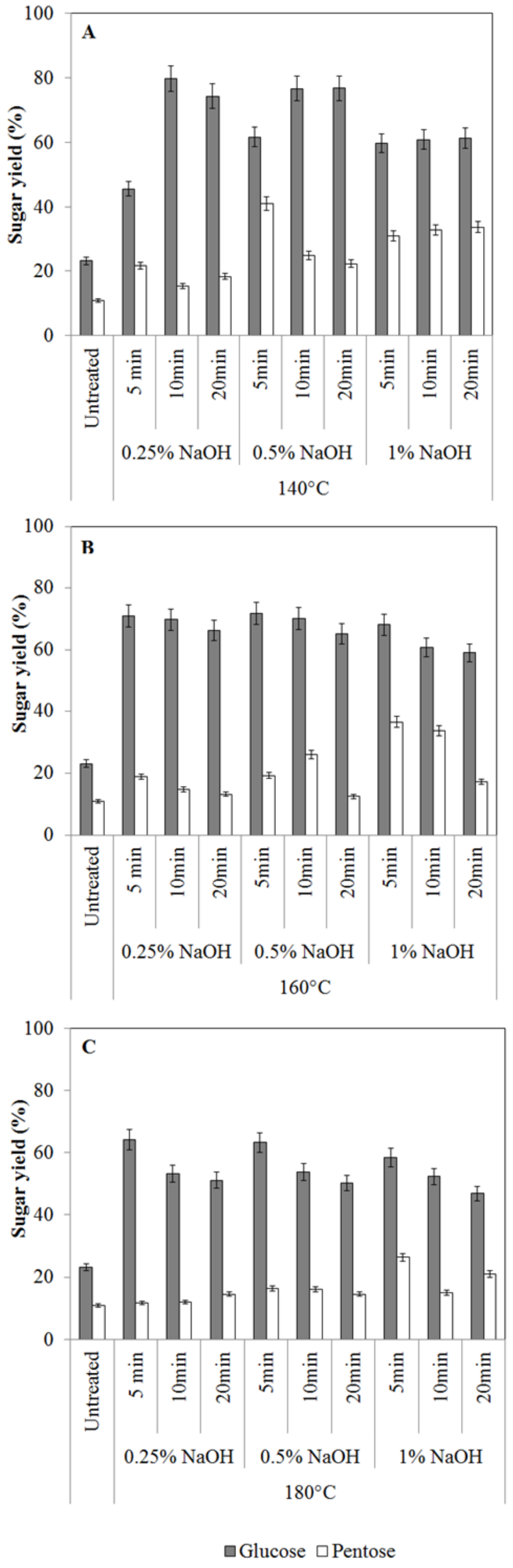


Figure 5.11 Effects of enzymatic hydrolysis on NaOH catalysts LHW pretreatment of rice straw. The biomass was pretreated at 10% (w/v) solid loading at 140-180°C at varying residence times in the presence of 0.25-1% (w/v) NaOH as the catalyst with the initial pressure at 25 bars: (A) 140°C, (B) 160°C and (C) 180°C.

5.2 Physicochemical analysis of pretreated solid residues

Physicochemical modifications of the biomass pretreated by LHW processes under the corresponding conditions were analyzed using BET and XRD (Table 5.1). Pretreated biomass under non-catalytic conditions led to increasing of surface area (3.2 m^2/g) as compared to the native rice straw (2.1 m^2/g). Addition of acid or alkaline catalysts showed further increase in the surface area. The highest surface area of 4.6 m^2/g was found with the reaction catalyzed by H_3PO_4. Increasing in crystallinity index (CrI) was observed for the rice straw pretreated using acid or alkaline catalytic process (68.6-70.6%) compared to the native biomass (60.3%) and rice straw pretreated by LHW under non-catalytic condition (63.6%). The increases in crystallinity could be due to the removal of the susceptible amorphous components in the biomass during the pretreatment step. This was correlated to solubilization of hemicellulose and removal of lignin.

The use of acids and alkalines as catalysts in LHW led to more pronounced changes in biomass physicochemical properties as compared to the non-catalytic LHW processes. Substantially increases in BET surface areas were observed compared to the native rice straw and the substrate pretreated in the absence of catalyst. The increase in internal surface areas could be related to the fragmentation and development of cracks as well as removal of the hemicelluloses and lignin which diminished shielding effects and open-up additional pores in the biomass structure. This is also related to the observed increase in crystallinity index due to higher efficiency on solubilization of the amorphous hemicelluloses and lignin fractions under the acid or alkaline catalytic conditions. The overall increase in enzyme accessibility to the cellulose fibers thus resulted in higher enzymatic susceptibility of the solid fraction, which led to improving glucose yield from the hydrolysis reaction [Gao *et al*. 2013].

Table 5.1 Physicochemical of the biomass pretreated by LHW processes presence of homogeneous acid and alkaline catalysts

Condition	Surface area (m^2/g)	Degree of crystallinity (%)
Native rice straw	2.1	60.3
Non-cat LHW	3.2	63.6
H_2SO_4	3.9	68.6
H_3PO_4	4.6	70.6
HCl	4.2	69.2
Oxalic acid	4.4	70.1
NaOH	4.2	70.4

5.3 Chapter conclusions

In this chapter, the effects of acids and NaOH as promoters for LHW pretreatment of rice straw were studied. The presences of acid (0.25-1% v/v H_2SO_4, HCl, H_3PO_4, and oxalic acid) and alkaline (0.25-1 w/v NaOH) efficiently promoted the hydrolysis of hemicellulose, improved enzymatic digestibility of the solids, and lower the required LHW temperature. Oxalic acid was a superior promoter under the optimal LHW conditions at 160°C, leading to the highest glucose yield from enzymatic hydrolysis (84.2%) and the lowest formation of furans. Combined with hydrolyzed glucose in the liquid, this resulted in the maximal 91.6% glucose recovery from rice straw. This was related to changes in surface area and crystallinity of pretreated biomass. The results displayed the efficiency of the external promoters on increasing sugar recovery and saving energy in LHW pretreatment.

CHAPTER 6

FRACTIONATION OF TROPICAL AGRICULTURAL RESIDUE BY SINGLE-STEP SOLVOTHERMAL PROCESS

Chapter Summary

An alternative approach to the pretreatment of lignocellulosic biomass is the complete fractionation of its main components cellulose, hemicellulose and lignin. This will allow maximum value-adding to biofuels and chemicals in an integrated biorefinery. Different organic solvents (methyl isobutyl ketone, MIBK; ethyl acetate, EA; toluene, TOL; and diethyl ether, DEE) in a ternary water/alcohol/organic solvent system and acid promoters (H_2SO_4, HCl, and H_3PO_4) were investigated. In addition, the use of microwave-assisted heating to replace conventional external heating was tested. The work provides an efficient alternative approach for efficient separation of primary lignocellulosic components with high recoverability and selectivity for further valorization in integrated biorefineries.

6.1 Influence of solvents on fractionation

The effects of reaction media on the separation of lignocellulosic components in rice straw were studied under non-catalytic conditions. The reactions containing rice straw in a ternary mixture solvent system containing water, ethanol, with a selected organic solvent with different polarity (*P´*) to the conventional solvent, MIBK. A single phase mixture was obtained using all solvent systems i.e. water/ethanol and MIBK (*P´* = 4.2), EA (*P´* = 4.4), TOL (*P´* = 2.4), or DEE (*P´* = 2.8). Addition of water to the mixture after the reaction was heated at 140-180^{o}C for 1 h in the absence of acid promoter led to phase separation to the upper organic phase and water/ethanol fraction only for the ternary mixture containing MIBK or EA but not for TOL and DEE. Analysis of lignocellulose components in the solid fraction obtained using different solvent systems is shown in Fig. 6.1 Enrichment of cellulose in the solid fraction was found which led to glucan recovery yield of 75.8% with a relative glucan content of 45.6% in the reaction containing MIBK. Slightly higher glucan yield and relative content of 77.8% and 49.0% were achieved in the reaction containing EA. This was correlated to the selective extraction of hemicellulose and lignin from the solid fractions. Slightly lower yield and selectivity was obtained with ternary mixture containing TOL or DEE. Lower solubilization of hemicellulose and lignin from the solid residues were also observed for these solvents. MIBK and EA were therefore chosen for further reaction optimization in subsequent experiments.

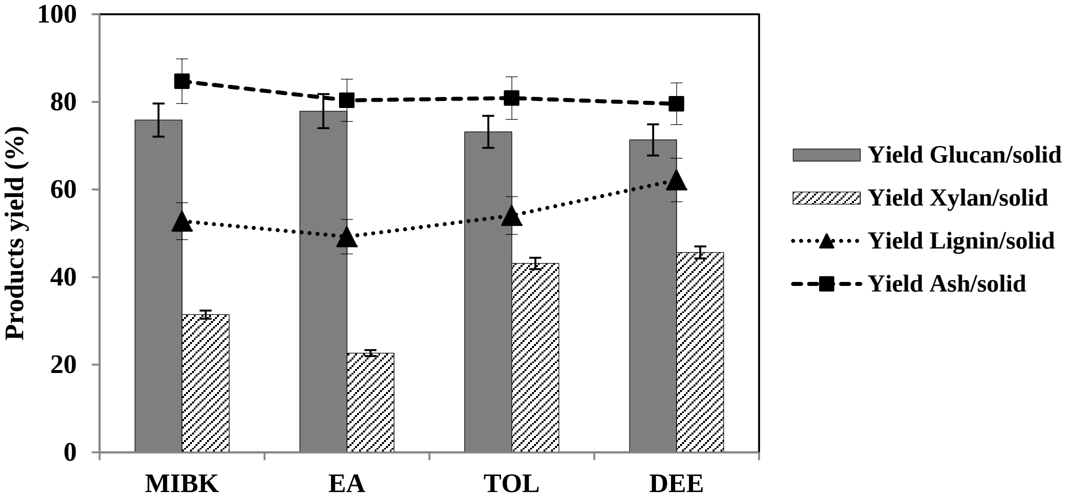


Figure 6.1 Components in solid phase obtained from fractionation of rice straw using different solvents. The reactions contained 10% (w/v) rice straw in different solvent systems and treated at 160°C for 1 h in the absence of an acid promoter. Solvent ratios: water/ethanol/MIBK (24%:32%:44%); water/ethanol/EA (62.5%:25%:12.5%); water/ethanol/TOL (10%:70%:10%); water/ethanol/DEE (50%:37.5%:12.5%).

In the next step, the effects of reaction temperature on lignocellulose fractionation was studied using the ternary mixture containing water/ethanol/MIBK (24%:32%:44%) or water/ethanol/EA (62.5%:25%:12.5%) with no acid promoter. Decreasing glucan yield in the solid phase from 81.0% to 71.4% was found with increasing temperature from 140-180°C using MIBK in the solvent mixture (Fig 6.2A). Similar trends in glucan yield in the range of 82.9-73.3% was found for the ternary system containing EA. This was correlated to higher solubilization of hemicelluloses and lignin from the solid fraction at higher temperatures. The highest recovery of hemicelluloses of 46.6% and 40.7% was obtained in the aqueous/ethanol phase at 160°C using MIBK and EA, respectively (Fig 6.2B) while decreasing yield

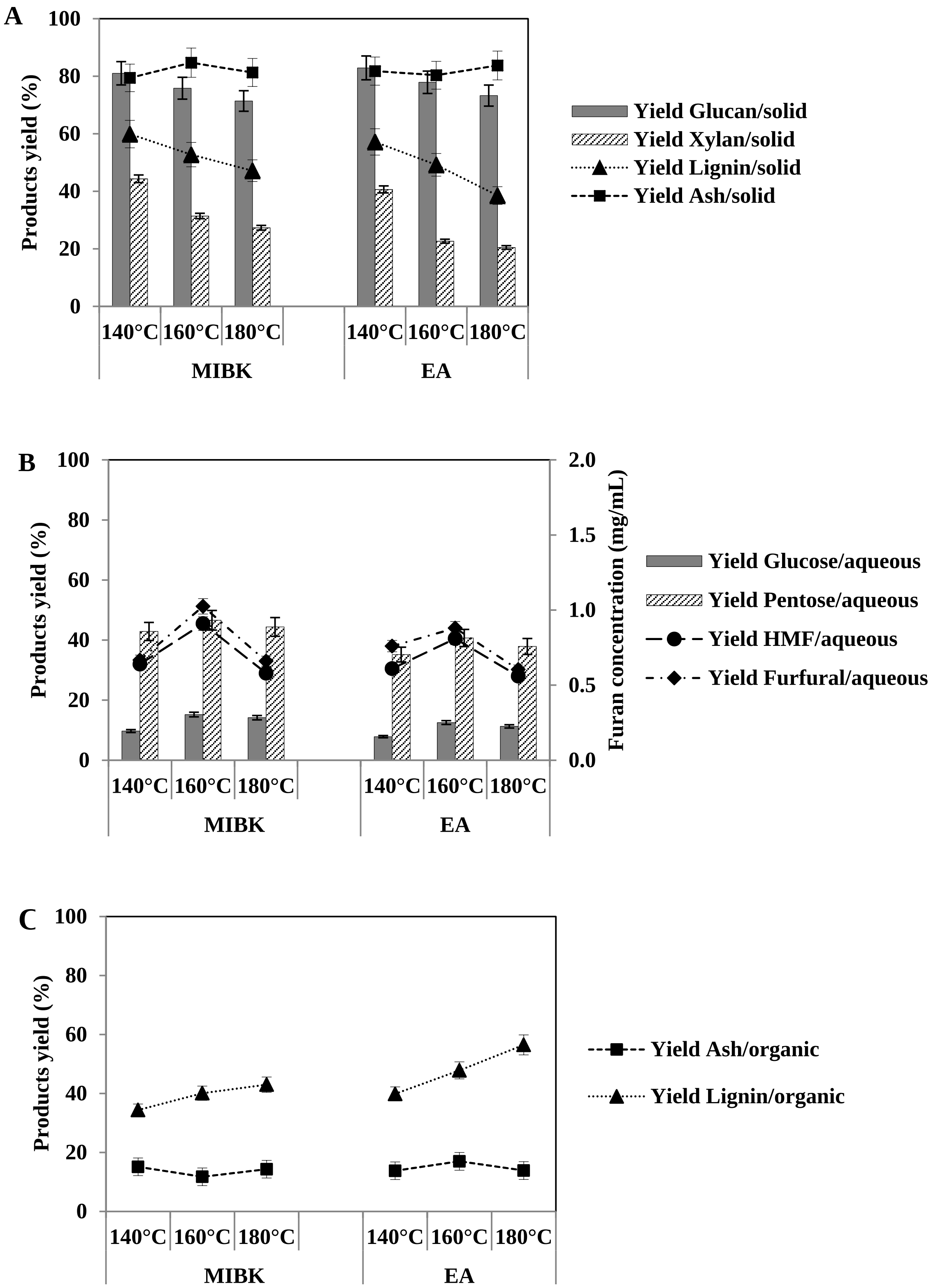


Figure 6.2 Effects of temperature on biomass fractionation. Reactions contained 10% (w/v) rice straw in water/ethanol/MIBK (24%:32%:44%) or water/ethanol/EA (62.5%:25%:12.5%) and treated at different temperatures for 1 h in the absence of an acid promoter: (A) solid phase; (B) aqueous phase; (C) organic phase.

Similar trends in glucan solubilization to hydrolysis products and the formation of sugar dehydration by-products in the aqueous phase in relation to the reaction temperature were also observed. Increasing lignin extraction into the organic phase was found with increasing temperature. The highest lignin yield of 43.0 and 56.5% was obtained at 180^{o}C using the ternary mixture containing MIBK or EA, respectively (Fig 6.2C), correlated to the decreasing lignin content in the solid phase. Majority of ash was still in the solid phase under the experimental conditions.

6.2 Effect of acid promoter

The influences of adding H_2SO_4, H_3PO_4, and HCl as acid promoters on the fractionation of rice straw were firstly studied at varying acid concentrations. Increasing the acid promoter concentration from 0.05 M to 0.2 M of H_2SO_4, H_3PO_4, and HCl led to lower glucan yield in the solid phase using MIBK and EA as the composite organic solvent with increasing acid concentration and operating temperature (Fig 6.3 and 6.6). Compared to MIBK, higher glucan yield was found using EA of which 78.2% glucan yield was recovered at 140°C, 0.05M H_3PO_4 (Fig 6.6B). Markedly increasing solubilization of glucan and xylan into the aqueous phase was observed in the presence of acid promoter. The highest xylose accumulation of 71.3 and 55.8% in the aqueous phase was found at 160^{o}C with 0.1 M H_2SO_4 in the MIBK (Fig 6.4A) and EA (Fig 6.7A) systems while their slight decreases were observed at higher temperature. Continual decreases in sugar recovery and furans in the aqueous phase were found at higher acid concentration. The highest accumulation of HMF of 0.94 mg/mL and furfural of 1.15 mg/mL at 160^{o}C was observed at 0.1 M HCl for MIBK (Fig 6.4C) while they were 0.78 mg/mL and 0.98 mg/mL (Fig 6.7C) for the reaction containing EA as the organic solvent, respectively. Higher recovery of lignin in the organic phase was found with higher concentration of the acid promoter. The highest lignin recovery of 80.9 and 84.9% was achieved at 0.2M HCl and 0.2 M H_2SO_4 using MIBK (Fig 6.5C) and EA (Fig 6.8C), respectively. The results suggested the marked effects of acid promoters on increasing solubilization and further conversion of lignocellulose components under the reaction conditions.

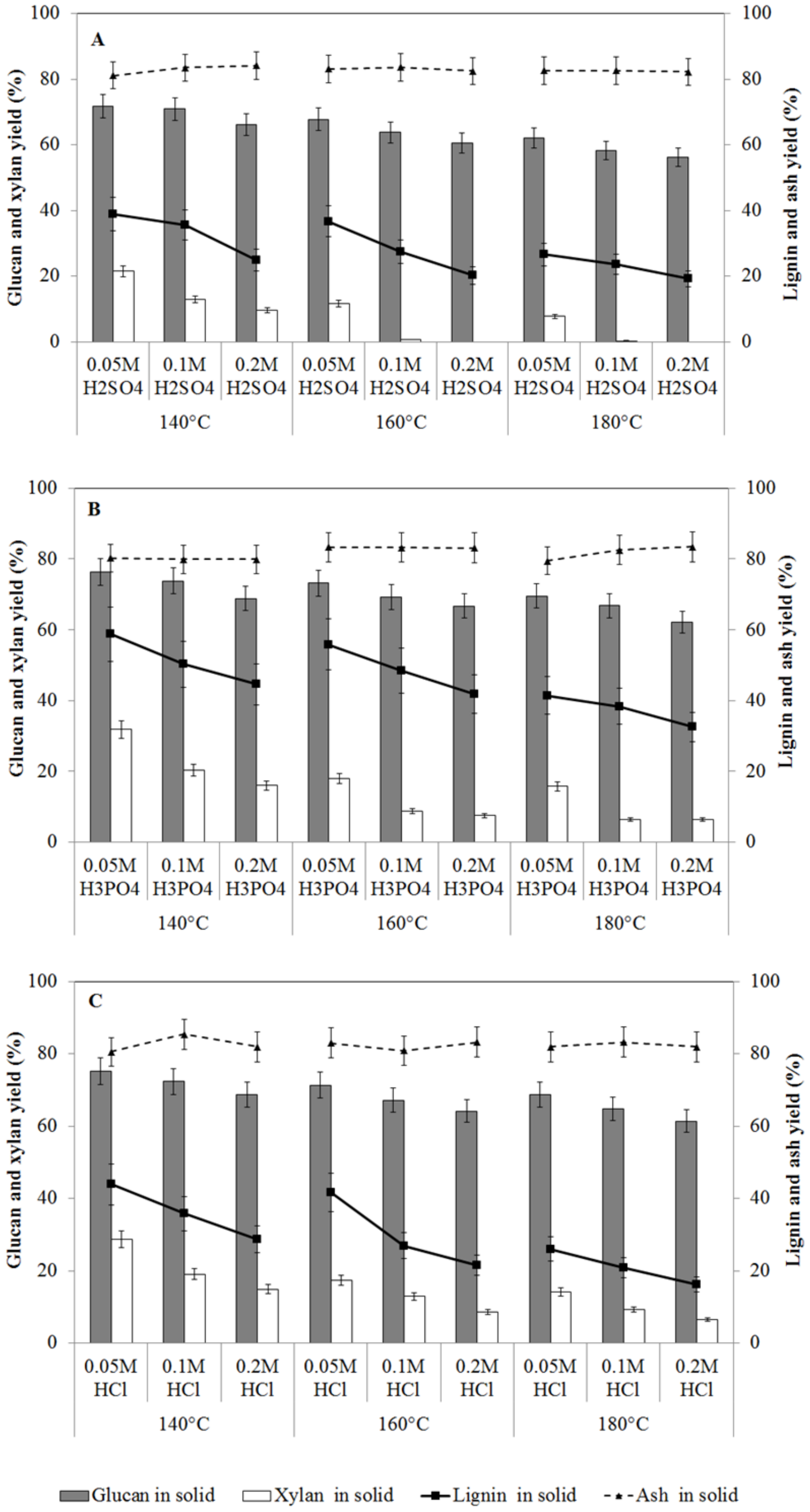


Figure 6.3 Effects of acid types on biomass fractionation. Reactions contained 10% (w/v) rice straw in water/ethanol/MIBK (24%:32%:44%) and treated at different temperatures for 1 h in the presence of varying acid concentration in the solid phase: (A) H_2SO_4, (B) H_3PO_4 and C) HCl.

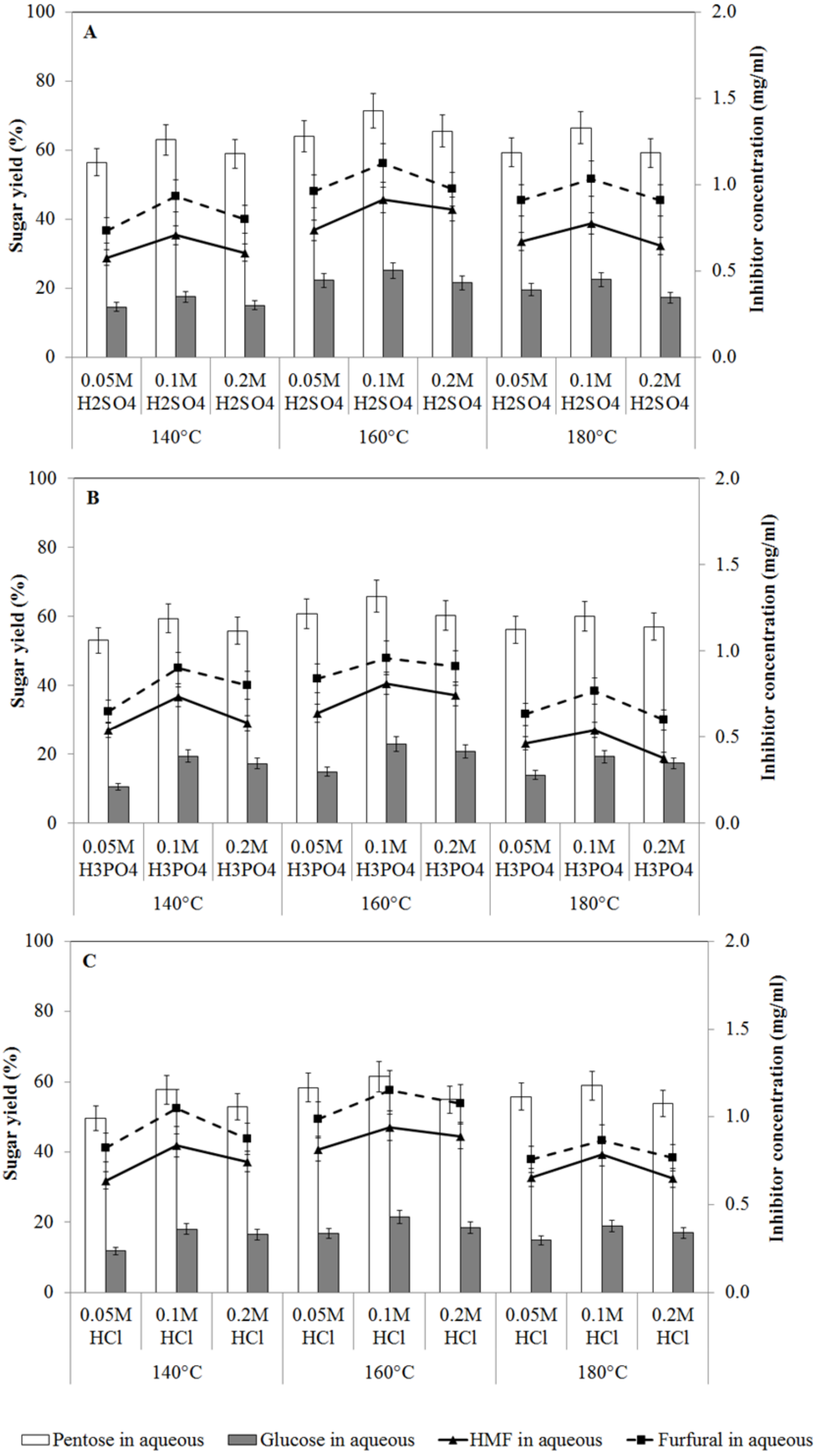


Figure 6.4 Effects of acid types on biomass fractionation. Reactions contained 10% (w/v) rice straw in water/ethanol/MIBK (24%:32%:44%) and treated at different temperatures for 1 h in the presence of varying acid concentration in the liquid phase: (A) H_2SO_4, (B) H_3PO_4 and C) HCl.

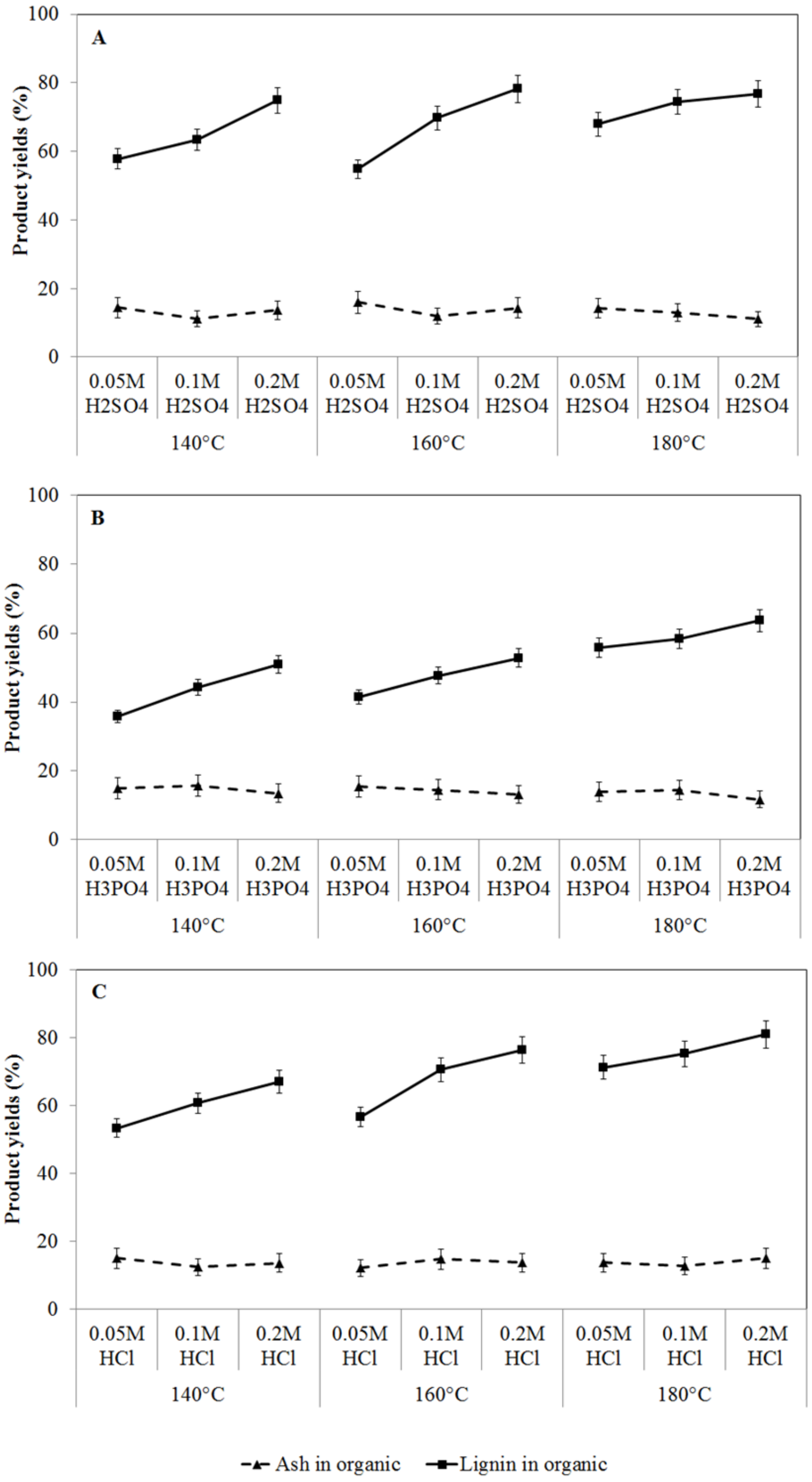


Figure 6.5 Effects of acid types on biomass fractionation. Reactions contained 10% (w/v) rice straw in water/ethanol/MIBK (24%:32%:44%) and treated at different temperatures for 1 h in the presence of varying acid concentration in the organic phase: (A) H_2SO_4, (B) H_3PO_4 and C) HCl.

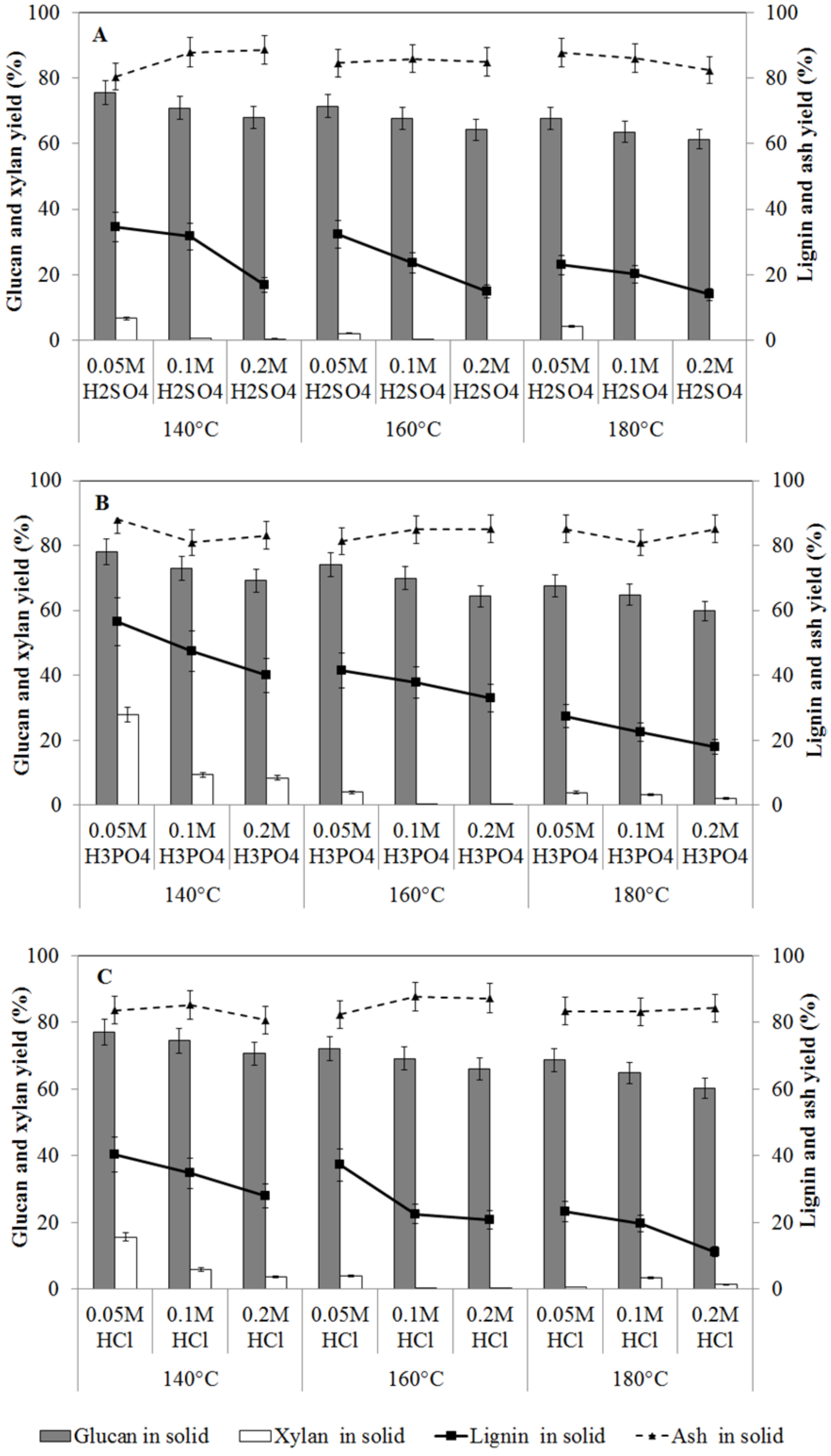


Figure 6.6 Effects of acid types on biomass fractionation. Reactions contained 10% (w/v) rice straw in water/ethanol/EA (62.5%:25%:12.5%) and treated at different temperatures for 1 h in the presence of varying acid concentration in the solid phase: (A) H_2SO_4, (B) H_3PO_4 and C) HCl.

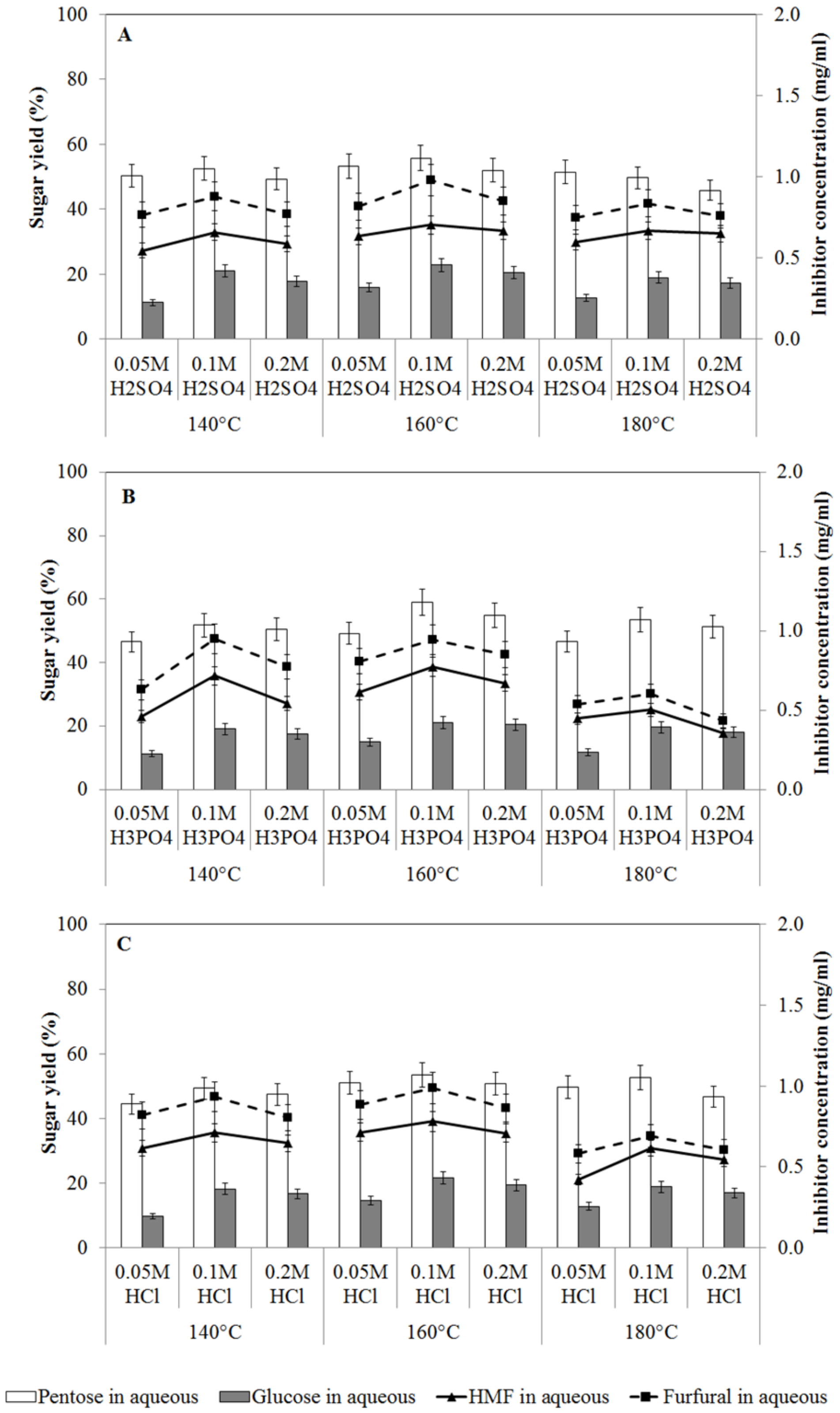


Figure 6.7 Effects of acid types on biomass fractionation. Reactions contained 10% (w/v) rice straw in water/ethanol/EA (62.5%:25%:12.5%) and treated at different temperatures for 1 h in the presence of varying acid concentration in the liquid phase: (A) H_2SO_4, (B) H_3PO_4 and C) HCl.

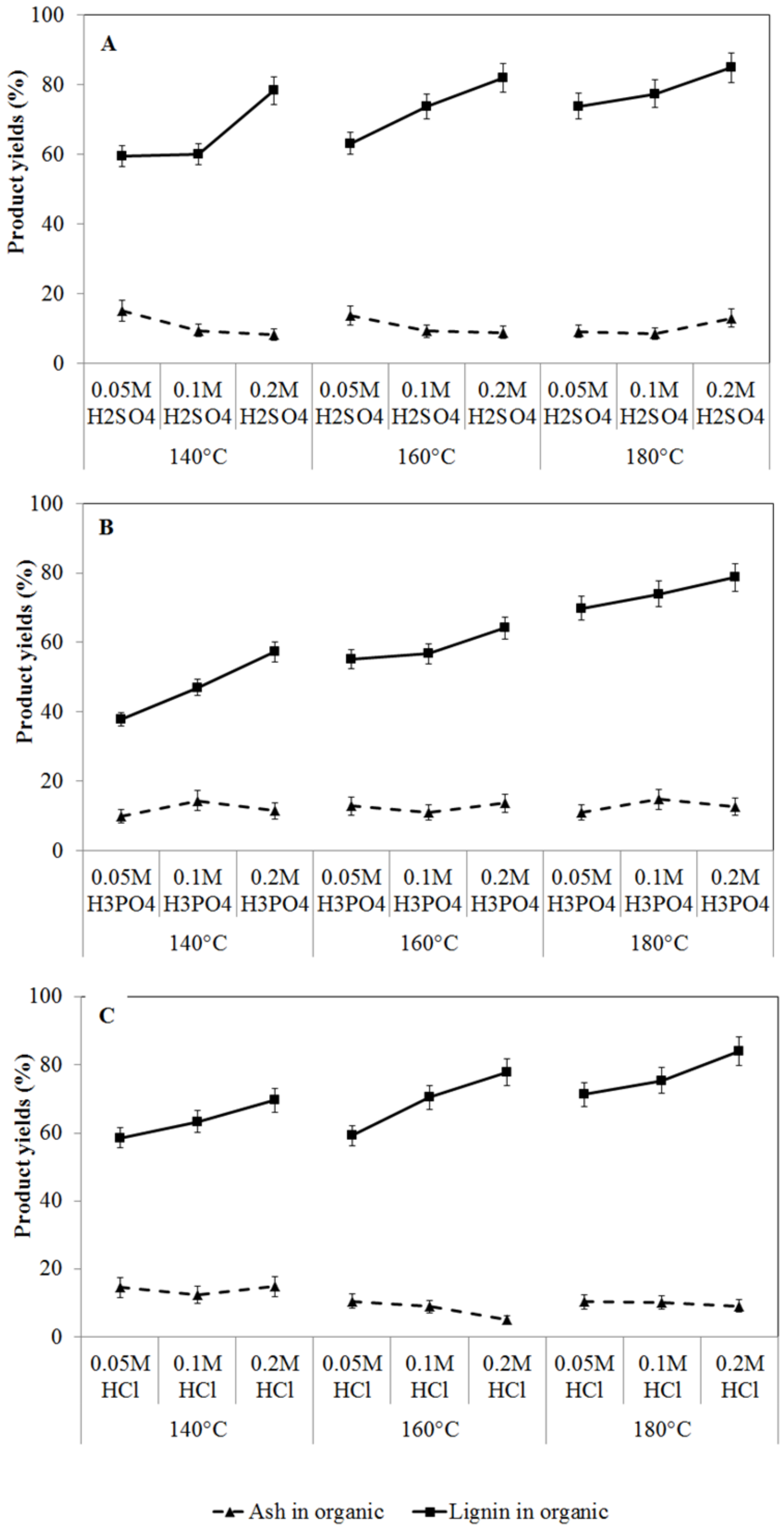


Figure 6.8 Effects of acid types on biomass fractionation. Reactions contained 10% (w/v) rice straw in water/ethanol/EA (62.5%:25%:12.5%) and treated at different temperatures for 1 h in the presence of varying acid concentration in the organic phase: (A) H_2SO_4, (B) H_3PO_4 and C) HCl.

Acid promoters play an important role on organosolv fractionation of lignocellulosic components in different systems e.g. clean fractionation process [Bozell *et al.* 2011]. The presence of acid led to the enhancing scission of glycosidic bonds, which are initially catalyzed by the action of proton (H^+) existing in the aqueous medium [Fengel *et al.* 1984]. The addition of homogeneous acid promoters led to increasing selectivity of fractionated biomass components from rice straw in both the MIBK and EA systems. The yield and homogeneity of lignin obtained was also higher due to the acid-catalyzed hydrolysis of the polysaccharide fractions. Different homogeneous mineral acid promoters were found to possess varying degree of activity on the CF-based fractionation reactions. H_2SO_4 was found to be the most effective promoter in term of reaction selectivity. The relatively higher activity of H_2SO_4 compared to other mineral acids were previously reported in pretreatment of rice husk using hydrothermal process [Ang *et al.* 2013]. Organic acids e.g. formic and oxalic were shown to enhance reaction selectivity on biomass component separation compared to mineral acids in various pretreatment systems e.g dilute acid and liquid hot water [Zhang *et al.* 2013]. The use of various heterogeneous acid promoters synthesized on different solid carbon supports on increasing selectivity of the CF process has also been recently reported, showing the potential of using solid acid promoters in the process with advantages on its reusability and formation of less degradation by-products [Klameassamee *et al.* 2013]. The results thus indicated the important role of acid promoters on enhancing product yield and reaction selectivity of the CF-based reactions.

6.3 Effect of microwave treatment

The use of microwave treatment as an internal heating process for the developed fractionation reaction was studied using MIBK or EA as the composite solvent. Reactions were performed in the ternary mixture of water/ethanol/MIBK (24%:32%:44%) (Figs 6.9-6.11) or water/ethanol/EA (62.5%:25%:12.5%) (Fig 6.12-6.14) in the presence of different acid concentration as promoter. The microwaves were applied at 200-450 W watts, corresponding to the temperature range of 140-180°C. Increasing microwave power led to higher solubilization of glucan, hemicelluloses, and lignin which resulted in their decreased recovery in the solid phase (Figs. 6.9 and 6.12). Increasing microwave power led to led to higher of glucan yield from 60.6-76.7% in the solid due to higher solubilization of polysaccharides similar to those observed using the conventional external heating.

Substantially higher accumulation of hemicellulose hydrolysis products in the aqueous phase was found in the microwave assisted reactions as compared to conventional heating with the highest pentose yield of 72.8% at 300 watts (0.1M H_2SO_4) (Fig 6.10A) and 62.7% at 300 watts (0.1M H_3PO_4) (Fig 6.13B) for MIBK and EA, respectively. The lower formations of HMF (0.18-0.94 mg/mL) and furfural (0.36-1.05 mg/mL) were also found compared to those with conventional heating. The highest lignin recovery of 80.4% (0.2M H_2SO_4) (Fig 6.11A) and 79.2% (0.2M HCl) (Fig 6.14C) was achieved at 450 watts for MIBK and EA, respectively, which was comparable to that obtained using the conventional heating process with no acid promoter at 180^{o}C. The results thus suggested the potential of using microwave as an alternative heating source for the developed fractionation reaction.

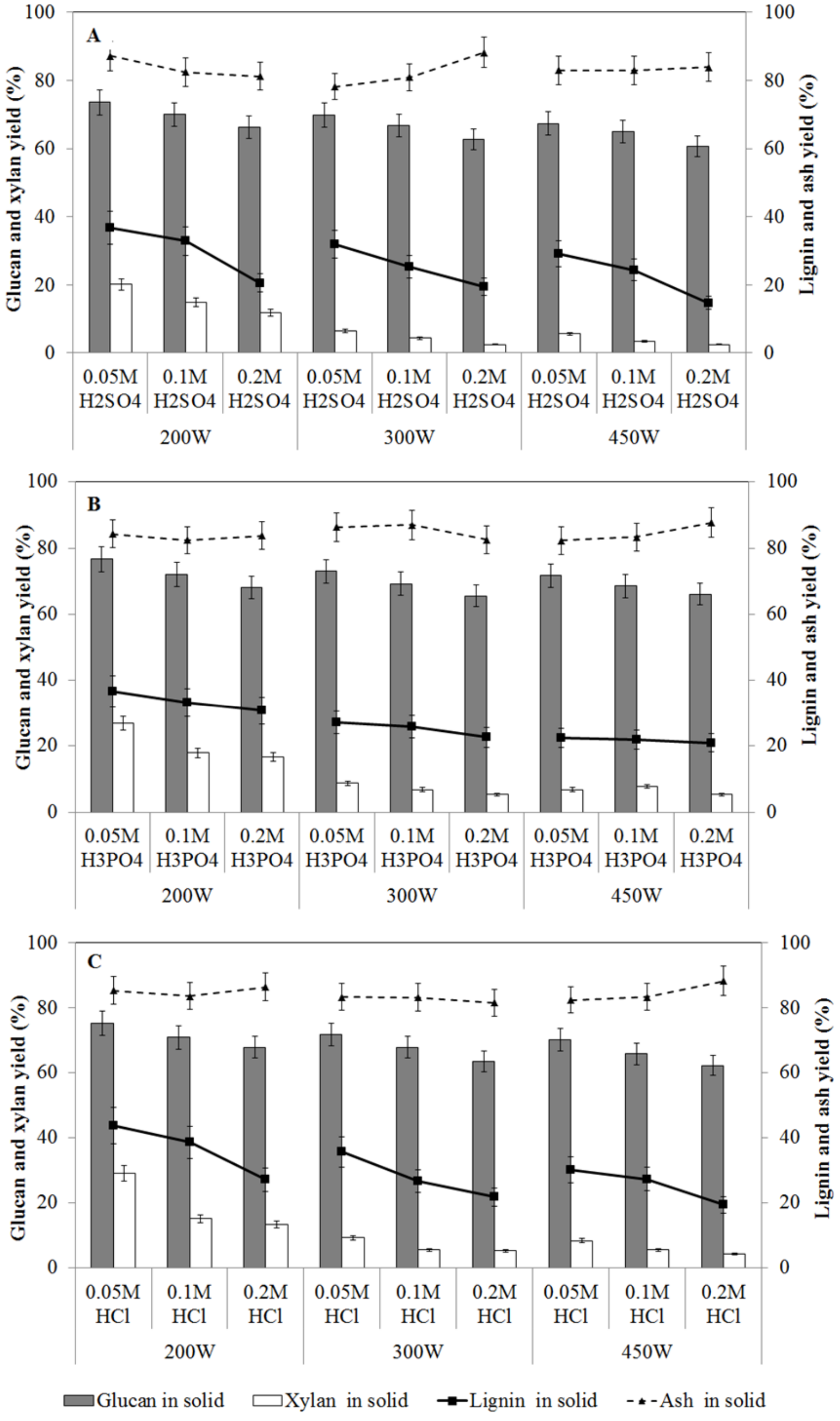


Figure 6.9 Effects of acid types on biomass fractionation. Reactions contained 10% (w/v) rice straw in water/ethanol/MIBK (24%:32%:44%) and treated at different temperatures for 1 h in the presence of varying acid concentration in the solid phase: (A) H_2SO_4, (B) H_3PO_4 and C) HCl.

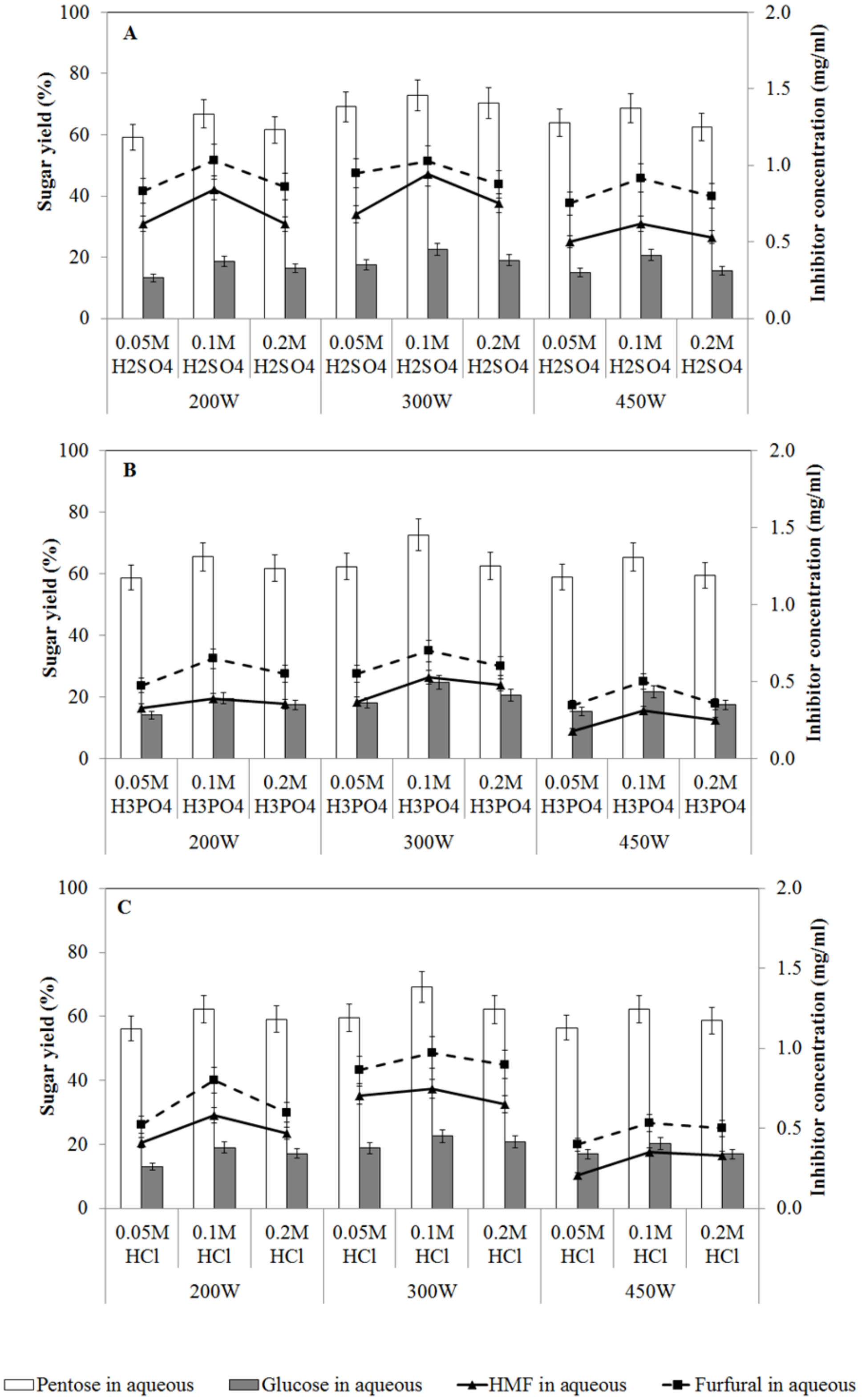


Figure 6.10 Effects of acid types on biomass fractionation. Reactions contained 10% (w/v) rice straw in water/ethanol/MIBK (24%:32%:44%) and treated at different temperatures for 1 h in the presence of varying acid concentration in the liquid phase: (A) H_2SO_4, (B) H_3PO_4 and C) HCl.

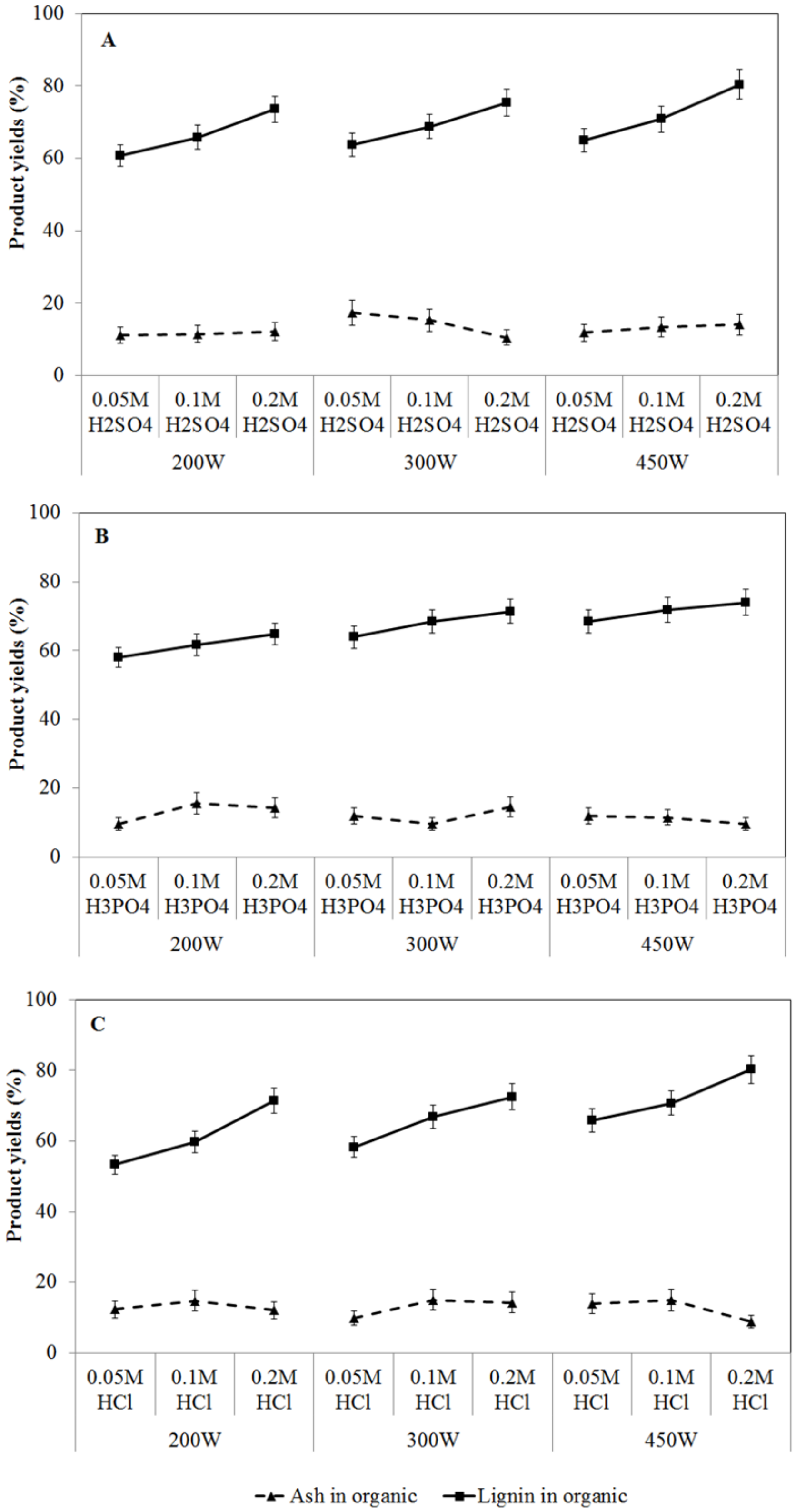


Figure 6.11 Effects of acid types on biomass fractionation. Reactions contained 10% (w/v) rice straw in water/ethanol/MIBK (24%:32%:44%) and treated at different temperatures for 1 h in the presence of varying acid concentration in the organic phase: (A) H_2SO_4, (B) H_3PO_4 and C) HCl.

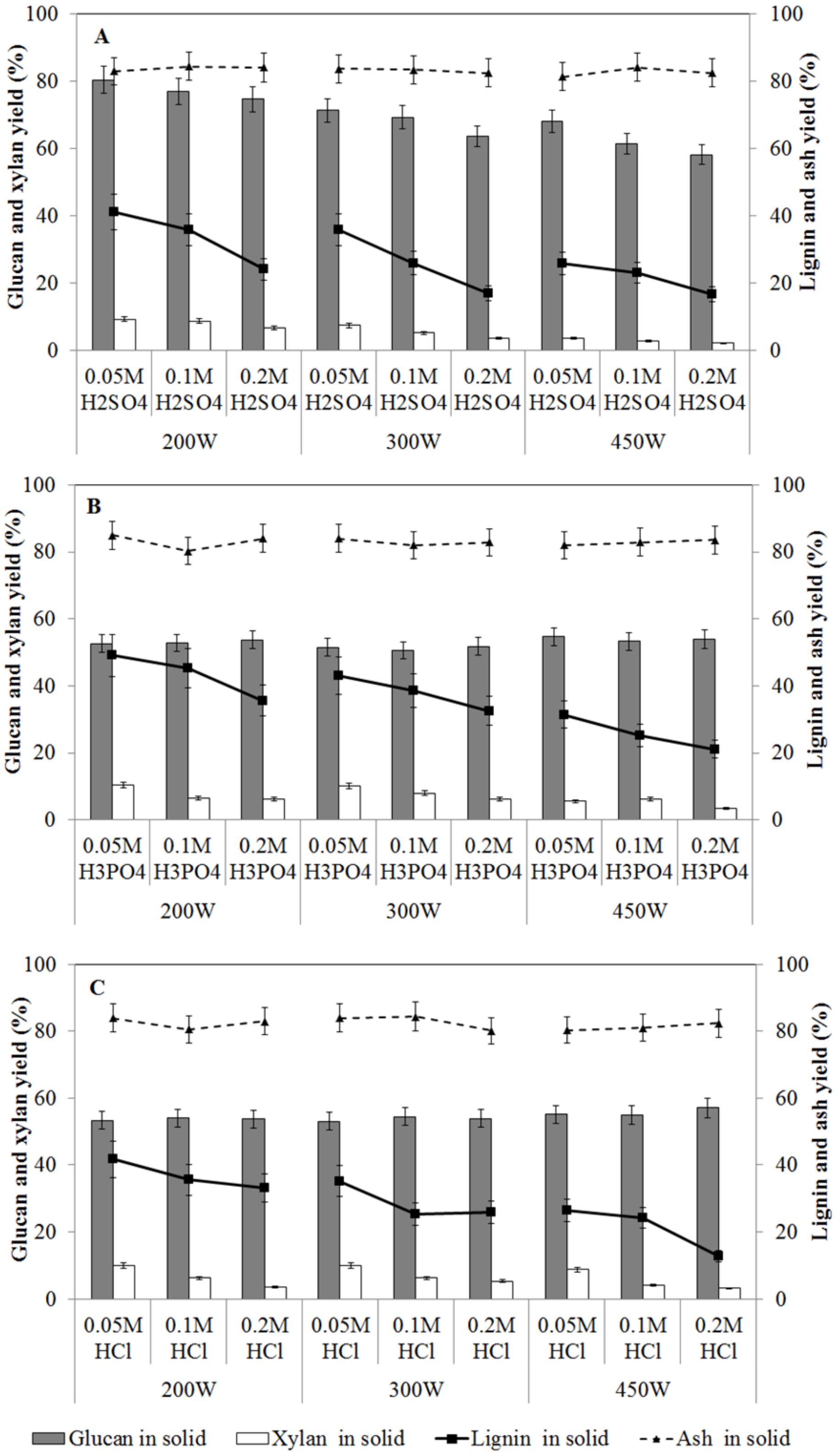


Figure 6.12 Effects of acid types on biomass fractionation. Reactions contained 10% (w/v) rice straw in water/ethanol/EA (62.5%:25%:12.5%) and treated at different temperatures for 1 h in the presence of varying acid concentration in the solid phase: (A) H_2SO_4, (B) H_3PO_4 and C) HCl.

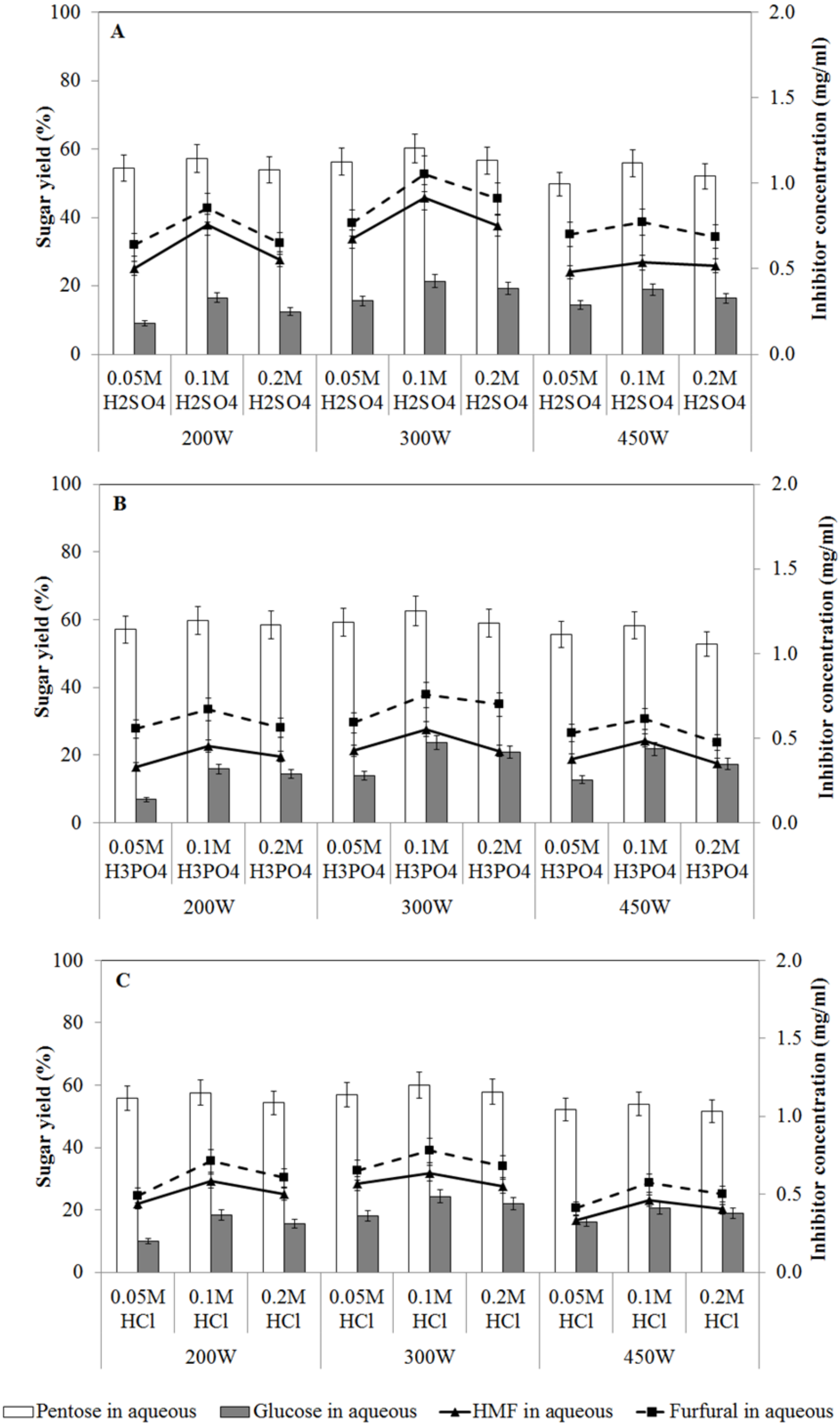


Figure 6.13 Effects of acid types on biomass fractionation. Reactions contained 10% (w/v) rice straw in water/ethanol/EA (62.5%:25%:12.5%) and treated at different temperatures for 1 h in the presence of varying acid concentration in the liquid phase: (A) H_2SO_4, (B) H_3PO_4 and C) HCl.

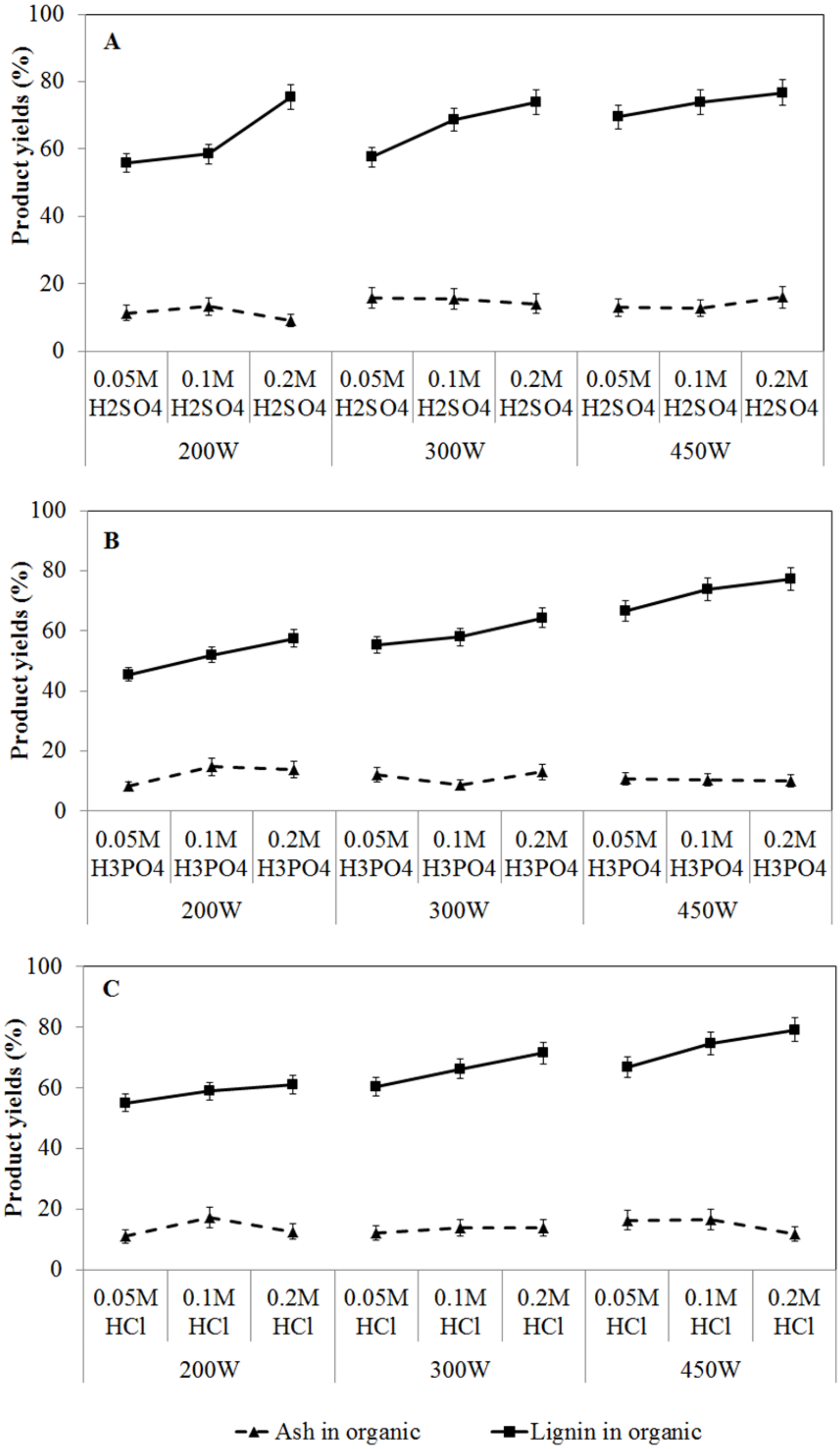


Figure 6.14 Effects of acid types on biomass fractionation. Reactions contained 10% (w/v) rice straw in water/ethanol/EA (62.5%:25%:12.5%) and treated at different temperatures for 1 h in the presence of varying acid concentration in the organic phase: (A) H_2SO_4, (B) H_3PO_4 and C) HCl.

The application of microwave treatment to the fractionation process under catalytic conditions resulted in comparable yields of glucan and lignin in their respective phases and increased pentose yield with lower furan accumulation in the aqueous phase as compared to the use of conventional heating. Compared to direct conventional heating, microwave irradiation supplies internal heat generation resulted from vibrations of polar bonds in the biomass and surrounding aqueous medium [Kappe *et al.* 2008]. The use of microwave to accelerate chemical reactions has been previously shown for pretreatment of lignocellulosic biomass using different processes including ethanosolv with presence of H_2SO_4 as an acid promoter [Monteil-Rivera *et al.* 2012] and enhanced enzymatic saccharification [Ma *et al.* 2009]. The results showed advantages of microwave treatment including higher reaction rate and shorter reaction time due to uniformity in heating of the reaction mixture and lower process energy input [Ma *et al.* 2009; Gabriel *et al.* 1998]. The uniformity in processing temperature of the reaction could result in lower dehydration of sugars to furans and hence led to increasing sugar yields from the microwave-assisted reactions. The result thus shows the potential of microwave treatment as an effective approach for accelerating the organosolv-based CF reactions.

6.4 Structural analysis of the fractionated solid residues and extracted lignin

The microstructures of the solid residues obtained from fractionation with conventional heating and the microwave-assisted reaction using EA as the composite organic solvent under non-catalytic conditions were compared to the native rice straw using scanning electron microscopy. The native biomass displayed regular, smooth, and intact surface, reflecting the highly ordered intact structure of the plant material (Fig 6.15A). The SEM micrographs were observed differences in biomass surface structures in the fractionated solid residues using conventional heating (Fig 6.15B) and microwave treatment (Fig 6.15C). These involved formation of cracks and papillae structure on the surface. The removal of wax coated on the biomass surface was observed which led to higher accessibility to the cellulose fibers.

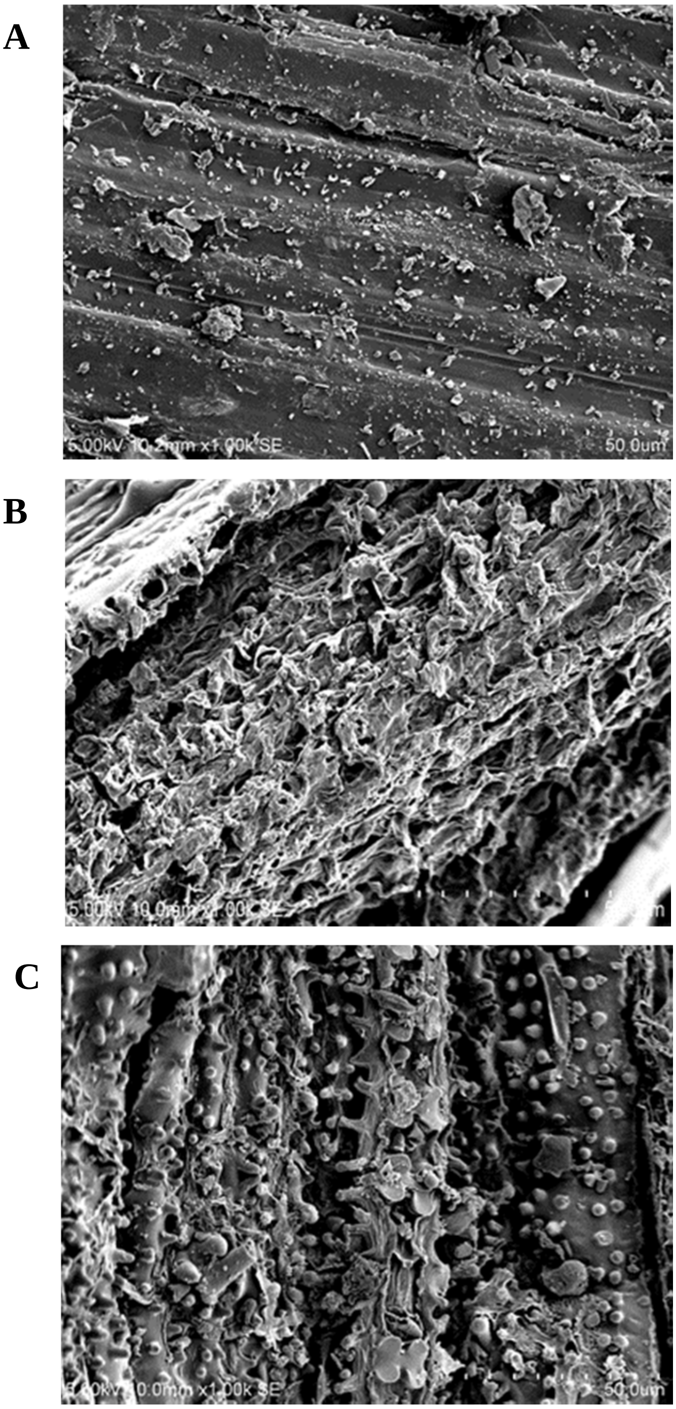


Figure 6.15 Scanning electron micrographs of native and solid residues from the fractionation of rice straw in water/ethanol/EA system under non-catalytic conditions. (A) native rice straw; (B) conventional heating process at 160°C; (C) microwave heating process at 300W in the absence of acid promoters.

In addition, structural modifications of the biomass by the fractionation process led to an increase in accessible surface area as observed by the BET analysis (Table 6.1). Increasing reaction temperature from 140-180°C led to increases in BET surface area to 7.5 m^2/g for conventional heating compared to the surface area of 2.4 m^2/g for the native rice straw. Similar trend in increasing surface area to 7.3 m^2/g was also found for the solid fractions obtained from the microwave-treated reaction with increasing power. Increasing crystallinity (CrI) was found for the fractionated solid using either conventional heating (74.9%) or microwave treatment (74.2%) compared to the native rice straw (63.4%). The increases in crystallinity could be due to the removal of the susceptible amorphous hemicellulose and lignin in the biomass during the fractionation process.

Table 6.1 Surface area and crystallinity index of native rice straw and solid residues from the fractionation process with different heating methods.

Condition	**Microwave heating**		**Conventional heating**	
	Surface area (m^2/g)	Degree of crystallinity (%)	Surface area (m^2/g)	Degree of crystallinity (%)
Native	2.4	63.4	2.4	63.4
140°C	5.8	68.6	6.1	70.8
160°C	6.7	71.3	6.8	72.1
180°C	7.3	74.2	7.5	74.9

The acid-catalyzed fractionation reactions led to remarkable changes in the overall biomass physical structure and physicochemical properties. These include the disruption of the highly ordered fibrous structure and degradation of the biomass surface. These effects were related to the increasing BET surface area of the fractionated solid residues both from conventional heating and microwave accelerated processes. Similar observations in biomass microstructure were earlier reported for corn stover and switch grass pretreated with hydrothermal pretreatment [Kumar *et al.* 2011] and sugarcane bagasse using dilute sulfuric acid pretreatment [Chen *et al.* 2011]. Increases in surface area could be due to the fragmentation and development of cracks and removal of hemicelluloses and lignin, which led to diminished shielding effects on cellulose microfibers [Zeng *et al.* 2007]. Cellulose crystallinity of the cellulose-enriched solid residues was increased due to the partial removal of the amorphous hemicellulose and lignin fractions. Increase in crystallinity index of acid pretreated samples has been reported for diluted formic acid pretreatment of sugarcane bagasse [Sindhu *et al.* 2010], acid-catalyzed steam explosion of eucalyptus

[Emmel *et al*. 2003] and non-catalytic hot compressed water pretreatment [Imman *et al*. 2013]. These changes altogether resulted in the alteration of the biomass microstructures, and hence increased, susceptibility to enzymatic hydrolysis.

For the extracted lignin, the chemical properties of isolated lignin in the MIBK phase obtained from the fractionation reactions using non-catalyzed conventional processes (Fig 6.16A), non-catalytic microwave treatment (Fig 6.16B), and commercial kraft lignin (Fig 6.16C) were determined by FTIR. Identical absorption spectra of lignin from both processes were observed compared to the commercial lignin. General bands previously reported for lignin were identified including aromatic phenypropane skeleton vibrations (1613, 1514 and 1427 cm^{-1}), aromatic and aliphatic hydroxyl groups (3373 cm^{-1}), C-H aliphatic bonds (2948, 2850 and 1462 cm^{-1}), and phenolic hydroxyl groups (1360 cm^{-1}). In addition, the spectra also showed other signals attributed to syringyl (S) and guaiacyl (G) groups: syringyl ring breathing with C–O stretching (1264 cm^{-1}), syringyl-type aromatic C–H in plane deformations (1120 cm^{-1}), and guaiacyl (835 cm^{-1}) units. The results thus showed high chemical homogeneity of lignin obtained from the fractionation processes.

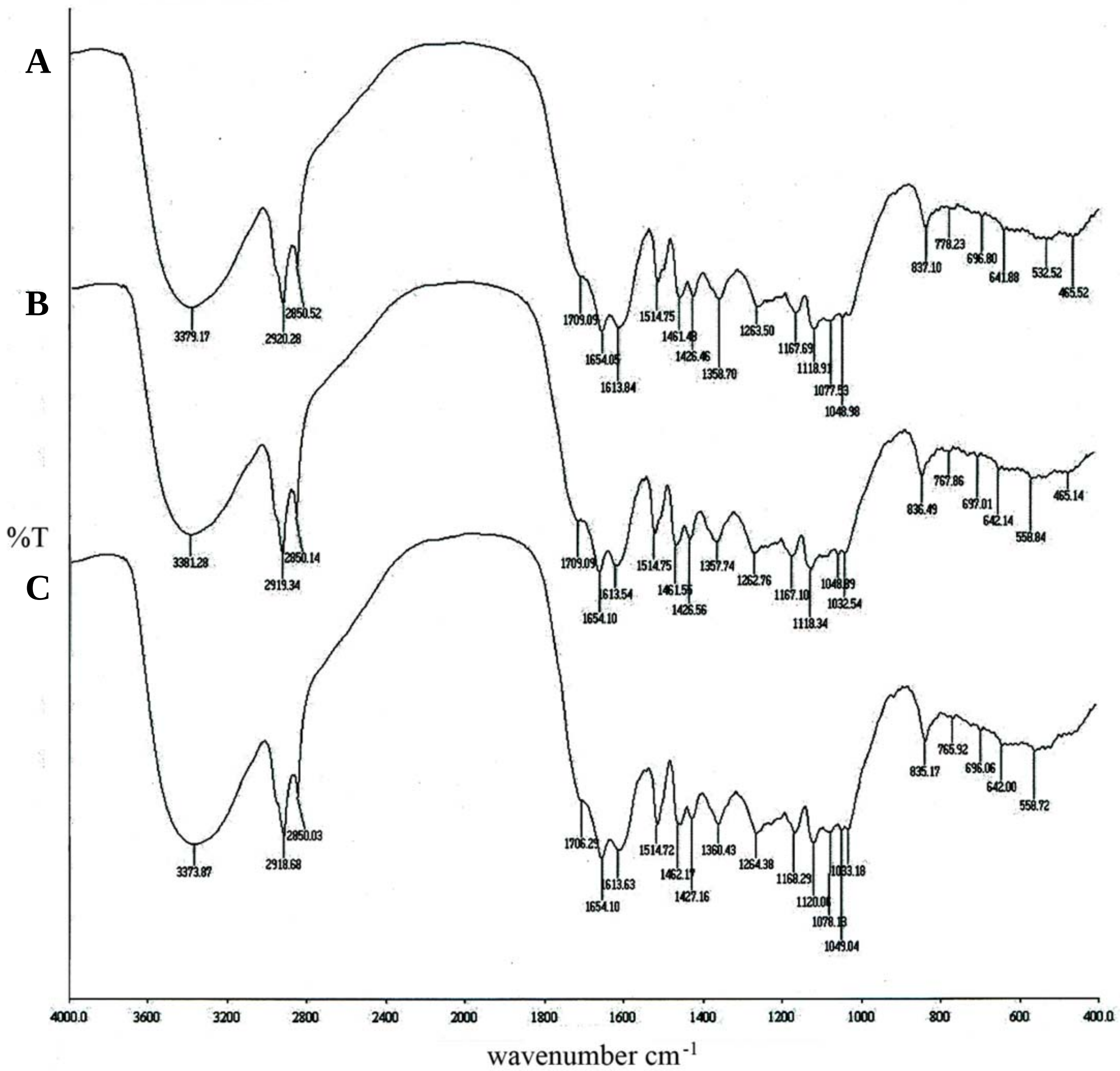


Figure 6.16 FTIR spectra of lignin obtained from (A) conventional heating process at 160^{o}C; (B) microwave heating process at 300W in the absence of acid promoter; and (C) commercial kraft lignin.

6.5 Chapter conclusions

In this chapter, different organic solvents (methyl isobutyl keone, ethyl acetate, toluene and diethyl ether) in the ternary water/alcohol/organic solvent system and acid promoters (H_2SO_4, HCl, and H_3PO_4) were studied by a single-step solvothermal fractionation of rice straw by the Clean Fractionation (CF) process. Ethyl acetate was found to be a superior composite solvent to the conventional solvent MIBK by providing higher glucan and lignin yields. Glucan and xylan could be further solubilized and lignin recovery increased at higher temperature and acid concentration. The optimal fractionation conditions were found to be water/ethanol/ethyl acetate (62.5%:25%:12.5%) mixture using 0.05 M H_2SO_4 at 160^{o}C for 1 h, which gave separation of cellulose-enriched solid with 71.4 wt% glucan yield. The maximum of hemicellulose of 71.3 wt% was obtained as sugars, and dehydration products in the aqueous/alcohol phase were minimal. 84.9 wt% lignin was recovered in the organic solvent phase with no cross-contamination of sugars. Finally, microwave-assisted heating at 300 W for 1 h resulted in comparable glucan and lignin yields in the respective phases to conventional heating with the advantage of increased pentose yield and lower furan formation in the aqueous phase.

CHARTER 7

CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

7.1 Conclusions

The optimal conditions for the maximum enzyme digestibility of the biomass, and the concentrations of HMF and furfural formed were investigated. The research activities were: (i) to study the effectiveness of LHW pretreatment on four agricultural residues. (RS, BG, CS, EPFB), (ii) to study the effect of acid catalyst and NaOH on LHW pretreatment of RS, and (iii) to study the effect of a ternary mixture in the presence and absence of promoters on the fractionation of RS. The use of microwave heating in the solvo-thermal process was also assessed.

This research focused on the studies of lignocellolosic biomass pretreatment and fractionation by the thermochemical processes (i.e. hydrothermal and solvothermal) with and without the presence of homogeneous catalysts. The optimal conditions on the enzymatic digestibility, HMF and furfural concentration were investigated. The research contains three parts; (**i**) to study the efficiency of LHW on improving digestibility of selected five lignocellulosic biomass as well as its impact on biomass structures and physicochemical properties were investigated based on hot compressed water processes in the absence of liquid-state acid and alkali on pretreatment in batch system under various conditions; (**ii**) to explore the use of external catalysts on LHW process, the comparative study of different homogeneous acid and alkalines on catalytic LHW pretreatment of rice straw. Effects of catalyst types on sugar recovery from hemicellulose hydrolysis and enzymatic digestibility of the pretreated biomass were investigated under different LHW conditions; and **(iii**) to study the effects of organic solvents and acid promoters on fractionation of rice straw using a modified clean fractionation process (CF) were study. In addition, the use of microwave-assisted heating to replace the conventional external heating was reported. The work provides an efficient alternative approach for efficient separation of primary lignocellulosic components with high recoverability and selectivity for further valorization in integrated biorefineries.

The results showed a modification of the biomass structural and chemical properties by the LHW pretreatment, which led to remarkable improvement in enzymatic biomass digestibility with high sugar yields from the starting native biomass. The results showed that the efficiency of LHW, which integrates the key pretreatment parameters, i.e.

temperature and time of pretreatment. The relationship between the glucose yields from enzymatic hydrolysis of the solid residues in response to the process severity factor was found. Increasing in pretreatment temperature and time led to increasing glucose yields, with the highest response for BG, followed by RS and CS, while EPFB was the most recalcitrant to LHW pretreatment. The relatively poor yield from EPFB could be due to the high lignin content of native EPFB and its rigid physical structure. Temperature, pH, and holding time are the major factors affecting the efficiency of LHW pretreatment in general. Our study indicates the potential for implementation of LHW as an efficient universal pretreatment method for different feedstocks.

The use of acid and alkali helped to promote the efficiency of the LHW process and lowering of the reaction temperature. It was found that homogenous strong acids and weak acids are effective catalysts for hemicellulose hydrolysis in the LHW process, in which the highest pentose yield achieved at 180°C for 5 min under the 0.5% v/v H_2SO_4 as the catalyst conditions. At 160°C for 10 min, oxalic acid was the best acid catalyst in that it led to highest glucose recovery from hydrolysis of the pretreated solids with low solubilized glucose level in the liquid phase. Adding acids in hydrothermal pretreatments has the drawback of increasing sugar dehydration to by-products inhibitory to subsequent fermentation. We found that sugar furfural and HMF by-products were greatest using HCl, followed by H_2SO_4, H_3PO_4 and oxalic acid. However, the accumulated inhibitory by-product levels for all catalyzed reactions were within the maximal tolerance threshold for ethanolgens (2 mg/mL for furfural and HMF). The use of low NaOH dosage led to pentose solubilization into the liquid fraction to an extent comparable to that obtained using acid catalysts, while more pronounced effects on improving cellulose digestibility was observed.

Alkali-catalysed pretreatment thus resulted in higher glucose yield from saccharification of the solid residues compared with the use of acids under LHW conditions at the same temperature. Overall, the use of acids and alkalis as catalysts in the LHW process led to enhanced efficiency on pretreatment of lignocellulose, which was reflected by increased yield of fermentable sugars in both the liquid phase and saccharification of the solid residues. Catalyzed pretreatment thus overall resulted in increased sugar recovery from the biomass. The catalyzed reactions gave high yields at a lower reaction temperature, which reduces process energy consumption and the formation of inhibitory sugar degradation by-products.

The fractionation of lignocellulosic components is a prerequisite step for the separation of individual biopolymers for further value-maximization. A modified CF process using ternary solvent systems has been reported in this study using a ternary solvent system composed of water, ethanol, and EA, which has been recently proposed as an alternative solvent to MIBK with its advantages on its lower cost and easy reusability due to its lower boiling point. Addition of acid promoters led to decreased glucan yield in the solid phase but with higher homogeneity which was correlated to the increasing pentose and glucose yield in aqueous and lignin yield in the organic phase compared to the non-catalyzed reactions. The efficient separation of lignocellulosic components from rice straw with high yields and selectivity under the optimal conditions results in the cellulose-enriched solid. The aqueous-alcohol fraction contained mainly composite hemicelluloses sugars and degradation by-products. The concentrations of furfural and HMF in the aqueous phase obtained in this study were lower than the inhibition level to ethanologens of 2.0 mg/ml. High-purity lignin was recovered in the organic phase with high yield and homogeneity as shown by FT-IR. Overall, different mineral acids showed differences in promoting product selectivity of the fractionation reaction. EA showed comparable efficiency in separation of biomass components compared to MIBK used in the conventional process with advantages on cost and energy used in recovery. Microwave treatment was also shown as an alternative to accelerate the CF reaction with advantages on lower formation of inhibitory by-products with less process energy. The work demonstrated the potential of the developed method for fractionation of lignocellulosic biomass components in integrated biorefinery process.

7.2 Suggestions for future work

This study indicated the major success of the LHW pretreatment methods (non-catalytic and catalytic LHW) and fractionation of lignocellulosic biomass, but some detailed problems need to be solved to reach the environmental friendly process for future applications. Since the organosolv pretreatment and fractionation processes generated several wastes (i.e. contaminated solvents, chemicals, acid/alkaline), which is unattractive to a green biorefinery application, the appropriate approaches for recycling and/or regeneration of solvents and chemical should be further studied to minimize the waste generation as well as to develop cost-effective methods.

In the fractionation of rice straw using a solvo-thermal process was studied. However, only homogeneous catalysts were studied in this work. To optimize the advantage of fractionation processes, future studies for fractionation under heterogeneous are required. The use of heterogeneous catalysts instead of homogeneous for fractionation system may have several advantages, such as easier recovery from the products and convenient regeneration. Furthermore, lignin characterization in the organic phase would be useful. Lignin has received an attention due to its potential for industrial application. However, complex structure of lignin still hindered the utilization of lignin for biorefinery application. Characterization of lignin structure will be benefit in order to achieve the advantages of biomass for biorefinery as well as the utilization of separated lignin for value-added production.

For large scale applications, it is necessary that other criteria are considered e.g. costs associated with handling hazardous toxic waste, such as liquid steam containing strong acids and bases, toxic gases, etc. Moreover, the methods which are based on the use of strong acids and bases require a much higher level of safety precautions and also material design and construction of equipment for the catalyst recycle are rather different. To ensure the environmental acceptance, catalytic pretreatment and fractionation process may require some process to recovery catalyst from hydrolyzate, moreover, also reduced operation cost and enhance efficiency on biorefinery application.

REFERENCES

Adney, B., Baker, J., 1996. Measurement of cellulase activities, Chemical Analysis and Testing Task., The National Renewable Energy Laboraory (NREL). December. pp 1-11. Report no.: NREL/TP-510-42628.

Alfani, A., Gallifuoco, F., Saporosi, A., Spera, A., Cantarella, M., 2000. Comparison of SHF and SSF process for the bioconversion of steam-exploded wheat straw, J. Ind. Microbiol. Biotechnol. 25, 184–192.

Allen, S., Kam, L., Zemann, A., Antal, M., 1996. Fractionation of sugar cane with hot, compressed, liquid water, Ind. Eng. Chem. Res. 35, 2709–2715.

Allen, S., Schulman, D., Lichwa, J., Antal, M., Laser, M., Lynd, L., 2001. A comparison between hot liquid water and steam fractionation of corn fiber, Ind. Eng. Chem. Res. 40, 2934–2941.

Ando, H., Sakaki, T., Kokusho, T., Shibata, M., Uemura, Y., Hatate, Y., 2000**.** Decomposition behavior of plant biomass in hot-compressed water, Ind. Eng. Chem. Res. 39**,** 3688–3693.

Ang, T.N., Ngoh, G.C., and Chua, A.S.M., 2013. Comparative study of various pretreatment reagents on rice husk and structural changes assessment of the optimized pretreated rice husk, Bioresource. Technol., 135, 116-119.

Avellar, B.K., and Glasser, W.G., 1998. Steam-assisted biomass fractionation. I. Process considerations and economic evaluation, Biomass. Bioenerg. 14, 205–218.

Ballesteros, I., Negro, M., Oliva, J.M., Cabaas, A., Manzanares, P., Ballesteros, M., 2006. Ethanol production from steam-explosion pretreated wheat straw, Appl. Biochem. Biotechnol. 130, 496–508.

Bhattacharya, S.C., Shrestha, R.M., Ngamkajornvivat, S., 1989. Potential of biomass residue availability: The case of Thailand, Energy. Sources. 11, 201-16.

Binod, P., Satayanagalakshmi, K., Sindhu, R., Janu, K.U., Sukumaran, R.K., Pandey, A., 2012. Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse, Renew. Energ. 37, 109-116.

Black, S.K., Hames, B.R., Myers, M.D., 1998. Midwest research institute, assignee. Method of separating lignocellulosic material into lignin, cellulose and dissolved sugars. United State patent US 5730837, Mar 24.

Bozell, J.J., Black, S.K., Myers, M., Cahill, D., Mille,r P.W., Park, S., 2011. Solvent fractionation of renewable woody feedstocks: Organosolv gerneration of biorefinery process streams for the production of biobased chemicals, Biomass. Bioenerg. 35, 4197-4208.

Brownell, H.H., Yu, E.K.C., Saddler, J.N., 1986. Steam-explosion pretreatment of wood: effect of chip size, acid, moisture content and pressure drop, Biotechnol. Bioeng. 28, 729–801.

Camacho, F., Gonzalez-Tello, P., Jurado, E., Robles, A., 1996. Microcrystallinecellulose hydrolyisis with concentrated sulphuric acid, J. Chem. Technol. Biotechnol. 67, 350–356.

Cara, C., Ruiz, E., Ballesteros, I., Negro, M.J., Castro, E., 2006. Enhanced enzymatic hydrolysis of olive tree wood by steam explosion and alkaline peroxide delignification, Process Biochem. 41, 423–429.

Cara, C., Ruiz, E., Oliva, J.M., Saez, F., Castro, E., 2008. Conversion of olive tree biomass into fermentable sugars by dilute acid pretreatment and enzymatic saccharification, Bioresour. Technol. 99, 1869–1876.

Carvalheiro, F., Duarte, L.C., Girio, F.M., 2008. Hemicellulose biorefineries: a review on biomass pretreatments, J. Sci. Ind. Res. 67, 849–864.

Chang, V.S., Nagwani, M., Kim, C.H., Holtzapple, M.T., 2001. Oxidative lime pretreatment of high-lignin biomass: poplar wood and newspaper, Appl. Biochem. Biotechnol. 94, 1–28.

Chen, B.Y, Chen, S.W. Wang, H.T., 2012. Use of Different Alkaline Pretreatments and Enzyme Models to Improve Low-cost Cellulosic Biomass Conversion, Biomass Bioenerg. 39, 182-191.

Chen, W.H., Pen B.L., Yu C.T., Hwang W.S., 2011. Pretreatment efficiency and structural characterization of rice straw by an integrated process of dilute-acid steam explosion for bioethanol production, Bioresource. Technol. 102, 2916-2924.

Chiesa, S. and Gnansounou, E., 2014. Use of Empty Fruit Bunches from the Oil Palm for Bioethanol Production: a Thorough Comparison Between Dilute Acid and Dilute Alkali Pretreatment, Bioresour. Technol. 159, 355-364.

Cortright, R., Davda, R., Dumesic, J., 2002. Hydrogen from catalytic reforming of biomass-derived hydrocarbons in liquid water, Nature. 418, 964–967.

Cybulska, I., Breudecki, G., Rosentrater, K., Lei, H., Julson, J., 2012. Catalyzed modified clean fractionation of prairie cordgrass integrated with hydrothermal post-treatment, Biomass. Bioenerg**.** 46, 389-401.

Dien, B.S., Sarath, G., Pedersen, J.F., Satler, S.E., Chen, H., Funnell-Harris, D.L., Nichols N.N., Cotta, M.A., 2009. Improved Sugar Conversion and Ethanol Yield for Forage Sorghum (Sorghum Bicolor L. Moench) Lines with Reduced Lignin Contents, Bioenergy. Res. 2(3), 153-164.

Ehara, K., and Saka, S., 2005. Decomposition behavior of cellulose in supercritical water, subcritical water, and their combined treatments, J. wood. Sci. 51(2), 148-153.

Ehara, K., and Saka, S., 2002. A comparative study on chemical conversion of cellulose between the batch-type and flow-type systems in supercritical water, Cellulose. 9, 301-311.

Emmel, A., Mathias, A.L., Wypych, F., Ramos, L., 2003. Fractionation of *Eucalyptus grandis* chips by dilute acid-catalysed steam explosion, Bioresour. Technol. 86, 105-115.

Fang, Z., 2003.Phase behavior and oxidation of organic wastes in supercritical water, PhD thesis, McGill University.

Fang, Z., Kozinski, J.A., 2000. Phase behavior and combustion of hydrocarbon-contaminated sludge in supercritical water at pressures up to 822 MPa and temperatures up to 535 ˚C, Proc. Combust. Inst. 28, 2717–2725.

Fengel, B.J., and Wegener, G., 1984. Wood. Chemistry, Ultrastructure, reactions. Berlin, New York: W. de Gruyter.

Gabriel, C., Gabriel, S., Grant, E.H., Halstead, B.S.J., Mingos, D.M.P., 1998. Dielectric parameters relevant to microwave dielectric heating, Chem. Soc. Rev. 27, 213-23.

Galbe, M. and Zacchi, G., 2012. Pretreatment: The Key to Efficient Utilization of Lignocellulosic Materials, Biomass. Bioenerg. 46, 70-78.

Galbe, M., Zacchi, G., 2007. Pretreatment of lignocellulosic materials for efficient bioethanol production, Adv. Biochem. Eng. Biotechnol. 108, 41–65.

Gao, Y., Xu, J., Zhang, Y., Yu, Q., Yuan, Z., Liu, Y., 2013. Effects of Different Pretreatment Methods on Chemical Composition of Sugarcane Bagasse and Enzymatic hydrolysis, Bioresour. Technol. 144, 396-400.

Garcia-Cubero, M.T., Gonzalez-Benito, G., Indacoechea, I., Coca, M., Bolado, S., 2009. Effect of ozonolysis pretreatment on enzymatic digestibility of wheat and rye straw, Bioresour. Technol. 100, 1608–1613.

Garlock, R. J., Balan, V., Dale, B. E., Pallapolu, V. R., Lee, Y. Y., Kim, Y., Mosier, N. S., Ladisch, M. R., Holtzapple, M. T., Falls, M., Sierra-Ramirez, R., Shi, J., Ebrik, M. A., Redmond, T., Yang, B.,Wyman, C. E., Donohoe, B. S., Vinzant, T. B., Elander, R. T., Hames, B., Thomas, S., Warner, R. E., 2012. Comparative Materials Balance Around Pretreatment Technologies for the Conversion of Switchgrass to Soluble Sugars, Bioresour. Technol. 102, 11063–11071.

Girio, F.M., Fonseca, C., Carvalheiro, F., Duarte, L.C., Marques, S., Bogel-Lukasik, R., 2010. Hemicelluloses for fuel ethanol: A review, Bioresour. Technol. 101, 4775-4800.

Goh, C. S., Tan, H. T., Lee, K. T., 2012. Pretreatment of Oil Palm Frond Using Hot Compressed Water: An Evaluation of Compositional Changes and Pulp Digestibility Using Severity Factors, Bioresour. Technol. 110, 662–669.

Goldstein, I.S., 1983. Acid processes for cellulose hydrolysis and their mechanisms. Wood and agricultural residues. Academic Press Inc., New York. 315–328.

Gomis, V., Font, A., Saqute, M.D., 2008. Homogeneity of the water + ethanol + toluene Azeotrop at 101.3 kPa, Fluid. Phase. Equilibria. 266, 8-13.

Guirong, B., Saka, S., Hua, W., 2008. Cellulose decomposition behavior in hot-compressed aprotic solvents, Science in China Series B, Chemistry. 51, 479-486.

Gupta, R., Lee, Y.Y., 2010. Investigation of Biomass Degradation Mechanism in Pretreatment of Switchgrass by Aqueous Ammonia and Sodium Hydroxide, Bioresour. Technol. 101, 8185-8191.

Hall, D.O., Rosillo-Calle, F., Williams, R.H., Woods J. In: Johansson TB, Kelly H, Reddy AKN, Williams RH, editors. Renewable Energy. Washington, DC: Island Press, 1993, 608.

Hamelinck, C.N., van Hooijdonk, G., Faaij, A.P.C., 2005. Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle- and long-term, Biomass. Bioenerg. 28, 384–410.

Harnpicharnchai, P., Champreda, V., Sornlake, W., Eurwilaichitr, L., 2009. A thermotolerant β-glucosidase isolated from an endophytic fungi, Periconia sp., with a possible use for biomass conversion to sugars, Protein. Express. Purif. 67, 61-69.

Hasegawa, I., Tabata, K., Okuma, O., Mae, K., 2004. New pretreatment methods combining a hot water treatment and water/acetone extraction for thermo-chemical conversion of biomass, Energ. Fuel. 18, 55-760

Hayes, D.J., 2009. An examination of biorefining processes, catalysts and challenges, Catal. Today. 145, 138–151.

Hendricks, A. T. W. M., Zeema, G., 2009. Pretreatments to Enhance the Digestibility of Lignocellulosic Biomass, Bioresour. Technol. 100, 10–18.

Hideno, A.H, Tsukahara, K., Fujimoto, S., Minowa, T., Inoue, S., Endo, T., Sawayama, S., 2009. Wet disk milling pretreatment without sulfuric acid for enzymatic hydrolysis of rice straw. Bioresour. Technol. 100(10), 2706-2711.

Hongzhang, C., Liying, L., 2007. Unpolluted fractionation of wheat straw by steam explosion and ethanol extraction. Bioresour. Technol. 98, 666-676.

Hsu, T.C., Guo, G.L., Chen, W.H. Hwang, W.S., 2010. Effect of Dilute Acid Pretreatment of Rice Straw on Structural Properties and Enzymatic Hydrolysis, Bioresour, Technol. 101(13), 4907-4913.

Huber, G.W., Corma, A., 2007. Synergies between bio- and oil refineries for the production of fuels from biomass, Angew. Chem. Int. Ed. Engl., 46, 7184-7201.

Huijgen, J.J.W., Reith, H.J., Uil, D.H., 2010. Pretreatment and Fractionation of Wheat Straw by an Acetone-Based Organosolv Process, Ind. Eng. Chem. Res. 49(20), 10132-10140.

Imman S., Arnthong J., Burapatana V., Laosiripojana N., and Champreda V., 2013, Autohydrolysis of tropical agricultural residues by compressed liquid hot water pretreatment, Appl. Biochem. Biotechnol. 170, 1982-1995.

Ishikawa, Y., Saks, S., 2001. Chemical conversion of cellulose as treated in supercritical methanol, Cellulose. 8, 189–195.

Jacobsen, E.S., Wyman, C., 2002. Xylose Monomer and Oligomer Yields for Uncatalyzed Hydrolysis of Sugarcane Bagasse Hemicellulose at Varying Solids Concentration, Ind. Eng. Chem. Res. 41, 1454-1461.

Jonsson, L.J., Alriksson, B. Nilvebrant, N.O., 2013. Bioconversion of Lignocellulosic: Inhibitors and Detoxification, Biotechnol. Biofuel. doi:10.1186/1754-6834-6-16.

Kabyemela, B.M., Takigawa, M., Adschiri, T., Malaluan, R.M., Arai, K., 1998. Mechanism and Kinetics of Cellobiose Decomposition in Sub- and Supercritical Water, Ind. Eng. Chem. Res, 37, 357-361.

Kappe, C.O., 200). Microwave dielectric heating in synthetic organic chemistry, Chem. Soc. Rev, 37, 1127-1139.

Karunanithy, C., Muthukumarappan, K., Julson, J.L., 2008. Influence of high shear bioreactor parameters on carbohydrate release from different biomasses, American Society of Agricultural and Biological Engineers Annual International Meeting 2008. ASABE 084114. ASABE, St. Joseph, Mich.

Kim, Y., Mosier, N.S., 2009. Enzymatic Digestion of Liquid Hot Water Pretreated Hybrid Poplar, Biotechnol. Prog. 25(2), 340-348.

Kim, Y., Hendrickson, R., Mosier, N. S., Ladisch, M. R., Bals, B., Balan, V., Dale B. E., 2008. Enzyme Hydrolysis and Ethanol Fermentation of Liquid Hot Water and AFEX Pretreated Distillers Grains at High-Solids Loadings, Bioresour. Biotechnol. 99, 5206–5215.

Kim, S., Holtzapple, M.T., 2006. Delignification kinetics of corn stover in lime pretreatment, Bioresour. Technol. 97, 778–785.

Kim, T.H., Lee, Y.Y., 2005. Fractionation of corn stover by hot-water and aqueous ammonia treatment, Bioresour. Technol. 97, 224-232.

Klameassamee, T., Champreda, V., Reunglek, V., Laosiripojana, N., 2013. Comparison of homogeneous and heterogeneous acid promoters in single-step aqueous-organosolv fractionation of eucalyptus wood chips, Bioresour. Technol. 147, 276-284.

Koide, S., Brooks, R. B., Vicharangsan, T., 1982. Regional Study on Production of Fuel Ethanol from Ago-Product, Economic and Social Commission for Asia and the Pacific, 75.

Kootstra, A.M., Beeftink, H.H., Scott, E.L., Sanders, J.P.M., 2009. Comparison of Dilute Mineral and Organic Acid Pretreatment for Enzymatic Hydrolysis of Wheat Straw. J. Biomed. Eng. 46, 126-131.

Kuhar, S., Nair, L.M., Kuhad, R.C., 2008. Pretreatment of lignocellulosic material with fungi capable of higher lignin degradation and lower carbohydrate degradation improves substrate acid hydrolysis and eventual conversion to ethanol, Can. J. Microbiol. 54, 305–313.

Kumar, S., Kothari, U., Kong, L., Lee, Y.Y., Gupta, R.B., 2011. Hydrothermal Pretreatment of Switchgrass and Corn Stover for Production of Ethanol and Carbon Microspheres, Biomass. Bioenerg. 35, 956–968.

Kumar, R., Wyman, C.E., 2009a. Effects of cellulase and xylanase enzymes on the deconstruction of solids from pretreatment of poplar by leading technologies. Biotechnol. Prog. 25, 302–314.

Kurmar, S., Kothari U., Kong, L., Lee, Y.Y., Gupta, R.B., 2011. Hydrothermal pretreatment of switchgrass and corn stover for production of ethanol and carbon microspheres, Biomass. Bioenerg, 35, 956-968.

Laser, M., Schulman, D., Allen, S., Lichwa, J., Antal, M., Jr., Lynd, L., 2002. A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol, Bioresour. Technol. 81, 33-44.

Lee, J.W., Jeffries, T.W., 2011. Efficiencies of Acid Catalysts in the Hydrolysis of Lignocellulosic Biomass over a Range of Combined Severity Factors, Bioresour. Technol. 102, 5884-5890.

Lee, S.H., Doherty, T.V., Linhardt, R.J., Dordick, J.S., 2009. Ionic liquid-mediated selective extraction of lignin from wood leading to enhanced enzymatic cellulose hydrolysis, Biotechnol. Bioeng. 102, 1368–1376.

Levan, S.L., 1989. In: Schniewind, A. P. (ed.), *Concise encyclopedia of wood and wood-based materials*, 1st edn (pp. 271–273). Elmsford: Pergamon.

Li, C., Knierim, B., Manisseri, C., Arora, R., Scheller, H.V., Auer, M., Vogel, K.P., Simmons, B.A., Singh, S., 2010. Comparison of dilute acid and ionic liquid pretreatment of switchgrass: biomass recalcitrance, delignification and enzymatic saccharification, Bioresour. Technol. 101, 4900-4906.

Li, Q., He, Y.C., Xian, M., Jun, G., Xu, X., Yang, J.M., Li, L.Z., 2009. Improving enzymatic hydrolysis of wheat straw using ionic liquid 1-ethyl-3-methyl imidazolium diethyl phosphate pretreatment, Bioresour. Technol. 100, 3570–3575.

Liu, C., Wyman, C.E., 2005. Partial flow of compressed-hot water through corn stover to enhance hemicellulose sugar recovery and enzymatic digestibility of cellulose, Biores Technol. 96, 1978–1985.

Liu, C., Wyman, C.E., 2003. The effect of flow rate of compressed hot water on xylan, lignin, and total mass removal from corn stover, Ind. Eng. Chem. Res. 42(21), 5409–5416.

Lu, Y.,Mosier, N.S., 2007. Biomimetic Catalysis for Hemicellulose Hydrolysis in Corn Stover, Biotechnol. Prog. 23, 116-123.

Ma, H., Liu, W.W., Chen, X., Wu, Y.J., Yu, Z.L., 2009. Enhanced enzymatic saccharifications of rice straw by microwave pretreatment, Bioresour. Technol. 100, 1279-1284.

McHardy, J., Sawan, S., 1998. Supercritical fluid cleaning – fundamentals, technology, and applications. New Jersey, Westwood: Noyes Publications.

Merzougui, A., Hasseine, A., Kabouch, A., Korichi, M., 2011. LLE for the extraction of alcohol from aqueous solutions with diethyl ether and dichloromethane at 293.15K, parameter estimation using a hybrid genetic based approach, Fluid. Phase. Equilibria. 309, 161-167.

Miller, G.L., 1995. Using dinitrosalicylic acid for determination of reducing sugar, Anal. Chem. 31, 426–428.

Mok, W., Antal, M., 1992. Uncatalyzed solvolysis of whole biomass hemicelluloses by hot compressed liquid water, Ind. Eng. Chem. Res. 31, 1157–1161.

Montane, D., Farriol, X., Salvado, J., Jollez, P., Chornet, E., 1998. Fractionation of Wheat Straw by Steam-Explosion Pretreatment and Alkali Delignification. Cellulose Pulp and by-products from hemicellulose and lignin, J. Wood. Chem. Technol. 18, 171-191.

Monteil-Rivera, F., Huang, G.H., Paquet, L., Deschamps, S., Beaulieu, C., Hawari, J. 2012. Microwave-assisted extraction of lignin from triticale straw: Optimization and microwave effects, Bioresour. Technol. 104, 775-782.

Mosier, N., Hendrickson, R., Ho, N., Sedlak, M., Ladisch, M.R., 2005a. Optimization of pH controlled liquid hot water pretreatment of corn stover, Bioresour. Technol. 96, 1986–1993.

Mosier, N., Wyman, C.E., Dale, B.D., Elander, R.T., Lee, Y.Y., Holtzapple, M., Ladisch, C.M., 2005b. Features of promising technologies for pretreatment of lignocellulosic biomass, Bioresour. Technol. 96, 673–686.

Naik, S.N, Goud, V.V, Rout, P.K, Dalai, A.K., 2010. Production of first and second generation biofuels: A comprehensive review, Renew. Sust. Energ. Rev. 14, 578-597.

National Statistics Office, 2007. “Environment Statics”. Available online. http://web.nso.go.th/en/survey/env/env_2008.htm

Oliva, J.M., Suez, F., Ballesteros, I., Gonzalez, A., Negro, M.J., Manzanares, P., Ballesteros, M., 2003. Effect of lignocellulosic degradation compounds from steam explosion pretreatment on ethanol fermentation by thermotolerant yeast *Kluyveromyces marxianus*, Appl. Microbiol. Biotechnol. 105, 141–154.

Palmqvist, E., Hahn-Hagerdal, B., 2000. Fermentation of lignocellulosic hydrolysates II: inhibitors and mechanism of inhibition, Bioresour. Technol. 74, 25–33.

Pan, X., Xie, D., Gilkes, N., Gregg, D.J., Saddler, J.N., 2005. Strategies to enhance the enzymatic hydrolysis of pretreated softwood with high residual lignin content, Appl. Biochem. Biotechnol. Part A. 124, 1069–1079.

Pang, F., Xue, S., Yu, S., Zhang, C., Li, B., Kang, Y., 2013. Effects of Combination of Steam Explosion and Microwave Irradiation (SE-MI) Pretreatment on Enzymatic Hydrolysis, Sugar Yields and Structural Properties of Corn Stover, Industrial Crops and Products. 42, 402–408.

Papatheofanous, M.G., Billa, E., Koullas, D.P., Monties, B., Koukios, E.G., 1995. Two stage acid-catalyzed fractionation of lignocellulosic biomass in aqueous ethanol systems at low temperatures, Bioresour. Technol. 54, 305–310.

Perez, J.A., Ballesteros, I., Ballesteros, M., Serez, F., Negro, M.J., Manzanares, P., 2008. Optimizing liquid hot water pretreatment conditions to enhance sugar recovery from wheat straw for fuel-ethanol production, Fuel. 87, 3640–3647.

Qin, L., Liu, Z.H., Li, B.Z., Dale, B.E., Yuan, Y.J., 2012. Mass Balance and Transformation of Corn Stover by Pretreatment with Different Dilute Organic Acid, Bioresour. Technol. 112, 319-326.

Ritchie, G.S. De, Long, E.A. 1990. Method for fractionation of lignins from steam exploded lignocellulosics to provide fractions with different, but reproducible properties, and some methods for their interconversions and the products so produced. CA, Patent 1278294.

Rocha, M.V., Rodrigues, T.H., de Macedo, G.R., Goncalves, L.R., 2009. Enzymatic hydrolysis and fermentation of pretreated cashew apple bagasse with alkali and diluted sulfuric acid for bioethanol production, Appl. Biochem. Biotechnol. 155, 407–417.

Rowland, A.P., Roberts, J.D., 1994. Lignin and cellulose fractionation in decomposition studies using acid-detergent fibre methods, Commun. Soil. Sci.plant. anal. 25, 269-277.

Saha, B.C., 2003. Hemicellulose bioconversion, J. Ind. Microbiol. Biotechnol. 3, 279–291. doi: 10.1007/s10295-003-0049-x.

Saha, B.C., Iten, L.B., Cotta, M.A., Wu, Y.V., 2005. Dilute Acid Pretreatment, Enzymatic Saccharification, and Fermentation of Rice Hulls to Ethanol, Biotechnol. Prog. 21(3), 816-822.

Saha, B.C., Cotta, M.A., 2006. Ethanol production from alkaline peroxide pretreated enzymatically saccharified wheat straw, Biotechnol. Prog. 22, 449–453.

Saha, B.C., Iten, L.B., Cotta, M.A., Wu, Y.V., 2005. Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol, Process. Biochem. 40, 3693–3700.

Saka, S., Ueno, T. 1999. Chemical conversion of various celluloses to glucose and its derivatives in supercritical water, Cellulose. 6, 177–191.

Sakaki, T., Shibata, M., Sumi, T., Yasuda, S., 2002. Saccharification of Cellulose Using a Hot-Compressed Water-Flow Reactor, Ind. Eng. Chem. Res. 41, 661-665.

Sanchez., B., 1998. Effects of Furfural and 5-Hydroxymethylfurfural on the Fermentation of Saccharomyces Cervisiae and Biomass Production from *Candida Guilliermondii*, Enzyme. Microb. Tech. 101, 4895-4899.

Sanchez, B., Bautista, J., 1988. Effects of Furfural and 5-Hydroxymethylfurfural on the Fermentation of S*accharomyces Cerevisiae* and Biomass Production from *Candida Guilliermondii*, Enzyme. Microb. Tech**.** 10, 315-318.

Sanchez, C., 2009. Lignocellulosic residues: biodegradation and bioconversion by fungi, Biotechnol. Adv. 27, 185–194.

Sasaki, M., Adsciri, T., Arai, K., 2002. Fractionation of sugarcane bagasse by hydrothermal treatment, Bioresour. Technol. 86, 301-304.

Schellm, D.J., Farmer, J., Newman, M., McMillan, J.D., 2003. Dilute-Sulfuric Acid Pretreatment of Corn Stover in Pilot-Scale Reactor: Investigation of Yields, Kinetics, and Enzymatic digestibilities of Solid, Appl. Biochem.Biotechnol. 105-108, 69-85.

Shelly, M., 2006. Alcoholic fuels. Boca Raton, FL 33487-2742, USA: CRC Taylor and Francis Group.

Shi, J., Chinn, M.S., Sharma-Shivappa, R.R., 2008. Microbial pretreatment of cotton stalks by solid state cultivation of *Phanerochaete chrysosporium*, Bioresour. Technol. 99, 6556–6564.

Sindhu, R., Binod, P., Satyanagalakshmi, P., Janu, K.U., Sajna, K.V., Kurien, N., Sukumaran, R.K., Pandey, A., 2010. Formic acid as a potential pretreatment agent for the conversion of sugarcane bagasse to bioethanol, Appl. Biochem. Biotechnol. 162, 2313–2323.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., 20008. Laboratory analytical procedure (LAP). National Renewable Energy Laboratory. Report no.: NREL/TP51042618.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D.. 2008. Laboratory analytical procedure (LAP). National Renewable Energy Laboratory. Report no.: NREL/TP51042622.

Soderstrom, J., Pilcher, L., Galbe, M., Zacchi, G., 2002. Two-step steam pretreatment of softwood with SO_2 impregnation for ethanol production, Appl. Microbiol. Biotechnol. 98–100, 5–21.

Sreenath, H.K., Koegel, R.G., Moldes, A.B. Jeffries, T.W., Straub, R.J., 1999. Enzymatic Saccharifications of Alfalfa Fiber after Liquid Hot Water Pretreatment, Process. Biochem. 35, 33-41.

Srinivasan, N., Ju, L.K., 2012. Pretreatment of Guayule Biomass Using Supercritical Carbon Dioxide-Based Method, Bioresour. Technol. 101, 9785–9791.

Sun, Y., Cheng, J., 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review, Bioresour. Technol. 83, 1–11.

Taherzadeh, M.J., Karimi, K., 2008. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review, Int. J. Mol. Sci. 9, 1621–1651.

Tengborg, C., Stenberg, K., Galbe, M., Zacchi, G., Larsson, S., Palmqvist, E., Hahn-Hagerdal, B., 1998. Comparison of SO_2 and H_2SO_4 impregnation of softwood prior to steam pretreatment on ethanol production, Appl. Microbiol. Biotechnol. 70–72, 3–15.

Thepkanjana, P., 2002. Government Policies on Deregulation and Liberalization of the Thai Energy Sector, paper presented at the Thai Gas Market 2002 Conference, November, Bangkok, Thailand, Available online: http://www.energy.go.th/speech/sp-2545-11-14-ThGas.ppt

Trofimova, M., Toikka, M., Toikka, A.. 2012. Solubility, liquid-liquid equilibrium and critical states for the quaternary system acetic acid-ethanol- ethyl acetate-water at 293.15 K, Fluid. Phase. Equilibria. 313, 46-51.

U.S. Department of Energy, Energy Information Administration, Annual Energy Review 2006 (Washington, D.C., June 2007), pg. 279, http://www.eia.doe.gov/aer/pdf/aer.pdf. (Last visited April 21, 2008.)

Varga, E., Reczey, K., Zacchi, G., 2004. Optimization of steam pretreatment of corn stover to enhance enzymatic digestibility, Appl. Biochem. Biotechnol. 113–116, 509–523.

Vessia, O., Finden, P. and Skreiberg O., 2005. Biofuels from Lignocellulosic material, Department engineering, Faculty of information technology, mathematics and electrical engineering, Norwegian University of Science and Technology, Norway.

Wan., C., Li Y., 2011. Effect of Hot Water Extraction and Liquid Hot Water Pretreatment on the Fungal Degradation of Biomass Feedstocks, Bioresour. Technol. 102, 9788–9793.

Wheals, A.E., Basso, Alves D.M.G. Amorim, H.V., 1999. Fuel ethanol after 25 year. TIBTECH December. 17, 483

Wyman, C.E., 1996. Handbook on Bioethanol: Production and Utilization. Taylor Francis, Washington, 417.

Wyman, C.E., Dale, B.E., Elander, R.T., Holtzapple, M., Ladisch, M.R., Lee, Y.Y., 2005a. Coordinated development of leading biomass pretreatment technologies, Bioresour. Technol. 96, 1959–1966.

Xiao., L.P., Shi., Z.S., Xu., F., Sun, R.C., 2013. Hydrothermal Treatment and Enzymatic Hydrolysis of Tamarix Ramosissima: Evaluation of the Process as a Conversion Method in a Biorefinery Concept, Bioresour. Technol, 135, 73-81.

Xu, J., Cheng, J.J., 2011. Pretreatment of Switchgrass for Sugar Production with the Combination of Sodium Hydroxide and Lime, Bioresourc. Technol. 102, 3861-3868.

Xu., J., Zhang, X., Cheng J.J., 2012. Pretreatment of Corn Stover for Sugar Production with Switchgrass-Derived Black Liquor, Bioresour Technol. 111, 255-20.

Xu, X., Matsumura, Y., Stenberg, J., 1996. Antal M. Carbon-catalyzed gasification of organic feedstocks in supercritical water, Ind Eng Chem Res. 35, 2522–2530.

Yang, B., Wyman, C.E., 2008. Pretreatment: the key to unlocking low-cost cellulosic ethanol, Biofuels. Bioprod. Bior. 2, 26–40.

Yu, Q., Zhuang, X., Yuan, Z., Wang, Q., Qi, W., Wang, W., Zhang, Y., Xu, H., 2010. Two-Step Liquid Hot Water Pretreatment of Eucalyptus Grandis to Enhance Sugar Recovery and Enzymatic Digestibility of Cellulose, Bioresour. Technol. 101(13), 4895-4899.

Yu, D., Aihara, M., Antal, M., 1993. Hydrogen production by steam reforming glucose in supercritical water, Energ. Fuel. 7, 574–577.

Yu, Y.Y., Lou, X., Wu, H., 2008. Some Recent Advances in Hydrolysis of Biomass in Hot-Compressed Water and Its Comparisons with Other Hydrolysis Methods, Energ. Fuel. 22, 46-60.

Zaldivar J., Nielsen J., Olsson L., 2001. Fuel Ethanol Production from Lignocellulose: A Challenge for Metabolic Engineering and Process Integration, Appl. Microbiol. Biot. 56(1-2), 17–34.

Zeng M., Mosier N.S, Huang C.P., Sherman D.M., Ladisch M.R., 2007. Microscopic examination of changes of plant cell structure in corn stover due to hot water pretreatment and enzymatic hydrolysis, Biotechnol. Bioeng. 97(2), 265-278.

Zhang T., Kumar R., Wyman C.E. 2013. Sugar yields from oxalic acid pretreatment of maple wood compared to those with other dilute acids and hot water, Carbohyd. Polym. 92, 334-344.

Zhang, Y.H.P., Ding, S.Y., Mielenz, J.R., Cui, J.B., Elander, R., Laser, M., Himmel, M.E., McMillan, J.R., Lynd, L.R., 2007. Fractionating recalcitrant lignocellulose at modest reaction conditions, Biotechnol. Bioeng. 97, 214–223.

Zhao Y., Wang Y., Zhu J. Y., Ragauskas A., Deng, Y., 2008. Enhanced enzymatic hydrolysis of spruce by alkaline pretreatment at low temperature, Biotechnol. Bioeng. 99, 1320–1328.

Zhao, H., Jones, C.L., Baker, G.A., Xia, S., Olubajo, O., Person, V.N., 2009b. Regenerating cellulose from ionic liquids for an accelerated enzymatic hydrolysis, J. Biotechnol. 139, 47–54.

Zhao, X., Cheng, K., Liu, D., 2009a. Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis, Appl. Microbiol. Biotechnol. 82, 815–827.

Zhu S., Wu Y., Chen Q., Yu Z., Wang C., Jin S., Ding Y., Wu G. 2006. Dissolution of Cellulose with Ionic Liquids and its Application: A Mini-Review, Green. Chem. 8, 325–327.

APPENDICES

APPENDIX A

CONDITION AND CALIBRATION CURVE OF DNS METHOD

A.1 DNS reagent, glucose, xylose, arabinose standard preparation

Table A.1 Chemicals for DNS reagent preparation.

No.	Chemicals	Quantities
1.	3,5-Dinitrosalicylis acid ($C_7H_4N_2O_7$)	10.6 g
2.	Sodium hydroxide (NaOH)	19.8 g
3.	Deionized water (H_2O)	1,416 ml
4.	Sodium potassium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$)	306 g
5.	Sodium metabisulfite ($Na_2S_2O_5$)	83 g
6.	Phenol (C_6H_6O)	7.6 ml

Table A.2 Preparing of standard of glucose concentration

Glucose concentration (mg/mL)		Volume of stock 10 mg/mL STD glucose (µl)	Volume of dH_2O	Total volume (µL)
mg/mL	mg/100 µL			
0	0	-	1000	1000
1	0.1	100	900	1000
2	0.2	200	800	1000
3	0.3	300	700	1000
4	0.4	400	600	1000
5	0.5	500	500	1000

A.2 Steps for DNS method

A.2.1 Mix of DNS reagent 600µl with sample 100µl

A.2.2 Boiling the solution for 5 minute

A.2.3 Mixing the solution with deionized water in the ratio of 1:9 for glucose analysis

A.2.4 Analyzing with spectrophotometer using wave length 540 nm

A.3 Calibration curve of glucose concentration

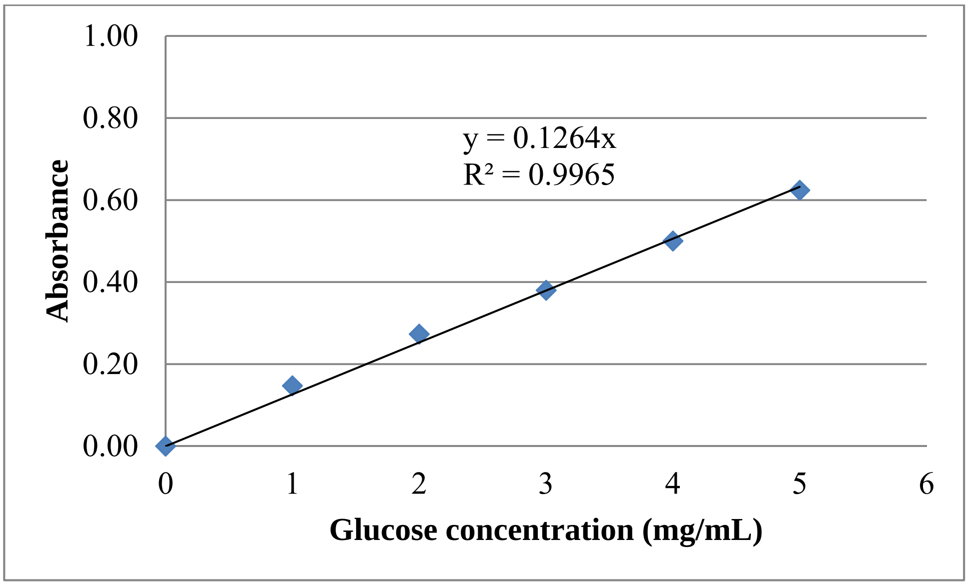


Figure A.1 Calibration curve of glucose concentration

APPENDIX B
DETERMINATION OF SUGARS, BYPRODUCTS, AND DEGRADATION PRODUCTS IN LIQUID FRACTION PROCESS SAMPLES

Procedure

B.1 Measure and record the pH of each sample to the nearest 0.01 pH unit

B.2 Analyze the sample for byproducts and degradation products as follows

B.2.1 Prepare 0.005 M (0.01 N) sulfuric acid for use as a HPLC mobile phase. In a 2L volumetric flask, add 2.00 mL of standardized 10 N sulfuric acid and bring to volume with HPLC grade water. Filter through a 0.2 µm filter and degas before use. If 10N sulfuric acid is not available, concentrated sulfuric acid may also be used. 278 µl concentrated sulfuric acid brought to volume in a 1L volumetric flask with HPLC grade water will also produce 0.005 M sulfuric acid.

B.2.2 Prepare a series of calibration standards containing the compounds that are to be quantified. Use a four-point calibration. If standards are prepared outside of the suggested ranges, the new range for these calibration curves must be validated. The linear range of HMF and furfural is limited by their solubility. Add these two components to the standards after the ethanol has been added to increase the HMF and furfural solubility. Filter the standard solutions through 0.2 µm filters into autosampler vials. Seal and label the vials.

B.2.2.1 Test the column to verify adequate peak separation and quantification. If adequate separation is not achieved, regenerate or replace the column and confirm improved separation.

B.2.2.2 A fresh set of standards is not required for every analysis. A large batch of standards may be produced, filtered through 0.2 µm filters into autosampler vials, sealed and labeled. The standards samples may be stored in a freezer and removed when needed. Thaw and vortex frozen standards prior to use. During every use, standards samples should be observed for unusual concentration behavior. Unusual concentrations may mean that the samples have been compromised or volatile components have been lost. Assuming sufficient volume, standard samples should not have more than 12 injections drawn from a single vial. In a chilled autosampler chamber, the lifetime of standards samples is approximately seven days.

B.3 Analyze the calibration standards, and samples by HPLC using a Biorad Aminex HPX-87H column. HPLC conditions:

- Sample volume: 10 - 25 µL, dependent on sample concentration and detector limits
- Mobile phase: 0.005 M sulfuric acid, 0.2 µm filtered and degassed
- Flow rate: 0.6 mL / minute
- Column temperature: 55 – 65 °C
- Detector temperature: as close to column temperature as possible
- Detector: refractive index
- Run time: 50 min

Table B.1 Approximate retention time for calibration standards

Component	Approximate Retention time (min)
Glucose	10.4
Xylose	11.1
Arabinose	12.0
HMF	29.4
Furfural	42.8

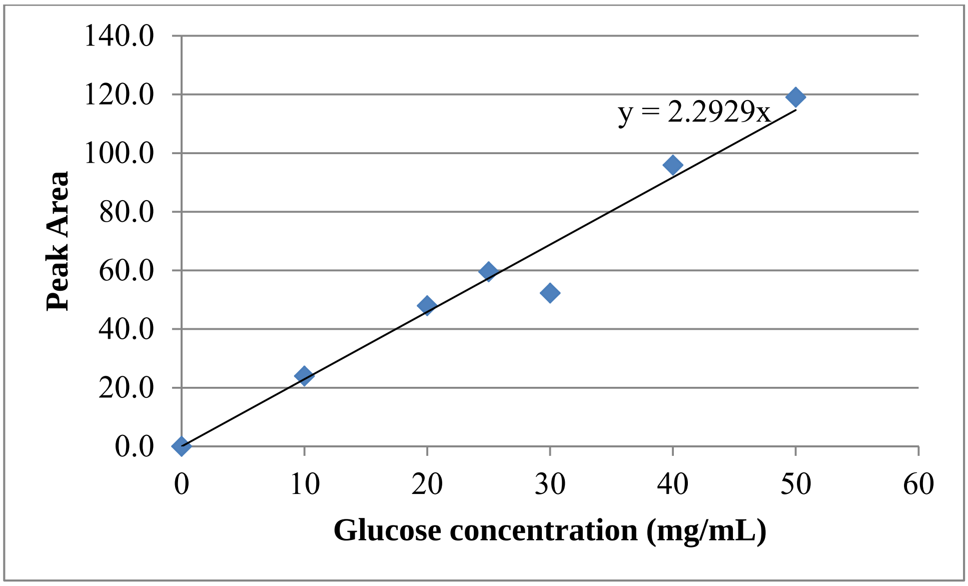


Figure B.1 Calibration curve of glucose concentration.

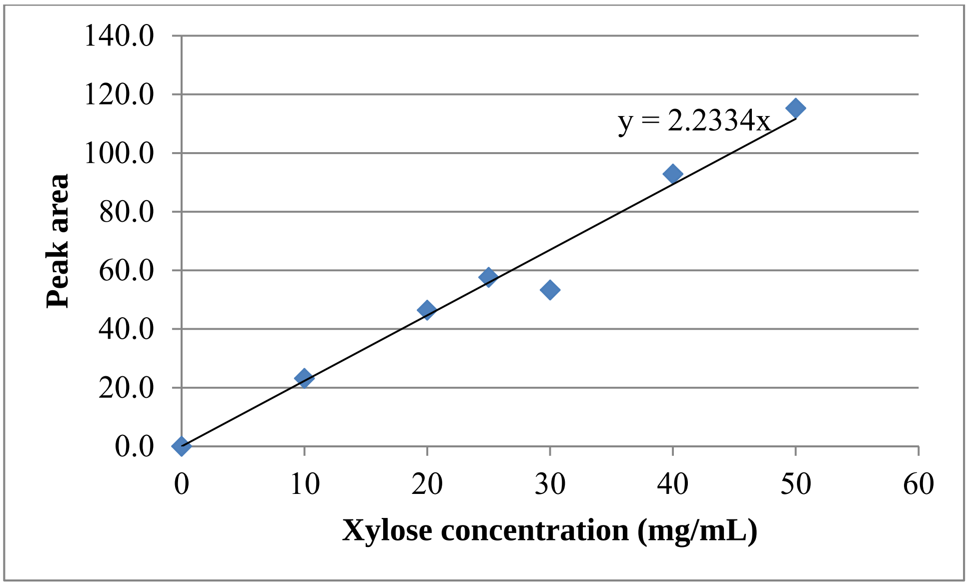


Figure B.2 Calibration curve of xylose concentration.

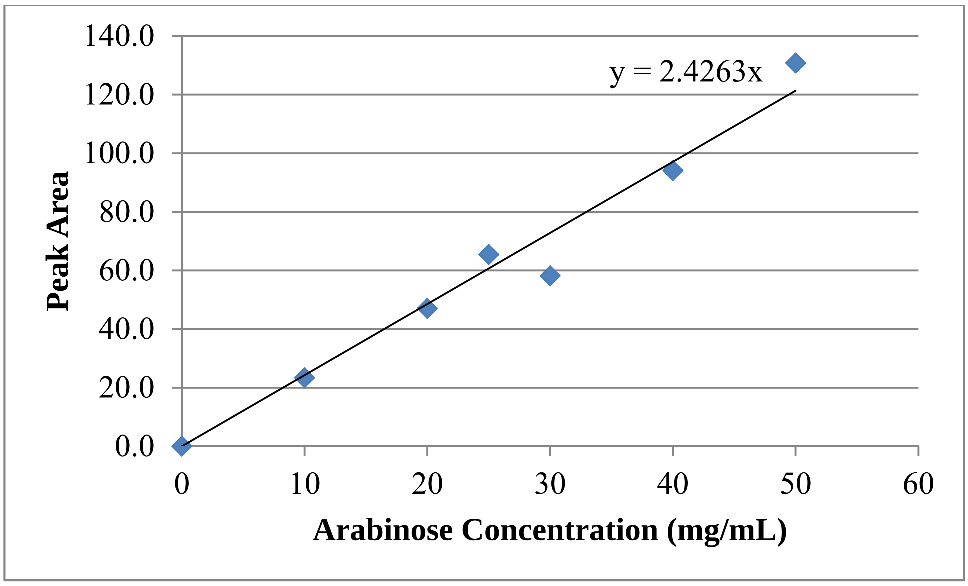


Figure B.3 Calibration curve of arabinose concentration.

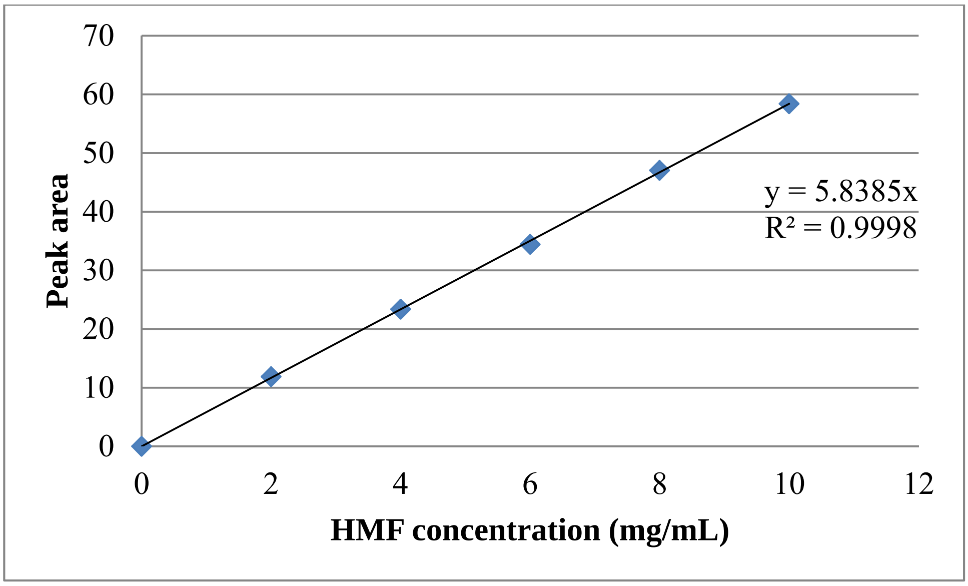


Figure B.4 Calibration curve of HMF concentration.

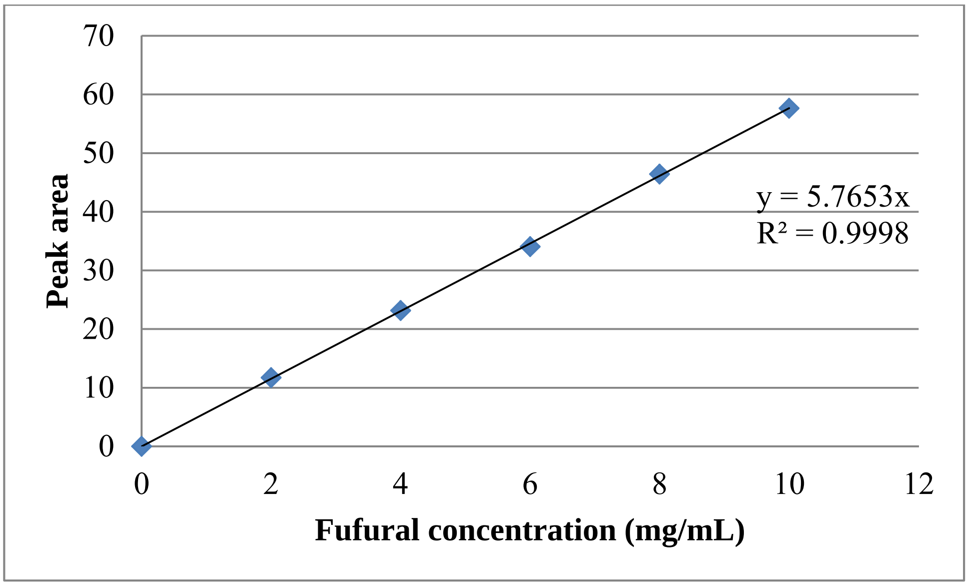


Figure B.5 Calibration curve of furfural concentration.

APPENDIX C

Determination of Structural Carbohydrates and Lignin in Biomass

Procedure

C.1 Prepare the sample for analysis and hydrolysis

C.1.1 Place an appropriate number of filtering crucibles in the muffle furnace at 575 ±25 °C for a minimum of four hours. Remove the crucibles from the furnace directly into a desiccator and cool for a specific period of time. One hour is recommended. Weigh the crucibles to the nearest 0.1 mg and record this weight. It is important to keep the crucibles in a specified order, if they are not marked with identifiers. Permanent marking decals are available from Wale Apparatus. Do not mark the bottom of the filtering crucible with a porcelain marker, as this will impede filtration.

C.1.2 Place the crucible back into the muffle furnace at 575 ± 25 °C and ash to constant weight. Constant weight is defined as less than ± 0.3 mg change in the weight upon one hour of re-heating the crucible.

C.1.3 Weigh 300.0 ± 10.0 mg of the sample or QA standards into a tared pressure tube. Record the weight to the nearest 0.1 mg. Label the pressure tube with a permanent marker. Each sample should be analyzed in duplicate, at minimum. The recommended batch size is three to six samples and a QA standard, all run in duplicate.

C.1.4 Add 3.00 ±0.01 mL (or 4.92 ± 0.01 g) of 72% sulfuric acid to each pressure tube. Use a Teflon stir rod to mix for one minute, or until the sample is thoroughly mixed.

C.1.5 Place the pressure tube in a water bath set at 30 ± 3 °C and incubate the sample for 60 ± 5 min. Using the stir rod, stir the sample every five to ten minutes without removing the sample from the bath. Stirring is essential to ensure even acid to particle contact and uniform hydrolysis. 0.01 mL (or 4.92 ± 0.01 g) of 72% sulfuric acid to each pressure tube. Use a Teflon stir rod to mix for one minute, or until the sample is thoroughly mixed.

C.1.6 Upon completion of the 60 min hydrolysis, remove the tubes from the water bath. Dilute the acid to a 4% concentration by adding 84.00 ± 0.04 mL deionized water using an automatic burette. Dilution can also be done by adding 84.00±0.04 g of purified water using a balance accurate to 0.01 g. Screw the Teflon caps on securely. Mix the sample by inverting the tube several times to eliminate phase separation between high and low concentration acid layers.

Note: The volume of the 4% solution will be 86.73 mL, as demonstrated in the following calculations. 0.04 g of purified water using a balance accurate to 0.01 g. Screw the Teflon caps on securely. Mix the sample by inverting the tube several times to eliminate phase separation between high and low concentration acid layers.

Density 72% H_2SO_4= $d_{72\%\ H2S04}$= 1.6338 g/mL
Density H_20= d_{H20}= 1.00 g/mL
Density 4% H_2SO_4 = $d_{4\%\ H2S04}$ = 1.025 g/mL

1. The weight of 3.00 mL 72% H_2SO_4 is:

3.00 mL 72% H_2SO_4 x d72% H_2S0_4= 4.90 g 72% H_2SO_4

2. The composition of 3 mL of 72% H_2SO_4 is:

4.90 g 72% H_2SO_4 x 72% (acid wt) = 3.53 g acid
4.90 g 72% H_2SO_4 x 28% (water wt) = 1.37 g water

3. The concentration of H_2SO_4 after dilution is:

3.53 g acid / (84.00 g H_20 + 4.90 g 72% H_2SO_4) = 3.97 % H_2SO_4 (w/w)

4. The total volume of solution present after dilution is:

(4.90 g H_2SO_4 + 84.00 g H_20) x (d4% H_2S0_4)-1 = 86.73 mL

C.1.7 Prepare a set of sugar recovery standards (SRS) that will be taken through the remaining hydrolysis and used to correct for losses due to the destruction of sugars during dilute acid hydrolysis. SRS should include D-(+)glucose, D-(+)xylose, D-(+)galactose, -L(+)arabinose, and D-(+)mannose. SRS sugar concentrations should be chosen to most closely resemble the concentrations of sugars in the test sample. Weigh out the required amounts of each sugar to the nearest 0.1 mg, and add 10.0 mL deionized water. Add 348 µL of 72% sulfuric acid. Transfer the SRS to a pressure tube and cap tightly.

C.1.7.1 A fresh SRS is not required for every analysis. A large batch of sugar recovery standards may be produced, filtered through 0.2 µm filters, dispensed in 10.0 mL aliquots into sealed containers, and labeled. They may be stored in a freezer and removed when needed. Thaw and vortex the frozen SRS prior to use. If frozen SRS are used, the appropriate amount of acid must be added to the thawed sample and vortexed prior to transferring to a pressure tube.

C.1.8 Place the tubes in an autoclave safe rack, and place the rack in the autoclave. Autoclave the sealed samples and sugar recovery standards for one hour at 121°C, usually the liquids setting. After completion of the autoclave cycle, allow the hydrolyzates to slowly cool to near room temperature before removing the caps.

C.2 Analyze the sample for acid insoluble lignin as follows

C.2.1 Vacuum filter the autoclaved hydrolysis solution through one of the previously weighed filtering crucibles. Capture the filtrate in a filtering flask.

C.2.2 Transfer an aliquot, approximately 50 mL, into a sample storage bottle. This sample will be used to determine acid soluble lignin as well as carbohydrates, and acetyl if necessary. Acid soluble lignin determination must be done within six hours of hydrolysis.

C.2.3 Use deionized water to quantatively transfer all remaining solids out of the pressure tube into the filtering crucible. Rinse the solids with a minimum of 50 mL fresh deionized water. Hot deionized water may be used in place of room temperature water to decrease the filtration time.

C.2.4 Dry the crucible and acid insoluble residue at 105 ±3 °C until a constant weight is achieved, usually a minimum of four hours.

C.2.5 Remove the samples from the oven and cool in a desiccator. Record the weight of the crucible and dry residue to the nearest 0.1 mg.

C.2.6 Place the crucibles and residue in the muffle furnace at 575 ± 25 °C for 24 ± 6 hours.

C.2.6.1 A furnace with temperature ramping may also be used for a Furnace Temperature Ramp Program:

- Hold at 105°C for 12 min
- Ramp to 250 °C at 10°C / min
- Hold at 250 °C for 30 min
- Ramp to 575 °C at 20 °C / min
- Hold at 575 °C for 180 min
- Allow temperature to drop to 105 °C
- Hold at 105 °C until samples are removed

C.2.7 Carefully remove the crucible from the furnace directly into a desiccator and cool for a specific amount of time, equal to the initial cool time of the crucibles. Weigh the crucibles and ash to the nearest 0.1 mg and record the weight. Place the crucibles back in the furnace and ash to a constant weight.

C.3 Analyze the sample for acid soluble lignin as follows

C.3.1 On a UV-Visible spectrophotometer, run a background of deionized water or 4% sulfuric acid.

C.3.2 Measure the absorbance of the sample at an appropriate wavelength on a UV-Visible spectrophotometer. Dilute the sample as necessary to bring the absorbance into the range of 0.7 – 1.0, recording the dilution. Deionized water or 4% sulfuric acid may be used to dilute the sample, but the same solvent should be used as a blank. Record the absorbance to three decimal places. Reproducibility should be + 0.05 absorbance units. Analyze each sample in duplicate at minimum. (This step must be done within six hours of hydrolysis.)

C.3.3 Calculate the amount of soluble acid.

APPENDIX D

CALCULATIONS

D.1 The percentage of product yield can be calculated as below

$$\text{Glucan content (\%)} = \frac{\text{Glucose concentration}\left(\frac{\text{g}}{\text{l}}\right) \times 0.087(\text{l}) \times 0.9}{\text{Mass of over dried (0.3g)}} \times 100$$

$$\text{Xylan content (\%)} = \frac{\text{Xylose concentration}\left(\frac{\text{g}}{\text{l}}\right) \times 0.087(\text{l}) \times 0.8}{\text{Mass of over dried (0.3g)}} \times 100$$

$$\text{Arabinan content (\%)} = \frac{\text{Arabinose concentration}\left(\frac{\text{g}}{\text{l}}\right) \times 0.087(\text{l}) \times 0.8}{\text{Mass of over dried (0.3g)}} \times 100$$

$$\text{Ash content (\%)} = \frac{\text{Weight crucible plus ash} - \text{Weight crucible}}{\text{Mass of over dried (0.3g)}} \times 100$$

Acid insoluble lignin, (ASL) (%) =

$$\frac{\text{Weight crucible plus solid after 105°C-Weight of crucible plus ash after 575°C}}{\text{Mass of over dried (0.3g)}} \times 100$$

$$\text{Acid soluble lignin, (ASL) (\%)} = \frac{\text{Uvabs} \times 0.087 \times \text{dilution}}{\varepsilon \times \text{Mass of over dried} \times \text{cell pathlength}} \times 100$$

$$\text{Lignin (\%)} = \text{\%ASL} + \text{\%AIL}$$

$$\text{Product yields (\%)} = \frac{\text{Products concentration}\left(\frac{\text{g}}{\text{L}}\right) \times \text{Vol. of liquid fraction(l)}}{\text{Amount of product in raw materials (g)}} \times 100$$