REACTION MECHANISMS OF HYDROTHERMAL LIQUEFACTION OF MODEL COMPOUNDS AND BIOWASTE FEEDSTOCKS

BY

ZHICHAO WANG

DISSERTATION

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Agricultural and Biological Engineering
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2011

Urbana, Illinois

Doctoral Committee:

Professor Yuanhui Zhang, Chair
Assistant Professor Ted L. Funk
Professor Steven R. Eckhoff
Assistant Professor Lance Schideman
Professor John R. Regalbuto, University of Illinois at Chicago

ABSTRACT

It has been demonstrated that hydrothermal liquefaction (HTL) could be an efficient technology that can not only reduce COD and pollutants in the bio-wastes but also generate valuable renewable bio-crude oil from many biomass sources. Previously, swine manure has been successfully converted into bio-crude oil through HTL with yield as high as 70% of the volatile solids at the University of Illinois at Urbana-Champaign. To further understand the fundamental mechanisms of HTL and to provide information for further developments of this technology, experiments with both model compounds and typical biomass (e.g. swine manure) as feedstock were conducted, the product distribution and the properties were analyzed. Some possible reaction pathways of specific compounds were proposed.

HTL tests were conducted for swine manure collected from nursery, grower-finisher and sow pigs. The testing conditions were: reaction temperature 305^{o}C, initial solid content 20% wt, retention time 30 min, initial N_2 gas pressure 0.65 MPa, and without catalysts or additives. Comparison between the bio-crude oil obtained from HTL of nursery, grower-finisher and sow pigs showed no significant differences. Length of manure storage time in a shallow pit did not profoundly affect the formation of bio-crude oil through HTL, although a slight decrease of refined oil yield from 42% to 35% was observed.

HTL tests of swine manure at transient temperatures (180-240^{o}C) and retention times of 0-60 min showed that the release of fatty acids, the hydrolysis of hemicellulose and proteins, and the Maillard reactions were the major reactions happened at temperatures lower than 240^{o}C. Maillard reaction started to occur at as low temperatures as 180^{o}C. The formed melanoidins further decomposed to gas, oil, char and aqueous products. Fatty acids were the major components in the bio-crude oil at temperatures lower than 240^{o}C, with some decomposition products from amino acids and melanoidins. At 200^{o}C, between 0-15 min, a rapid and intensive hydrolysis of hemicellulose occurred and caused the decrease of pH in the aqueous phase. The decreased pH probably enhanced the decomposition of melanoidins.

A refined oil yield of 20% was obtained at 300^{o}C from the HTL of egg albumin. Tests conducted between 200-300^{o}C showed that degradation of large molecules and re-combination of small molecules generated in the degradation process were probably the two major pathways for bio-crude oil formation from protein. HTL of carbohydrates generated large amount of solid residue

(more than 40%), with the refined oil yield lower than 25%. However, addition of base catalysts (e.g. Na_2CO_3), lipid and protein into carbohydrates could significantly decrease the solid residue yield. Addition of protein into lipid caused the increase of refined oil yield and the decrease of aqueous products with the main interaction probably being the formation of amides.

Fifteen tests were conducted for regression between the refined oil yield and the lipid, protein and carbohydrates content in the feedstocks. A positive linear relationship was observed between the refined oil yield and the lipid content, as well as between the refined oil yield and the protein content. A negative linear relationship was found between the refined oil yield and the carbohydrates content in the volatile solids in the feedstocks. Based on the regression results, a series of formulae were obtained which could be used to predict the refined oil yield from HTL of biomass. The errors for two predictions for examining purpose were -1.68% and 14.74% respectively.

Based on the results and from both model compounds and real biomass, a scheme of the reaction pathways during the HTL of biomass was proposed. The scheme included the reactions which could possibly happen to lipid, protein and carbohydrates.

To my wife and family

ACKNOWLEDGEMENTS

My sincere thanks go to many people who have helped me and supported me through this study. I would like to express my deepest gratitude to my advisor, Dr. Yuanhui Zhang. Without his guidance, support and patience, this study would not have been possible. His brilliant ideas and positive thinking, and more importantly, his optimist attitude and constant encourage have been most helpful in the completion of this study. I would like to thank Dr. Ted Funk, Dr. Steven R. Eckhoff, Dr. Lance Schideman, and Dr. John R. Regalbuto for their generous help serving as committee members in my Doctoral Committee.

I would like to thank my fellow researchers (past and present) in the Bioenvironmental Engineering group including Dr. Les L. Christianson, Dr. Brian He, Dr. Rong Dong, Dr. Shuangning Xiu, Dr. Yigang Sun, Guo Yu, Mitchell J. Minarick, Yan Zhou, Dr. Xufei Yang, Dr. Wei Yan, Dr. Hai Wu, Chi-Ting Kuo, Beni Brem, and anyone else who has worked with me and provided me valuable support.

I am grateful to the Department of Agricultural and Biological Engineering. I have enjoyed five wonderful years as a member of the department and have always been feeling that I am living in a big warm family. I would like to thank all the people in the department, faculty and staff, undergrads and graduates, who have helped me a lot in completing this study.

My family, more than 6,000 miles away back in China, has always been supportive and encouraging. My dad and mom always think their son is the best, which is not true but did lift me past some of those dark days. I love you so much and miss you all the time. My wife, Huafeng Shi, has provided me every kind of support she could have. She is a wonderful woman, and living with her has made my life colorful and has made being away from home a lot easier. Huafeng has always been my strength and inspiration that keeps me going forward. Thank you and I love you.

TABLE OF CONTENTS

CHAPTER 1. INTRODUCTION

1.1. BACKGROUND

Environment and energy are two of the most concerned global issues in recent decades. They have been widely researched from many different aspects, and yet are still in demand of more studies.

1.1.1. Bio-wastes treatment demand and environmental protection

Bio-wastes, although so called "wastes", could be valuable resources for renewable energy, specific chemicals or fertilizers. However, if not utilized and treated properly, it could cause adverse environmental problems. Every year, a large amount of bio-wastes is produced and needs to be treated. In U.S., annual biomass resource potential from forest and agricultural resources was estimated to be over 1.3 billion dry tons, and the amount of biomass currently available for bioenergy and bioproducts was about 194 million dry tons per year (Perlack et al., 2005). The amount of livestock manure generated in the U.S. at animal feeding operations (AFOs) and concentrated animal feeding operations (CAFOs) was estimated to exceed 335 million tons of dry matter per year, and often sufficient agricultural land is not available within the vicinity of animal operations to safely use all the manure produced (USDA, 2006). More than 2 billion US dollars is spent annually treating and managing approximately 5-7 million dry metric tons of biosolids from over 13,000 publicly owned treatment works (Meeroff, 2001). In 2007, about 250 million tons of municipal solid wastes were generated in U.S., disposal of these wastes cost billions of dollars (USEPA, 2008). Algae also present a challenge to our society in many areas. Microalgal blooms cover extensive body of natural water, such as the lakes and sea shores around the world due to eutrophication almost every year (Graneli et al., 2008).

Traditional waste treatment methods are facing more challenges than before. Taking livestock manures as an example, land application is the primary and oldest treatment method. However, intensive livestock production today is producing more manure than what the local land can use without over fertilization locally. Over-application may cause excess of NO_3^- and PO_4^{3-} and furthermore pollute the surface and groundwater (Mahro and Timm, 2007). Lagoon storage is

another widely-used method for livestock manure treatment in the United States. Building and managing lagoons increases costs and eventually the manures in lagoons still need to be disposed.

On the other hand, biomass is a potential source of renewable energy. Biomass-derived energy is considered as "carbon-neutral", which could dramatically decrease the carbon dioxide emission. Therefore, appropriate treatment of bio-wastes could not only protect the environment from many perspectives but also generate renewable energy.

1.1.2. Renewable energy demand

USA now is heavily relying on fossil fuels for most of its energy consumption. Currently fossil fuels, including petroleum, natural gas and coal, contribute more than 80% in the U.S. daily energy consumption (USDOE, 2009a). More strikingly, about 2/3 of crude oil consumed in the U.S. is imported (USDOE, 2009b).

Fossil fuel resources are facing limited reserves that are estimated to be sufficient to supply energy and chemicals for only about another 40 years, while coals and natural gases could supply for about another 70 and 150 years, respectively (Davies, 2006). Therefore, alternative energy resources other than fossil fuels are highly demanded.

Researchers believe biomass and biomass only, can serve as the sustainable source of liquid fuel and organic carbon for the industrial society (Chheda and Dumesic, 2007). By 2030, 20% of transportation fuel and 25% of chemicals is predicted to be produced from biomass in the U.S. (USDOE, 2002)

1.1.3. HTL as a method solving both environmental and energy problems

Considering the demands of bio-wastes treatment and renewable energy production, hydrothermal liquefaction (HTL) provides a possible promising solution for solving both environmental and energy problems. Hydrothermal liquefaction, also called hydro-pyrolysis by some researchers, is considered attractive for bio-fuel production which could be an economical alternative to fossil fuels in the future. Moreover, HTL has advantages in processing wet biomass with high moisture content and organic residues that are commonly considered "bio-wastes",

which makes it a suitable technology to produce renewable fuel and at the same time to protect the environment, hereafter called Environment-Enhancing Energy (E^2-Energy).

The E^2-energy potential from bio-wastes and algae is enormous. With an energy content of about 13.4 MJ/kg (Klass, 1998), presently collected manure itself represents an annual renewable energy resource of approximately $7*10^{11}$ MJ, which is about 110 million barrels of oil equivalent. Based on the estimation of total bio-wastes produced in the United States (ASABE, 2005; USEPA, 2008) and the potential for algae growth (NREL, 1998) in wastewater, it could produce enough biocrude oil to meet the entire national need for transportation fuel, approximately 1.2 billion tons of crude oil per year in 2007.

1.2. RESEARCH NEED

Research on HTL can date back to the 1930s and there is an extensive body of literatures in U.S. and world-wide. Despite this, HTL has not been developed as a commercially viable energy-making technology. It is largely limited by relatively low cost of crude petroleum and the high costs to purchase, transport and process feedstocks. However, with the increase of the price of petroleum, proper use of low cost bio-wastes and further development of the HTL technology, the HTL could be economically viable in the future.

Reaction mechanisms study is critical in understanding the process so as to better design the reactors and control the process. At present, understanding of HTL mechanisms is largely qualitative and indicative although efforts have been made through years by many researchers (Balat, 2008; Demirbas, 2000a; Demirbas, 2000b; Elliott et al., 1987; Minowa et al., 1998; Yuan et al., 2009). Generally, biomass is first broken up into fragments by hydrolysis, and then degraded into smaller compounds by dehydration, dehydrogenation, deoxygenation and decarboxylation. At the end, some complex chemicals may be synthesized by repolymerization. Biocrude product generated in the repolymerization process usually contains acids, alcohols, aldehydes, esters, ketones, phenols and other aromatic compounds (Chornet and Overend, 1985). However, the exact mechanisms or pathways of the HTL still remain unclear mainly due to the complexity of the feedstocks and HTL products, as well as the seemingly-infinite possible intermediate reactions.

Hydrothermal liquefaction now is in the transient state from lab-pilot scale to pilot-industrial scale, which makes the mechanisms study more important and urgent. With the support of mechanisms work, development of large-scale units could be more reasonable and high-grade products may be produced, adding a solution to current environmental and energy problems.

1.3. OBJECTIVES

The ultimate goal of this research is to develop economically sustainable hydrothermal liquefaction systems to produce bio-based crude oil from biomass, especially bio-wastes such as animal, human and food processing wastes and algae.

This research conducts studies to understand the fundamental mechanisms of HTL, including the elemental distribution and transfers during the process, and the functions of specific key components in actual biomass during HTL. Pathways of specific compounds are analyzed. Both model compounds and selected biomass are used as feedstock during the study. The specific objectives of the proposed work are listed below.

1. Investigate the effects of variation of swine manure feedstock on the HTL bio-crude conversion efficiency, to provide data for the set-up of on-farm HTL systems.
2. Investigate the HTL of swine manure in transient temperature range, to explore the oil formation process from real biomass with complicated chemical compositions.
3. Examine the pathways of biocrude oil conversion of typical model compounds (including egg albumin, butter, cellulose microcrystalline, glucose and xylose) and their mixtures during HTL, focusing on the conversion of protein and its effects on other compounds.
4. Explore the relationships between bio-crude oil yield and specific chemical compounds and study the effects of alkali catalyst on individual chemical compounds.
5. Investigate the reaction pathways of different chemical compounds based on the HTL tests of model compounds and some real biomass.

CHAPTER 2. LITERATURE REVIEW

2.1. THERMOCHEMICAL CONVERSION PROCESSES

To produce liquid transportation fuels from biomass using thermochemical processes, three major conversion technologies are widely studied and developed: pyrolysis, gasification (followed by syn-gas synthesis), and direct liquefaction.

Pyrolysis is the thermal decomposition of materials in the absence of oxygen or when significantly less oxygen is present than required for complete combustion. Usually, pyrolysis is divided into conventional pyrolysis (slow pyrolysis), fast pyrolysis and flash pyrolysis, based on the reaction temperature, heating rate and residence time. The range of the main operating parameters for different pyrolysis processes are listed in Table 2.1. Researchers have done immense amount of work on the development of pyrolysis, and a critical review on the pyrolysis technologies could be found elsewhere (Mohan et al., 2006). Some researchers were in favor of fast or flash pyrolysis at high temperatures with very short residence times (Elliott et al., 1991). Products including oil, water-soluble products, char, permanent gases and C_1-C_4 hydrocarbon gases could be obtained from biomass pyrolysis. Bio-oil (tar) is the product of greatest interest during fast and flash pyrolysis.

Table 2-1. Range of the main operating parameters for pyrolysis processes.

	Conventional pyrolysis	Fast pyrolysis	Flash pyrolysis
Operating temperature (°C)	300-700 [a]	600-1000 [a]	800-1000 [a]
Heating rate (°C/s)	0.1-1 [a]	10-200 [a]	≥ 1000 [a]
Solid residence time (s)	600-6000 [a] 450-550 [b]	0.5-5 [a]	< 0.5 [a]
Particle size (mm)	5-50 [a]	< 1 [a]	<0.2 [b]

[a]-(Maschio et al., 1992).

[b]-(Demirbas, 2006).

Gasification is a process in which solid or liquid carbonaceous material, such as biomass, coal, or oil, react with air, oxygen, and/or steam to produce a syn-gas product or produce gas that contains CO, H_2, CO_2, CH_4, and N_2 in various proportions (Huber et al., 2006). Then syn-gas could be converted to liquid fuel through Fisher-Tropsch Synthesis (FTS) or methanol synthesis. Since this is a two-step process to liquefy the feedstock, it is also called indirect liquefaction in

some cases. Generally speaking, gasification needs higher temperature than fast pyrolysis (Goyal et al., 2008). Gasification usually prefers high temperature, low heating rate and long gas residence time (Demirbas, 2006). The gasification process consists of drying, devolatilization, oxidation and reduction. Detailed description of each stage, as well as the classification of gasification reactors, could be found in the study of Puig-Arnavat et al. (2010). Gasification of biomass can also be accomplished in supercritical and near-supercritical temperatures in water (Kruse et al., 2005; Yanik et al., 2007).

Direct liquefaction is first used to generate liquid fuel from coal under increased pressure in the presence of hydrogen and catalysts, and it has a history of more than 100 years. Direct liquefaction of biomass came into focus as one potential path again since the oil crisis (Behrendt et al., 2008). Chornet and Overend (1985) suggested the following classification of direct liquefaction: solvolysis, aqueous medium, organic medium and thermal decomposition under reducing atmosphere. Based on this classification, hydrothermal liquefaction is defined as the direct liquefaction using aqueous medium. In this study, we will focus on hydrothermal liquefaction (HTL) process.

HTL of biomass produces a water-insoluble bio-oil by treatments at high pressure (50-200 atm) and relatively low temperature (250-450 °C) compared to pyrolysis and gasification. It usually happens in an anoxic or very low oxygen environment. The bio-oil produced by liquefaction has a lower oxygen content and therefore higher energy content than pyrolysis-derived oils. (Huber et al., 2006)

Each thermochemical process has its advantages and disadvantages, and judgment on which process to choose is largely based on the optimization of factors including feedstock cost and availability, capital cost, upgrading requirements, marketing, etc. Products, development stage and challenges for these technologies are summarized in Table 2-2.

Table 2-2. Brief overview of pyrolysis, gasification and hydrothermal liquefaction.

	Gasification (followed by syn-gas synthesis)	Fast Pyrolysis	Hydrothermal liquefaction (HTL)
Temperature	800-1200^{o}C [a]	600-1000^{o}C [b]	150^{o}C-420^{o}C [c]
			250-450^{o}C [d]
Pressure	<20 bar [c]	<5 bar [c]	<240 bar [c]
Catalysts	Usually unnecessary	Usually unnecessary	Low oil yield without catalysts
			Usually used: Alkalines and Heterogeneous catalysts
Products	Liquid Alkanes,	Bio-oils	Bio-oils
	Methanol and derivatives	Water soluble organics	Water soluble organics
	(Both synthesized from syn-gas (CO+ H_2))	Bio-char	Solid residue
		Gaseous products	Gaseous products
Development Stage	Large scale pilot plant [d]	Commercialized [d]	Pilot plant [d]
PTE	0.2-0.4 [d]	>0.6 [d]	>0.6 [d]
Advantages	-Short residence time	-Lower capital cost compared to HTL	-High throughputs
	-High octane gasoline is possible	-Companionable to many industrial-scale reactors/equipments	-High energy and separation efficiency
	-The high-temperature combustion refines out corrosive ash elements		-Offers possibilities in coordination with other biofuel processing techniques [d]
Disadvantages	*Low selectivity during FTS process	*Unstable oil products, needs upgrading for transportation use	*Unstable oil products, high viscosity
	*Tar removal/ conversion from syn-gas is difficult	*High drying cost for high moisture feedstocks	*High operation pressure
			*Unknown or largely uncharacterized reaction pathways and kinetics
		*Low heating value and high oxygen content of bio-oils	*Inadequate catalysts, and deactivation of heterogeneous catalysts

Note: Summarized according to varies sources. [a]: (Laird et al., 2009); [b]: (Maschio et al., 1992); [c]: (Behrendt et al., 2008); [d]: (Huber et al., 2006)

2.2. DEVELOPMENT OF HYDROTHERMAL LIQUEFACTION TECHNOLOGY

As mentioned in section 1.1.3, this research focuses on the hydrothermal liquefaction due to its advantages and potential of utilizing biowastes and algae as feedstocks. A historical development of hydrothermal liquefaction will be introduced in this section.

Berl (1934) has reported production of "proto product" containing aliphatic, naphthenic, and aromatic substances from cellulose and other carbohydrates in hot water with sufficient alkaline materials. Later he suggested that cornstalks, corn cobs, sugar cane and any other "carbohydrate-containing materials" could be turned into a petroleum-like product by a controlled internal combustion (Berl, 1944). Researches thereafter have confirmed Berl's conclusion using a wide range of biomass feedstocks.

In 1970s and 1980s, research on the HTL was widely conducted in seeking of alternative energy due to the oil crisis (Appell et al., 1970; Beckman and Elliott, 1985; Boocock and Sherman, 1985; Fu et al., 1974; Nelson et al., 1984). In general, hydrothermal liquefaction conditions range from 280-380^{o}C, 7 to 30 MPa pressure with liquid water, 10 to 60 min retention time, often with catalysts present (which are generally alkali), and sometimes with reducing gases such as CO or H_2. The oil produced generally has a heating value of 30 to 36 MJ/kg and oxygen contents of 10 to 20% (Peterson et al., 2008b).

Eighteen kinds of agricultural and forest residues in Indonesia were hydrothermally liquefied by Minowa et al. (1998). Tests were run in a 300 ml stainless steel autoclave with a magnetic mixer, at 300^{o}C temperature and 30 min retention time. Nitrogen gas was used as initial gas and added to a head-space pressure 3 MPa at the beginning. Five percent (wt) Na_2CO_3 was used as catalyst and acetone was used to extract oil product. Oil yields were in the range of 21-36%, depending on the species and parts of feedstock. Obtained oils had almost the same properties, C ~70%, H ~7%, N <1%, O~20%, calorific value around 30 kJ/g and viscosity >105 mPa.s. Gas yields were around 20%. A high correlation between lignin and residue was observed, with which a similar finding was also reported by Demirbas (2000b).

Various kinds of sewage sludge were also tested at 300°C, 12 MPa, 0 retention time and a catalyst loading of 0-20 wt % (Suzuki et al., 1988). Nature of sludge had no significant influence on the elemental composition and heating value of oils obtained. The reaction proceeded satisfactorily without adding any catalyst, and catalyst loading also had no significant influence on the properties of the oil products. A demonstration plant with a capacity for processing up to 5 t/d as dewatered sludge was operated at 300°C, 10 MPa, (feedstock moisture content ~80%, VS ~80%) (Itoh et al., 1994). As a result, 48% (maf-moisture and ash free) of the organic materials in the sludge were converted into heavy oil, and a quarter of the oil was separated from the reaction mixture by high pressure distillation with a distillate ratio of 0.33. Energy balance analysis showed the treatment of sewage sludge by this method could be sufficiently profitable.

Dote et al. (1994) were among the earliest researches who studied the hydrothermal liquefaction of algae. They liquefied a strain of micro-algae and were able to yield 64% (mass basis) oil from it at 300°C with Na_2CO_3 as catalyst. (Ross et al., 2010) recently investigated the hydrothermal conversion of microalgae (*Chlorella vulgaris*) and cyanobacteria (*Spirulina*) in a batch reactor at 300°C and 350°C. Bio-crude yield (daf) of 11.6-20.0% and 19.1-27.3% were obtained from *Spirulina* and *Chlorella vulgaris*, respectively when different species of catalysts were added (Na_2CO_3, KOH, HCOOH, CH_3COOH). The higher heating values (HHV) of bio-crude ranged from 33.4 to 39.9 MJ/kg. Analysis by GC-MS indicated that the bio-crude contained aromatic hydrocarbons, nitrogen heterocycles and long chain fatty acids and alcohols. The difference of the yields between the researches of Dote et al. and Ross et al. were probably due to the differences in bio-crude definition and collection methods.

Much of the pioneering hydrothermal liquefaction work was done by Appell and coworker at the Pittsburgh Energy Research Center in the 1970s (Appell et al., 1970; Appell et al., 1980). Then the process was demonstrated at a pilot plant in Albany, Oregon.

In the 1980s, Shell developed a hydrothermal liquefaction process known as Hydrothermal Upgrading, or the HTU process. It was concluded that for high HTU conversion, selectivity and yield, essential conditions are: temperature>300°C, liquid water present and reaction time>5min. (Goudriaan and Peferoen, 1990). Unfortunately the oil company abandoned the process in 1989.

However, later on in 1995 a technical and economic feasibility study was carried out by Stork Comprimo (now Stork Engineers & Contractors) to investigate the perspectives for the future of HTU. The results of the study led to the formation of a Dutch consortium which resumed the study in 1997. Main purpose of the R&D that would run till mid-2000 was the validation of the HTU Process on 20 kg (dry matter) biomass/hr pilot plant scale and the development of the necessary design data for the first commercial applications. Good preliminary results in the conversion of biocrude from HTU with hydrodeoxygenation were reported (Goudriaan et al., 2001).

Changing World Technologies Inc., founded in 1997, tried to develop and commercialize the hydrothermal liquefaction process (which they called thermal conversion process). A plant in Carthage, Missouri opened in 2004, mainly treating turkey wastes. However, it was closed in 2009. Up to the finish of this dissertation, no other commercialized plant has been reported using the hydrothermal liquefaction process to produce large amount of transportation fuel from biomass.

2.3. MECHANISM STUDY ON HTL

Investigations into the mechanisms of hydrothermal liquefaction is not a new topic, yet many questions remain unanswered, mainly due to the complex natures of both the feedstocks and the resulting products, not to mention the extremely large number of possible intermediate chemical reactions.

The chemistry behind reactions of individual biochemicals under hydrothermal conditions is widely studied for some materials, such as glucose and triacylglycerides. However, the chemical pathways of, kinetics of, and interactions between components of a biomass at these conditions are largely uncharacterized (Peterson et al., 2008a).

Proteins and Amino Acids

Amino acids are the building blocks of proteins. All amino acids have carboxyl and amine groups. It is known that C-N bond, the structural bond that links amino acids together into

proteins, will rapidly hydrolyze in a hydrothermal system. However, the amino acids produced will subsequently decompose to form other chemicals. This is the reason that researchers could not get high amino acids yields using hydrothermal methods.

Klingler et al. (2007) studied the hydrothermal degradation of glycine and alanine and found the primary mechanisms of degradation to be decarboxylation and deamination. Through decarboxylation and deamination, the amino acids lose the functional group –COOH and $–NH_2$ respectively, and therefore carbonic acid and ammonia are formed. Sato et al. (2004) studied the hydrothermal decomposition of alanine and its derivatives leucine, phenylalanine, serine, and aspartic in the temperature range of 200-340^{o}C at a pressure of 20 MPa and similar conclusion was drown that deamination and decarboxylation are the two main pathways during the process. Abdelmoez et al. (2007) investigated the reactions of amino acids under subcritical water conditions in the temperature range of 230-290^{o}C using a pressure value corresponding to the saturated vapor pressure of water at the reaction temperature. They found that most amino acids are labile at acidic and near-natural pH and more stable in highly basic pH.

The distribution of nitrogen in the products for direct liquefaction of protein-contained biomass was studied by other researchers using egg albumin as the feedstock (Dote et al., 1996). The tests were run at 150^{o}C-340^{o}C, 0.5 h and 2 h retention time, w/o Na_2CO_3 as catalyst. The maximum oil yield was 10% (wt), much less than the oil yield obtained from real biomass feedstocks (all above 30%). Nitrogen distributed (ND) to oil was 5% at most, much less than that for practical feedstocks (30%-45%). Other biomass feedstocks contain other elements such as cellulose and lipid, which may increase the amount of oil converted from protein or react with nitrogen-containing compounds produced from protein during the conversion. No distribution of nitrogen to oil occurred below 150^{o}C, and the distribution was completed by 250^{o}C. The majority of the nitrogen in albumin (80%) was distributed to the aqueous phase, and albumin was decomposed to ammonia, not to amino acids. Sodium carbonate seemed to prevent the distribution of nitrogen to oil.

Kruse et al. (2007) studied both real biomass and the model compounds including alanine and glucose, and found some significant effects of proteins on hydrothermal conversion. They concluded that the effects found for the presence of proteins or alanine are a result of the reaction

of glucose or its consecutive products with amino acids or its consecutive products via the Maillard reaction. These types of reactions lead to nitrogen containing cyclic organic compounds, which are more or less strong free radical scavengers and inhibit free radical chain reactions.

Cellulose

Cellulose is a polysaccharide composed of units of glucose. Usually cellulose could be classified into amorphous and crystalline cellulose. Although its crystalline structure is relatively strong due to the β- (1→4)-glycosidic bonds, cellulose can be hydrolyzed to oligomers and glucose before they are further reacted to other derivative compounds under hydrothermal conditions.

Peterson et al. (2008b) summarized several kinetics studies (Adschiri et al., 1993; Mochidzuki et al., 2000; Sasaki et al., 2000; Schwald and Bobleter, 1989) and obtained an activation energy of 215 kJ/mol cellulose through the best-fit line.

Minowa et al. (1998) studied the decomposition of microcrystalline cellulose in the hot-compressed water from 200-350^{o}C, using a sodium carbonate catalyst, a reduced nickel catalyst or catalyst-free. At catalyst-free condition, the cellulose was found to be decomposed quickly between 240 and 270°C, and no cellulose remained after the reaction at over 280°C. Below 240°C, only water-soluble products were obtained, and the water-soluble products obtained at 200 and 220°C (short reaction time) were almost glucose/oligomer, and those at over 240°C contained non-glucose products. A role of the alkali catalyst in inhibiting the formation of char from oil (stabilization of oil) was suggested. On the other hand, the nickel catalyst catalyzes the steam reforming reaction of aqueous products and the methanation reaction.

Nelson et al. (1984) also studied the mechanisms of direct liquefaction of cellulose. At 250-400^{o}C, a pressure up to 20.7MPa, and with the presence of Na_2CO_3, pure cellulose was converted in a 300ml autoclave, to a mixture of phenols, cyclopentanones and hydroquinones as well as other components. At 300^{o}C, 1 h retention time, most of the oil components are present in amounts of 0.1 wt% or less in the oil product, which makes it very complicated to analyze the oil product. The use of alkaline catalysts at 300^{o}C was shown to shift the mechanism from one

involving aqueous pyrolysis (predominant furan formation) to one incorporating aldol and related condensations.

Russell et al. (1983) used selected aldehydes and ketones which might have formed from cellulose degradation as model compounds to study the formation of aromatic compounds during cellulose liquefaction, under the same conditions as those of cellulose liquefaction. Many of the same aromatic compounds were formed from these reactions as were found in cellulose derived oils. The condensation and cyclisation of aldehydes and ketones is apparently involved in the formation of aromatic compounds in cellulose liquefaction oils.

In the study of Karagoz et al. (2005) using cellulose at hydrothermal conditions, they extracted the liquid phase of the product with diethyl ether and identified the compositions of the extracted fraction using GC-MS. 5-Methyl-2-Furancarboxaldehyde and 2-Furancarboxaldehyde were found counting 48% of the total peak area, and they proposed a reaction pathway which involved 2-formyl-5-(hydroxymethyl)-tetrahydrofuran-3-one as an intermediate.

Glucose/Fructose

Glucose and fructose are isomers which can reversibly isomerize into each other. The hydrolysis of glucose and fructose has been studied for a long time and the rapid degradation at hydrothermal conditions has been confirmed. Many researchers agree that glucose degrades mostly to fragmentation products (glycolaldehyde, pyruvaldehyde, glyceraldehyde, etc.) (Bonn and Bobleter, 1983; Srokol et al., 2004), while fructose will react to a higher amount of dehydration product 5-HMF (Antal Jr et al., 1990; Bonn and Bobleter, 1983; Srokol et al., 2004).

Temperature and heating rate can have a profound impact on the reaction pathway. It was reported that more than 80% of the glucose was consumed above 300^{o}C and 60 s heating period in hot compressed water (Kumar and Gupta, 2008; Watanabe et al., 2005). Retention time also influences the degradation products. A longer residence time enhances the degradation rate of converted hydrolysis products (Kumar and Gupta, 2008). Longer retention time may cause the formation of more complex compounds.

Luijkx et al. (1993) found that aromatic compound 1, 2, 4-benzenetriol could be formed in significant yields from fructose, which shows that aromatics can be formed from cellulosic sugars. Catallo et al. (2010) concluded that the generation of semi-volatile compounds (e.g. phenolics, substituted benzenes, and substituted cycloalkenes) was not primarily the result of desaturation and aromatization of intact glucose ring backbones, and was more likely through the reactions between reactive molecular fragments generated during the process. The lower temperature (300^{o}C) gave rise to distributions of oxygenated species including aldehydes, ketones, diones, and furans with lesser amounts of phenol and substituted phenols, while the major products at higher temperature (400^{o}C) were aromatic hydrocarbons (e.g. alkyl-substitued benzenes and furans), phenol and alkyl-substituted phenols, and acetylcyclohexene isomers.

It was also concluded that the medium H_2O is the most important reactant in the generation of oxidized gaseous products from the substrate as they found the hydrothermal processing of glucose in H_2O^{18} produced mainly $CO_2{}^{18}$ with lesser amounts of $CO^{16}O^{18}$ and $CO_2{}^{16}$. Also, the O^{18} were well presented in the semi-volatile products, indicating the influence of hydration/oxidation reactions (Catallo et al., 2010).

Knezevic et al. (2009) performed a thorough study on the hydrothermal conversion of glucose at 250-350^{o}C, using batch quartz capillary reactors. They used acetone to extract the products, and water-solvent soluble (WSS) fraction, water-solvent insoluble (WSIS) fraction and gas were the three product streams they defined. Reaction pathways were proposed, and a formal reaction mechanism was also presented (Figure 2-1). In the reaction mechanism they stated, glucose first dehydrates to form WSS, and then WSIS and gas are formed from a certain fraction of WSS. During the decay of WSS, two separate reactions happen at different rates.

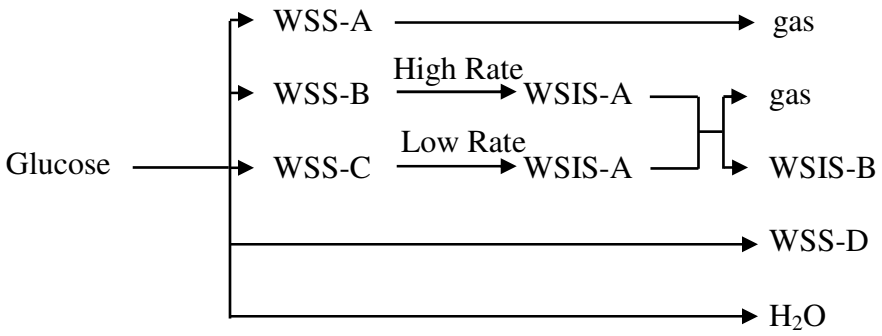
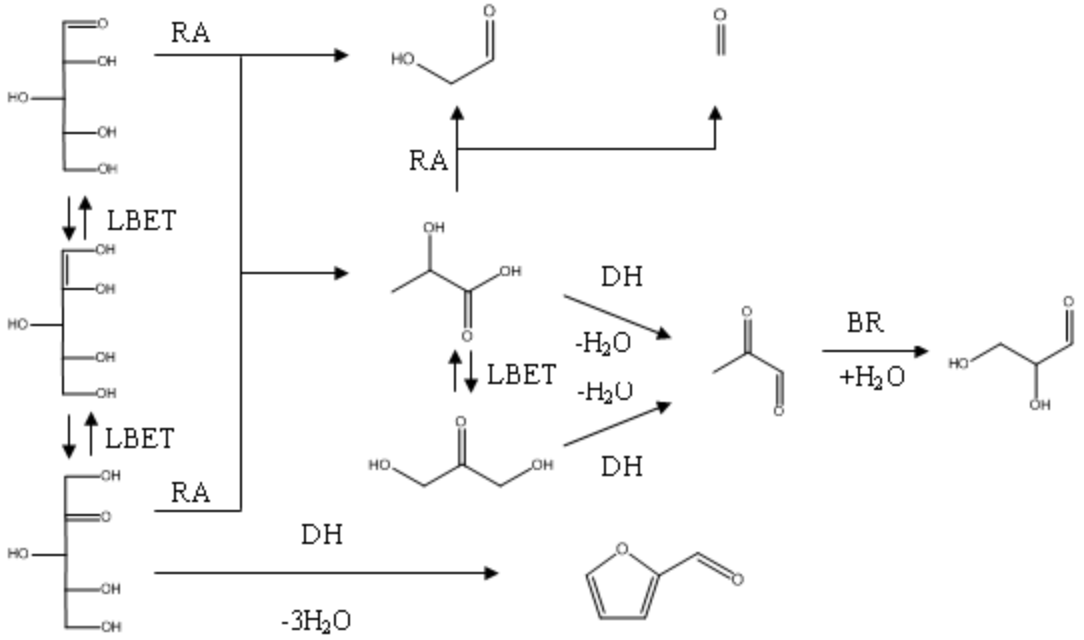


Figure 2-1. Reaction mechanisms of glucose during HTL, adapted from Knezevic et al. (2009).

Hemicellulose and xylose

Hemicellulose is a heteropolymer composed of sugar monomers (e.g. arabinose, mannose, galactose, rhamnose, xylose, glucose, etc.), while xylose is a five-carbon sugar that is one of the most common monosaccharide residues in hemicellulose. Bobleter (1994) suggested that hemicellulose is easily dissolved in water at temperatures above 180^{o}C, forming monosaccharides. Mok and Antal (1992) reported that they could extract an average of 95% of hemicellulose as monomeric sugars at 34.5 MPa and 200-230^{o}C over a span of only a few minutes.

Xylose can exist in water as a pyranose ring, a furanose ring, or an open-chain structure. Most of furfural is produced from hemicellulose-derived xylose. Antal et al. (1991) explored how furfural is formed directly from xylose. However, furfural also degrades under hydrothermal conditions (Jing and Lv, 2007). Aida et al. (2010) studied the reaction kinetics and pathways of D-xylose in sub-and supercritical water and based on their results and also the results from (Antal et al., 1991; Luijkx et al., 1994; Oefner et al., 1992), they proposed a reaction pathway scheme (Figure 2-2). At temperatures of 360-420^{o}C, Sasaki et al. (2003) reported that quantity of fragmentation products dominated the measured quantity of furfural after reactions. On the other hand, aromatic compounds may be formed through hydrothermal liquefaction of xylose, as observed by Nelson et al. (1988). The above compounds generated in hydrothermal liquefaction, could probably react with other intermediates results from hydrolysis of other biomass components, forming more complex chemicals.



Note: LBET: Lobry de Bruyn-Alberda van Ekenstein Transformation
RA: Retro Aldol
DH: Dehydration
BR: Benzilic acid rearrangement

Figure 2-2. Reaction pathway of D-xylose during HTL, adapted from Aida et al. (2010).

Lipids

Lipids are a broad group of many categories of molecules, including fats, oils, waxes, sterols and phospholipids. The reactions of lipids and water are strongly influenced by their phase behavior. The increase in temperature causes fats and oils to become increasingly soluble in water as its temperature rises under hydrothermal conditions. Mills and Microcrystalline celluloselain (1949) reported the complete miscible to occur at 293^{o}C for coconut-oil derived fatty acids and 321^{o}C for tallow-derived fatty acids. This property may indicate an increasingly important role of water during the HTL of lipids as the temperature increases to the range of higher than 300^{o}C.

Triacylglycerides (TAGs), the most common form of lipid in biological systems, can be hydrothermally split to form free fatty acids and glycerol. Hydrolysis reactions occur primarily in the oil phase, and proceed to an increasing equilibrium level with increasing water-to-oil ratio.

King et al. (1999) found that they could achieve rapid hydrolysis of fatty acids in liquid water at temperature of 330-340^{o}C, giving 90-100% yields of free fatty acids.

Free fatty acids can degrade in hydrothermal systems and produce long-chained hydrocarbons. Watanabe et al. (2006) studied stearic acid decomposition in a batch reactor. It was found that more alkene was made than alkane in the hydrothermal experiments, and production of hydrocarbons with fewer than 16 carbons was suppressed. However, when NaOH or KOH was added, decomposition of stearic acid increased significantly and alkane again became dominant product.

He et al. (2001) studied the element transfer, characteristic conversion temperature and conversion reaction mechanism of the thermochemical conversion (TCC) process for sewage sludge and concluded that fatty acid and lipid are the main reactants of the thermochemical conversion reaction. The predominant thermochemical conversion reaction below 300^{o}C is considered to be distillation of aliphatic compounds. Suzuki et al. (1988) also found a nearly linear relationship between crude fat content and amount of oil fraction in the starting materials.

Lignin

Lignin is a complex high molecular-weight compound with a random structure. The typical monomers in lignin are aromatic subunits.

Funazukuri et al. (1990) studied the hydrothermal liquefaction of lignin sulphonate with both subcritical and supercritical water. An oil yield of 25% was obtained at 623K and 673K. At 673K, the oil decomposed rapidly as the retention time increased, from 25% at 3 min to below 10% at 10min. A higher water density was found to be favorable for the formation of oil products. ^{1}H-NMR spectra showed that oil obtained at short reaction times had relatively high methoxyl hydrogen contents.

A high correlation between lignin and residue was observed by both Minowa et al. (1998) and Demirbas (2000b), when real biomass containing lignin was liquefied to produce biocrude oil.

2.4. HTL OF LIVESTOCK MANURE

Livestock manure, as a representative bio-waste has been studied as feedstock in the hydrothermal liquefaction. It has been proven that hydrothermal treatment of swine manure not only produces bio-crude oil but can be an efficient way to reduce COD and pollutants in the manure (He et al., 2000). Previous research on hydrothermal treatment of swine manure investigated the effects of feedstock pH, total solids content, initial CO process gas addition, operating temperature, retention time and alternative process gases. The raw oil product was characterized by analyzing the elemental contents, benzene solubility, viscosity and heating values; COD reduction efficiency was also studied (He, 2000). A continuous reactor system was also developed and tested, demonstrating raw oil yields ranging from 62.0%-70.4% (Ocfemia et al., 2006).

Dong et al. (2009) studied the transition of the feedstock from slurry to separated product streams including bio-oil, aqueous phase, solid residues and gaseous products. At shorter reaction times, the raw oil product appears to consist of numerous small spherical particles of a homogeneous size. As temperature and time increase, the sphere particles disappear and become a tar-like fluid with an estimated pour point higher than 80°C. Under scanning electron microscopy (SEM), similar spherical structures were also observed as a poplar stick was exposed to about 290°C steam/water for an extremely short time in a tube reactor without agitation (Boocock and Kosiak, 1988). The spherical particles are believed to indicate primary oil formation resulting from partial depolymerization of the biopolymers even while well defined biomass structures are still present (Boocock and Kosiak, 1988).

Dong et al. (2009) also developed and modified the experimental and analytical method for quantifying the product yields from the HTL batch experiments, collected a series of kinetic data from hydrothermal conversion of swine manure. He also proposed a hypothesis to explain the mechanism of the oil formation based on the experimental observation/facts. It was found that except for oil all products derived from swine manure were significantly influenced by the reaction temperature, time, and pressure. It was believed that the formation of oil was directly related to the original compositions of swine manure rather than the intermediates such as sugars

derived from decomposition of swine manure under the hydrothermal conditions. The experimental results imply that the lipid component is favorable for oil formation in hydrothermal conversion of swine manure.

Previous studies provide some important insight on bio-oil production through hydrothermal conversion of swine manure. The swine manure was in its "fresh" state as the feedstock since fresh manure is more consistent in properties. In reality, properties of swine manure may vary with the management as well as manure storage time and some other factors. It is difficult to obtain fresh swine manure as the only feedstock source for commercial HTL plants. The mechanism of bio-oil formation from swine manure was still not well understood due to the limited knowledge regarding the hydrothermal reactions. Therefore, it is necessary to further explore the fundamental mechanisms during hydrothermal liquefaction-- both for swine manure and for other biomass. Studies on different feedstocks- both real biomass and model compounds– will be important to further understand the mechanisms of HTL and for future HTL reactor design.

CHAPTER 3. EXPERIMENTAL DESIGN AND PROCEDURES

3.1. HTL TESTS OF SWINE MANURE

The purpose of this part of research is to study the effects of feedstock variation on bio-crude oil production from HTL of swine manure, to discover the bio-crude oil formation process through transient temperature range, and to explore the pathways of different components in the swine manure during the HTL process.

3.1.1. Effects of variation of swine manure feedstock on bio-crude production

Swine manure samples collected from nursery pigs, grower-finishers and sows were used as feedstock to test the effects of growing stages on the bio-crude production. Comparison of the fresh and different-aged pit manure from grower-finisher pigs was performed to study the effects of swine manure storage time on bio-crude production. Fresh swine manure was collected from the partially slotted floor, while the pit manure was collected from a shallow pit during a 39-day span at the Grein's Farm, University of Illinois at Urbana-Champaign. During the 39-day period, the manure, urine and modest spillage from the waterers accumulated continuously in the pit without any treatments. Typical solid content for the pit manure was 5-10% which was relatively low for HTL tests. So after collection, pit manure samples were air-dried for 5-7 days until its solid content reached 20% or higher. All the manure samples were stored in a refrigerator below 4 ^{o}C before being used in the HTL tests.

HTL tests at the same reaction conditions were conducted for the fresh swine manure from different growing stages. For each test, 3 replicates were run, and a statistical analysis was performed to examine the differences between these manure samples. For the pit manure tests, the same conditions were used, and duplicates were conducted for selected conditions.

In this study, unless otherwise specified, the solid content of the feedstock was 20%, the operating temperature was 305^{o}C, the retention time in the reactor at temperature was 30 minutes, the N_2 initial pressure was 0.65 MPa, and no catalyst was added.

Properties of feedstock and the products were analyzed, including component characterization of feedstocks, yields of products, raw oil quality (toluene solubility of raw oil products), GC

analysis for the gaseous products and the refined oil products, etc. GC-MS analysis for selected samples was also perfumed. Details of analysis methods could be found in section 3.4.

3.1.2. HTL of swine manure in transient temperature range

Previously, a series of tests have been done by (Dong et al., 2009) to investigate the product distribution of hydrothermal conversion of swine manure at 240-280^{o}C and they concluded that the significant amount of oil produced at lower temperatures was probably related to a decarboxylation reaction involving some components present in the swine manure (e.g. the fat an protein). However, the transition period of the oil formation was not observed in this temperature range so the kinetic analysis became impossible from the data obtained. They suggested that to evaluate the hydrothermal process, it would be necessary to collect more data at reaction temperatures below 240^{o}C if a similar batch reactor system is used. Therefore, it is necessary to conduct tests at lower temperature to discover the complete formation process of bio-crude oil.

A series of HTL tests were carried out at 180, 200, 220 and 240 ^{o}C at 0, 15, 30, 45 and 60 min. Three replicated tests were run at 200 ^{o}C, 15min to verify the repeatability of the tests. No replicates were run at other conditions since the repeatability obtained was satisfactory enough. Operating parameters other than temperature and retention time were the same as those described in section 3.1.1. Product distributions and oil product were analyzed and efforts were made to explain the observed phenomena and their implications on the mechanisms.

3.2. HTL TESTS OF MODEL COMPOUNDS

This part of research work conducted studies on understanding the roles of specific key components in actual biomass during the hydrothermal liquefaction, which remain unclear despite of having been studied by researchers during past decades (Appell et al., 1980; Balat, 2008; Chornet and Overend, 1985; Demirbas, 2000a; Elliott et al., 1987; Minowa et al., 1998; Yuan et al., 2009). Most previous research work on hydrothermal liquefaction focuses on lignocellulosic materials and carbohydrates (Catallo et al., 2010; Kumar and Gupta, 2008; Sasaki et al., 1998). Transformations of lipids are relatively straightforward, -- fats decompose to free fatty acids and then to alkanes and alkenes and other smaller molecules. However, relatively little research has been conducted on the performance and pathways of proteins and their

interactions with other chemicals including lipids under hydrothermal processing conditions, especially in sub-critical water. Proteins are recognized to be an important factor affecting the bio-crude formation from biomass, especially non-cellulosic biomass with high protein content (Kruse et al., 2005; Matsui et al., 1997). In this study, more efforts were put on studying how the temperature influences the protein conversion and its pathways. On the other hand, the conversions of cellulose, lipid, glucose and xylose were also examined with or without catalysts.

To investigate the functionality and pathways of key components in HTL, egg albumin, microcrystalline cellulose, xylose, D-glucose and butter was used as model compounds, representing the corresponding component categories (protein, cellulose, hemicellulose, monosaccharide and lipid) in real biomass such as swine manure and algae. HTL tests were performed for each of these model compounds and also for some combinations between these model compounds.

Product analysis included product distribution analysis, toluene solubility analysis, elemental distribution analysis (C, H, N and O), heating value measurement, and GC-MS analysis of selected refined oil products.

3.2.1. HTL of Single-model-compounds

As mentioned above, egg albumin, microcrystalline cellulose, xylose, D-glucose and butter were chosen as model compounds representing protein, cellulose, hemicellulose, monosaccharide and lipid. More HTL tests were run using egg albumin then others since it was the main focus of this study, which could be seen in Table 3-1.

Table 3-1. Tests to investigate performances of single-model-compounds in HTL.

Test No.	feedstock	Temp (^oC)	Catalyst	Notes
1	Protein	300	None	
2	Protein	300	None	Replicate of test #1
3	Protein	300	None	Replicate of test #1
4	Protein	300	Na_2CO_3(5% w/w)	
5	Protein	280	None	
6	Protein	260	None	
7	Protein	230	None	
8	Protein	200	None	
9	Cellulose	300	None	
10	Cellulose	300	None	Replicate of test #9
11	Cellulose	300	None	Replicate of test #9
12	Cellulose	300	Na_2CO_3(5% w/w)	
13	Glucose	300	None	
14	Glucose	300	None	Replicate of test # 13
15	Glucose	300	Na_2CO_3(5% w/w)	
16	Xylose	300	None	
17	Xylose	300	Na_2CO_3(5% w/w)	
18	Lipid	300	None	

-Unless otherwise specified, tests were run at a retention time of 30 min, a N_2 initial pressure of 0.65 MPa, a loading mass of 800g and a solid content of ~20%.

3.2.2. HTL of mixed model compounds

Interactions between protein/cellulose, protein/lipid, proteins/xylose, protein/glucose and lipid/cellulose were investigated. Furthermore, mixture of the five representatives was used as feedstock to simulate the real biomass (e.g. swine manure). Detailed tests are shown in table 3-2.

Table 3-2. Tests to investigate interactions between model compounds in HTL.

Test No.	Feedstock	Temp (^oC)	Catalyst	Notes
1	P:C=1:1	300	None	
2	P:C=3:1	300	None	
3	P:C=1:3	300	None	
4	P:C=1:1	300	Na_2CO_3(5% w/w)	
5	P:C=1:1	300	None	Retention time: 120min
6	P:L=1:1	300	None	
7	P:X=1:1	300	None	
8	P:G=1:1	300	None	
9	C:L=1:1	300	None	
10	P:C:L:G:X= 30:10:15:10:25	300	Na_2CO_3(10% w/w)	A simulation of real manure
11	P:C:L=50:20:20	300	Na_2CO_3(10% w/w)	A simulation of real manure

-P =Protein, C =Cellulose, G =Glucose, X =Xylose, L =Lipid

-Unless otherwise specified, tests were run at a retention time of 30 min, a N_2 initial pressure of 0.65 MPa, a loading of 800g and a solid content of ~20%.

3.3. GENERAL EXPERIMENTAL PROCEDURES

Usually, for feedstock preparation, samples (swine manures, or model compounds) were blended with tap water (if necessary) using a commercial Warning® blender and then homogenized by a high-shear mixer.

A 2-liter cylindrical batch reactor (Model 4534, Parr Instrument Company, Moline, Illinois) was used for the HTL tests. Feedstock was continuously agitated during tests by an agitator driven by magnetic drive. In a typical test, 800 g of feedstock was loaded into the reactor. After sealing the reactor and purging the air with nitrogen gas 3 times, an initial pressure of 0.65 MPa was built up by nitrogen. Electric resistance heating was used to achieve the designated temperature. After being held at the designated temperature for a certain retention time, the reactor was cooled rapidly by a cooling coil located inside the reactor. When the temperature inside the reactor reached room temperature, the gas phase was carefully released through a control valve except that in some cases it was collected using a gas sampling bag for further analysis.

3.4. ANALYTICAL METHODS

3.4.1. Characterization of feedstock

The real biomass feedstock samples (majorly swine manure) were sent to the Midwest Laboratories, Inc. (Omaha, Nebraska) for basic analysis using the methods of the Association of Official Analytical Chemists (AOAC). The analysis package is in the “feeds” section on the website of Midwest Laboratories and is listed as package F9. Additionally to the package F9, the analysis of NDF (neutral detergent fiber) and lignin were performed. A typical analysis result is shown in Table 3-3.

Table 3-3. A typical feedstock analysis result from Midwest laboratories.

Component	As Sent	Of Dry Wt.
Moisture (vacuum oven) 70c (%)	79.15	
Dry Matter (%)	20.85	
Crude Protein (%)	5.61	26.9
Acid Hydrolysis Fat (%)	3.91	18.8
Acid Detergent Fiber (%)	1.48	7.08
Ash (%)	2.38	11.4
Neutral Detergent Fiber (%)	7.29	35.0
Total digestible nutrients (%)	19.5	93.6
Net energy-lactation (Mcal/lb)	0.21	0.99
Net energy-maint. (Mcal/lb)	0.22	1.04
Net energy-gain (Mcal/lb)	0.14	0.68
Digestible energy (Mcal/lb)	0.39	1.87
Metabolizable energy (Mcal/lb)	0.35	1.7
Sulfur (%)	0.07	0.32
Phosphorus (%)	0.39	1.87
Potassium (%)	0.30	1.42
Magnesium (%)	0.17	0.79
Calcium (%)	0.42	2.02
Sodium (%)	0.05	0.25
Iron (ppm)	322	1544
Manganese (ppm)	51	245
Copper (ppm)	19	91
Zinc (ppm)	152	729
Lignin (%)	1.11	5.32

Total solid content (TS) - measured by heating at 105^{o}C for 24 hours in a Mechanical Convection Oven (DKN 400, Yamato Co.).

Volatile solid content (VS) - measured by burning the feedstock in a furnace (Barnstead Thermolyne Co.) at 600^{o}C for over 3 hours till the weight was stable.

3.4.2. Elemental analysis

Elemental analysis in this study was carried out by the Microanalysis Laboratory, University of Illinois at Urbana-Champaign. A CHN analyzer (Model CE440, Exeter Analytical, Inc. N. Chelmsford, MA) was used to measure the carbon, hydrogen and nitrogen contents. The oxygen content was therefore calculated by difference. The results were reported in percentages by weight.

3.4.3. Product distribution and the analysis of raw oil

Typically four streams of products were produced when oily product was generated in a test: raw oil product, aqueous phase product, gas product and visible solid residue. This kind of tests was

named type I tests. Sometimes no oily product was generated during the tests. In these cases, three streams of product were obtained: raw solid product, aqueous phase product and gas product. This kind of tests was named type II tests. Slightly different collection and separation methods were used for different types of tests.

In type I tests, after releasing the gas product, what remained in the reactor was called reaction mixture. The raw oil product was scooped off the top of the aqueous phase and also scraped from the wall of the reactor and the blades of the mixer. Then the aqueous phase product, which also contained some visible solid residue, was poured into a bottle. After removing all the products from the reactor as much as possible, the leftover in the reactor was regarded as raw oil. Visible solid residue was obtained by filtration of the aqueous phase using a Whatman filter paper. Moisture content of the raw oil product was determined by distillation, based on ASTM Standard D95-99 (ASTM, 2004a). Sediment content of the raw oil product is measured by using Soxhlet extraction, according to ASTM Standards D473-02 (ASTM, 2004b) and D4072-98 (ASTM, 2004c). Note that the sum of the sediment in raw oil and the visible solid residue was defined as the "total solid residue."

Product yields in type I tests are defined in the following manner:

The percentage of raw oil yield:

$$\text{Raw oil yield}(\%)=\frac{\text{weight of raw oil product}}{\text{weight of dry feedstock}}\times 100$$

The percentage of gas yield:

$$\text{Gas yield}(\%)=\frac{\text{weight of (dry feedstock-reaction mixture)}}{\text{weight of dry feedstock}}\times 100$$

The toluene solubility of raw oil yield:

$$\text{Toluene Solu bility}(\%)=\frac{\text{weight of raw oil-sediment in raw oil}}{\text{weight of (raw oil-moisture)}}\times 100$$

The percentage refined oil yield:

$$\text{Refined oil yield}(\%)=\frac{\text{weight of (raw oil-moisture-sediment)}}{\text{weight of dry feedstock}}\times 100$$

The percentage solid residue yield:

$$\text{Solid residue yield}(\%)=\frac{\text{weight of (filtration solid}+\text{sediment in raw oil)}}{\text{weight of dry feedstock}}\times 100$$

The percentage aqueous product yield:

$$\text{Aqueous product yield}(\%)=\frac{\text{weight of (total feedstock - gas - solid residue - refined oil - water in feedstock)}}{\text{weight of dry feedstock}}\times 100$$

In type II tests, three streams of product were produced: raw solid product, aqueous phase product and gas product. The reaction mixture of raw solid and aqueous product was removed from the reactor and separated by filtration using a Whatman filter paper. It is necessary to mention that the raw solid product contained moisture and possibly some organic compounds adsorbed on the surface of the solid product. After removing the reaction mixture from the reactor, any remaining products in the reactor were regarded as raw solid. Moisture content of the raw solid product was determined by heated at 105ºC for 24 hours in a Mechanical Convection Oven (DKN 400, Yamato Co.). Toluene solubility of the raw solid product was measured by using Soxhlet extraction and was regarded as solid residue, according to ASTM Standards D473-02 and D4072-98.

Yields of products are defined in the following manner:

The percentage gas yield:

$$\text{Gas yield}(\%)=\frac{\text{weight of (total feedstock - reaction mixture)}}{\text{weight of dry feedstock}}\times 100$$

The percentage solid residue yield:

$$\text{Solid residue yield}(\%)=\frac{\text{weight of toluene insoluble fraction in the raw solid}}{\text{weight of dry feedstock}}\times 100$$

The percentage oil yield:

$$\text{Oil yield}(\%)=\frac{\text{weight of (raw solid - moisture- solid residue)}}{\text{weight of dry feedstock}}\times 100$$

The percentage aqueous product yield:

$$\text{Aqueous product yield}(\%)=100 - \text{gas yield} - \text{solid residue} - \text{refined oil yield}$$

3.4.4. Gas Chromatography (GC) Analysis

Some of the gaseous and refined oil product samples were analyzed by a Varian CP-3600 gas chromatograph (GC). For gaseous product analysis, the GC was coupled with a thermal conductivity detector (TCD) to determine the amount of several important gas components (methane, carbon dioxide, hydrogen, nitrogen, oxygen, and carbon monoxide). The GC analysis

used a Haysep D 100/120 column (20-ft, 1/8-in diameter), with an injection temperature of 120 °C and a filament temperature of 140 °C. The carrier gas was Helium at 30 mL/min.

For the refined oil product analysis, a capillary column was used. The capillary column (Alltech Associates, Inc., IL) has AT^{TM}-5 as stationary phase with film thickness of 1.00 µm, a length of 30 m and an inner diameter of 0.32 mm. Helium was used as the carrying gas at a constant flow rate of 1 ml/min. Column temperature was programmed from 30^{o}C to 340^{o}C, at a rate of increase of 5^{o}C/min. The holding times of initial and final temperature were 2 min and 6 min, respectively. Total time required for a test was thus 70 min. The flame ionization detector (FID) was used, with a temperature setting of 350^{o}C.

3.4.5. GC-MS Analysis

The toluene soluble fraction of selected oil samples was identified using GC-MS. Samples (3µL) were injected in split mode (10:1) to the GC/MS system consisted of an Agilent 6890N gas chromatograph, an Agilent 5973 mass selective detector and HP 7683B (Agilent Inc, Palo Alto, CA, USA) autosampler. Injections were performed on a 30 m ZB-WAX Plus column with 0.32 mm I.D. and 0.25 µm film thickness (Phenomenex, Torrance, CA, USA) with an injection port temperature of 250ºC, the interface set to 250ºC, and the ion source adjusted to 230ºC. The carrier gas (Helium) was set at a constant flow rate of 3 ml min^{-1}. The temperature program was 5 min isothermal heating at 65ºC, followed by an oven temperature increase of 5ºC min^{-1} to 265ºC for final 2 min. The mass spectrometer was operated in positive electron impact mode (EI) at 69.9 eV ionization energy in m/z 30-800 scan range. The spectra of all chromatogram peaks were evaluated using the HP Chemstation (Agilent, Palo Alto, CA, USA) and AMDIS (NIST, Gaithersburg, MD, USA) programs. The spectra of all chromatogram peaks were compared with electron impact mass spectrum from NIST Mass Spectral Database (NIST08) and W8N08 library (John Wiley & Sons, Inc., USA).

Samples for metabolite profiling were dried and derivatized according to (Roessner et al., 2000) with minor modifications: 90 min at 50^{o}C with 80 µl of methoxyamine hydrochloride in pyridine (20 mg/ml) with following 60 min treatment at 50^{o}C with 80 µl MSTFA. A 10 µl of an internal standard (C31 fatty acid) was added prior to trimethylsilylation. Sample volume of 1 µL was

injected with a split ratio of 1:5. The GC-MS system consisted of an Agilent 7890A (Agilent Inc, Palo Alto, CA, USA) gas chromatograph, an Agilent 5975C mass selective detector and Agilent 7683B autosampler. Gas chromatography was performed on a 60 m HP-5MS column with 0.25 mm inner diameter and 0.25 µm film thickness (Agilent Inc, Palo Alto, CA, USA) with an injection temperature of 250^{o}C, the interface set to 250^{o}C, and the ion source adjusted to 230^{o}C. The helium carrier gas was set at a constant flow rate of 1.5 ml/min. The temperature program was 5-min isothermal heating at 70^{o}C, followed by an oven temperature increase of 5^{o}C/min to 310^{o}C and a final 20 min at 310^{o}C. Mass spectra were recorded in the m/z 50-800 scanning range at 69.9 eV ionization energy.

Volatile metabolites were analyzed as follow: 1µl sample was injected in split mode (1:10) to the GC/MS system consisted of an Agilent 6890N gas chromatograph, an Agilent 5973 mass selective detector and HP 7683B (Agilent Inc, Palo Alto, CA, USA) autosampler. Injections were performed on a 30 m ZB-WAX column with 0.32 mm I.D. and 0.25 µm film thickness (Phenomenex, Torrance, CA, USA) with an injection port temperature of 250^{o}C, the interface set to 250^{o}C, and the ion source adjusted to 230^{o}C. The helium carrier gas was set at a constant flow rate of 3 ml/min. The temperature program was 5-min isothermal heating at 65^{o}C, followed by an oven temperature increase of 5^{o}C/min to 265^{o}C for 2 min. The mass spectrometer was operated in positive electron impact mode (EI) at 69.9 eV ionization energy in m/z 50-550 scan range.

The spectra of all chromatogram peaks were compared with electron impact mass spectrum libraries NIST08 (NIST, MD, USA), WILEY08 (Palisade Corporation, NY, USA), and the custom library. To allow comparison between samples all data were normalized to the internal standard in each chromatogram and fresh weight of each sample. The chromatograms and mass spectra were evaluated using the MSD ChemStation (Agilent, Palo Alto, CA, USA) and AMDIS (NIST, Gaithersburg, MD, USA) programs. The retention time and mass spectra were implemented within the AMDIS method formats.

3.4.6. Heating value

High heating values (HHVs) of feedstock and raw oil products were measured using a bomb calorimeter (Parr, USA). About 0.5g sample was used in a test, and about 0.2g benzoic acid was used to ignite the sample.

3.4.7. Nitrogen in the aqueous phase

The total nitrogen in the aqueous phase was analyzed by the following method: Samples are oxidized in an autoclave at 120ºC and 15 psi with an alkaline persulfate mixture. The oxidation process converts all nitrogen-containing compounds to nitrate. The nitrate is subsequently determined colorimetrically by continuous-flow autoanalysis using the hydrazine sulfate – sulfanilamide reduction method.

Ammonia nitrogen (NH_4^+-N), nitrate (NO_3^--N) and nitrite (NO_2^--N) were analyzed by a Technicon AA II Continuous-flow Autoanalyzer (TechniCon, USA), according to standard methods 4500-NH_3 G, 4500-NO_3 H. The automated procedure for the determination of ammonia (NH_3 -N) in water utilizes the Berthelot Reaction in which the formation of a green-colored compound believed to be closely related to indophenol occurs when the solution of an ammonium salt is added to sodium phenoxide followed by the addition of sodium hypochlorite (bleach). A solution of potassium sodium tartrate (Rochelle Salt) is added to the sample stream to eliminate the precipitation of the hydroxides of heavy metals which may be present. Ammonia concentration is determined colorimetrically at 630nm.

The determination of nitrate is utilized by a reaction whereby nitrate (NO_3^-) is reduced to nitrite (NO_2^-) by an alkaline solution of hydrazine sulfate containing a copper catalyst. The solution is then treated with sulfanilamide under acidic conditions to form a soluble azo dye which is measured colorimetrically at 520 nm. The final product measured represents the nitrite ion originally present plus that formed from the nitrate.

CHAPTER 4. HTL OF SWINE MANURE

In this chapter, the effects of the variation of swine manure feedstock on bio-crude oil production during HTL are reported. The results of HTL tests with fresh grower-finisher swine manure at transient temperature range (180-240^{o}C) are analyzed and discussed.

Figure 4-1 shows the temperature profiles of 3 replicated HTL tests of fresh swine manure at 200^{o}C, 15 min and 1 HTL test of fresh swine manure at 300^{o}C, 30 min. It represents the stability of the reactor and the repeatability of the same reaction conditions.

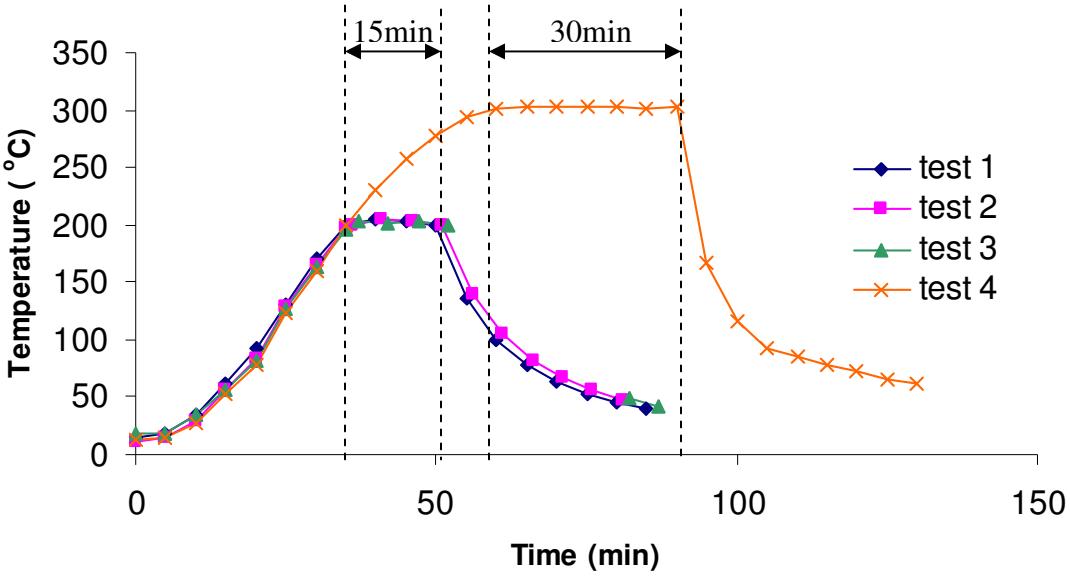


Figure 4-1. Temperature profiles of typical HTL tests: Tests 1-3, replicated tests at 200^{o}C, 15min; Test 4, 300^{o}C, 30min.

4.1. EFFECTS OF VARIATION OF SWINE MANURE FEEDSTOCKS

4.1.1. Effects of swine growing stages

The feedstock components analysis results are shown in Table 4-1.

Table 4-1. Feedstock components analysis results.

Components, dry weight	Fresh (G-F)	14 day (G-F)	21 day (G-F)	Fresh Sow	Fresh Nursery
Crude Protein (Kjeldahl) (%)	23.1	37.9	43.3	23.5	23.8
Acid Hydrolysis Fat (%)	20.3	16.7	17.9	22.3	20.7
Acid Detergent Fiber (%)	8.25	13.6	8.73	10.1	11.4
Ash (%)	15.2	18.3	18.3	26.1	14.2
Neutral Detergent Fiber (%)	32.6	35.8	35.5	31.1	31.7
Total digestible nutrients (%)	92.1	81.0	83.1	83.8	92.3

Note: G-F stands for Grower-finisher pig

In all the tests, after reactions, there was a black oil layer on top of the aqueous phase, except the tests with sow manure as feedstock, in which instead a black, adhesive char-like solid product was produced. To avoid confusion and perform the comparison, we still call this water-insoluble product “raw oil” even though it is not oily or tarry.

Figure 4-2 shows the comparison of product distribution from the HTL of the swine manure from three growing stages. There was no significant difference for the gas and aqueous product yields, while the product of sow manure did contain more solid residue and slightly lower refined oil yield. Toluene solubility results in Figure 4.2 showed that only 53% of the raw oil product could be dissolved into toluene. This toluene solubility was much lower than the 82.3% and 79.5% of raw oils generated from grower-finishers and nursery pigs, respectively. One explanation for this difference could be the higher ash content in the sow manure compared to the other two. Another possible reason for the difference is sow diet: there was more fiber in the sow feed and therefore higher fiber content in the sow manure was resulted.

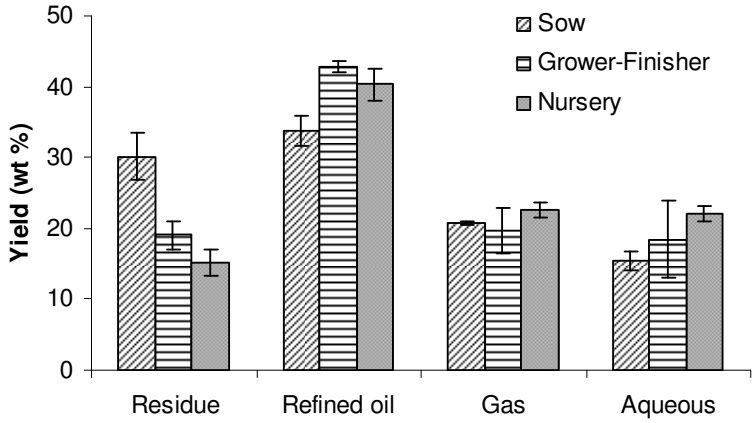


Figure 4-2. Product distribution of HTL of swine manures from different growing stages.

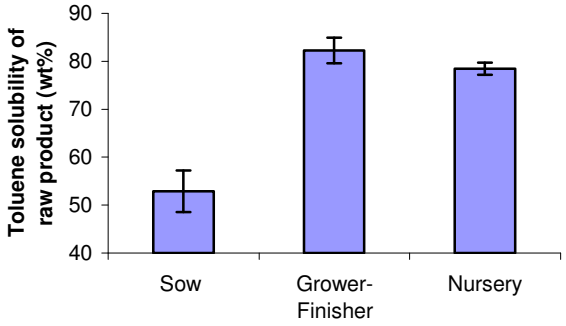


Figure 4-3. Toluene solubility of raw oil products of swine manure from different growing stages.

To remove the effect of ash content, refined oil yield was recalculated based on the volatile solid content in the feedstock (Figure 4-4). ANOVA analysis, a method of analyzing the difference between means of several sets of data, was also performed based on those data. Results show that there was no significant difference (at α=0.05) among the refined oil yield from the three feedstocks, based on the volatile solid content in the feedstock.

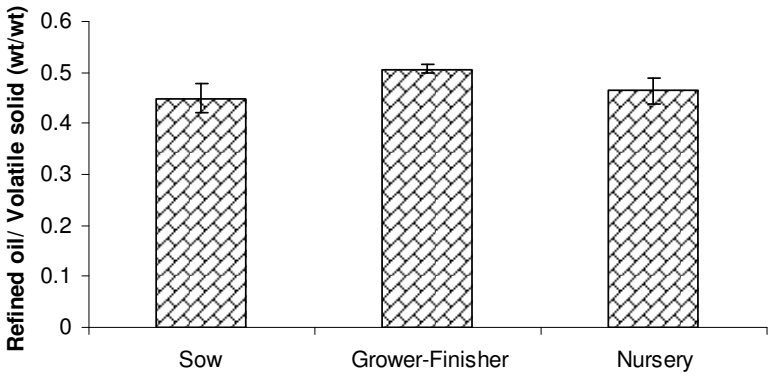


Figure 4-4. Refined oil yield based on the volatile solid content of feedstock.

GC analysis results (Figure 4-5) showed a high similarity among the spectra of all three oil samples, which means that the composition of refined oil is relatively independent of the swine manure growing stages. This result confirmed, from another perspective, the earlier conclusion.

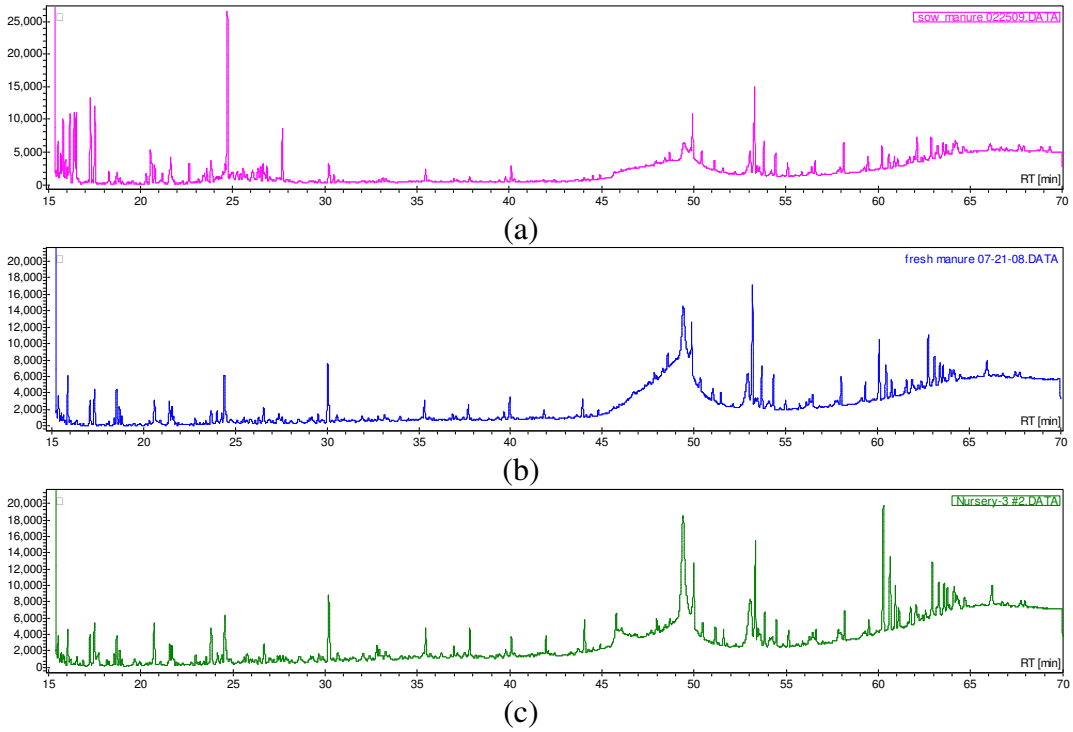


Figure 4-5. GC of refined oil products: (a) Sow (b) Grower-finisher (c) Nursery.

4.1.2. Effects of storage time in pit

In all the tests with different aged manure as feedstock, after reactions, a black oil layer was formed on top of the aqueous phase. As shown in figure 4-5, a slight decrease of refined oil yield occurred when the storage time of manure increased, but the lowest refined oil yield was still

greater than 32%. One possible reason for the lower refined oil yield could be the excessive protein content in the older manure. Dote et al. (1996) also observed oil yield as low as 10% when using egg albumin as feedstock, which contained mainly proteins. Protein is easier to decompose and more likely to be transferred into water soluble materials than into bio-crude oil. The corresponded increase between aqueous products and the crude protein content in the manure as manure age increased could substantiate this theory. On the other hand, bio-degradation and the feed spillage may also affect the composition of the feedstock, and therefore affect oil production. These possible influences were not included in this study.

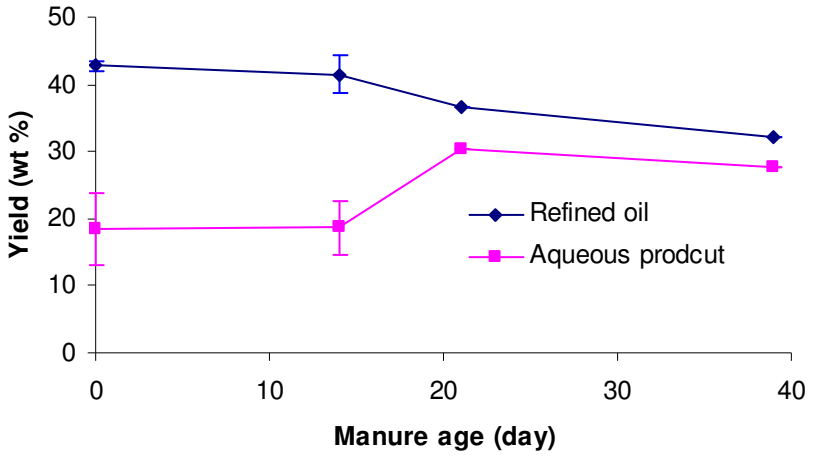


Figure 4-6. Effect of manure storage time on the refined oil and aqueous product yield.

GC analysis results of different aged manure product in Figure 4-7 look similar; it would be hard to conclude from a visual inspection of the spectra that there was significant difference among the refined oil compositions.

Usually, in a barn having a shallow pit, the pit is emptied (and sometimes flushed and recharged) monthly or more frequently. Based on interpolation of our results, at one month the refined oil yield only dropped from 43% to 35%, which is less than a 20% decrease. As long as the composition and characteristics of the refined oil do not change too much- which could be seen from the GC analysis above- storage time of 1 month or so should be acceptable, which is to say that it is not critical that the manure be “fresh”.

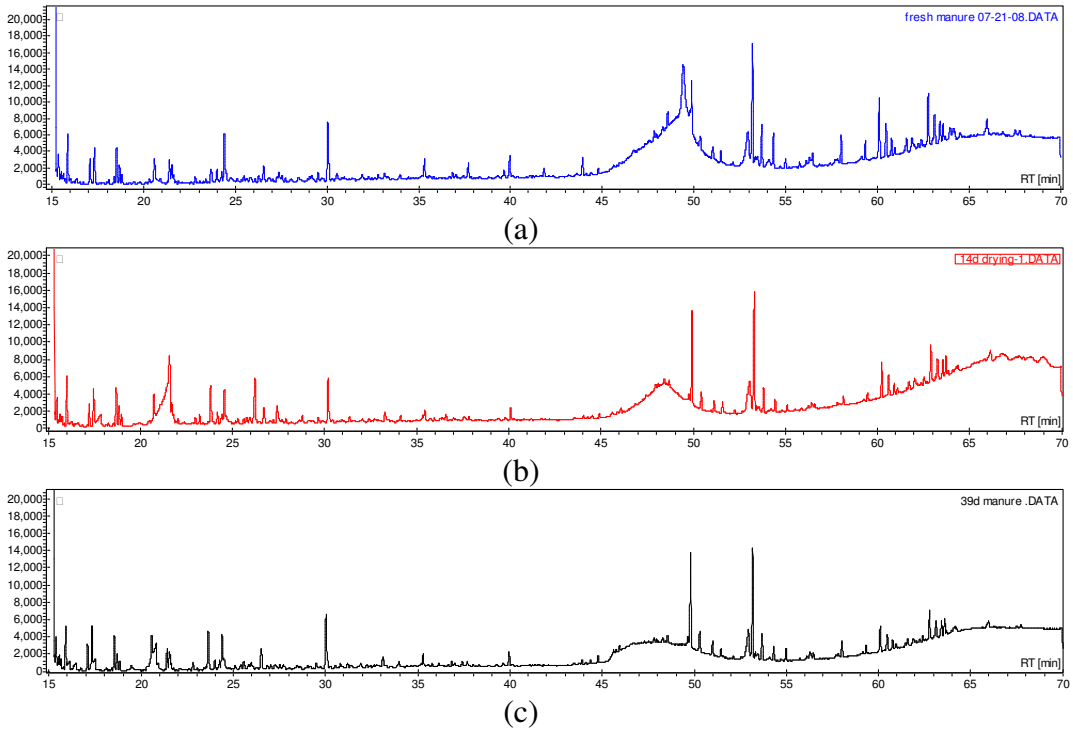
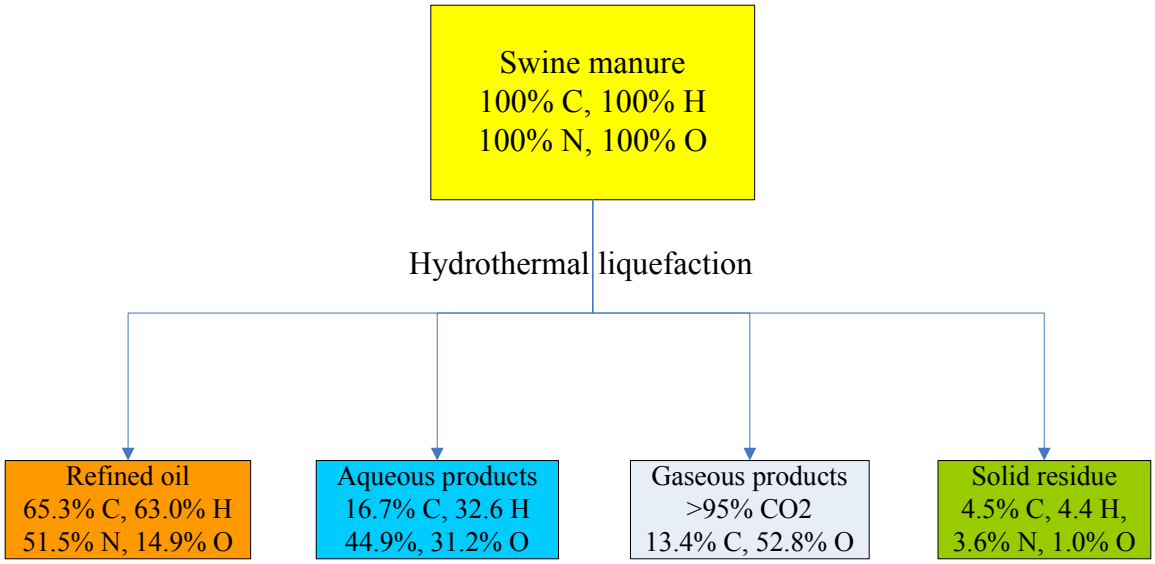


Figure 4-7. GC of refined oil product from different aged manures: (a) Fresh (b) 14 day (c) 39 day.

4.1.3. Elemental distribution of Swine manure to products

Elemental analysis provided the elemental distribution of swine manure to products (Figure 4-8). Over 60% of the carbon and hydrogen, 50% of the nitrogen and 15% of the oxygen was transferred into refined oil product. About 13% of the carbon and over 50% of the oxygen was transferred into gas phase product-carbon dioxide. There is still about 17% of the carbon, over 30% of the hydrogen, about 45% of the nitrogen and over 30% of the oxygen remaining in the aqueous phase. As of phosphorous, more than 85% wt of the phosphorous in the feedstock was transferred into the solid residue fraction.



Note: The corresponding percentage of element in the products is the fraction of the element in the feedstock

Figure 4-8. Total elemental mass balance of HTP of swine manure.

4.1.4. GC-MS analysis for the refined oil from fresh grower-finisher manure

GC-MS analysis result for the refined oil product from the test with fresh grower-finisher manure at 300^{o}C, 30min was shown in Figure 4-9. Major compounds identified which have an area of more than 1% of the total area are listed in Table 4-2. Large amount of cycloalkanes and their derivatives were found at shorter elusion time. Alkanes such as heptane, nonane, and their derivatives with side-chains were also found. These alkanes were probably the decarboxylation product of fatty acids. Fatty acids (C16:0, C18:0) and their amides were in the far-end of the elusion. Large amount of phenols and derivatives were also found.

Table 4-2. Major compounds in the refined oil from the HTL fresh grower-finisher manure.

#	Retention time	Name	Area (%)
1	3.27	Cyclohexane, 1,3-dimethyl-, cis-	5.2
2	3.44	Cyclohexane, 1,2-dimethyl-,-cis-	8.0
3	3.58	Heptane, 3-ethyl-	3.9
4	3.70	Cyclohexane, ethyl-	5.6
5	3.84	Nonane	1.4
6	4.64	Nonane, 4-methyl-	1.6
7	14.23	Acetic Acid	5.8
8	22.73	Hexanoic Acid	4.9
9	25.42	Phenyl-β-D-glucoside ??	1.5
10	25.88	Phenol, 4-ethyl-2-methoxy-	1.8
11	30.76	Phenol, 2,4-bis (1,1-dimethylethyl)	1.4
12	39.66	n-Hexadecanoic Acid	5.1
13	42.38	Octadecanoic Acid	9.4
14	43.25	Hexadecanamide	1.3
15	45.94	Octadecanamide	4.0
Total			60.9

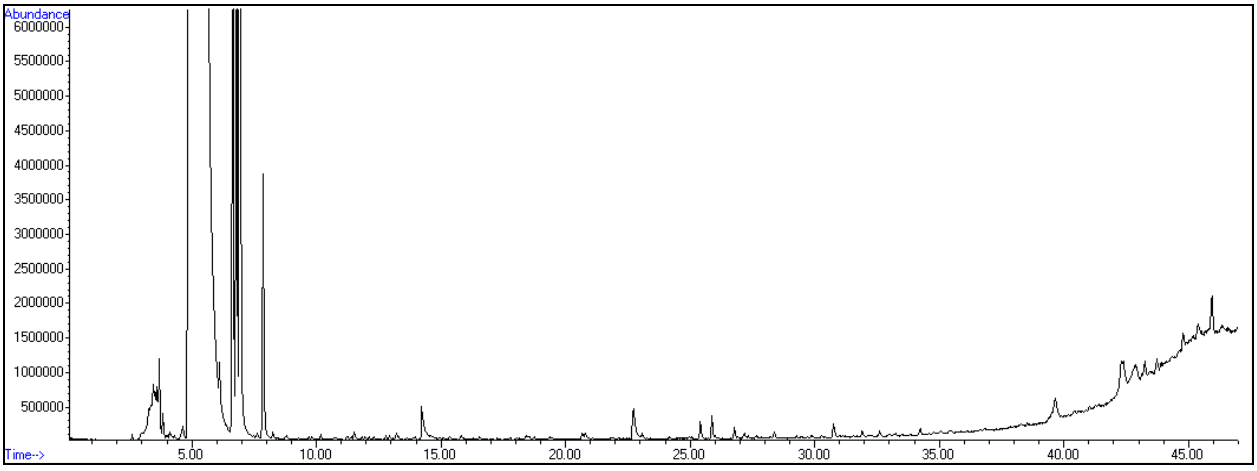


Figure 4-9. GC-MS for the refined oil product from fresh grower-finisher manure at 300°C, 30min.

4.2. HTL OF SWINE MANURE IN THE TRANSIENT TEMPERATURE RANGE

The composition of the swine manure used in this part of study is show in Table 4-3. It can be seen that the mass percentage contents of each chemical category are similar to those presented

in section 4.1, and fresh swine manure could be regarded as an ideal real biomass feedstock for HTL research.

Table 4-3. Swine manure composition used in this study.

Total Solid (wt %)	20.0
Components, dry weight (%)	
Volatile Solid	88.6
Ash	11.4
Crude Protein (Kjeldahl)	26.9
Acid Hydrolysis Fat	18.8
Acid Detergent Fiber (ADF)	7.1
Neutral Detergent Fiber (NDF)	35.0
Elemental composition (%)	
C	44.22
H	6.27
N	3.55
O[a]	45.96

a- calculated by difference, containing other elements including ash

4.2.1. Experiment results

Figure 4-10 shows the results obtained from the triplicate experiments at 200ºC, 15min retention time. Average yields and the standard deviations of solid residue, refined oil, gas and aqueous products were 38.51±0.37%, 33.18±1.20%, 8.90±0.80% and 19.41±0.65%, respectively. The standard deviations relative to corresponding product yields were small, demonstrating the consistency of the experimental results.

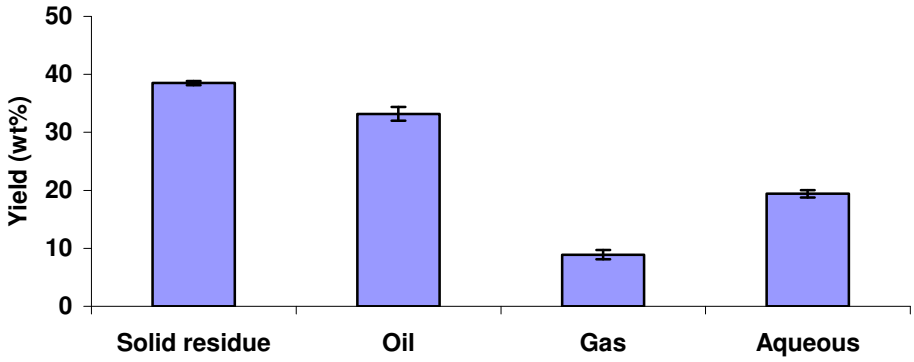


Figure 4-10. Product distribution from triplicate experiments (n=3) at 200°C, 15min retention time (error bar represents standard deviation).

In most of above experiment cases, no oil-like or tarry products were produced, unlike the results from high temperature (300^{o}C) in section 4.1. This does not mean that no oil fraction was produced according to our previous definition of oil product. At 240 ^{o}C, 60 min, a sticky tarry product was formed and automatically separated from the aqueous phase. Researchers used severity factor which combined the effects of retention time and reaction temperature to measure the treatment intensity in liquid hot water (Miyazawa et al., 2008; Overend and Chornet, 1987; Rogalinski et al., 2008a). Basically, the higher temperature and longer retention time lead to higher severity factor. In this study, 240 ^{o}C, 60 min was the harshest condition with the highest severity factor. Seemingly, at less severe condition than 240 ^{o}C and 60 min, no apparent oil product could be converted from HTL of swine manure.

At 180 ^{o}C, a brown emulsion-like mixture was obtained after reaction at all the retention time tested. It was very difficult to separate this mixture since the filter was clogged rapidly. Maillard browning reaction apparently happened during the both heating period and the holding time (retention time). Maillard reaction usually occurs at low temperatures. The swine manure used in this study has a protein content of 26.9%. And the water-soluble oligo- and monosaccharides content is calculated as about 8 wt%. In the study by Hrubrant et al. (1978) a monosaccharide content of 29.8 wt% and a glucose content of 25.5 wt% were reported. Although the manure composition may be influenced by many factors, it contains a certain amount of oligo- and monosaccharides. Therefore the Millard reaction between the monosaccharides and the hydrolyzed amino acids from protein becomes very possible. The brown color and coffee-like smell of the products observed are significant characteristics of Maillard reaction. Changing World Technologies Inc., (CWT) also reported similar phenomenon under certain conditions when they tried to hydrothermally convert and separate turkey processing waste products (Adams, T., unpublished data, from (Peterson et al., 2010)).

At 200 ^{o}C, 0 min, a similar emulsion-like mixture was obtained. However, at 200 ^{o}C, 15min, the brown color disappeared, and a mixture of char and black liquid which was fairly easy to be separated by filtration. Just by observation, some fundamental reactions could have happened between 0-15min at 200^{o}C.

The pH value of aqueous product at 180 °C decreased monotonously from 5.20 to 4.59 as the retention time increased (Figure 2). However, for all three temperatures at 200, 220 and 240 °C, another trend was observed, as the pH first decreased slightly at 15min and increased thereafter. One interesting observation needs to be mentioned is that, at 200 °C, the pH value decreased dramatically from 4.88 to 4.24 in 15 minutes and then only slowly increased to 4.32 in the following 45min. This dramatic drop of pH coincided with the disappearance of the brown emulsion-like mixture at the same stage of hydrothermal liquefaction. The decrease of pH could probably enhance the decomposition of Maillard products.

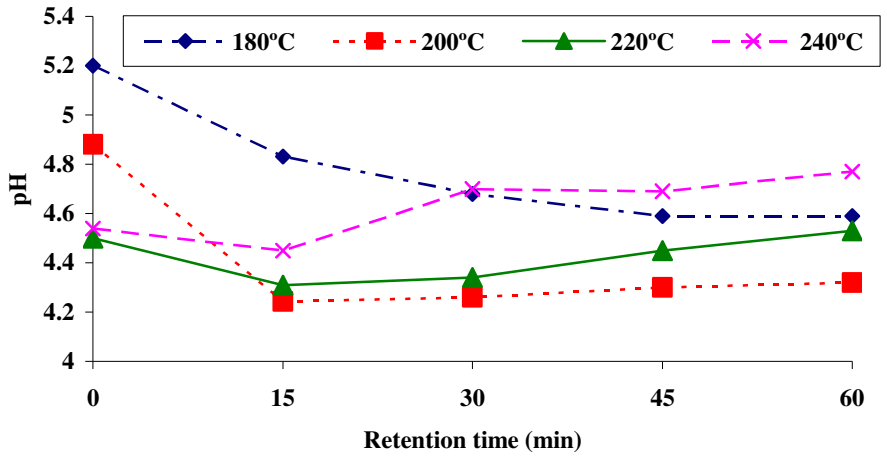


Figure 4-11. The pH of aqueous products HTL of swine manure at temperatures of 180 to 240°C and retention times of 0 to 60 min.

4.2.2. Product distribution analysis

4.2.2.1. Solid residue

Figure 4-12 shows the solid yields at different experimental conditions. At 180 °C, the yield of solid residue kept decreasing as the retention time increased. At 200 °C, from 0min to 15 min, a rapid decrease of solid residue yield occurred and remained almost constant thereafter. This rapid decrease happened at the same time period of the disappearance of emulsion-like mixture mentioned previously. At 220°C and 240°C, the solid residue yield also tended to level off when retention time was longer than 15min. Generally, higher temperature resulted in lower solid residue yield. This phenomenon indicates that at a given temperature, only certain amount of swine manure could be solublized and a barrier exists which prevents the further solublization even with prolonged retention time.

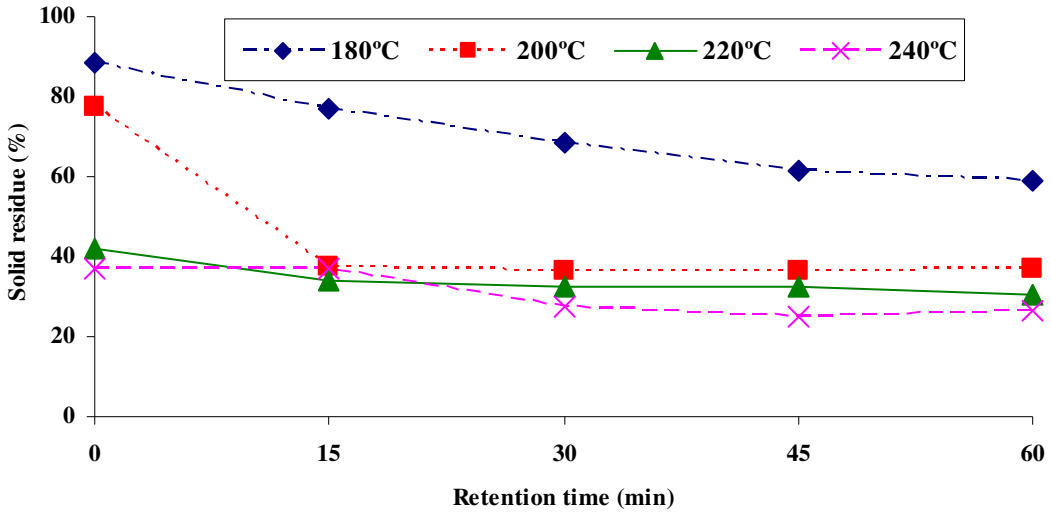


Figure 4-12. The solid yield from HTL of swine manure at temperatures of 180 to 240°C and retention times of 0 to 60min.

The remained solid residue probably included fractions of the ash and the unreacted organic matters, as well as char formed from the intermediates. Yu and Wu (2010) studied the hydrolysis of microcrystalline cellulose in the hot-compressed water and concluded that the short chain segments (C4-C13) hinged in the microcrystalline cellulose structure can be extracted or hydrolyzed at relatively low temperatures (e.g., <150°C) while the crystalline portion has very low specific reactivity at 200°C or lower. In the absence of a catalyst, hydrothermal degradation of cellulose is usually very slow at low temperatures. It was demonstrated that at temperatures lower than 240°C, the cellulose (filter paper) decomposition in hot compressed liquid water at 10 MPa was less than 10% (Mochidzuki et al., 2000). Yuan et al. (2009) investigated the thermochemical liquefaction of straw by hot compressed water and also found that the decompositions of hemicellulose and cellulose were very low at 200°C, while the raw straw structure was completely destroyed between 220 and 250°C. However, the lignocellulosic structure in swine manure may be damaged in some degree due to the digestion of swine, making it possible to decompose at lower temperatures than 220°C. Minowa et al. (2004) studied the hydrothermal reaction of glucose and glycine representing carbohydrates and proteins respectively, as model compounds of biomass at 150-350°C. They concluded that at 150°C Maillard products, melanoidins were produced and the char was formed between 150°C and

200°C from the decomposition of melonoidins. The char yield remained almost constant through 200-350°C.

In general, the decrease of solid residue yield with time at 180°C is probably due to the gradual release of fat and its derivatives, the release of oligo- and monosccharides, the gradual degradation of protein and hemicellulose, and the formation of water soluble melanoidins. It is also possible that some solid residue was formed through the decomposition of melonoidins.

The sharp decrease of solid residue at 200°C, 15min is probably due to the rapid and intensive hydrolysis of hemicellulose. Acetyl groups are known to be present as substituents on various plant polysaccharides, notably hemicelluloses and pectins (Bacon et al., 1975; Bouveng, 1961; Wood and McCrae, 1986). In swine manure, hemicellulose content is about 28% (estimated by subtracting ADF from NDF in Table 4-3). When hemicelluose is hydrolyzed, acetic acids will be formed through deacetylation and the pH value of the solution will dramatically decrease. This drop of pH value was observed as expected and shown previously in Figure 4-11.

The hydrolysis of protein is also a gradual process, instead of an instant one. (Rogalinski et al., 2008b) studied the hydrolysis kinetics of bovine serum albumin in subcritical water and found that the maximum amino acid yield is not achieved even after a residence time of 6000 s. They predicted, based on the data from 230-290 °C, that at a low temperature of 190 °C the optimum amino acid yield is reached only after 350 h. Moreover, the produced amino acids are fairly unstable and decompose to further degradation products. Sato et al. (2004) studied the decomposition of five selected amino acids in high-temperature and high-pressure water, and concluded that the general reaction network includes two pathways: deamination to produce ammonia and organic acids, and decarboxylation to produce carbonic acid and amines.

4.2.2.2. Gas product

Figure 4-13 shows the gas product yields at different experimental conditions. Both the increases of temperature and retention time caused the increase of gas product yield. Gas chromatography analysis showed that more than 95% of the gas product was CO2 in all the tests. Carbon dioxide could be formed through many pathways, including the decarboxylation of amino acids, fatty acids, decomposition of monosaccharides, decomposition of the intermediates formed during the

hydrothermal liquefaction (e.g. melanoidins), etc. Dong et al. (2009) found that the gas yields from hydrothermal liquefaction of swine manure were around 15% of the total solids and changed slightly with time at temperatures of 240°C and 260°C. The formation of CO_2 is a slow and gradual process and could reach equilibrium at certain temperature after certain reaction time.

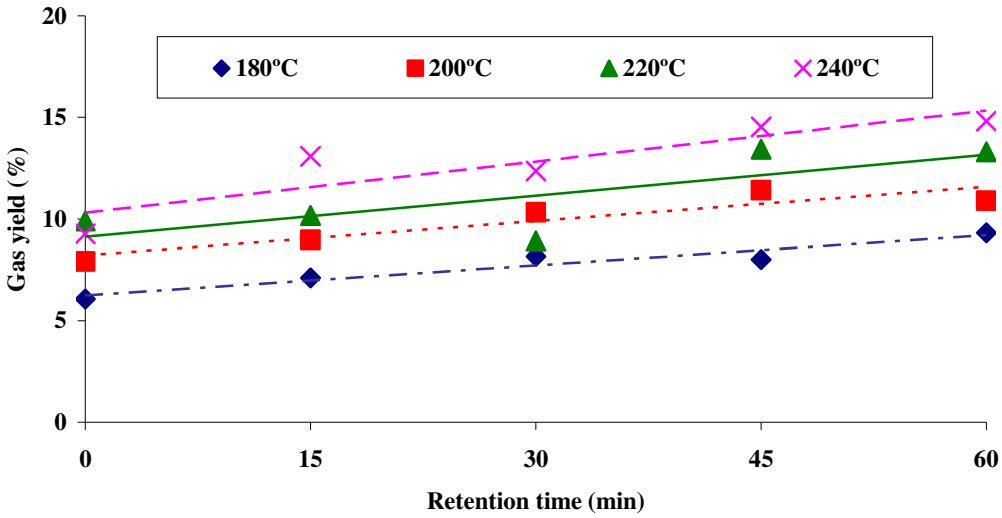


Figure 4-13. The gas yield from HTL of swine manure at temperatures of 180 to 240°C and retention time of 0 to 60 min.

4.2.2.3. Oil product

Figure 4-14 shows the oil product yield at low-temperature experimental conditions. The major components of refined oil are concluded to be fatty acids. GC-MS analysis results supported this conclusion, as large amount of fatty acids were found in the refined oil products in all the tests and most of the peak areas belong to fatty acids (see Figure 4-16 (a-d) in the GC-MS analysis section). The lipid and its derivatives were released from the feedstock gradually at 180°C. In the swine manure feedstock, acid hydrolysis fat content is 18.8%, which is very close to the maximum refined oil yield of 17.3% at 180°C. When the temperature was higher than 200°C, the lipid had already been released completely.

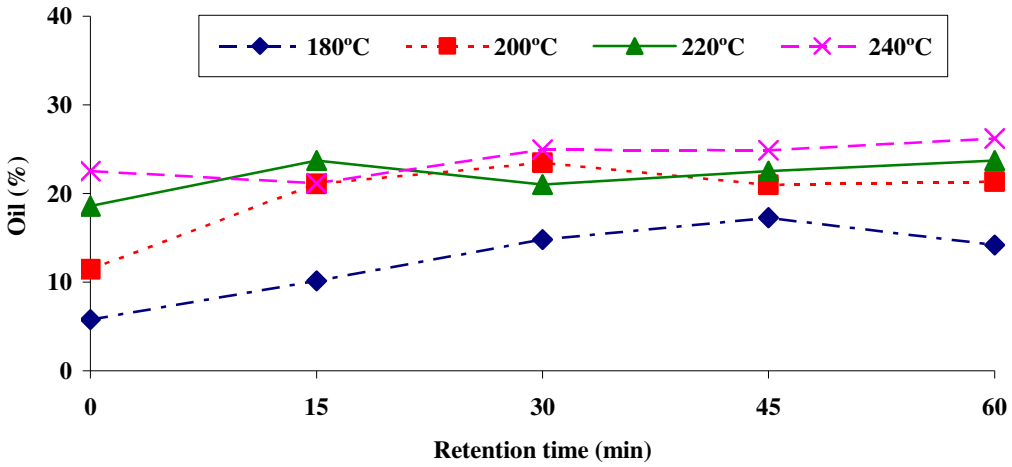


Figure 4-14. The oil yield from HTL of swine manure at temperatures of 180 to 240°C and retention times of 0 to 60min.

Since the content of acid hydrolysis fat in the feedstock is only 18.8%, while the oil yields were about 20-25%, other compounds also contributed to the oil generation at temperatures 240 °C or below. The decomposition products of melanoidins and protein are likely the other contributors to the oil product. Minowa et al. (2004) observed the generation of oil product at 200°C using the mixture of glucose and glycine as feedstock and they concluded that the oil product was generated from the decomposed products of melanoidins. When the C-C bonds in specific amino acids are broken (e.g. phenylalanine, tryptophan, tyrosine, etc.) hydrophobic compounds such as phenols, toluene, indoles and other N-heterocyclic compounds can be generated. Furthermore, the hydrolysis products from a multitude of amino acids could react with each other to form hydrophobic chemicals which resulted in the oil product.

No strong evidence shows that significant amount of oil product were generated directly from hemicellulose/cellulose at the temperatures below 240 °C. The decomposition products of hemicellulose/cellulose were mainly sugars, furans, furfurals and organic acids, etc. It was stated that with filter paper as feedstock, production of oily substances was observed only when the temperature was higher than 250 ºC (Mochidzuki et al., 2000).

4.2.2.4. Aqueous product

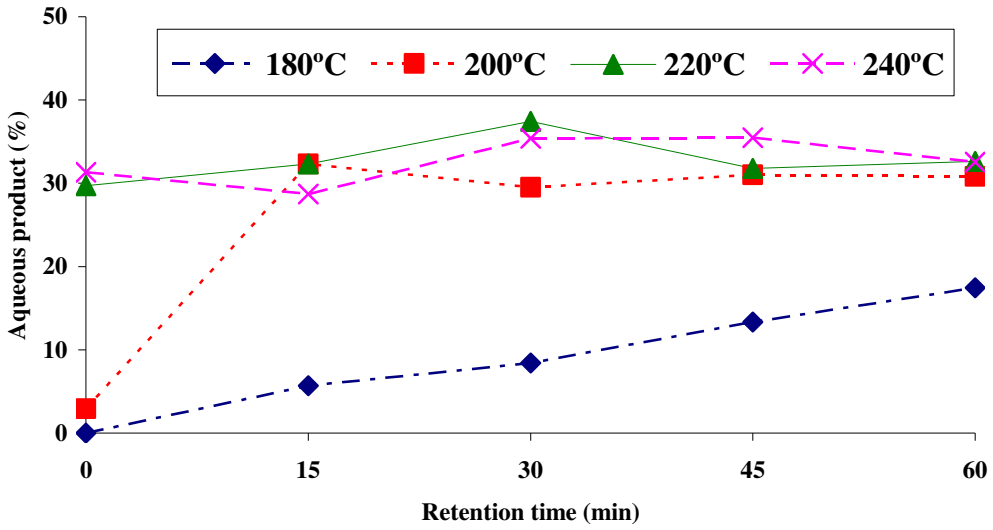


Figure 4-15. The aqueous product yield from HTL of swine manure at temperatures of 180 to 240°C and retention times of 0 to 60min.

Figure 4-15 shows the aqueous product yield at different experimental conditions. At 200°C, after 15min, and at 220°C and 240°C, the aqueous product yield was about 30-35% with slight increase with time and temperature. This again proved that under a given temperature, solubilization of feedstock to aqueous phase could only reach a certain percentage.

The nitrogen distribution of selected aqueous samples was analyzed (Table 4-4). Since the nitrogen in feedstock mainly, if not solely, came from protein, the existence of organic nitrogen and the increase of the total nitrogen (TN) concentration of the aqueous phase with temperature and retention time confirmed the gradual decomposition of protein to amino acids and amino acids to further degradation products. Particularly, ammonia was generated through the deamination of the amino acids. Retention time plays a significant role in the generation of ammonia from amino acids. When we compare the results of 200°C, 60min and 240°C, 0min, we can see that although the TN was almost identical, the NH_4^+-N at 200°C, 60 min was much higher than that at 240°C, 0min. This result indicates that the generation of ammonia from amino acid is not an instant reaction.

Table 4-4. Nitrogen distribution in aqueous products from selected reaction conditions.

Reaction condition		Nitrogen concentration (mg/L)			
Temperature (^{o}C)	Retention time (min)	TN[a]	NH_4^+-N	NO_3^--N+NO_2^--N	Org-N[b]
200	60	5735	1982	<MDL[c]	3753
220	60	6505	2162	<MDL	4343
240	60	7213	2068	<MDL	5145
240	0	5706	1170	2	4534

a- total Kjeldahl nitrogen
b- organic nitrogen, calculated by Org-N=TN- (NH_4^+-N)- (NO_3^--N+NO_2^--N)
c- MDL, minimum detection limit

An interesting observation about the product distribution (Figures 4-12 to Figure 4-15) is the similarity between 200^{o}C, 0min and 180^{o}C, 15min, 220^{o}C, 0min and 200^{o}C, 15min, 240^{o}C, 0min and 220^{o}C, 15min, respectively. To summarize, similar product distribution could be obtained at higher temperature and shorter retention time, or vise versa. It is difficult to calculate the severity factor in this study due to the heating period from room temperature to the designed reacting temperature. However, this similarity indicates that the liquefaction process could be controlled by adjusting the reaction temperature and retention time which is very important for large-scale production of bio-crude oil.

4.2.3. GC-MS analysis

4.2.3.1. GC-MS of oil products

Figure 4-16 (a-d) shows the GC-MS analysis results of oil obtained from tests at 180-240^{o}C. In each of the figures, a comparison of two spectra, one from 0 min and the other from 60min, are shown. A trend of more complex product system at higher temperature was found as more peaks were shown. However, the peaks with larger areas were almost identical in all the spectra. Table 4-5 summarizes the major identified compounds at 180 ^{o}C, 0min. Since the raw solid product was directly dissolved into toluene, some of the water-soluble organic matters adsorbed on the solid product could also been detected. However, this should not fundamentally influence the detection of oil product.

At 220 and 240^{o}C, octadecanamide, a combination product of octadecanoic acid and ammonia, was detected. This means at higher temperature, fatty acids could react with ammonia formed from amino acids, introducing more nitrogen into the oil product.

No quantitative analysis has been conducted in this study. It is needed for further quantifying the reactions between compounds in a complicated system. If this goal is achieved, product distribution and quality could be predicted using the mechanism and kinetics models.

Table 4-5. Major compounds in oil product of 180 ^{o}C, 0min identified by GC-MS.

#	Retention time (min)	Name
1	9.89	Pyrazine, methyl-
2	14.27	Acetic acid
3	15.83	Benzaldehyde
4	16.23	Propanoic acid
5	16.94	Propanoic acid, 2-methyl-
6	18.17	Butanoic acid
7	18.84	2-Furanmethanol
8	19.09	Butanoic acid, 3-methyl-
9	20.44	Pentanoic acid
10	22.58	Hexanoic acid
11	24.61	Heptanoic acid
12	24.97	1-Dodecanol
13	25.39	Phenol
14	26.55	Octanoic acid
15	26.76	Phenol, 4-methyl-
16	27.66	2-Piperidinone
17	28.35	Phenol, 4-ethyl-
18	30.15	n-Decanoic acid
19	33.22	1H-indole, 3-methyl-
20	33.50	Dodecanoic acid
21	34.31	Vanillin
22	35.34	Benzenepropanoic acid
23	36.59	Tetradecanoic acid
24	38.07	Pentadecanoic acid
25	39.50	n-Hexadecanoic acid
26	40.88	Heptadecanoic acid
27	42.27	Octadecanoic acid
28	42.63	Cis-13-octadecanoic acid
29	43.28	9,12-Octadecadienoic acid [Z,Z]-

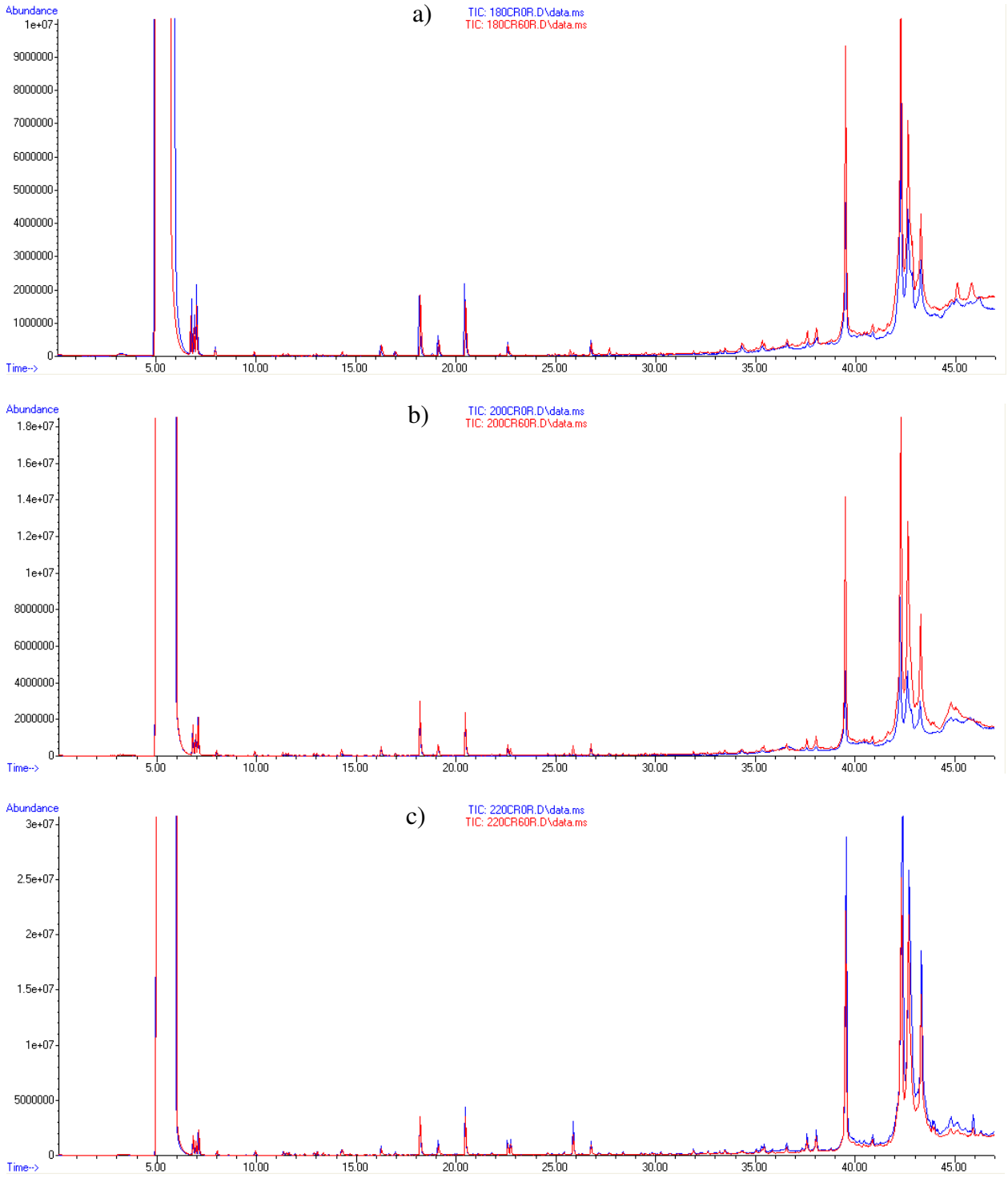


Figure 4-16. GC-MS analysis results for oil product at different conditions: a) 180^{o}C, 0 and 60min; b) 200^{o}C, 0 and 60min; c) 220^{o}C, 0and 60min.

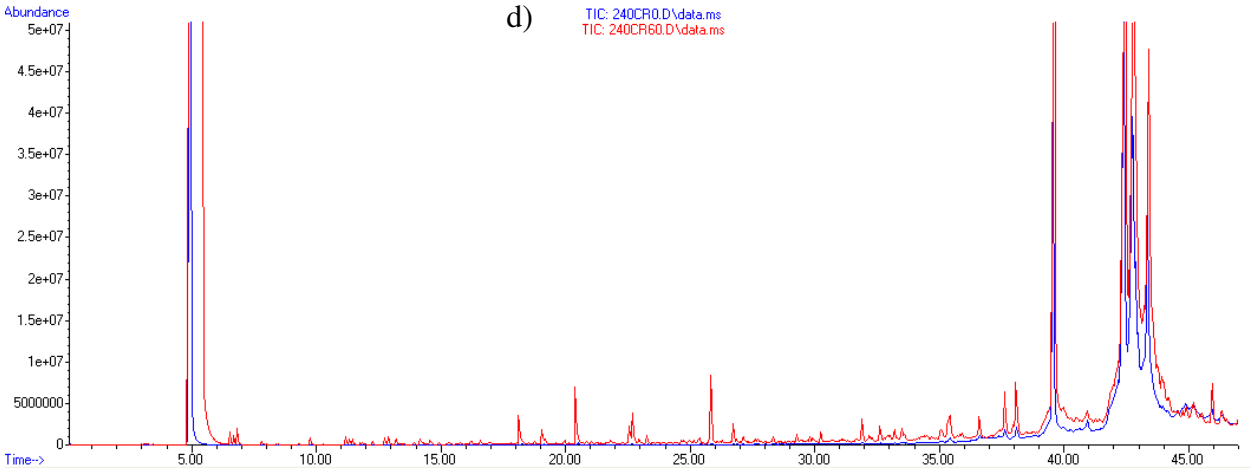


Figure 4-16 (cont.). GC-MS analysis results for oil product at different conditions: d) 240°C, 0 and 60 min.

4.2.3.2.GC-MS of aqueous products

Figure 4-17 shows the GC-MS spectra of the aqeous phase product from HTL of swine manure at 240°C, 60min. It could be seen that the compositions of the aqueous product are very complicated. The major compounds identified are listed in Table 4-6. Large amount of organic acids (C2-C6) were found in the aqueous product, especially acetic acids which accounts 14.98% of the total peak area. N-containing chemicals, such as pyridine, pyrazine, pyrrolo and their derivatives existed in the aqueous product.

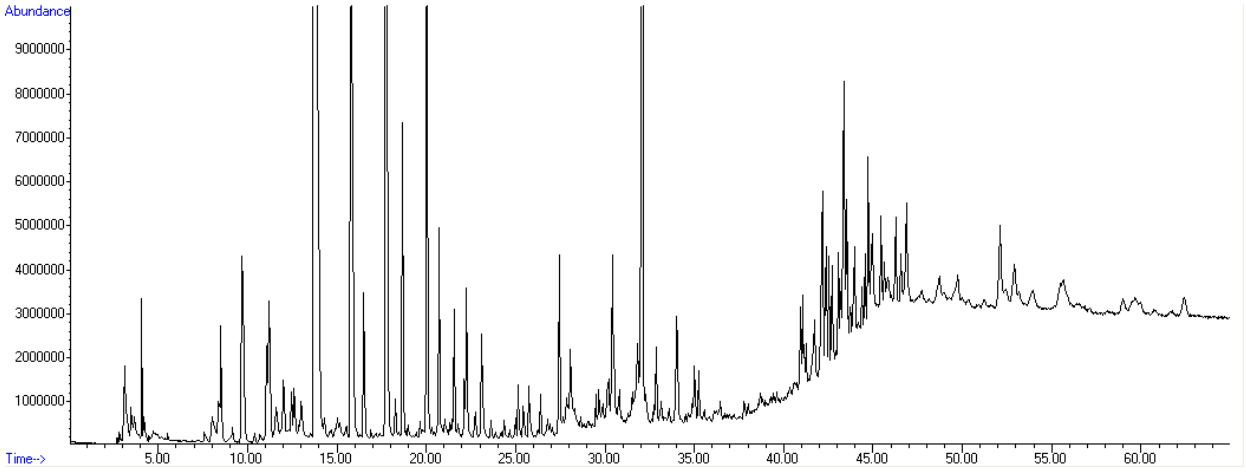


Figure 4-17. GC-MS of aqueous phase product from HTL of swine manrue at 240°C, 60 min.

Table 4-6. GC-MS of volatile compounds analysis in the aqueous phase product from HTL of swine manure at 240°C, 60min.

#	Retention time	Name	Area (%)
1	3.12	Acetone	0.74
2	3.48	2-Butanone	0.33
3	4.09	Trimethylamine	0.13
4	8.00	Pyridine	0.46
5	8.39	Pyrazine	0.42
6	8.52	Pyridine	0.58
7	9.68	Pyrazine,methyl-	2.28
8	11.06	Pyrazine, 2,5-dimethyl-	0.78
9	11.20	Pyrazine,ethyl-	1.35
10	11.60	Pyrazine, 2,3-dimethyl-	0.51
11	12.02	2-Cyclopenten-1-one, 2-methyl-	0.58
12	12.46	Pyrazine, 2-ethyl-6-methyl-	0.41
13	12.62	Pyrazine, 2-ethyl-5-methyl-	0.48
14	13.01	Pyrazine, trimethyl-	0.50
15	13.66	Acetic Acid	14.98
16	15.76	Propanoic Acid	6.17
17	16.50	Propanoic acid, 2-methyl-	0.93
18	17.75	Butanoic Acid	7.77
19	18.28	Acetamide, N-ethyl-	0.31
20	18.66	Butanoic acid, 3-methyl-	1.79
21	20.02	Pentanoic Acid	3.51
22	20.74	Acetamide	1.26
23	21.56	Propanamide	0.72
24	22.13	Hexanoic acid	0.30
25	22.26	Phenol, 2-methoxy-	0.93
26	23.10	Butanamide	0.80
27	24.99	Phenol	0.11
28	25.14	Pentanamide	0.34
29	25.43	Phenol, 4-ethyl-2-methoxy-	0.22
30	25.74	2-Pyrrolidinone	0.38
31	26.39	Phenol, 4-methyl-	0.28
32	27.44	2-Piperidinone	1.06
33	28.07	S)-2-Hydroxypropanoic acid	0.85
34	30.42	Glycerin	1.12
35	30.49	Pentanoic acid, 4-oxo-	0.69
36	31.83	3-Pyridinol, 6-methyl-	1.16
37	32.10	3-Pyridinol	5.41
38	32.73	2,5-Pyrrolidinedione	0.12
39	32.87	5-Hydroxymethyldihydrofuran-2-one	0.51
40	34.03	Benzeneacetic acid	1.04
41	35.02	Benzenepropanoic acid	0.43
42	35.24	1-(2,4-Dihydroxy-3-methylphenyl)ethanone	0.34
43	40.95	Butanoic acid, 2-oxo-	0.49

Table 4-6 (cont.). GC-MS of volatile compounds analysis in the aqueous phase product from HTL of swine manure at 240°C, 60min.

#	Retention time	Name	Area (%)
44	41.06	3,6-Diisopropylpiperazin-2,5-dione	0.44
45	42.17	3-Isopropyl-6-methyl-piperazine-2,5-dione	1.58
46	42.39	Uric acid	1.09
47	42.53	3,6-Diisopropylpiperazin-2,5-dione	0.54
48	43.07	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	0.62
49	43.35	3-Isobutylhexahydropyrrolo[1,2-a]Pyrazine-1,4-dione	2.34
50	43.55	dl-Alanyl-l-leucine	0.85
51	43.96	2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-	0.80
52	44.54	3-Isobutylhexahydropyrrolo[1,2-a]Pyrazine-1,4-dione	0.32
53	44.73	3-Isobutylhexahydropyrrolo[1,2-a]Pyrazine-1,4-dione	0.84
54	44.95	Uracil	0.83
55	45.46	Cycloglycylvaline	0.49
56	45.63	2,5-Piperazinedione, 3-methyl-	0.22
57	45.82	4-Methyleneproline	0.34
58	46.27	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	0.52
59	46.56	3-Hydroxymethyl-piperazine-2,5-dione	0.31
60	46.86	Cyclo-(glycyl-l-leucyl)	0.96
61	49.75	Thymine	0.44
62	52.13	Uracil	1.02
63	52.92	l-Pyrrolid-2-one, N-carbamoyl-	0.68
64	55.65	3-Benzyl-6-isopropyl-2,5-piperazinedione	1.04
65	62.39	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	0.39
Total			80.24

From Table 4-7, the concentration of lactic acid, acetic acid and pyroglutamic acid decreased significantly as the reaction temperature increased from 240°C to 300°C. This observation explains why the pH value of the aqueous products increased with the increase of temperature. The organic acids formed during the HTL were not stable as the temperature increased, and were consumed to form other products, probably gas and oil products. The concentration of phosphoric acid, succinic acid, 2-Pyrrolindione significantly increased as the reaction temperature increased. Phosphoric acids were probably generated from the decomposition of phosphate ester which is also a kind of lipid.

Table 4-7. A comparison between the major compounds in the aqueous products from HTL of swine manure at 240°C, 60min and 300°C, 30 min .

Retention Time (min)	Name	Area (%)		Relative concentration	
		300CR30	240CR60	300CR30	240CR60
16.21	Glycolic acid	1.49	0.27	0.51	0.14
16.44	Lactic acid	2.98	12.74	1.02	6.54
16.68	Hexanoic acid	1.70	0.14	0.58	0.07
16.91	Acetic acid	8.36	8.01	2.85	4.11
18.81	3-Hydroxypyridine	6.57	6.13	2.24	3.14
19.07	2-Pyrrolidinone	6.56	0.21	2.23	0.11
21.59	Butyric acid	1.05	0.28	0.36	0.14
22.84	Glycerol	7.57	10.56	2.58	5.42
22.87	Phosphoric acid	16.58	4.36	5.64	2.24
23.83	Succinic acid	17.24	2.05	5.87	1.05
24.54	2,4-Dihydroxypyrimidine	N/A	2.13	N/A	1.09
29.33	Pyroglutamic acid	11.14	22.51	3.79	11.55
32.24	3,4,5-Trihydroxypentanoic acid	N/A	2.64	N/A	1.35
32.59	2,4,5-Trihydroxypentanoic acid	N/A	3.20	N/A	1.64
36.87	2,4,5,6-tetrahydroxy-hexenoic acid	N/A	3.26	N/A	1.67
41.45	Inositol	1.01	2.50	0.34	1.28
	Total	82.26	80.99		

Some of the findings and their speculations in section 4.2 are listed below.

1. Maillard reaction occurred at low temperatures during the hydrothermal liquefaction of swine manure, and the formed melanoidins further decomposed to gas, oil, char and aqueous products.
2. Fatty acids are the major components in the oil product obtained through hydrothermal liquefaction of swine manure at 180-240 °C. The decomposition products of melanoidins and protein may be the other contributors to the oil product. At temperatures higher than 220 °C, fatty acids may react with ammonia to form amide products.
3. The decrease of solid residue yield with time at 180°C is probably due to the gradual release of fatty acids, the release of oligo- and monosccharides, the gradual degradation of protein and hemicellulose, and the formation of water soluble melanoidins. At a given temperature, only certain amount of swine manure could be solubilized and a barrier (likely a needed critical temperature to cause chemical conversion) exists which prevents the further solubilization even with prolonged retention time.

4. At 200^{o}C, between 0-15 min, a rapid and intensive hydrolysis of hemicellulose occurred and caused the decrease of pH in the aqueous phase. The decreased pH probably enhanced the decomposition of melanoidins.
5. Similar product distribution could be obtained at higher temperature and shorter retention time, or vice versa.

CHAPTER 5. HTL OF MODEL COMPOUNDS

5.1. HTL OF SINGLE MODEL COMPOUNDS

In this section, the results of HTL of single model compounds (e.g. egg albumin, microcrystalline cellulose, glucose, xylose and butter) are reported and discussed. Since the focus of this section was put on the protein (egg albumin), more tests have been run with it. And more details are presented and discussed for the protein than for other feedstocks.

5.1.1. Protein

5.1.1.1. Repeated experiment

Figure 5-1 shows the HTL results of egg albumin obtained from the triplicate experiments at 300^{o}C. Average yields and the associated standard deviations of solid residue, refined oil, gas and aqueous products were 1.18±0.11%, 19.92±0.66%, 9.29±1.05% and 69.61±1.37%, respectively. The standard deviations relative to corresponding product yields were small, and should not fundamentally influence the reliability of the experimental results.

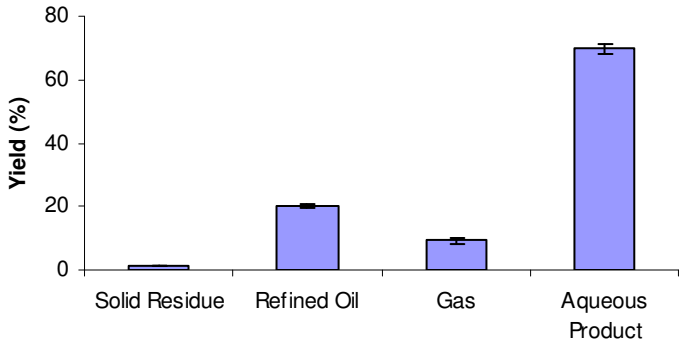


Figure 5-1. Product distribution from triplicate HTL tests with egg albumin (n=3) at 300^{o}C (error bar represents standard deviation).

5.1.1.2. Effects of operating temperature on the product distribution

Figure 5-2 shows the product distribution at different temperatures. As the temperature increased from 200^{o}C to 300^{o}C, the refined oil yield increased from 3.2% to 19.9%, the aqueous product yield decreased from 81.0% to 69.6%, and the solid residue yield decreased from 11.7% to 1.2%. The gas product yield increased from 4.1% at 200^{o}C to 8.8% at 230^{o}C and changed slightly thereafter, with a highest value of 10.5% at 260^{o}C.

One observation is that when reaction temperature was higher than 230°C, the aqueous product yield, the gas yield and the sum of refined oil and solid residue yields were almost constant, with only very small changes. One possible explanation for this observation is that the certain portion of solid residue was gradually converted to refined oil product as the temperature increased. This reduction of solid residue could be seen in figure 5.3 which shows the increase of the toluene solubility of the raw oil product as the reaction temperature increased. (Dote et al., 1996), who also used egg albumin as feedstock in HTL, concluded that the oil production was complete at about 300°C, as they found an almost negligible increase of the oil yield at 340°C. Our results and explanation support this conclusion. According to our presumption, at 300°C the solid residue yield was close to zero and no more oil would be formed from the solids fraction, indicating the completion of the oil production. Biller and Ross (2011) obtained a very similar product distribution with albumin at higher temperature and longer retention time (350°C, 1h), validating this conclusion from another angle. To confirm that the oil product was not generated directly from the aqueous product, the aqueous product was re-heated at 300°C for 30min after the hydrophobic products were removed. No oil product was generated in that test.

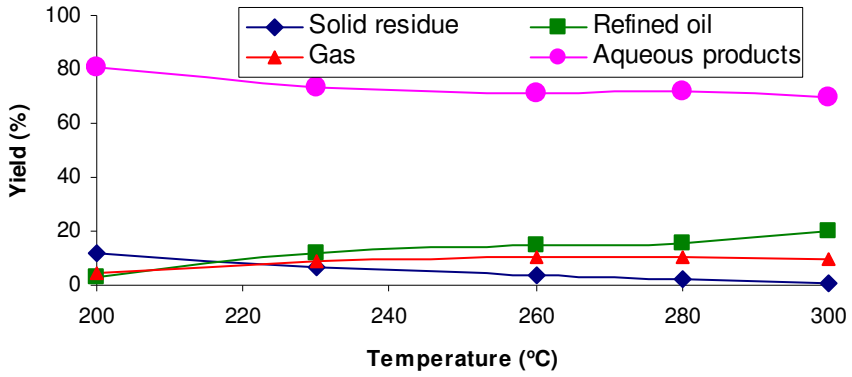


Figure 5-2. Effects of temperature on the product distribution in HTL of egg albumin.

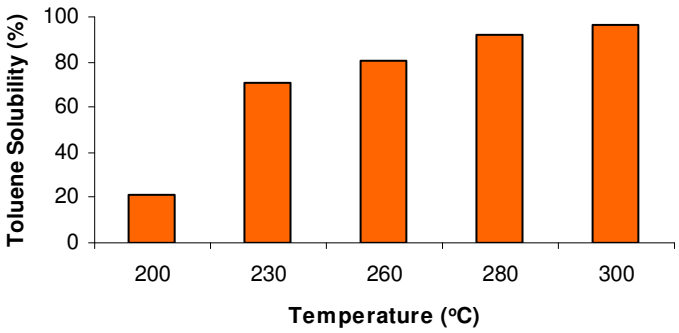


Figure 5-3. Toluene solubility of raw oil products at different temperatures.

5.1.1.3. Elemental analysis and heating values of the raw oil products

Elemental analysis and heating values of raw oil products and feedstock are shown in Table 5-1. The data shown here is adjusted by removing the effect of moisture in the raw oil product. The result showed a continuous increase of carbon content in the oil as the temperature increased, at the same time the oxygen and nitrogen contents decreased. The heating value increased from 17.09 MJ/kg (feedstock) to 32.15MJ/kg at 300°C.

Table 5-1.Elemental distribution and heating values of raw oil products (moisture free).

	Elemental compositions (wt %)				H/C	O/C	HHV (MJ/kg)
	C	H	N	O[a]			
Egg Albumin	46.07	6.55	12.94	34.45	1.71	0.56	17.09
230	66.75	7.93	11.40	13.92	1.43	0.16	28.66
260	67.67	8.54	10.02	13.76	1.51	0.15	29.89
280	72.53	7.45	8.88	11.14	1.23	0.12	31.86
300	73.41	8.82	8.83	8.95	1.44	0.09	32.15

[a]- Calculated by difference

The dramatic decrease of oxygen content in the raw oil could be explained in several possible ways. Hydrolysis of protein could produce amino acids whose carboxyl groups are hydrophilic and would more likely dissolve in the aqueous phase than remain in the oil phase. The CO_2 could then be formed through further decarboxylation of amino acids. Another possible pathway is dehydration, forming H_2O to eliminate the oxygen. Formation of peptide bonds when carboxyl groups and amino groups react with each other will cause dehydration. H_2O could also be generated when amino acids containing hydroxyl groups lose their hydroxyl groups.

When the C-C bonds in an amino acid are broken (e.g. phenylalanine, tryptophan, tyrosine, etc.) hydrophobic compounds such as phenols, toluene, indoles and other N-heterocyclic compounds can be generated. Furthermore, the hydrolysis products from a multitude of amino acids could react with each other to form hydrophobic chemicals. As we will discuss in the GC-MS analysis session, many aromatic compounds and N-heterocyclic compounds were found in the oil product. These compounds also caused the decrease of the H/C ratio in the oil.

Nitrogen content in the oil product decreased mainly due to the depolymerization and hydrolysis of proteins and the following deamination of amino acids. A large portion of the nitrogen was found in the aqueous phase. The total N in the aqueous phase was almost constant at all the temperatures at about 28,000mg/L. The ammonia-N increased as the temperature increased from 200-300^{o}C. This phenomenon showed the promoted deamination process of amino acids to ammonia as the temperature increased.

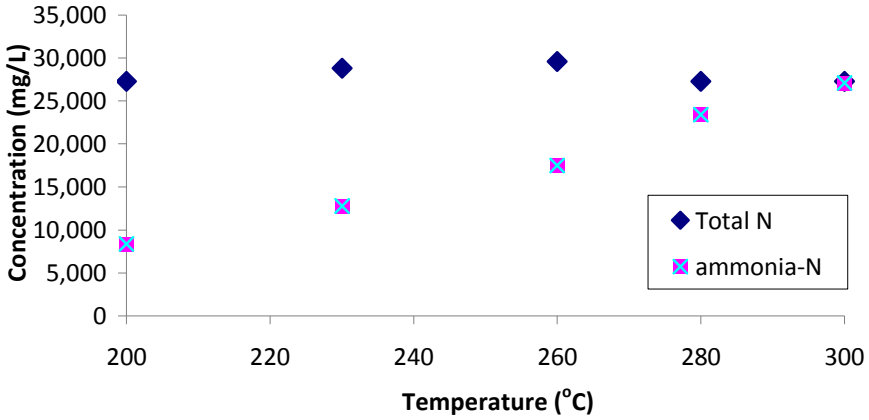


Figure 5-4. Total N and ammonia-N in the aqueous products after HTL of egg albumin.

Generally, higher temperature is beneficial in terms of bio-oil quality because of the removal of oxygen and nitrogen from oil at high temperatures. When bio-oil is considered for fuel for direct combustion, oxygen in the fuel will decrease the heating value, and nitrogen may increase the emission of harmful gases NOx. However, higher temperature will also increase the cost of production. Therefore, in the real production of bio-crude oil, the process reaction temperature should consider both oil quality and production cost.

5.1.1.4. GC-MS analysis

Refined oil samples from 230, 260, 280 and 300^{o}C were dissolved in toluene and analyzed by GC-MS. As the temperature increased, the composition of refined oil became more complex and more peaks were shown in the chromatograph. Almost all the peaks in the chromatographs of lower temperatures could be found in those of higher temperatures, with rare exceptions that will be discussed later. The concentrations of major compounds were directly dependent on temperature, based on observation of the relative peak areas.

At 230^{o}C, several major peaks could be seen (Figure 5-5 a) and identified (Table 5-2). These molecules have relatively higher molecular weight, and their concentrations at 230^{o}C were obviously higher than those at higher temperatures. The existence of these large molecules indicated the incomplete hydrolysis of protein. Abdelmoez and Yoshida (2006) studied the plasticization of serum albumin from bovine blood (BSA) and found that at 200^{o}C to 300^{o}C, within only 0.5 min almost all the water-soluble BSA molecules were aggregated and polymerized into water-insoluble solids (Plastic BSA). However, after 0.5 min, as the temperature and reaction time increased, the formed solids degraded again to water-soluble amino acids and further to other compounds. In this study, similar aggregation and polymerization phenomena were also observed when the reactor was opened at 230^{o}C, 0 min. We can conclude that there was a process of aggregation and polymerization before the hydrolysis of protein to amino acids and further degradation. At 300^{o}C, these large molecules almost disappeared, indicating the completed hydrolysis of the polymerized protein.

The chromatograph at 280^{o}C is very similar to that at 300^{o}C. However, one significant difference is that at 300^{o}C large amounts of long-chain amides were found. These amides apparently formed through the combination of long-chain fatty acids and ammonia (amines). Egg albumin contains small amount of lipids and is not a pure source of protein. This observation indicated that at higher temperatures, depolymerized products of protein and lipids could react with each other and possibly recombine.

Table 5-2. Large molecules identified in GC-MS Chromatograph at 230°C.

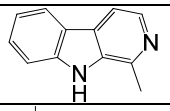
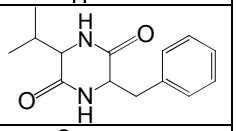
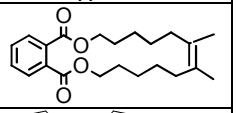
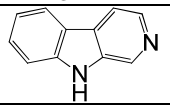
#	Retention time	Name	Structure
a	37.73	9H-Pyrido[3,4-b]indole, 1-methyl-	
b	40.37	2,5-Piperazinedione, 3-benzyl-6-isopropyl-	
c	42.84	1,2-benzenedicarboxylic acid diisooctyl ester	
d	44.48	9H-Pyrido[3,4-b] indole	

Table 5-3 lists the major peaks identified at 300°C (Figure 5-5 d). Between the retention time 10 min and 22 min, many small-area peaks were shown. These peaks represent smaller molecules containing nitrogen. In our analysis, many types of pyrazine-derivatives were found. These smaller molecules were probably the hydrolysis residues of amino acid or their re-combination products.

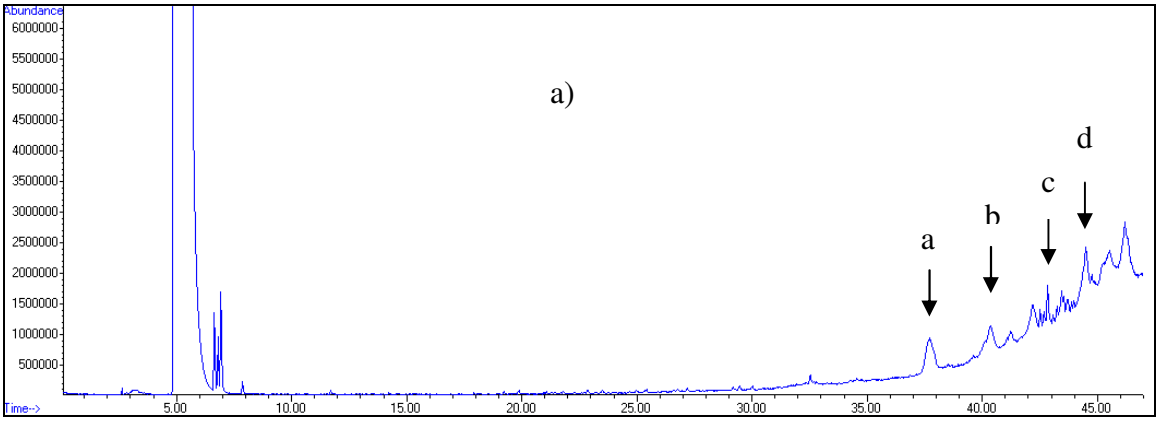


Figure 5-5. GC-MS chromatograms of refined oil at a) 230°C; b) 260°C; c) 280°C and d) 300°C.

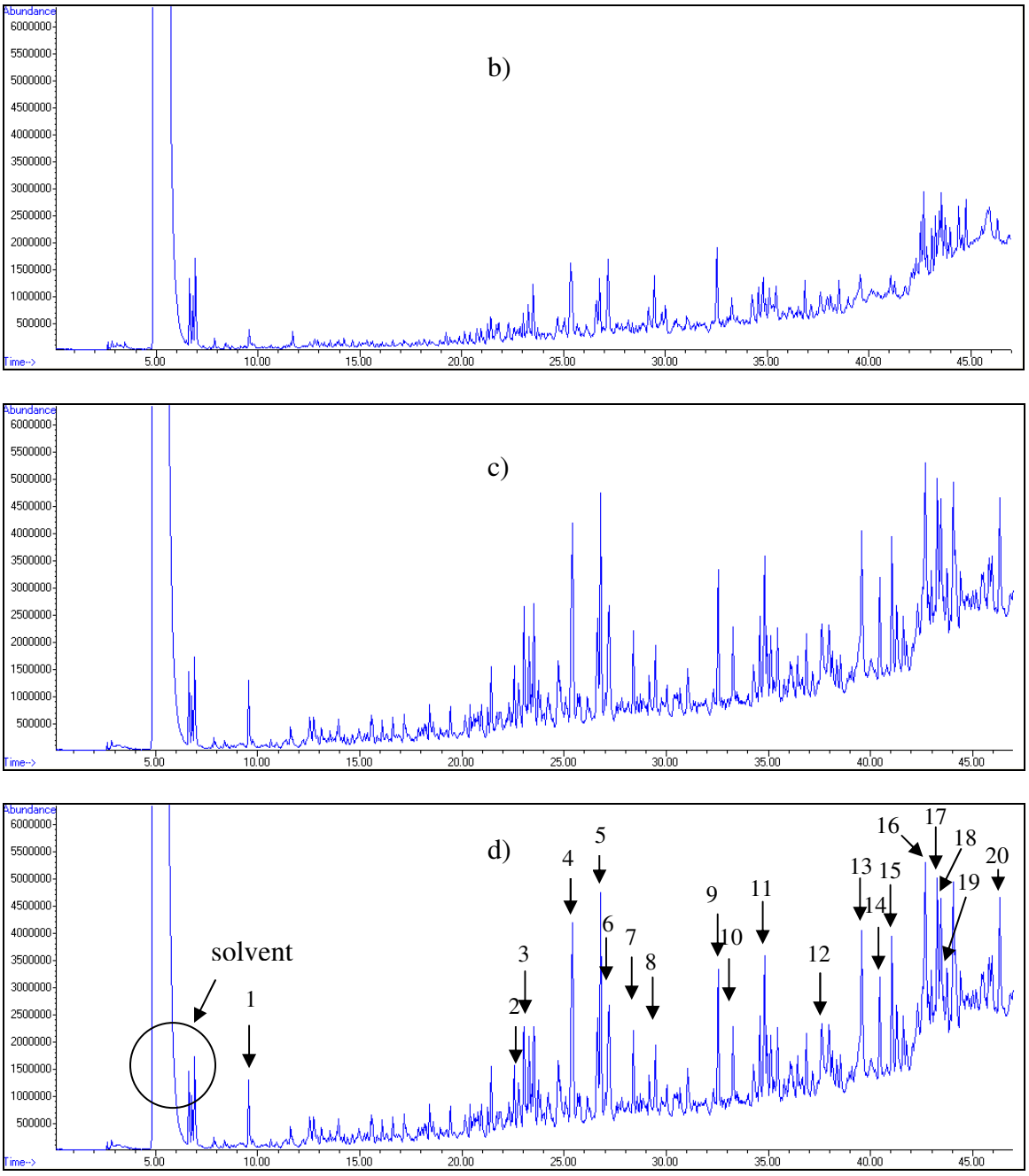
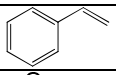
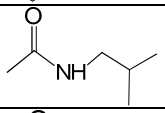
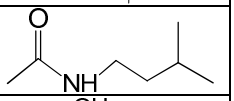
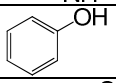
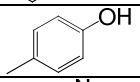
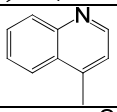
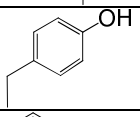
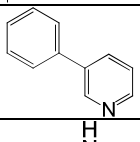
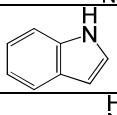
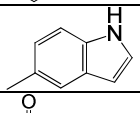
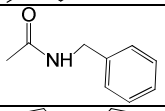
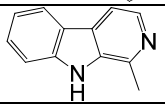
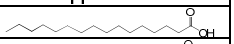
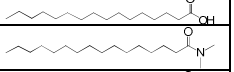
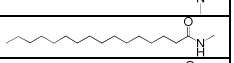
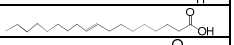
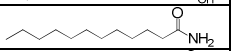
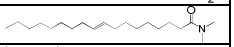
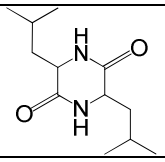
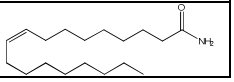


Figure 5-5 (cont.). GC-MS Chromatograms of refined oil at: b) 260°C; c) 280°C; d) 300°C.

Table 5-3. Major compounds in the refined oil at 300°C reaction temperature.

#	Elusion time (min)	Name	Structure
1	9.55	Styrene	
2	22.56	Acetamide, N-[2-methylpropyl-]	
3	23.02	Acetamide, N-[3-methylbutyl-]	
4	25.42	Phenol	
5	26.79	Phenol, 4-methyl-	
6	27.25	Quinoline, 4-methyl-	
7	28.39	Phenol, 4-ethyl-	
8	29.47	Pyridine, 3-phenol-	
9	32.54	Indole	
10	33.27	1H-indole, 2-methyl-	
11	34.80	Acetamide, N-[2-phenylethyl-]	
12	37.73	9H-Pyrido[3,4-b]indole, 1-methyl-	
13	39.55	n-hexadecanoic acid	
14	40.45	N,N-dimethyl-hexadecanamide	
15	41.05	N-methyl-hexadecanamide	
16	42.68	Octadec-9-enoic acid	
17	43.27	dodecanamide	
18	43.44	9-octadecenamide, N,N-dimethyl-	
19	43.57	3,6-bis(2-ethylpropyl)piperazine-2,5-dione	
20	46.38	9-octadecenamide [Z]-	

5.1.1.5. Hypothetical pathways for formations of identified compounds

Two major pathways are hypothesized for the formation of the chemicals identified by GC-MS: hydrolysis of larger molecules from the feedstock and re-combination of small molecules generated through the protein depolymerization. It is likely that the hydrolysis of polymers would take place first. However, once the small molecules such as organic acids, ammonia, pyrroles and phenols are formed, a re-combination among them may occur. Shock (1993) stated that amide formation and peptide formation may proceed at elevated temperatures, and condensation of complex organic molecules may be energetically favored in hydrothermal solutions. The detection of more amides and amino acid dimers at higher temperature in this study agreed with this statement. It is unclear whether these two kinds of reactions happened concurrently or sequentially.

Based on the HTL product analysis, a hypothesized reaction scheme is shown in Figure 5-6. Formulae (A)-(G) describe possible formation pathways for several identified chemicals. The numbers in the parentheses indicate the position of corresponding compounds in Table 5-3. Formulae (A)-(E) describe the first pathway: degradation from amino acids to aromatics and N-heterocyclic compounds. Formulae (F) and (G) describe the second pathway: re-combination of small molecules.

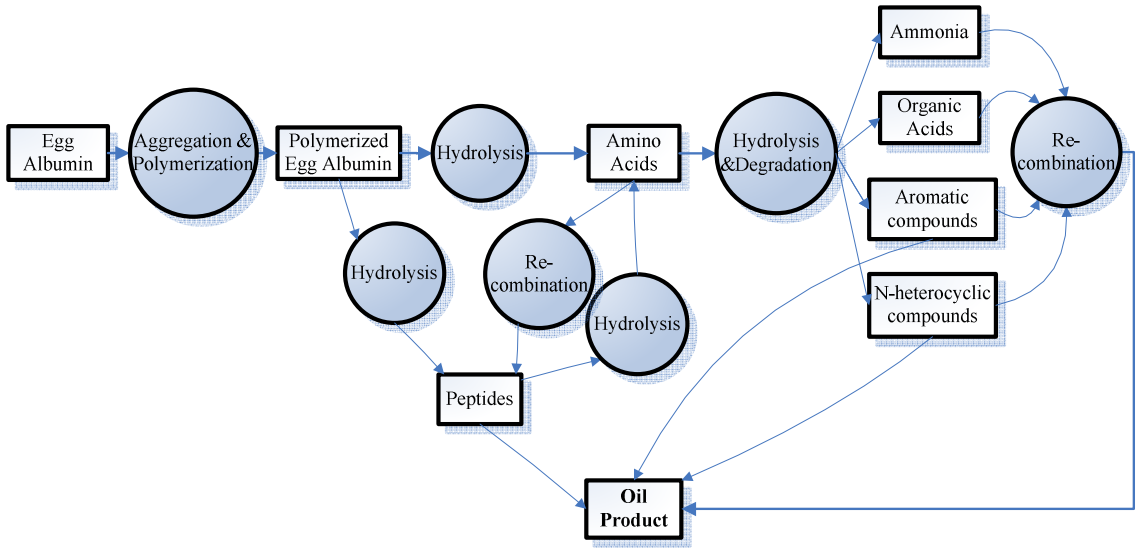
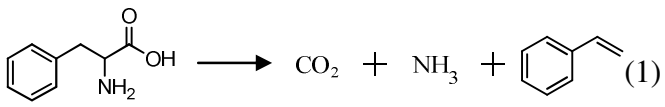
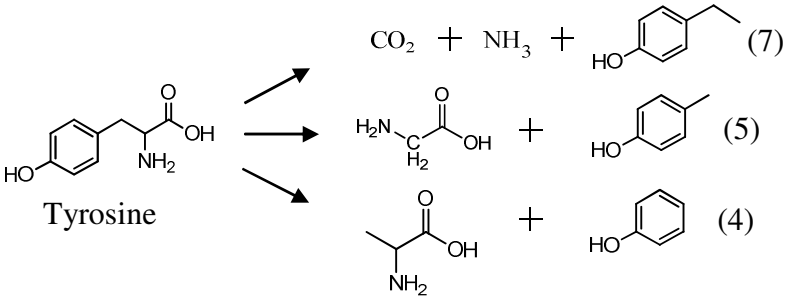


Figure 5-6. A simplified possible reaction scheme for HTL of egg albumin.



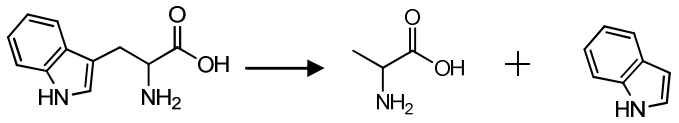
Phenylalanine (A)



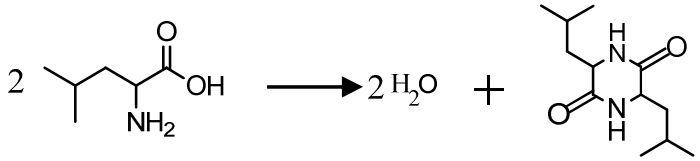
(B)

(C)

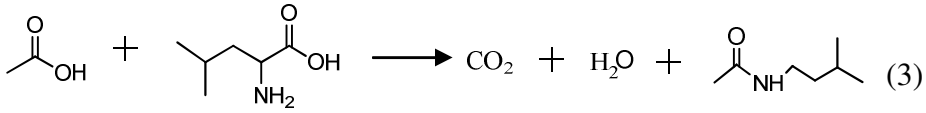
(D)



Tryptophan (E)



Leucine (F)



Leucine (G)

It is necessary to point out that these formulae did not consider the origin of the reactants or the destination of the products generated; they are not the concern here as long as the reactants could be obtained in the process and the reactions could happen according to thermodynamic principles. These pathways are only some of the possible pathways which could reasonably explain the formation of specific chemicals. These chemicals could be formed through other pathways. However, the egg albumin system is still too complicated since it contains all 20 essential amino acids as well as other impurities. Therefore, numerous reactions could actually happen, and no quantitative analysis has been done to identify these specific chemicals. Further studies are needed to quantify these possible pathways.

5.1.2. Cellulose

After the HTL of microcrystalline cellulose, three streams of product were obtained: gas, solid (char-like) and aqueous product. No tarry product like that in the HTL of swine manure and egg albumin was formed. After vacuum filtration for 3 hours, the solid obtained was black and still contain high moisture content. This property of the solid product shows its potential to be used in the soil modification since it has the ability to hold the moisture efficiently.

Figure 5-7 shows the results obtained from the triplicate experiments at 300°C using microcrystalline cellulose as feedstock. Average yields and the associated standard deviations of solid residue, refined oil, gas and aqueous products were 40.03±0.87%, 25.99±4.29%, 21.43±1.83% and 12.55±3.17%, respectively. The standard deviations relative to corresponding product yields were small, and should not fundamentally influence the reliability of the experimental results.

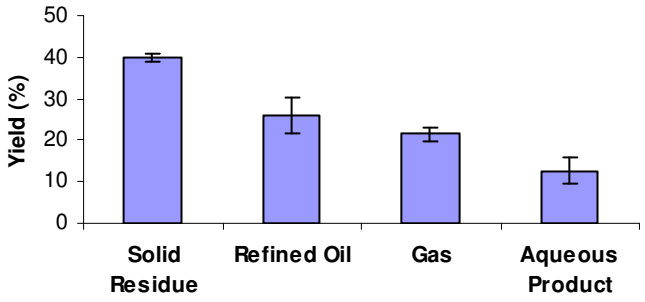


Figure 5-7. Product distribution from triplicate HTL tests with microcrystalline cellulose (n=3) at 300°C (error bar represents standard deviation).

Minowa et al. (1998) used microcrystalline cellulose as feedstock at 200-350°C. They analyzed the product distribution based on the carbon mass balance. Although the mass balance was not so satisfactory (only about 80% of the carbon could be recovered), the yields of oil, gas, char and aqueous product at 300°C, 0min were 20%, 5%, 42% and 15%, respectively. This result is comparable with our product distribution, except for a lower gas yield in their study. They used a higher nitrogen initial pressure (3MPa compared to the 0.65MPa in our study), which could be a factor inhibiting the generation of more gaseous products according to the chemical equilibrium theory.

Nelson et al. (1984) also studied the direct thermal liquefaction of cellulose, and in their study, after treatment of 2.5min at 300°C, the major products were 30% 5-HMF, 13% furfural, and about 30% saccharides with the remainder as an acetone-insoluble solid. The lower solid residue they obtained compared to our study, is very possibly due to the difference of solvent.

The elemental analysis results of the raw oil, dried raw oil (dried at 105°C overnight), toluene insoluble solid residue, and refined oil are shown in Table 5-4. Theoretically, cellulose does not contain nitrogen. However, nitrogen is present in the measurement results, probably due to: 1) the impurity of feedstock; 2) the uncleaned residue sticked to the reactor from previous HTL tests and 3) the measurement error.

From the Table 5-4, the refined oil has slightly lower carbon content and higher hydrogen content than the solid residue, while the oxygen contents of these two are similar. This result agrees with the other researchers (Nelson et al., 1984).

Table 5-4. Elemental analysis of oil and solid residue products from HTL of cellulose[a].

	Raw oil		Dried raw oil		Solid residue		Refined oil [b]	
	Weight Percentage (%)	Mass (g)	Weight Percentage (%)	Mass (g)	Weight Percentage (%)	Mass (g)	Weight Percentage (%)	Mass (g)
C	17.98	0.18	71.53	0.17	72.24	0.11	70.34	0.06
H	7.75	0.08	4.44	0.01	3.81	0.01	5.49	0.01
N	0.23	0.00	0.40	0.00	0.33	0.00	0.50	0.00
O	74.05	0.74	23.64	0.06	23.63	0.04	23.66	0.02
Total	100.00	1.00	100.00	0.24	100.00	0.15	100.00	0.09

[a]- the mass balance is based on assuming the mass of raw oil =1g.

[b]- calculated based on the measurement of other fractions.

The purpose of running these HTL tests with microcrystalline cellulose is to verify the comparability of our study with other researchers, and to provide basic information for the interaction tests between single model compounds. The effects of operating parameters and the optimization of them are not the purpose in this study.

5.1.3. Glucose

The apparent products obtained from the HTL of glucose were similar to those from microcrystalline cellulose: gas, solid (char-like) and aqueous product. The yields of solid residue, refined oil, gas and aqueous products were 42.09%, 11.79%, 25.15% and 20.97%, respectively (Figure 5-8). Although cellulose is the polymer of glucose, the product distribution of these two showed differences after the HTL process. The HTL of glucose yielded similar amount of solid residue, more gas and aqueous products, but less refined oil. In the study of Chareonlimkun et al. (2010), the conversion of glucose and cellulose at 523K, 5min without catalysts was also different- 40% and 27%, respectively. It is widely recognized that the hydrolysis of cellulose is the first step during the HTL process (Kruse et al., 2007; Minowa et al., 1998). However, the hydrolysis of cellulose is very slow at the temperatures below 200^{o}C (Mochidzuki et al., 2000; Yu and Wu, 2010; Yuan et al., 2009b). It was found that in hot-compressed water, the epimerization of glucose to fructose was promoted at higher temperature (623K vs. 573K) (Kabyemela et al., 1997). When cellulose is hydrolyzed to glucose, glucose is generated at high temperature, which means more fructose would be produced from cellulose than from glucose during the HTL process. Research showed that glucose degrades mostly to fragmentation products while fructose will react to a higher amount of dehydration product 5-HMF. The fragmentation products tend to exist in the aqueous phase and form CO_2 gas, while 5-HMF tend to form polymers and oil products. This is a reasonable explanation why less oil product was produced from glucose than cellulose, since the fragmentation of glucose happened at lower temperature and the fragments formed less oil than the fructose did.

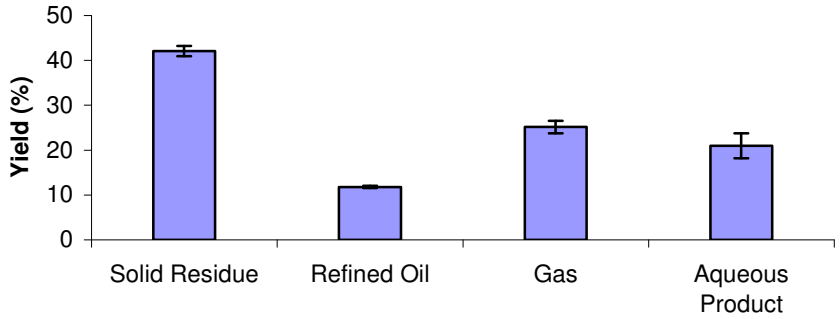


Figure 5-8. Product distribution from HTL tests with glucose (n=2) at 300^{o}C (error bar represents standard deviation).

5.1.4. Xylose

The yields of solid residue, refined oil, gas and aqueous products were 41.41%, 13.04%, 23.20% and 22.34%, respectively. This product distribution is very similar to that of the glucose test (See the comparison in Figure 5-9). The pH value of aqueous product is 2.69, which is also close to the 2.77 of the aqueous product of glucose test. These similarities mean although the chemical structure and reaction pathways may be different for carbohydrate monomers, their product distribution could be very similar. This info could be very useful since probably no strict separation and selection of feedstock is necessary during the real large-scale production. Goudriaan et al. (2000) studied the deoxygenation selectivity of hydrothermal upgrading (HTU) process for several different feedstocks (feedstocks were not specified in the paper). The decarboxylation selectivity is defined as the ratio (oxygen removed as CO_2) : (total of oxygen removed as CO_2 and H_2O). They found that the decarboxylation selectivity was almost constant, in the range of 0.49-0.54.

A high similarity was also found in the GC-MS analysis results of the refined oil obtained from glucose and xylose (Figure 5-10). A significant difference, though, is the oil from xylose contained more furfural than that from glucose.

It needs to be mentioned that there could and would be differences between the HTL products of hemicellulose and xylose, according to the results from cellulose and glucose. Due to the availability and feasibility, the HTL of bulk hemicellulose was not investigated in this study. However, with the microreactor system, it would be possible to study the HTL of hemicellulose directly.

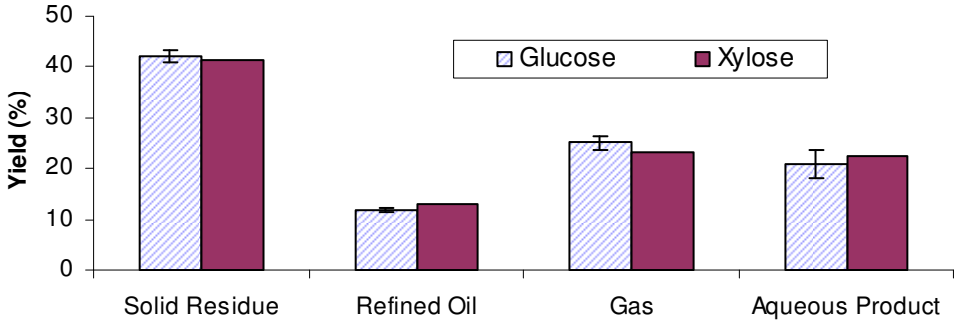


Figure 5-9. Comparison of product distribution between glucose and xylose at 300°C.

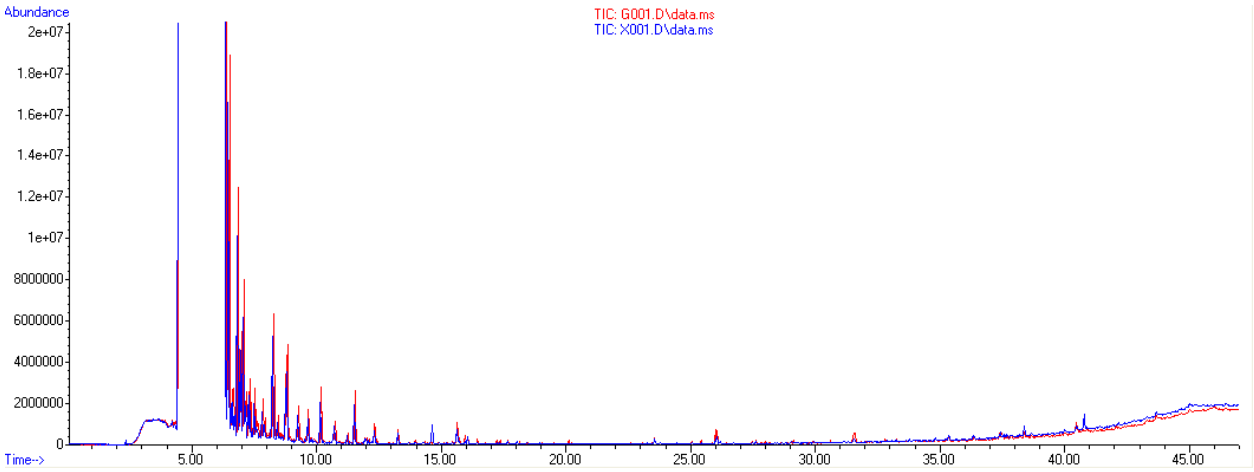


Figure 5-10 Comparison of GC-MS with oil from HTL of glucose and xylose.

5.1.5. Lipid

The yields of solid residue, refined oil, gas and aqueous product from HTL of butter were 0, 90.5%, 5.6% and 3.9%, respectively (Figure 5-11). There is no surprise that a high refined oil yield was obtained since the butter itself contains mainly fat.

The GC-MS spectra of refined oil from HTL of butter is shown in Figure 5-11. The major compounds identified by GC-MS were carboxylic acids, which accounted more than 90 percents of the total peak area (Table 5-5). The presence of large amount of carboxylic acids proved the hydrolysis of fat under hydrothermal condition.

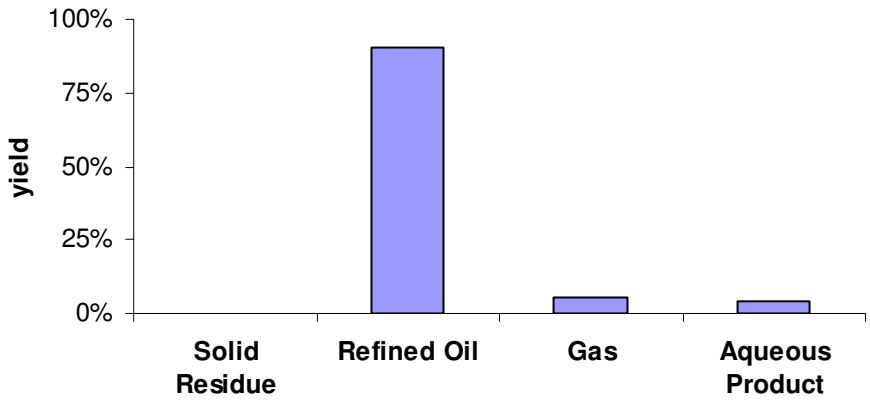


Figure 5-11. Product distribution of HTL test with butter at 300°C.

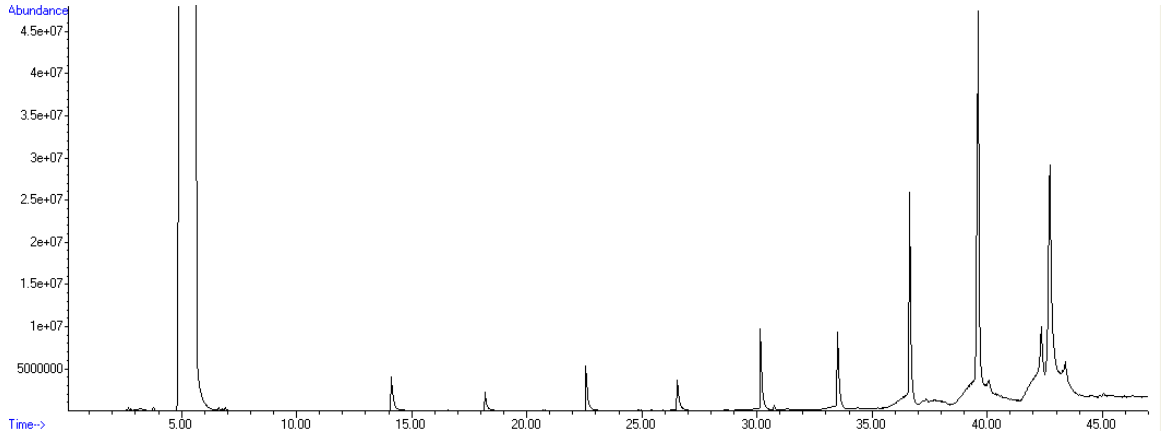


Figure 5-12. GC-MS chromatograph of the refined oil from the HTL of butter.

Table 5-5. Major compounds in the refined oil from HTL of butter at 300°C.

Elusion time (min)	Compound	Area (%)
14.10	Acetic Acid	2.00
18.18	Butanoic Acid	0.80
22.58	Hexanoic Acid	1.78
26.55	Octanoic Acid	1.40
30.15	Decanoic Acid	3.73
33.52	Dodecanoic Acid	4.04
36.64	Tetradecanoic Acid	11.67
39.62	Hexadecanoic Acid	27.00
42.35	Octadecanoic acid	10.81
42.72	Oleic acid	24.45
43.38	9,12-octadecadienoic acid [Z,Z]-	3.91
Total		91.59

5.2. HTL OF MIXTURES OF MODEL COMPOUNDS

5.2.1. Protein and Cellulose

Figure 5-13 shows the product distribution after HTL of the mixtures of egg albumin and microcrystalline cellulose with different mixing ratios. The non-linear trendlines for the product yields clearly indicate the interactions between these two feedstocks and/or between their decomposition products during the HTL process.

It could be seen that the gas production was obviously promoted when the mixture was used compared to when either egg albumin or microcrystalline cellulose was used solely as the

feedstock. The highest gas yield was obtained at the mixing ratio of 1:1. The major fraction of the gas products was CO_2 (>95% v.v.). Typically, CO_2 is generated through decarboxylation process during the hydrothermal liquefaction. And it appears this decarboxylation process was promoted when egg albumin and microcrystalline cellulose were mixed. It is hard to say that significant changes have happened to the yields of aqueous products or toluene soluble oil (refined oil).

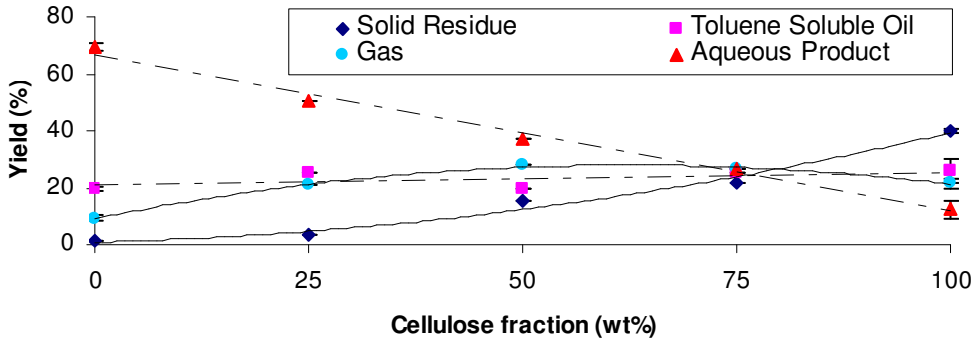


Figure 5-13. Product distribution of HTL tests with mixtures of egg albumin and microcrystalline cellulose at different ratio.

When microcrystalline cellulose alone was hydrothermally converted, a large amount of solid residue (~40% yield) was generated. However, with the increase of egg albumin fraction in the mixture, a pronounced decrease of solid residue yield was observed.

It is widely accepted that hydrolysis is the first step of cellulose decomposition, and glucose is the major hydrolysis product of cellulose. (Bobleter, 1994; Kruse et al., 2007; Minowa et al., 1998) Glucose, under hydrothermal conditions, first dehydrates to form water-solvent soluble (WSS) fraction, and then water-solvent insoluble (WSIS) products and gas are formed from a certain fraction of WSS. And WSIS is considered mainly the polymerization product of WSS with similar elemental compositions. (Knezevic et al., 2009) This WSIS is very possible the resinification product of furan derivatives as this polymerization phenomenon has been proved for decades.(Dunlop and Peters, 1953; Gandini and Belgacem, 1997)

The reasons leading to the reduction of solid residue when egg albumin was added into microcrystalline cellulose may include:

1. The influence of ammonia generated during the decomposition of egg albumin;
2. Maillard reactions between amino acids and glucose or between the decomposition products of them.

It has been recognized that alkali materials could reduce the formation of solid residue from the HTL of cellulose and glucose (Minowa et al., 1998; Nelson et al., 1984). And it seemed the addition of alkali catalysts shifted the mechanisms from one involving aqueous pyrolysis (predominant furan formation) to one incorporating aldol and related condensations. (Nelson et al., 1984) Ammonia was used in the HTL of cellulose and demonstrated to function as both basic catalyst and reactant with cellulose. (Inoue et al., 1999) And in our previous study, it has also been demonstrated that ammonia could be readily produced through the deamination process when egg albumin was converted under 200-300^{o}C. Therefore the influence of ammonia on the reduction of solid residue is expected.

On the other hand, Maillard reactions could also play an important role in reducing the formation of solid residue. Firstly, it could in a certain level protect the glucose from decomposition to furan derivatives and therefore avoid the polymerization between the furan derivatives to form solid residue. At temperatures as low as 150^{o}C, glucose monomer could have already been generated from microcrystalline cellulose in hot compressed water (HCW).(Yu and Wu, 2010) In the presence of egg albumin, Maillard reaction became highly possible and in consequence avoided the formation of furan derivatives. Secondly, Maillard reactions have also been observed to happen between the degradation products of sugars and amino acids. For example, Peterson et al. confirmed that pyruvic aldehyde, as the degradation product of glucose can also participate in Maillard-type reactions with glycine, and appeared to do so even more aggressively than glucose. Also, ethanolamine, a surrogate for glycine, also reacts with glucose and in a much more dramatic manner than glycine does. (Peterson et al., 2010)

It was observed that when microcrystalline cellulose was hydrothermally converted at 200^{o}C and 220^{o}C without catalyst, the water-soluble products were predominantly glucose/oligomers. (Minowa et al., 1998) However, at higher temperature, glucose and its isomer fructose decompose to form other compounds very quickly under hydrothermal conditions and the major

reactions include dehydration and retro-aldol condensation. (Kabyemela et al., 1999). In our study, it took about 30 min for the temperature raises from 150°C to 300°C and the reaction temperature was maintained at 300°C for another 30 min. So it is very possible that Maillard reactions happened both between glucose/ amino acids and between their decomposition products.

When pure glucose is hydrothermally decomposed to fragments, usually organic acids (e.g. lactic acid, formic acid, acetic acid, levulinic acid, etc.) are produced which causes the low pH of the aqueous phase. (Kabyemela et al., 1999) In our study, the pH of aqueous phase from HTL of microcrystalline cellulose was 2.42. However, when the mixture of egg albumin and with mixing ratio of 3:1 and 1:1 were used, the pH values of aqueous phase were 7.95 and 7.66 respectively (Figure 5-14). This increase of pH could also be explained by the influence of ammonia and Maillard reactions. As mentioned previously, ammonia was generated through the deamination process of amino acids, and could shift the pathways of glucose decomposition and therefore reduce the generation of organic acids. On the other hand, ammonia could also neutralize the acids in case they were generated. When Maillard reactions happened, the pathways producing acidic compounds were suppressed. Instead, pyrroles, pyridines, pyrazines and other less or non- acidic compounds were generated, which could be seen in the GC-MS analysis section. When the mixing ratio of cellulose and protein was greater than 1:1, it appears that the excessive cellulose took the pathways of pure cellulose and more acidic chemicals were formed, resulting in the dramatic decrease of pH (Figure 5-14).

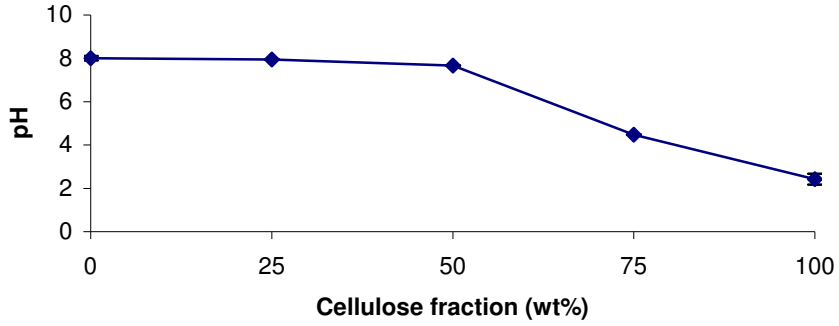


Figure 5-14. pH value of the aqueous products from HTL tests with mixtures of egg albumin and microcrystalline cellulose at different ratio.

When the retention time was prolonged from 30 min to 120 min for the mixture of egg albumin and microcrystalline cellulose (mixing ratio=1:1), a significant change of product distribution was observed (Figure 5-15). The toluene soluble oil yield increased about 10%, and the aqueous product yield decreased about 10%. The solid residue and gas yield both changed less than 5%. This phenomenon showed that 30 min retention time is not long enough for the reaction system to reach equilibrium. And it is very possible that as the retention time increased the refined oil product was generated from the aqueous phase, because the only other product with a decreased yield was solid residue, but the decrease of solid residue yield itself was less than the increase of the refined oil yield.

When 5% Na_2CO_3 was added into the mixture of egg albumin and microcrystalline cellulose (mixing ratio=1:1), a significant decrease of solid residue yield and a significant increase of toluene soluble oil yield were observed (Figure 5-14). No pronounced influence was shown on the gas and aqueous product yield. As mentioned previously, ammonia could be generated from EA and functions as an alkali catalyst and also a reactant. However, when Na_2CO_3 was added here, the solid residue was further reduced and the toluene soluble oil yield increased about 10%. This observation shows that to obtain maximum oil yield using the mixture of egg albumin and microcrystalline cellulose under current conditions, the catalytic effect of ammonia generated during the reaction itself is probably not sufficient and a certain amount of additional catalyst is necessary. Otherwise, different reaction conditions are needed.

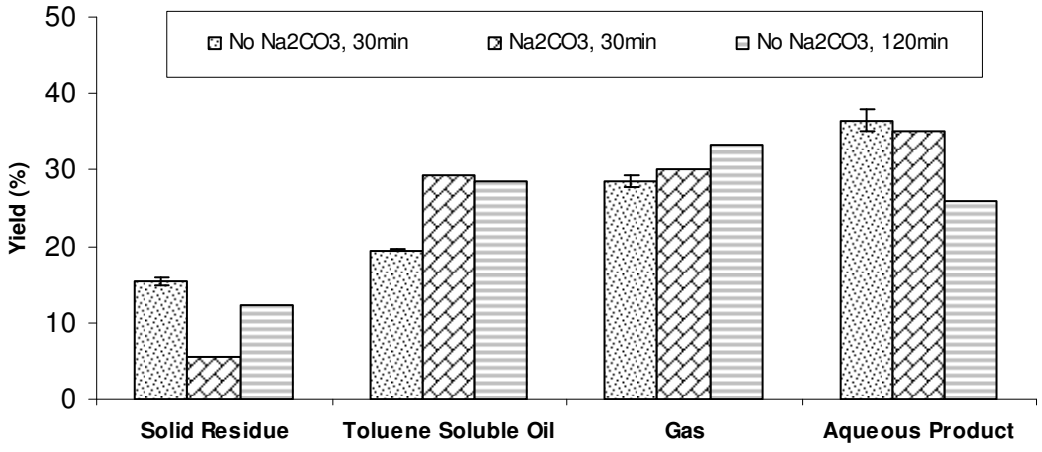


Figure 5-15 Product distribution after the HTL of the mixture of egg albumin and microcrystalline cellulose under different conditions.

Figure 5-16 is the GC-MS chromatogram for the refined oil from HTL of mixture of egg albumin and microcrystalline cellulose at a weight ration of 1:1 under 300°C. And Table 5-6 lists the major compounds in the refined oil. Several major groups were identified in the refined oil:

1. pyrazine and its derivatives
2. pyridine and its derivatives
3. pyrrole and its derivatives
4. indole and its derivatives
5. phenol and its derivatives
6. cyclopentanone/cyclopentenone and their derivatives

It is obvious that the first four groups are somewhat related to the egg albumin since they all contain nitrogen and egg albumin is the only significant nitrogen source in this study. Glucose could dehydrate or isomerise to form other C6 compounds, or degrade through retro-aldol condensation to form C1/C5, C2/C4 and C3/C3 products depending on the cracking of C-C bond at deferent locations. And the dicarbonyl structure could be easily formed in these C2-C6 compounds. (Kabyemela et al., 1999) Therefore Maillard reactions of -NH_2 containing compounds with these C2-C6 compounds could generate many kinds of substituted pyrazines, pyrroles and pyridines.

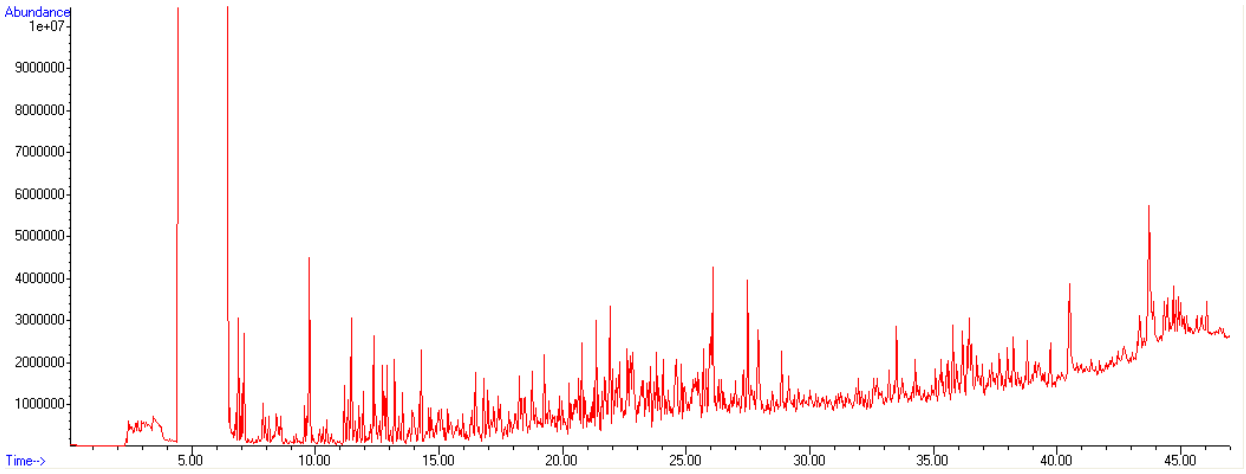


Figure 5-16 GC-MS chromatograph of the refined oil from the HTL of mixture of egg albumin and microcrystalline cellulose at 300°C.

The formation mechanisms of pyrazines have been proposed by other researchers. (Hwang et al., 1994; Milić and Piletić, 1984; Rizzi, 2003) Typically the formation of pyrazines needs the dehydration and cyclization of two Maillard reaction products. These two compounds could both be formed through the reaction between an N-containing compound (amine, amino acids, ammonia, etc.) and a-dicarbonyl compound which could be easily formed through the retro-aldol condensation of glucose under hydrothermal conditions.

The formation of pyrroles and pyridines does not necessarily need two individual N-containing maillard reaction products. However, Maillard reaction is still important and the dehydration and cyclization are necessary to form the ring structures. Three major pathways were previously reported to form pyrroles. The first one results from the interaction between an amino acid and a 3-deoxyhexosone through the Strecker degradation followed by dehydration and ring closure. (Kato and Fujimaki, 1968; Milić and Piletić, 1984; Njoroge et al., 1987) The second one is the reaction of furans with amines or amino acids, which requires a carbonyl function in position 2 of the furan derivative.(Rizzi, 1974) And the third pathway is the condensation of α-aminocarbonyls with ketones or aldehydes, known as the Knorr pyrrole synthesis. (Yaylayan and Keyhani, 2001) Since the reactants for these three pathways were all possibly generated during the HTL, it is unclear that which pathway was the predominant one and it is possible that all these three pathways existed during the process. The formation of pyridines may involve the condensation of aldehydes, ketones, or α, β-unsaturated carbonyl compounds with ammonia which is degraded from amino acids. (Hwang et al., 1995; Suyama and Adachi, 1980) A detailed mechanism describing the formation of 2-methyl-5-hydroxy-6propylpyridine through condensation of crotonal, glyoxal and amino acids was proposed previously. (Milić and Piletić, 1984)

On the other hand, these compounds were also found generated through the degradation of melanoidins, which are macromolecules formed at lower temperature through Maillard reaction.(Yaylayan and Keyhani, 2001) So, condensation and decomposition could both contribute to the formation of pyrazines, pyrroles and pyridines.

Indole was found generated through the decomposition of Tryptophan Amadori Rearrangement Products (Trp-ARP), which is a meladonin product produced through maillard reactions between tryptophan and glucose at over 110^{o}C. (Yaylayan and Forage, 1991) And also, indole could be generated directly through the decomposition of egg albumin itself, which was proved in previous study. The same mechanism could be utilized to speculate the formation of phenols since phenyl is a functional group in amino acid tyrosine, similar to the situation of indole group in tryptophan. On the other hand, phenols could also be formed from glucose. The formation mechanisms were proposed by researchers preveiously. (Nelson et al., 1984; Williams and Onwudili, 2005)

Group 6 compounds are found in the HTL products of cellulose and glucose in many other researches without addition of amino acids or protein. (Karagoz et al., 2005; Kruse and Gawlik, 2003; Srokol et al., 2004) The presence of these compounds indicates that part of the cellulose still underwent the pathways it takes when it is solely converted, which means the addition of protein shifted the pathways of cellulose, but not completely.

Other compounds, including fatty acids, furans, amides, Piperazinedione derivatives were also detected. Fatty acids should have originated from the lipid impurity in egg albumin. Furans are the common decomposition product of cellulose/glucose. Amides could easily be formed through acids with amines/ammonia. And Piperazinediones are typical cyclization products of amino acids. The presence of all these compounds indicates that even though most of the identified compounds are related to Maillard reactions during the HTL of egg albuminand microcrystalline cellulose, many other types of reactions also happened at the same time. The pathways in individual HTL of egg albumin and microcrystalline cellulose could not be completely suppressed. The whole reaction system possibly includes at least hydrolysis, isomerization, dehydration, cyclization, deamination, decarboxylation, condensation, etc.

Compared to the toluene soluble oil from HTL of egg albumin itself (Figure 5-5), it could be seen that the peaks were shifted from the longer retention time to shorter retention time, which means more molecules with lower boiling point were generated. The interactions between egg albumin and microcrystalline cellulose show double-folded effects on the production of biofuel

through hydrothermal conversion. On the one hand, it could generate less solid residue and more lower boiling point products, which is a positive effect. On the other hand, the introduction of nitrogen into the biofuel could cause potential upgrading problems or combustion problems. How to remove the nitrogen will be an important issue for future study.

Table 5-6. Major compounds in the refined oil from HTL of mixture of egg albumin and microcrystalline cellulose at 300°C.

#	Elusion time (min)	Compound	Matching Index
1	6.51	Thiophene, 2-methyl-	90
2	6.77	Thiophene, 3-methyl-	91
3	7.06	1H-Pyrrole, 1-methyl-	93
4	7.44	Thiophene, 2,5-dimethyl-	90
5	7.85	Pyridine	89
6	7.87	1H-Pyrrole, 1-ethyl-	93
7	7.94	benzene, 1,3-dimethyl-	94
8	7.96	Cyclopentanone	95
9	8.29	1-butanol, 2-methyl-	89
10	8.12	Cyclopentanone, 2-methyl-	96
11	8.42	Thiophene, 3-ethyl-	93
12	8.46	Pyrazine	92
13	8.61	Pyridine, 2-methyl-	84
14	9.15	2-Heptanone, 6-methyl-	93
15	9.23	2(1H)-Pyridinone, 5-methyl-	90
16	9.57	Styrene	94
17	9.68	1H-Pyrrole, 1-butyl-	82
18	9.76	Pyrazine, methyl-	96
19	9.90	1H-Pyrrole, 2,5-dimethyl-	92
20	10.17	Pyridine, 2-ethyl-	91
21	10.30	Cyclopentanone, 2-ethyl-	94
22	10.45	Pyridine, 3-methyl-	91
23	10.63	Pyridine, 2-ethyl-6-methyl-	88
24	11.16	Pyrazine, 2,5-dimethyl-	96
25	11.32	Pyrazine, 2,6-dimethyl-	93
26	11.45	Pyrazine, ethyl-	93
27	11.62	1H-Pyrrole, 3-ethyl-2,4-dimethyl-	72
28	11.76	Pyrazine, 2,3-dimethyl-	96
29	11.93	2-Isopropylpyrazine	89
30	12.35	2-Cyclopenten-1-one, 3,4-dimethyl-	95
31	12.58	Dimethyl trisulfide	95
32	12.59	Pyridine, 3-ethyl-	91
33	12.73	Pyrazine, 2-ethyl-6-methyl-	92
34	12.79	Pyrazine, 2-ethyl-5-methyl-	92
35	13.04	1,2,5-Trimethylpyrrole	89
36	13.20	Pyrazine, 2-ethyl-3-methyl-	91
37	13.53	Pyrazine, 2-(n-propyl)-	96

Table 5-6 (cont.). Major compounds in the refined oil from HTL of mixture of egg albumin and microcrystalline cellulose at 300°C.

#	Elusion time (min)	Compound	Matching Index
38	13.93	Pyrazine, 2,6-diethyl-	90
39	14.20	Pyrazine, 3-ethyl-2,5-dimethyl-	91
40	14.28	2-Cyclopenten-1-one, 2,3-dimethyl-	92
41	14.42	2,3-Dimethyl-5-ethylpyrazine	91
42	14.95	Pyrazine, 2-methyl-5-propyl-	89
43	15.15	1H-Pyrrole, 2,3,4,5-tetramethyl-	91
44	15.35	Pyrazine, 3,5-diethyl-2-methyl-	87
45	15.46	2-Cyclopenten-1-one, 2,3,4-trimethyl-	91
46	15.96	2-Cyclopenten-1-one, 3-methyl-	94
47	16.46	2-Cyclopenten-1-one, 2,3-dimethyl-	92
48	16.80	Pyrrole, 1-methyl-3-(1,1-dimethylethyl)-	82
49	16.98	3-Cyclohexen-1-one, 3,5,5-trimethyl-	85
50	17.11	1H-Pyrrole, 3-ethyl-2,4,5-trimethyl-	87
51	17.40	2-Isoamylpyrazine	86
52	17.47	5-Ethyl-2-furaldehyde	81
53	18.25	5H-5-Methyl-6,7-dihydrocyclopentapyrazine	93
54	18.76	Pyrazine, (1-methylethenyl)-	85
55	19.26	1H-Pyrrole, 2,3,5-trimethyl-	89
56	19.43	5H-Cyclopentapyrazine, 6,7-dihydro-2,5-dimethyl-	85
57	19.86	1H-Pyrrole, 2-ethyl-3,5-dimethyl-	93
58	19.97	2-Cyclopenten-1-one, 2-pentyl-	83
59	20.26	Pyrazine, 2-methyl-5-(1-propenyl)-, (E)-	80
60	20.64	1H-Pyrrole, 2-ethyl-3,5-dimethyl-	85
61	20.77	1H-Pyrrole, 3-ethyl-2,4-dimethyl-	93
62	21.24	1H-Pyrrole, 3,4-diethyl-2-methyl-	87
63	21.36	1H-Pyrrole, 2,3,4,5-tetramethyl-	89
64	21.92	1H-Pyrrole, 3-ethyl-2,4,5-trimethyl-	94
65	22.01	N-Ethyl-3-methoxyaniline	82
66	22.29	1H-Pyrrole, 2-ethyl-3,4,5-trimethyl-	93
67	22.61	Phenol, 3-(ethylamino)-4-methyl-	81
68	23.27	2-Butanone, 4-phenyl-	83
69	25.38	Ethanone, 1-(1H-pyrrol-2-yl)-	93
70	25.68	1-Adamantanamine, N,N-dimethyl-	79
71	26.07	Phenol	93
72	27.49	Phenol, 4-methyl-	95
73	27.92	N-[2-Hydroxyethyl]succinimide	84
74	29.14	Phenol, 4-ethyl-	90
75	32.10	Pyrazine, (2-phenylethyl)-	87
76	33.51	Indole	95
77	34.36	1H-Indole, 2-methyl-	94
78	38.23	1H-Pyrrole, 3,5-dimethyl-2-phenyl-	87
79	40.51	n-Hexadecanoic acid	90
80	43.34	Octadecanoic acid	88
81	43.73	cis-13-Octadecenoic acid	80
82	44.81	2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-	88

5.2.2.Protein and Glucose

When egg albumin and glucose was mixed, the solid residue yield slightly decreased, the refined oil yield increased, the aqueous product yield decreased, and the gas yield increased (Figure 5-17). The pH value of the aqueous product for the mixture feedstock (P:G=1:1) was 7.66, which is exactly the same as that of the mixture of protein and cellulose at the same mixing ratio.

Minowa et al. (2004) studied the HTL of glucose and glycine at 150-350^{o}C with a mixing ratio of 2.4:1 (mass ratio). At 150^{o}C, no oil or char was formed but the maillard reaction has already started. They observed the formation of oil product starting from 250^{o}C and the oil yield increased with the reaction temperature.

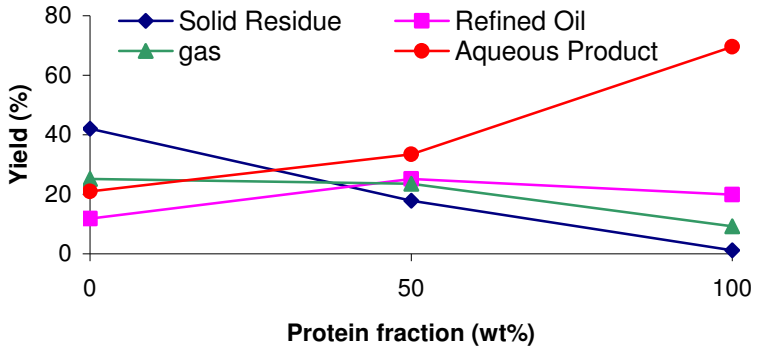


Figure 5-17. Product distribution of HTL tests with mixtures of egg albumin and glucose at different ratio.

5.2.3. Protein and Xylose

Based on the product distribution (Figure 5-17), xylose behaved similar as glucose during the HTL when mixed with egg albumin. This similarity has already occurred in previous section (5.1.4) when they were hydrothermally liquefied individually. Xylose could also react with amino acids through maillard reaction to form melanoidin. The pH value of the aqueous product for the mixture feedstock (P:G=1:1) was 7.88, compared to 7.66 from the HTL of mixture of egg albumin and glucose.

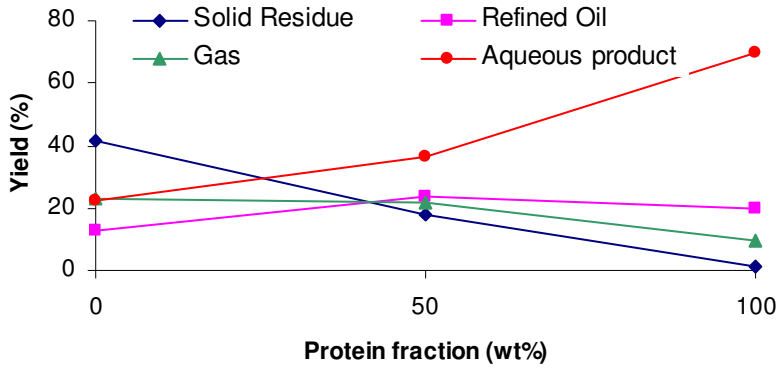


Figure 5-18. Product distribution of HTL tests with mixtures of egg albumin and xylose at different ratio.

5.2.4. Protein and Lipid

When protein and lipid was mixed as the feedstock in the HTL process, an increase of refined oil yield, a decrease of aqueous product yield and a slightly increase of gas yield were observed (Figure 5-19). The main interaction between these two feedstocks is possibly the formation of amides.

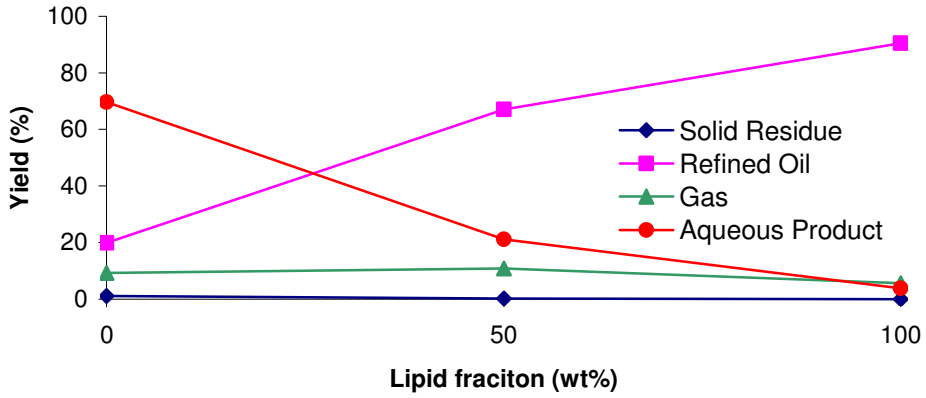


Figure 5-19. Product distribution of HTL tests with mixtures of egg albumin and butter at different ratio

5.2.5. Lipid and Cellulose

When the mixture of lipid and cellulose was used as feedstock during the HTL process, an increase of refined oil and a decrease of solid residue were observed (Figure 5-20). Compared to the individual HTL results of them, the oil product from the mixture contained more short chain alkanes and cyclic alkanes. Also, more long chain alkanes were formed through the HTL. This

results show the promotion of these two compounds to each other towards favorable products since to get more oil and less solid residue are two of the major goals of the HTL process.

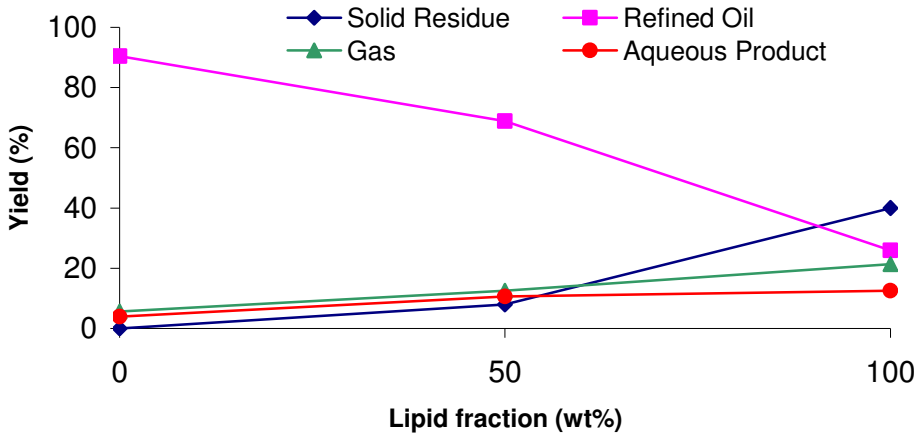


Figure 5-20.Product distribution of HTL tests with mixtures of butter and microcrystalline cellulose at different ratio.

The detailed mechanisms are not very clear yet. However, the generation of organic acids may be one of the possible reasons promoting this process. The hydrolysis of cellulose could generate acidic products, which can be seen from the pH value (2.42) after HTL of cellulose. Under acidic conditions, the HTL of lipid will generate long chain alkanes, which is shown in the GC-MS analysis. Minami and Saka (2006) stated that proton has catalytic effects on the hydrolysis of triglycerides. And our study supported this conclusion.

CHAPTER 6. ANALYSIS OF HTL MECHANISMS

6.1. RELATIONSHIP BETWEEN THE REFINED OIL YIELD AND THE INDIVIDUAL COMPOSITIONS IN THE REAL BIOMASS

Real biomass usually has very complicated compositions. Biowastes such as livestock manure and sewage sludge don't have dominant compounds like lignocellulosic biomass does. The major components in biomass could be roughly classified into protein, lipid, cellulose, hemicellulose, lignin, sugars, and ash. Neutral detergent fiber (NDF) is regarded as the sum of lignin, cellulose and hemicellulose, and acid detergent fiber (ADF) usually refers to the sum of lignin and cellulose. Total carbohydrate is defined as the sum of NDF and soluble carbohydrates. Based on the HTL test results of swine manure, cattle manure, sewage sludge, sawdust, algae, specific fractions of swine manure, mixtures of swine manure and sawdust with specific ratio, etc., a relationship between some of the major categories and the refined oil yield was obtained. The feedstocks used are listed in Table 6-1, together with the weight percentage of specific compounds and the refined oil yields.

Table 6-1. The compositions and refined oil yield from HTL for different feedstocks.

#	Feedstock	Weight Percentageof TS (%)						Weight Percentage of VS (%)			
		Lipid	Protein	NDF	CH[a]	Ash	Refined Oil Yield	Lipid	Protein	NDF	CH
1	Fresh swine manure #1	18.8	26.9	35.0	42.9	11.4	38.0	21.2	30.4	39.5	48.4
2	Nursery swine manure	15.9	29.4	35.3	40.5	12.3	38.7	18.1	33.5	40.3	46.2
3	Fresh swine manure #2 [b]	16.4	25.3	35.4	44.1	14.2	36.4	19.1	29.5	41.3	51.4
4	Soluble fraction in fresh swine manure [b]	29.1	46.2	15.7	15.7	20.9	62.6	36.8	58.4	19.8	19.9
5	Solid fraction in fresh swine manure [b]	5.0	11.4	54.4	71.7	11.9	14.5	5.7	12.9	61.7	81.4
6	Sawdust	0.8	1.5	88.7	88.7	1.2	10.0	0.8	1.5	89.8	89.8
7	Fresh sowmanure	20.0	22.6	22.2	32.5	24.9	32.3	26.6	30.1	29.6	43.3
8	Fresh cattle manure	6.8	26.6	52.5	52.5	23.5	22.9	8.9	34.8	68.6	68.6
9	14d pit swine manure +sawdust #1	7.0	15.6	63.6	66.2	7.84	22.6	7.6	17.0	69.1	71.8
10	14d pit swine manure +sawdust #2	10.2	23.1	54.3	56.0	11.4	27.9	11.6	26.1	61.2	63.1
11	Mixture of model compounds #1	15.0	30.0	35.0	45.0	10.0	33.0	16.7	33.3	38.9	50.0
12	Mixture of model compounds #2	20.0	50.0	20.0	20.0	10.0	29.9	22.2	55.6	22.2	22.2
13	Diatom [c]	5.6	40.0	10.1	18.7	32.6	32.1	8.3	59.3	15.0	27.8
14	Chlorella [c]	0.5	71.3	1.0	23	5.6	35.4	0.5	75.5	1.1	24.4
15	Spirulina [c]	5.1	64.4	2.1	21	9.5	37.3	5.6	71.2	2.3	23.2
16	Algae from Algaewheel [d]	3.7	29.0	29.9	29.9	25.7	31.0	5.0	39.0	40.2	40.3
17	Sewage sludge	0.0	41.6	51	41.1	31.2	32.5	0.0	60.5	74.1	59.7

[a]- CH = Carbohydrates; [b]- Obtained from Dong (2009); [c]- Obtained from Yu (2011); [d]- Obtained from Zhou (2011)

A prediction of refined oil yield based on the linear combination of refined oil yields of individual model compounds (lipid, protein and carbohydrates) are performed (Table 6-2). However, the results are not satisfactory and the errors range from -58.48% to 83.52%. The errors for the algal feedstocks and sewage sludge are relatively higher than others and are not acceptable. Possible reasons for these large errors may include: 1) Not all the compositions in the feedstocks are considered during the prediction; 2) There are some problems directly transferring the results of simple model compounds to complex real biomass.

Table 6-2. A prediction of refined oil yield based on the linear combination of individual refined oil yield of model compounds.

		Weight Percentageof TS (%)			Refined Oil yield (%)		
#	Feedstock	Lipid	Protein	CH[a]	Measured	Predicted	Error (%)
1	Fresh swine manure #1	18.8	26.9	42.9	38.0	33.5	13.4
2	Nursery swine manure	15.9	29.4	40.5	38.7	30.8	25.8
3	Fresh swine manure #2	16.4	25.3	44.1	36.4	31.3	16.1
4	Soluble fraction in fresh swine manure	29.1	46.2	15.7	62.6	39.6	58.0
5	Solid fraction in fresh swine manure	5.0	11.4	71.7	14.5	25.4	-43.0
6	Sawdust	0.8	1.5	88.7	10.0	24.1	-58.5
7	Fresh sow manure	20.0	22.6	32.5	32.3	31.1	4.0
8	Fresh cattle manure	6.8	26.6	52.5	22.9	25.1	- 8.9
9	14d pit swine manure +sawdust #1	7.0	15.6	66.2	22.6	26.6	-15.1
10	14d pit swine manure +sawdust #2	10.2	23.1	56.0	27.9	28.4	-1.8
11	Mixture of model compounds #1	15.0	30.0	45.0	33.0	31.3	5.6
12	Mixture of model compounds #2	20.0	50.0	20.0	29.9	33.3	-10.1
13	Diatom	5.6	40.0	18.8	32.1	17.9	79.6
14	Chlorella	0.5	71.3	23.0	35.4	20.6	71.7
15	Spirulina	5.1	64.4	21.0	37.3	22.9	63.0
16	Algae from Algaewheel	3.7	29.0	29.9	31.0	16.9	83.5
17	Sewage sludge	0.0	41.6	41.1	32.5	19.0	71.4

To further develop the methods for refined oil prediction, linear regression analysis is performed. Figures 6-1 shows the relationship between refined oil yield and the weight percentage of lipid in the total solid of the feedstocks. With tests 1-10, the linear regression between the lipid content in the feedstock and the refined oil yield is good, with R^2=0.9177. The main difference between the feedstock 13-17 and 1-10 is that samples 1-10 have a more balanced distribution of bulk carbohydrates, proteins and crude lipids. To predict the oil yield from swine-manure-like feedstocks, it is better to use the formula:

$$Y_{11} = 1.6701X_L + 8.8709 \quad (6\text{-}1)$$

While to predict the oil yield from algae or biomass similar to algae, the formula 6-2 performs better:

$$Y_{21} = 0.0333\ X_L + 33.565 \quad (6\text{-}2)$$

Figure 6-2 shows the relationship between refined oil yield and the weight percentage of protein in the total solid of the feedstocks. Similar trend was obtained as from the regression of lipid. With the tests 1-10, a regression between the protein content in the feedstock and the reined oil yield is good, with R^2=0.8877. Formula 6-3 could be used to predict the refined oil yield based on the protein content in feedstock.

$$Y_{12} = 1.1828\ X_p + 3.5485 \quad (6\text{-}3)$$

Again, for tests 12-17, a much smaller slope was obtained. When protein content is higher than a certain level (roughly 30% of TS) and becomes the dominant position in the feedstock, it is better to use the formula 6-4 to predict the oil yield based on protein content.

$$Y_{22} = 0.1341\ X_p + 27.059 \quad (6\text{-}4)$$

Figure 6-3 shows the relationship between refined oil yield and the weight percentage of carbohydrates in the total volatile solid of the feedstocks. It needs to be mentioned that in this regression, weight percentage of volatile solid is used instead of total solid. Carbohydrates showed a negative effect on refined oil production in this study. In a co-existing system of carbohydrates, protein, lipids and other chemicals, less carbohydrates content meant more refined oil formation. There are many interactions among the hydrolysis products of carbohydrates and those of protein/lipid, making the reaction pathway different from the reaction mechanisms of those feedstocks containing little or no protein and lipid at all.

With tests 1-10, a good linear regression is obtained, which gives R^2 =0.9450. The formula is as formula 6-5

$$Y_{13} = -0.7014\ X_c + 71.543 \quad (6\text{-}5)$$

With tests 13-17, the refined oil yield could be predicted with the formula 6-6

$$Y_{23} = -0.0984\ X_c + 37.114 \quad (6\text{-}6)$$

Summarizing the regressions in Figure 6.1-6.3, when a feedstock with balanced distribution of bulk carbohydrates, protein and lipid (X_p <30, X_L <20 and X_c <80), the refined oil product yield could be predicted with formula 6-7:

$$Y_1 = (Y_{11}+Y_{12}+Y_{13}) / 3 \quad (6\text{-}7)$$

When Xp >30, X_L < 30, using formula 6-8 gives more accurate result.

$$Y_2= (Y_{21} + Y_{22} +Y_{23}) / 3 \quad (6\text{-}8)$$

When Xp >30, X_L > 30, formula 6-7 could also be used. But this prediction is rather shaky, since not enough tests have been done to examine it.

When X_c > 80 which means the carbohydrates are the dominant composition in the feedstock, using formula 6-5 gives the closest results between predicted yield and actual yield.

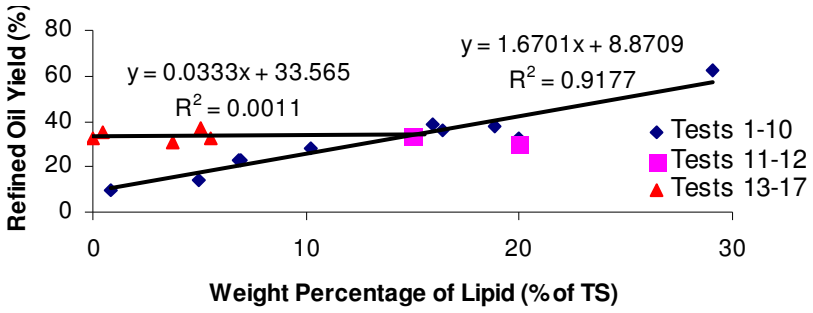


Figure 6-1. Relationship between refined oil yield and the lipid content of TS in the feedstocks.

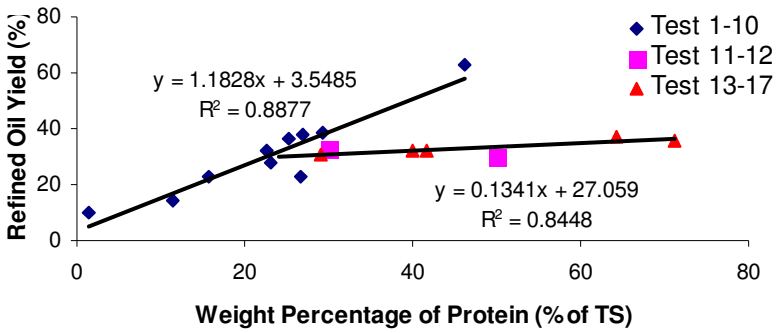


Figure 6-2. Relationship between refined oil yield and the protein content of TS in the feedstocks.

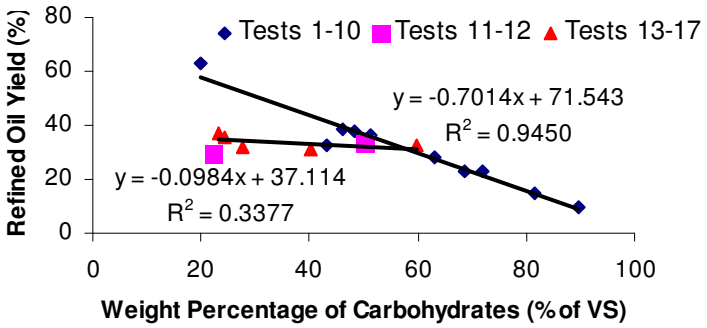


Figure 6-3. Relationship between refined oil yield and the carbohydrates content of VS in the feedstocks.

Table 6-3 shows the prediction results using the formulae above for different feedstocks. The errors listed show that for tests 11 and 12, the errors are -1.68% and 14.74%, respectively.

The refined oil yield analysis shows that, in a feedstock system that contains protein, lipids and carbohydrates, protein and lipids are two of the most important positive factors for the bio-crude oil formation. Less carbohydrates content is beneficial in terms of refined oil yield, but not necessary the oil quality. The oil quality is not discussed here.

Table 6-3. Prediction of refined oil yield based on the compositions of feedstocks.

Test#	X_L	X_P	X_c	Y_{11}	Y_{12}	Y_{13}	Y_1	Y_{21}	Y_{22}	Y_{23}	Y_2	Refined oil yield (%)		Formula	Error (%)
												Measured	Predicted		
1	18.8	26.9	48.4	40.27	35.37	37.58	37.82	34.19	30.67	32.35	32.40	38.00	37.82	6-7	-0.48
2	15.9	29.4	46.2	35.43	38.32	39.15	36.87	34.09	31.00	32.57	32.56	38.70	36.87	6-7	-4.72
3	16.4	25.3	51.4	36.26	33.47	35.49	34.87	34.11	30.45	32.06	32.21	36.40	34.87	6-7	-4.21
4	29.1	46.2	19.9	57.47	58.19	57.62	57.83	34.53	33.25	35.16	34.32	62.60	57.83	6-7	-7.62
5	5.0	11.4	81.4	17.22	17.03	14.46	17.13	33.73	28.59	29.11	30.47	14.50	14.46	6-5	-0.28
6	0.8	1.5	89.8	10.22	5.26	8.59	7.74	33.59	27.25	28.28	29.71	10.00	8.59	6-5	-14.08
7	20.0	22.6	43.3	42.27	30.28	41.19	36.28	34.23	30.09	32.86	32.39	32.30	36.28	6-7	12.31
8	6.8	26.6	68.6	20.28	35.01	23.41	27.64	33.79	30.63	30.36	31.59	22.90	21.84	6-1&6-5	-4.62
9	7.0	15.6	71.8	20.53	22.06	21.16	21.29	33.80	29.16	30.05	31.00	22.62	21.29	6-7	-5.86
10	10.2	23.1	63.1	25.97	30.92	27.27	28.45	33.91	30.16	30.90	31.66	27.90	28.45	6-7	1.96
11	15.0	30.0	50.0	33.92	39.03	36.47	36.48	34.06	31.08	32.19	32.45	33.00	32.45	6-8	-1.68
12	20.0	50.0	22.2	42.27	62.69	55.96	52.48	34.23	33.76	34.93	34.31	29.90	34.31	6-8	14.74
13	5.6	40.0	27.8	18.17	50.86	52.03	34.52	33.75	32.42	34.38	33.52	32.10	33.52	6-8	4.41
14	0.5	71.3	24.4	9.71	87.88	54.45	48.79	33.58	36.62	34.72	34.97	35.40	34.97	6-8	-1.21
15	5.1	64.4	23.2	17.39	79.72	55.27	48.55	33.73	35.70	34.83	34.75	37.30	34.75	6-8	-6.83
16	3.7	29.0	40.3	15.07	37.85	43.31	26.46	33.69	30.95	33.15	32.60	31.02	32.60	6-8	5.08
17	0.0	41.6	59.7	8.87	52.75	29.64	30.81	33.57	32.64	31.24	32.48	32.50	32.48	6-8	-0.06

6.2. EFFECTS OF SODIUM CARBONATE ON THE HTL OF MODEL COMPOUNDS

Figure 6-4 compared the product distribution of HTL tests of several model compounds with and without Na_2CO_3 as catalysts. It can be seen that the addition of Na_2CO_3 did not show profound effects on the hydrothermal liquefaction of protein based on the product distribution. However, significant distribution changes occurred for cellulose, glucose and xylose. And the trends of changes are rather similar for these three compounds. The solid residue decreased more than a half from about 40% to less than 20%. The refined oil slightly decreased. Gas product yield increased 5-10%. And the aqueous product also increased from ~20% to 40% for glucose and xylose, and from 15% to 35% for cellulose.

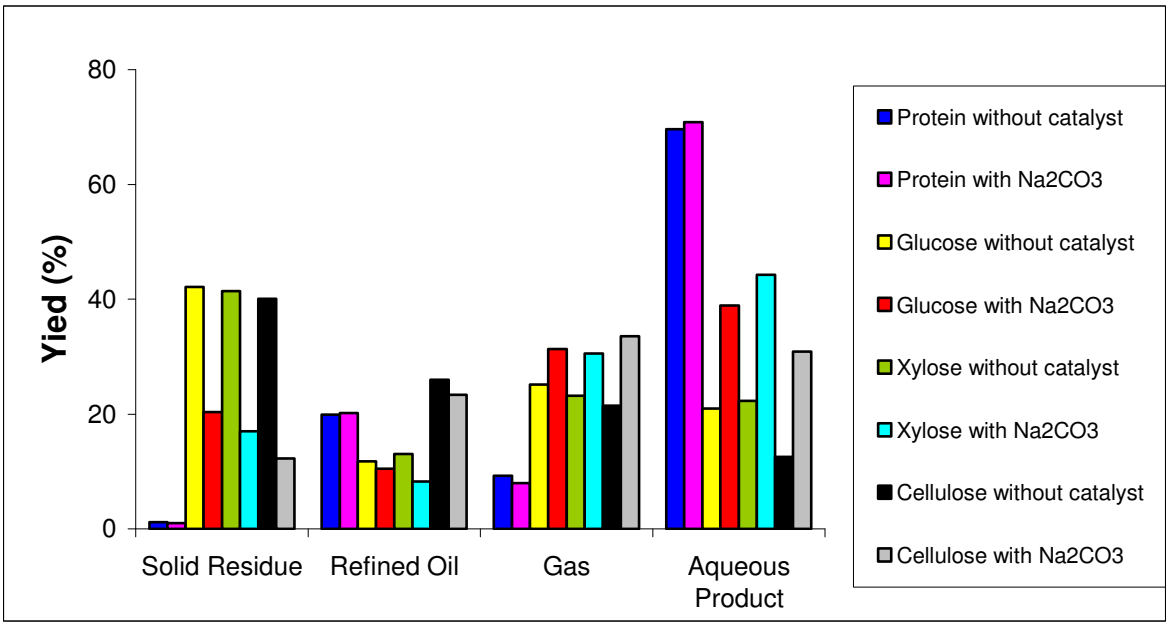


Figure 6-4. Effects of Na_2CO_3 (wt 5%) on the product distribution for HTL of model compounds.

Researchers previously have concluded that alkaline catalysts have profound effects on the HTL of cellulose and cellulosic biomass. It was observed that the presence of alkali salts, (e.g. Na_2CO_3) led to an increased rate of degradation and gasification of cellulose and to a decreased char formation (Minowa et al., 1998). Yokoyama et al. (1985) used 3% Na_2CO_3 as catalyst during the HTL of wood at 300°C, and found that the heavy oil yield increased dramatically from 5% to 50.8%, and the solvent they used to extract the heavy oil product was acetone. They liquefied 5g of wood with in 30ml of water. At 350°C, 30min retention time, and 2MPa initial argon pressure, they found when 0.1g of K_2CO_3 was added, the heavy oil yield increased from

5.0% to 21.4%, but further increasing the concentration of catalyst had little effect on the heavy oil yield. A maximized yield of 47.6% was obtained at 300^{o}C, 0min, 4-5% wt% catalyst and 2.0MPa initial pressure.

In our study, the addition of 5% Na_2CO_3 also significantly reduced the yield of solid residue, increased the yield of gas and aqueous product yield. However, no significantly positive effect was found for the oil formation, which is quite different from the results of other researchers. The difference of oil yield change may be due to the different solvent used in the studies and sometimes the different definition of "oil". Toluene, a solvent considered non-polar has a much lower ability to dissolve polar molecules than acetone does. Therefore, more polar products could be extracted using acetone. According to Nelson et al. (1984), the confirmed components in cellulose oil they listed were all polar compounds.

When 5% Na_2CO_3 was added to the mixture of protein and cellulose (1:1), it showed different effects on the product distribution than it did on protein and cellulose individually. A significant decrease of solid residue yield and a significant increase of refined oil yield were observed. However, no profound influence was shown on the gas and aqueous product yield.

Alkali acts as a catalyst for hydrolysis of cellulose into small fragments and for prevention of undesirable reactions, such as polymerization. Ammonia could be generated from protein and functions as a basic catalyst and prevent the occurrence of undesirable reactions (Inoue et al., 1999). In the previous chapter (5.2.1), this catalytic effect of protein (ammonia) on the decrease of solid residue has already been described. However, no significant oil yield increase was found. When additional Na_2CO_3 was added here, the solid residue was further reduced and the refined oil yield increased about 10%. This observation shows that to obtain maximum oil yield using the mixture of cellulose and protein under current conditions, the ammonia generated during the reaction itself is probably not sufficient and certain amount of catalyst is necessary. Otherwise, different reaction conditions are needed.

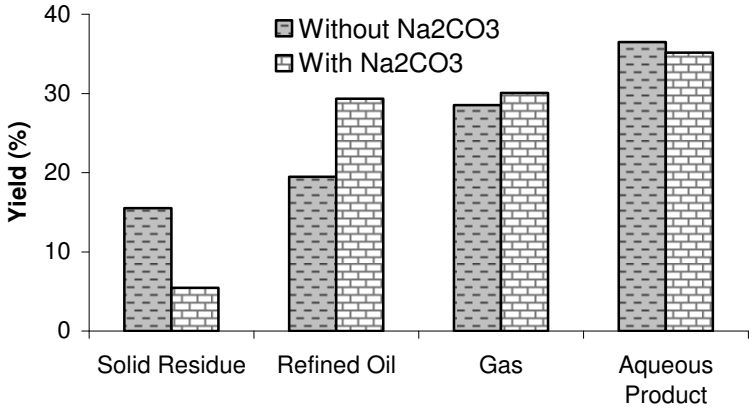


Figure 6-5. Effects of adding Na_2CO_3 on the HTL of mixture of protein and cellulose.

A widely accepted reaction mechanism describing the catalytic effects of sodium carbonate with the presence of CO is proposed by Appell (1967):

$$Na_2CO_3 + 2CO + H_2O \rightarrow 2HCOONa + CO_2 \quad (6\text{-}9)$$

$$\text{-CH(OH)-CH(OH)-} \rightarrow \text{-CH=C(OH)-} \rightarrow CH_2\text{-CO-} \quad (6\text{-}10)$$

$$HCOO^- + \text{-}CH_2\text{-CO-} \rightarrow \text{-}CH_2\text{-}CH(O^-)\text{-} + CO_2 \quad (6\text{-}11)$$

$$\text{-}CH_2\text{-}CH(O^-)\text{-} + H_2O \rightarrow \text{-}CH_2\text{-CH(OH)-} + OH^- \quad (6\text{-}12)$$

$$OH^- + CO \rightarrow HCOO^- \quad (6\text{-}13)$$

No significant amount of CO was found in the gas product after HTL. However, it was possible that the CO was formed during the HTL and then consumed. So it is hard to conclude if this reaction mechanism of sodium carbonate is the major one or not. Further study is still needed to understand the functionality of alkali catalysts.

6.3. A COMPREHENSIVE ROUTE-MAP OF REACTION PATHWAYS IN HTL

Based on the tests of both real biomass and model compounds, the build-up of a comprehensive rout-map of reaction pathways of different chemical compounds becomes possible.

The pathways of lipid are relatively straightforward. At lower temperature, the major reaction for lipid is hydrolysis. For example, triglycerols could hydrolyze to glycerol and fatty acids, and phosphate ester could hydrolyze to release phosphoric acid and fatty acids. As temperature increases, alkanes and alkenes could be formed from fatty acids selectively under different

conditions. And with the presence of ammonia, amides could be generated though the combination of fatty acids and ammonia.

Pathways of protein during HTL are relatively more complicated than those of lipids. First of all, the hydrolysis of protein is relatively slow compared to lipids. From the investigation of HTL of swine manure, the hydrolysis of protein to amino acids is not an instant reaction that could happen in seconds. In stead, this hydrolysis process could last for minutes to hours, and may easily keep happening during the entire HTL process. As the hydrolysis happens, the products could further decompose, react with each other, or react with other chemicals in the reaction system. This makes the reaction system even more complicated.

The major reaction pathways of protein, amino acids and their derivatives include:
1. Gradual hydrolysis from protein to amino acids
2. Deamination of amino acids to form organic acids and ammonia
3. Decarboxylation of amino acids to form carbon dioxide and amines
4. Re-combination of amino acids to form dimers
5. Maillard reaction at the presence of reductive sugars to form melanoidins
6. C-C bond scission of amino acids to form fragment products
7. Other reactions

The reaction pathways of cellulose and glucose and their derivatives include
1. Hydrolysis of cellulose to oligomers and glucose, releasing acetic acids at the same time
2. Fragmentation of oligomers and glucose to C_2,C_3,C_4,C_5 chemicals, including ketones, acids, aldehydes, alcohols, etc.
3. Maillard reaction in the presence of protein (amino acids)
4. Isomerization of glucose to fructose
5. Dehydration of glucose to 5-HMF, furfural, etc.
6. Re-polymerization of fragmented intermediates to form oil products and char
7. Transformation between ketones, acids, aldehydes, alcohols, etc.
8. Decarboxylation to form carbon dioxide or sometimes carbon monoxide

The reaction pathways of hemicellulose are somewhat similar to those of cellulose, with some specific reactions solely belonging to hemicellulose and its derivatives. The major reaction may include:

1. Hydrolysis to oligomers and monosugars (e.g. xylose, arabinose, glucose, etc.), releasing acetic acids at the same time
2. Fragmentation of oligomers and monosugars to C_2,C_3,C_4,C_5 chemicals, including ketones, acids, aldehydes, alcohols, etc.
3. Maillard reaction in the presence of protein (amino acids)
4. Dehydration of xylose to furfural
5. Re-polymerization of fragmented intermediates to form oil products and char
6. Transformation between ketones, acids, aldehydes, alcohols, etc.
7. Decarboxylation to form carbon dioxide or sometimes carbon monoxide

Usually it is difficult, if not impossible, to define all the reaction pathways for the HTL of real biomass. However, it is possible to explore the major reaction pathways for different feedstocks. Take the HTL of swine manure as an example. The release of fatty acids, the hydrolysis of hemicellulose and proteins and the Maillard reactions are identified as the major reaction pathways at low temperature (<240^{o}C). When temperature increases to 300^{o}C or even higher, the reaction could include the formation of amides, the formation of alkanes and alkenes from fatty acids, the intensive hydrolysis of cellulose, and the further decomposition of melanodins to form all kinds of products.

Based on the above results and discussion, a scheme of the reaction pathways during the HTL of biomass is proposed in Figure 6-6.

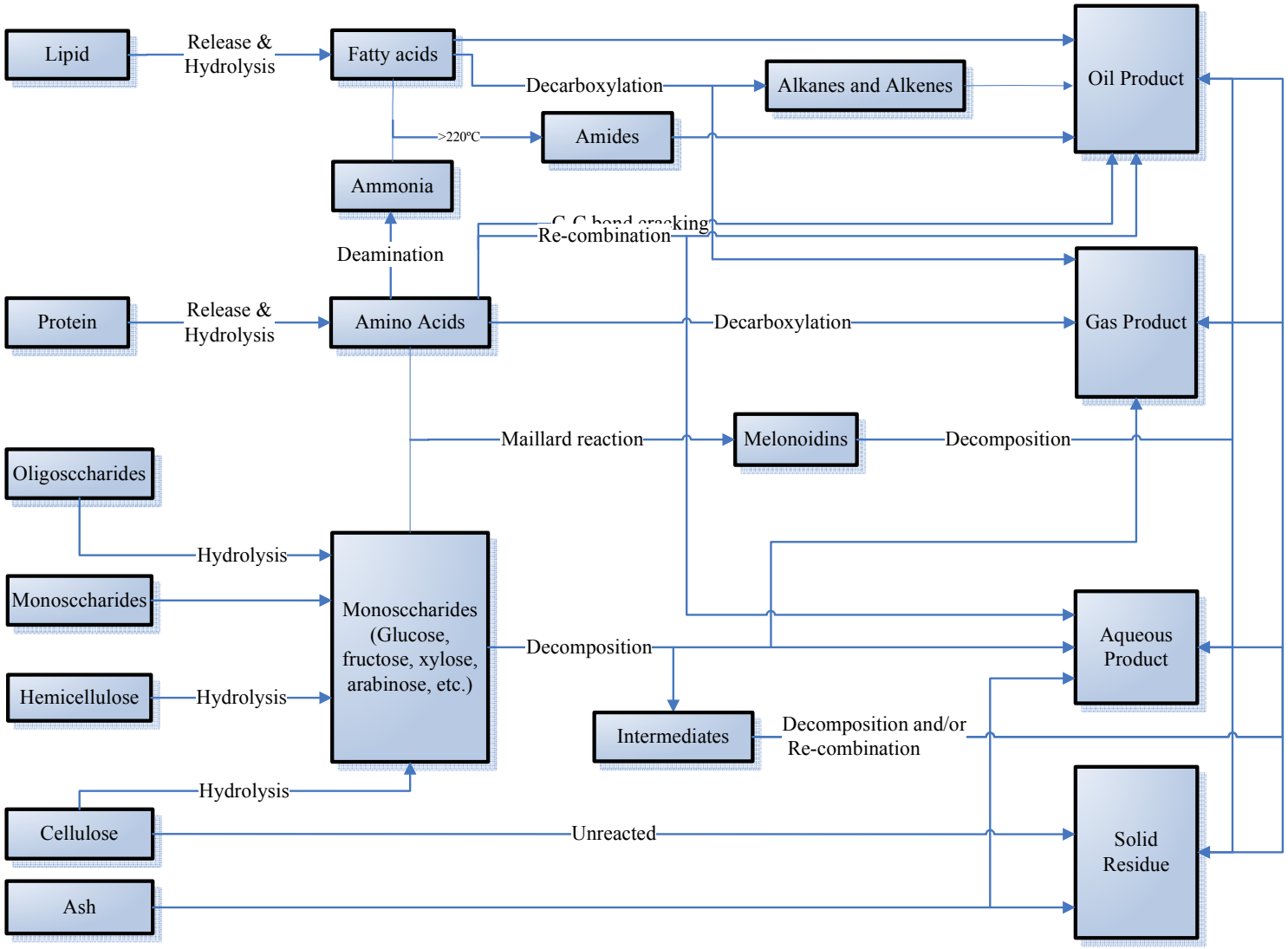


Figure 6-6. A proposed scheme of reaction pathways during the HTL of swine manure at low temperature.

CHAPTER 7. SUMMARY AND RECOMMENDATIONS

7.1. SUMMARY

It has been demonstrated that hydrothermal liquefaction (HTL) could efficiently reduce COD and pollutants in the bio-wastes and generate valuable renewable bio-crude oil from various biomass sources. Previously, swine manure has been successfully converted into bio-crude oil through HTL with yield as high as 70% of the volatile solids. To further understand the fundamental mechanisms of HTL and to provide information for further developments of this technology, experiments with both model compounds and real biomass (e.g. swine manure) as feedstocks were conducted, the product distribution and the properties were analyzed, and the reaction pathways of specific compounds were analyzed. Major findings and conclusions are summarized as followed.

1) HTL tests were conducted using swine manure collected from different sources. The testing conditions were: reaction temperature 305^{o}C, initial solid content 20% wt, retention time 30 min, initial N_2 gas pressure 0.65 MPa, and without catalysts or additives. Comparison between the bio-crude oil obtained from HTL of nursery, grower-finisher and sow pigs showed no significant differences regarding oil yield and compositions. Length of manure storage time in a shallow pit did not profoundly affect the formation of bio-crude oil through HTL, although a slight decrease of refined oil from 42% to 35% was observed.

2) HTL tests of swine manure at transient temperatures (180-240^{o}C) and retention times of 0-60 min showed that the release of fatty acids, the hydrolysis of hemicellulose and proteins and the Maillard reactions were the major reactions happened at temperatures lower than 240^{o}C. Maillard reaction occurred at low temperatures during the HTL of swine manure, and the formed melanoidins are further decomposed to gas, oil, char and aqueous products. At temperature higher than 220^{o}C, fatty acids may react with ammonia to form amide products. At a certain temperature, only certain amount of swine manure could be solubilized and a barrier exists which prevents the further solubilization even with prolonged retention time. At 200^{o}C, between 0-15 min, a rapid and intensive hydrolysis of hemicellulose occurred and caused the decrease of

pH in the aqueous phase. The decreased pH probably enhanced the decomposition of melanoidins. Similar product distribution could be obtained at higher temperature and shorter retention time, or vice versa.

3) A refined oil yield of 20% was obtained at 300^{o}C from the HTL of egg albumin. Tests conducted at 200-300^{o}C showed that refined oil yield increased with the increase of temperature, and degradation of large molecules and re-combination of small molecules generated in the degradation process were probably the two major pathways for bio-crude oil formation from protein. A brief process of aggregation and polymerization appeared to occur during initial heating in the egg albumin before the polymer hydrolyzed and degraded again. Increased temperature also improved bio-crude oil quality by increasing the carbon content and heating value of the oil, and decreasing the nitrogen and oxygen contents in the oil. HTL of carbohydrates generated large amount of solid residue (more than 40%), with the refined oil yield lower than 25%. However, addition of base catalysts (e.g. Na_2CO_3), lipid and protein into carbohydrates could significantly decrease the solid residue yield. Compared to the HTL of carbohydrates and protein individually, more small molecules with lower boiling point were generated during the HTL of the mixtures of them. These small molecules include pyrazine and its derivatives, pyridine and its derivatives, pyrrole and its derivatives, indole and its derivatives, phenol and its derivatives and 2-Cyclopenten-1-one and its derivatives. Addition of protein into lipid resulted in an increase of refined oil yield and the decrease of aqueous products. The main interaction probably is the formation of amides.

4) Fifteen tests data were used for regression between the refined oil yield and the lipid, protein and carbohydrates content in the feedstocks. A positive linear relationship was observed between the refined oil yield and the lipid content, as well as between the refined oil yield and the protein content. A negative linear relationship was found between the refined oil yield and the carbohydrates content in the volatile solids in the feedsocks. Based on the regression results, a series of formulae were obtained which could be used to predict the refined oil yield from HTL of biomass. The errors for two predictions for examining purpose were -1.68% and 14.74% respectively.

5) Based on the results and from both model compounds and real biomass, a scheme of the reaction pathways during the HTL of biomass was proposed. The scheme included the reactions which could possibly happen to lipid, protein, carbohydrates, etc.

7.2. RECOMMENDATIONS

The major work in this study focused on the general exploration of the mechanisms of HTL. It is more like a scouting procedure which put more efforts on the width of the study rather than the depth. Although some reaction pathways were proposed and examined, further investigations are needed to better understand the reaction mechanisms.

7.2.1. Kinetics study

Chemical kinetics is always essential for the development of a chemical process. In this study, no kinetics study was performed with swine manure or model compounds due to the larger retention time scale. It was observed that the major reaction at each reaction temperature primarily happened during the first 15 min. So to obtain the kinetics parameters, more tests are needed for shorter retention time. The batch reactor used in this study was an obstacle for kinetics study since it is relatively time consuming to use. If a continuous reactor with sampling ports which allow periodically sampling could be used, it will be much easier and probably more accurate to collect the kinetics data.

7.2.2. Quantification of the compositions in the HTL products

The major work conducted in this study regarding to the analysis of HTL products is the product distribution analysis and the identification of the chemicals in the products. Some of the compositions in the products were quantified. However, to confirm the proposed reaction pathways, it is necessary to further quantify the chemicals in all product streams as much and as accurate as possible. Once the chemicals were identified, internal and external standards could be used in the GC-MS, HPLC or other analysis to quantify them.

The quantification itself is a major task since typically in the refined oil product of real biomass (e.g. swine manure) hundreds to thousands of compounds would be observed. Even with pure

model compounds like glucose, still, many compounds would be generated. Another problem is how to bridge the results from the model compound and those from the real biomass.

7.2.3. Yield prediction model

The refined oil yield prediction model set up in this study is a regression model. Although it could predict the refined oil with relatively small error, more data are needed to further improve it. Real biomass such as poultry manure, different species of algae, crop residues, woody biomass, and sewage sludge from different sources could all be used in the HTL tests to collect data. Also, mixtures of model compounds could be used for data collection and model demonstration. However, it is necessary to mention that the "artificial" biomass is different from the real biomass and researchers always observed lower oil yield from the former one. Some investigations are needed to explain this phenomenon.

In the current model, the reaction condition was fixed, and the compositions of feedstock are the only considered factors. However, more parameters could and should be involved in this model, such as reaction temperature, retention time, catalyst used, etc.

REFERENCES

Abdelmoez, W. and H. Yoshida. 2006. Synthesis of a novel protein-based plastic using sub-critical water technology. *AIChE Journal.* 52(7): 2607-2617.

Abdelmoez, W., T. Nakahasi and H. Yoshida. 2007. Amino acid transformation and decomposition in saturated subcritical water conditions. *Industrial & Engineering Chemistry Research*. 46(16): 5286-5294.

Adschiri, T., S. Hirose, R. Malaluan and K. Arai. 1993. Noncatalytic conversion of cellulose in supercritical and subcritical water. *Journal of Chemical Engineering of Japan*. 26(6): 676-680.

Aida, T. M., N. Shiraishi, M. Kubo, M. Watanabe and R. L. Smith. 2010. Reaction kinetics of D-xylose in sub- and supercritical water. *Journal of Supercritical Fluids*. 55(1): 208-216.

Antal Jr, M. J., W. S. L. Mok and G. N. Richards. 1990. Mechanism of formation of 5-(hydroxymethyl)-2-furaldehyde from D-fructose and sucrose. *Carbohydrate Research*. 199(1): 91-109.

Antal, M. J., T. Leesomboon, W. S. Mok and G. N. Richards. 1991. Kinetic-studies of the reactions of ketoses and aldoses in water at high-temperature. 3. Mechanism of formation of 2-furaldehyde from D-xylose. *Carbohydrate Research*. 217: 71-85.

Appell, H. R. 1967. Fuels from waste. *Anderson, L., and Tilman, D. A., (Eds.). New York: Academic Press*.

Appell, H. R., I. Wender and R. D. Miller. 1970. Conversion of urban refuse to oil. Bureau of Mines solid waste program technical progress report. U.S. Department of the Interior.

Appell, H. R., Y. C. Fu, S. Friedman, P. M. Yavorsky and I. Wender. 1980. Converting organic wastes to oil: A replenishable energy source. Report of investigations 7560. Washington, D.C.: U.S. Bureau of Mines.

ASABE. 2005. Manure production and characterization, American Society of Agricultural and Biological Engineers Standard D384.2. Mar. 2005.

Bacon, J. S. D., A. H. Gordon, E. J. Morris and V. C. Farmer. 1975. Acetyl groups in cell-wall preparations from higher-plants. *Biochemical Journal.* 149(2): 484-487.

Balat, M. 2008. Mechanisms of thermochemical biomass conversion processes. Part 3: Reactions of liquefaction. *Energy Sources Part A-Recovery Utilization and Environmental Effects*. 30(7): 649-659.

Beckman, D. and D. C. Elliott. 1985. Comparisons of the yields and properties of the oil products from direct thermochemical biomass liquefaction processes. *Canadian Journal of Chemical Engineering*. 63(1): 99-104.

Behrendt, F., Y. Neubauer, M. Oevermann, B. Wilmes and N. Zobel. 2008. Direct liquefaction of biomass. *Chemical Engineering & Technology*. 31(5): 667-677.

Berl, E. 1934. Origin of asphalts, oil, natural gas and bituminous coal. *Science*. 80(2071): 227-228.

Berl, E. 1944. Production of oil from plant material. *Science*. 99(2573): 309-312.

Biller, P. and A. B. Ross. 2011. Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content. *Bioresource Technology.* 102(1): 215-225.

Bobleter, O. 1994. Hydrothermal degradation of polymers derived from plants. *Progress in Polymer Science*. 19(5): 797-841.

Bonn, G. and O. Bobleter. 1983. Determination of the hydrothermal degradation products of D-(U-^{14}C) glucose and D-(U-^{14}C) fructose by TLC. *Journal of Radioanalytical Chemistry*. 79(2): 171-177.

Boocock, D. G. B. and K. M. Sherman. 1985. Further aspects of powdered poplar wood liquefaction by aqueous pyrolysis. *Canadian Journal of Chemical Engineering*. 63(4): 627-633.

Boocock, D. G. B. and L. Kosiak. 1988. A scanning electron-microscope study of structural-changes during the liquefaction of poplar sticks by rapid aqueous thermolysis. *Canadian Journal of Chemical Engineering*. 66(1): 121-126.

Bouveng, H. O. 1961. Studies on arabogalactans. 5. Barry degradation of arabogalactans from western larch- a kinetic study of mild acid hydrolysis of araboglactan. *A. Acta Chem. Scand.* 15(1): 78-86.

Catallo, W. J., T. F. Shupe, J. L. Comeaux and T. Junk. 2010. Transformation of glucose to volatile and semi-volatile products in hydrothermal (HT) systems. *Biomass & Bioenergy*. 34(1): 1-13.

Chareonlimkun, A., V. Champreda, A. Shotipruk and N. Laosiripojana. 2010. Reactions of c5 and c6-sugars, cellulose, and lignocellulose under hot compressed water (HCW) in the presence of heterogeneous acid catalysts. *Fuel*. 89(10): 2873-2880.

Chheda, J. N. and J. A. Dumesic. 2007. An overview of dehydration, aldol-condensation and hydrogenation processes for production of liquid alkanes from biomass-derived carbohydrates. *Catalysis Today*. 123(1-4): 59-70.

Chornet, E. and R. Overend. 1985. Biomass liquefaction: An overview. In*: Proceedings of International Conference on Fundamentals of Thermochemical Biomass Conversion, New York, NY, Elsevier Applied Science.*: 967-1002.

Davies, P. 2006. Quantifying energy. BP Statistical Review of Energy 2006. Available at Http://www.Bp.Com/liveassets/bp_internet/globalbp/globalbp_uk_english/publications/energy_reviews2006/staging/local_assets/downloads/powerpoint/statistical_review_full_report_slidepack_2006.ppt. Accessed on Feb. 25, 2010.

Demirbas, A. 2000a. Mechanisms of liquefaction and pyrolysis reactions of biomass. *Energy Conversion and Management*. 41(6): 633-646.

Demirbas, A. 2000b. Effect of lignin content on aqueous liquefaction products of biomass. *Energy Conversion and Management.* 41: 1601-1607.

Demirbas, M. F. 2006. Current technologies for biomass conversion into chemicals and fuels. *Energy Sources Part A-Recovery Utilization and Environmental Effects*. 28(13): 1181-1188.

Dong, R., Y. Zhang, L. L. Christianson, T. L. Funk, X. Wang, Z. Wang, M. Minarick and G. Yu. 2009. Product distribution and implication of hydrothermal conversion of swine manure at low temperatures. *Transaction of the American Society of Agricultural and Biological engineers.* 52(4): 1239-1248.

Dote, Y., S. Inoue, T. Ogi and S. Yokoyama. 1996. Studies on the direct liquefaction of protein-contained biomass: The distribution of nitrogen in the products. *Biomass Bioenergy*. 11(6): 491-498.

Dote, Y., S. Sawayama, S. Inoue, T. Minowa and S.-y. Yokoyama. 1994. Recovery of liquid fuel from hydrocarbon-rich microalgae by thermochemical liquefaction. *Fuel*. 73(12): 1855-1857.

Dunlop, A. P. and F. N. Peters. 1953. The furans. Reinhold Publishing Co., New York

Elliott, D. C., L. J. Sealock Jr and R. S. Butner. 1987. Product analysis from direct liquefaction of several high-moisture biomass feedstocks. In *ACS Division of Fuel Chemistry, Preprints*, 186-194.

Elliott, D. C., D. Beckman, A. V. Bridgwater, J. P. Diebold, S. B. Gevert and Y. Solantausta. 1991. Developments in direct thermochemical liquefaction of biomass - 1983-1990. *Energy & Fuels*. 5(3): 399-410.

Fu, Y. C., E. G. Illig and S. J. Metlin. 1974. Conversion of manure to oil by catalytic hydrotreating. *Environmental Science & Technology*. 8(8): 737-740.

Funazukuri, T., N. Wakao and J. M. Smith. 1990. Liquefaction of lignin sulfonate with subcritical and supercritical water. *Fuel*. 69(3): 349-353.

Gandini, A. and M. N. Belgacem. 1997. Furans in polymer chemistry. *Progress in Polymer Science*. 22(6): 1203-1379.

Goudriaan, F. and D. G. R. Peferoen. 1990. Liquid fuels from biomass via a hydrothermal process. *Chemical Engineering Science.* 45(8): 2729-2734.

Goudriaan, F., B. van de Beld, F. R. Boerefijn, G. M. Bos, J. E. Naber, S. van der Wal and J. Zeevalkink. 2000. Thermal efficiency of the HTU process for biomass liquefaction. Paper presented at *Progress in Thermochemcial Biomass Conversion Conference, Bridgwater, A. V. (Ed.) Tyrol, Austria*: 1312-1325.

Goudriaan, F., B. v. d. Beld, F. R. Boerefijn, G. M. Bos, J. E. Naber, S. v. d. Wal and J.A.Zeevalkink. 2001. Thermal efficiency of the HTU process for biomass liquefaction, in: Proceedings of progress in thermochemical biomass conversion, Bridgewater A. (Ed.), Oxford, U.K., IEA Bioenergy, Blackwell Science.

Goyal, H. B., D. Seal and R. C. Saxena. 2008. Bio-fuels from thermochemical conversion of renewable resources: A review. *Renewable & Sustainable Energy Reviews*. 12(2): 504-517.

Graneli, E., M. Weberg and P. S. Salomon. 2008. Harmful algal blooms of allelopathic microalgal species: The role of eutrophication. *Harmful Algae*. 8(1): 94-102.

He, B. J. 2000. Thermochemical conversion of swine manure to produce oil and reduce waste. Phd Diss. Urbana, IL.: University of Illinois at Urbana-Champaign.

He, B. J., Y. Zhang, T. L. Funk, G. L. Riskowski and Y. Yin. 2000. Thermochemical conversion of swine manure: An alternative process for waste treatment and renewable energy production. *Transactions of the American Society of Agricultural Engineers*. 43(6): 1827-1833.

He, P., L. Shao, G. Gu and G. Li. 2001. Mechanism and kinetics of low-temperature thermo-chemical conversion process for sewage sludge. *Water Science and Technology*. 44(10): 341-347.

Hrubrant, G. R., R. A. Rhodes and G. H. Sloneker. 1978. Specific composition of representative feedlot wastes: A chemcial and microbial profile. Washington, D.C.: Science and Education Administration, U.S. Departmenta of Agriculture.

Huber, G. W., S. Iborra and A. Corma. 2006. Synthesis of transportation fuels from biomass: Chemistry, catalysts, and engineering. *Chemical Reviews*. 106(9): 4044-4098.

Hwang, H. I., T. G. Hartman and C. T. Ho. 1995. Relative reactivities of amino-acids in the formation of pyridines, pyrroles, and oxazoles. *Journal of Agricultural and Food Chemistry*. 43(11): 2917-2921.

Hwang, H. I., T. G. Hartman, R. T. Rosen, J. Lech and C. T. Ho. 1994. Formation of pyrazines from the maillard reaction of glucose and lysine-α-amine-^{15}N. *Journal of Agricultural and Food Chemistry*. 42(4): 1000-1004.

Inoue, S., K. Okigawa, T. Minowa and T. Ogi. 1999. Liquefaction of ammonia and cellulose: Effect of nitrogen carbon ratio in the feedstock. *Biomass & Bioenergy*. 16(5): 377-383.

Itoh, S., A. Suzuki, T. Nakamura and S. y. Yokoyama. 1994. Production of heavy oil from sewage sludge by direct thermochemical liquefaction. *Desalination*. 98(1-3): 127-133.

Jing, Q. and X. Lv. 2007. Kinetics of non-catalyzed decomposition of D-xylose in high temperature liquid water. *Chinese Journal of Chemical Engineering*. 15(5): 666-669.

Kabyemela, B. M., T. Adschiri, R. M. Malaluan and K. Arai. 1997. Kinetics of glucose epimerization and decomposition in subcritical and supercritical water. *Industrial & Engineering Chemistry Research*. 36(5): 1552-1558.

Kabyemela, B. M., T. Adschiri, R. M. Malaluan and K. Arai. 1999. Glucose and fructose decomposition in subcritical and supercritical water: Detailed reaction pathway, mechanisms, and kinetics. *Industrial and Engineering Chemistry Research*. 38(8): 2888-2895.

Karagoz, S., T. Bhaskar, A. Muto and Y. Sakata. 2005. Comparative studies of oil compositions produced from sawdust, rice husk, lignin and cellulose by hydrothermal treatment. *Fuel*. 84(7-8): 875-884.

Kato, H. and M. Fujimaki. 1968. Formation of n-substituted pyrrole-2-aldehydes in browning reaction between D-xylose and amino compounds. *Journal of Food Science*. 33(5): 445-&.

King, J. W., R. L. Holliday and G. R. List. 1999. Hydrolysis of soybean oil: In a subcritical water flow reactor. *Green Chemistry*. 1(6): 261-264.

Klass, D. K. 1998. *Biomass for renewable energy, fuels and chemicals*. San Diego, CA: Academic Press.

Klingler, D., J. Berg and H. Vogel. 2007. Hydrothermal reactions of alanine and glycine in sub- and supercritical water. *Journal of Supercritical Fluids*. 43(1): 112-119.

Knezevic, D., W. P. M. van Swaaij and S. R. A. Kersten. 2009. Hydrothermal conversion of biomass: I, glucose conversion in hot compressed water. *Industrial & Engineering Chemistry Research*. 48(10): 4731-4743.

Kruse, A. and A. Gawlik. 2003. Biomass conversion in water at 330-410 °C and 30-50 MPa. Identification of key compounds for indicating different chemical reaction pathways. *Industrial & Engineering Chemistry Research*. 42(2): 267-279.

Kruse, A., P. Maniam and F. Spieler. 2007. Influence of proteins on the hydrothermal gasification and liquefaction of biomass. 2. Model compounds. *Industrial and Engineering Chemistry Research*. 46(1): 87-96.

Kruse, A., A. Krupka, V. Schwarzkopf, C. Gamard and T. Henningsen. 2005. Influence of proteins on the hydrothermal gasification and liquefaction of biomass. 1. Comparison of different feedstocks. *Industrial & Engineering Chemistry Research*. 44(9): 3013-3020.

Kumar, S. and R. B. Gupta. 2008. Hydrolysis of microcrystalline cellulose in subcritical and supercritical water in a continuous flow reactor. *Industrial & Engineering Chemistry Research*. 47(23): 9321-9329.

Laird, D. A., R. C. Brown, J. E. Amonette and J. Lehmann. 2009. Review of the pyrolysis platform for coproducing bio-oil and biochar. *Biofuels, Bioproducts and Biorefining*. 3(5): 547-562.

Luijkx, G. C. A., F. Van Rantwijk and H. Van Bekkum. 1993. Hydrothermal formation of 1,2,4-benzenetriol from 5-hydroxymethyl-2-furaldehyde and d-fructose. *Carbohydrate Research*. 242: 131-139.

Luijkx, G. C. A., W. Vanderhorst, S. O. A. Koskinen, F. Vanrantwijk and H. Vanbekkum. 1994. Hydrothermal formation of hydroxylated benzenes from furan-derivatives. *Journal of Analytical and Applied Pyrolysis*. 28(2): 245-254.

Mahro, B. and M. Timm. 2007. Potential of biowaste from the food industry as a biomass resource. *Engineering in Life Sciences*. 7(5): 457-468.

Maschio, G., C. Koufopanos and A. Lucchesi. 1992. Pyrolysis, a promising route for biomass utilization. *Bioresource Technology*. 42(3): 219-231.

Matsui, T. O., A. Nishihara, C. Ueda, M. Ohtsuki, N. O. Ikenaga and T. Suzuki. 1997. Liquefaction of micro-algae with iron catalyst. *Fuel*. 76(11): 1043-1048.

Meeroff, D. E. 2001. Effects of ionizing radiation in wastewater treatment and residuals processing. Phd Diss. Coral Gables, FL.: University of Miami.

Milić, B. L. and M. V. Piletić. 1984. The mechanism of pyrrole, pyrazine and pyridine formation in non-enzymic browning reaction. *Food Chemistry*. 13(3): 165-180.

Mills, V. and H. McClain. 1949. Fat hydrolysis. *Industrial & Engineering Chemistry*. 41(9): 1982-1985.

Minowa, T., T. Kondo and S. T. Sudirjo. 1998. Thermochemical liquefaction of indonesian biomass residues. *Biomass and Bioenergy*. 14(5-6): 517-524.

Minowa, T., S. Inoue, T. Hanaoka and Y. Matsumura. 2004. Hydrothermal reaction of glucose and glycine as model compounds of biomass. *Nihon Enerugi Gakkaishi/Journal of the Japan Institute of Energy*. 83(10): 794-798.

Miyazawa, T., S. Ohtsu and T. Funazukuri. 2008. Hydrothermal degradation of polysaccharides in a semi-batch reactor: Product distribution as a function of severity parameter. *Journal of Materials Science.* 43(7): 2447-2451.

Mochidzuki, K., A. Sakoda and M. Suzuki. 2000. Measurement of the hydrothermal reaction rate of cellulose using novel liquid-phase thermogravimetry. *Thermochimica Acta*. 348(1-2): 69-76.

Mohan, D., C. U. Pittman and P. H. Steele. 2006. Pyrolysis of wood/biomass for bio-oil: A critical review. *Energy & Fuels*. 20(3): 848-889.

Mok, W. S. L. and M. J. Antal. 1992. Uncatalyzed solvolysis of whole biomass hemicellulose by hot compressed liquid water. *Industrial & Engineering Chemistry Research*. 31(4): 1157-1161.

Nelson, D., R. Hallen and O. Theander. 1988. Formation of aromatic compounds from carbohydrates: Reaction of xylose, glucose, and glucuronic acid in acidic solution at 300^{o}C, in pyrolysis oils from biomass (ACS symposium series 376), chapter 11, Ed. E. Soltes and t. Milne, American Chemical Society. 113–118.

Nelson, D. A., P. M. Molton, J. A. Russell and R. T. Hallen. 1984. Application of direct thermal liquefaction for the conversion of cellulosic biomass. *Industrial & Engineering Chemistry Product Research and Development.* 23(3): 471-475.

Njoroge, F. G., L. M. Sayre and V. M. Monnier. 1987. Detection of D-glucose-derived pyrrole compounds during maillard reaction under physiological conditions. *Carbohydrate Research*. 167: 211-220.

NREL. 1998. A look back at the U.S. Department of Energy's aquatic species program—biodiesel from algae. NREL/TP-580-24190. National Renewable Energy Laboratory. 1617 Cole Boulevard, Golden, Colorado.

Ocfemia, K. S., Y. Zhang and T. Funk. 2006. Hydrothermal processing of swine manure into oil using a continuous reactor system: Development and testing. *Transaction of the American Society of Agricultural and Biological Engineers.* 49(2): 533-541.

Oefner, P. J., A. H. Lanziner, G. Bonn and O. Bobleter. 1992. Quantitative studies on furfural and organic-acid formation during hydrothermal, acidic and alkaline-degradation of deuterium-xylose. *Monatshefte Fur Chemie*. 123(6-7): 547-556.

Overend, R. P. and E. Chornet. 1987. Fractionation of lignocellulosics by steam-aqueous pretreatments. *Philosophical Transactions of the Royal Society of London Series A-Mathematical Physical and Engineering Sciences*. 321(1561): 523-536.

Perlack, R. D., L. L. Wright, A. F. Turhollow, R. L. Graham, B. J. Stokes and D. C. Erbach. 2005. Biomass as feedstock for a bioenergy and bioproducts industry: The technical feasibility of a billion-ton annual supply. Washington, D.C: U.S. Department of Agriculture and U.S. Department of Energy.

Peterson, A., F. Vogel, R. Lachance, M. Froling, M. Antal and J. Tester. 2008a. Thermochemical biofuel production in hydrothermal media: A review of sub- and supercritical water technologies. *Energy Environmental Science.* 1(1): 32-65.

Peterson, A. A., R. P. Lachance and J. W. Tester. 2010. Kinetic evidence of the maillard reaction in hydrothermal biomass processing: Glucose-glycine interactions in high-temperature, high-pressure water. *Industrial & Engineering Chemistry Research.* 49(5): 2107-2117.

Peterson, A. A., F. Vogel, R. P. Lachance, M. Froling, M. J. Antal and J. W. Tester. 2008b. Thermochemical biofuel production in hydrothermal media: A review of sub- and supercritical water technologies. *Energy Environmental Science.* 1(1): 32-65.

Puig-Arnavat, M., J. C. Bruno and A. Coronas. 2010. Review and analysis of biomass gasification models. *Renewable & Sustainable Energy Reviews*. 14(9): 2841-2851.

Rizzi, G. P. 1974. Formation of n-alkyl-2-acylpyrroles and aliphatic aldimines in model nonenzymic browning reactions. *Journal of Agricultural and Food Chemistry*. 22(2): 279-282.

Rizzi, G. P. 2003. Free radicals in the maillard reaction. *Food Reviews International*. 19(4): 375-395.

Roessner, U., C. Wagner, J. Kopka, R. N. Trethewey and L. Willmitzer. 2000. Simultaneous analysis of metabolites in potato tubers by gas chromatography-mass spectrometry. *The Plant Journal*. 23: 131-142.

Rogalinski, T., T. Ingram and G. Brunner. 2008a. Hydrolysis of lignocellulosic biomass in water under elevated temperatures and pressures. *Journal of Supercritical Fluids*. 47(1): 54-63.

Rogalinski, T., K. Liu, T. Albrecht and G. Brunner. 2008b. Hydrolysis kinetics of biopolymers in subcritical water. *Journal of Supercritical Fluids*. 46(3): 335-341.

Ross, A. B., P. Biller, M. L. Kubacki, H. Li, A. Lea-Langton and J. M. Jones. 2010. Hydrothermal processing of microalgae using alkali and organic acids. *Fuel*. 89(9): 2234-2243.

Russell, J. A., R. K. Miller and P. M. Molton. 1983. Formation of aromatic compounds from condensation reactions of cellulose degradation products. *Biomass*. 3(1): 43-57.

Sasaki, M., T. Hayakawa, K. Arai and T. Adschiri. 2003. Measurement of the rate of retro-aldol condensation of D-xylose in subcritical and supercritical water, in hydrothermal reactions and techniques: The proceedings of the seventh international symposium on hydrothermal reactions, Ed. S. Feng, J. Chen and Z. Shi.

Sasaki, M., Z. Fang, Y. Fukushima, T. Adschiri and K. Arai. 2000. Dissolution and hydrolysis of cellulose in subcritical and supercritical water. *Industrial and Engineering Chemistry Research*. 39(8): 2883-2890.

Sasaki, M., B. Kabyemela, R. Malaluan, S. Hirose, N. Takeda, T. Adschiri and K. Arai. 1998. Cellulose hydrolysis in subcritical and supercritical water. *Journal of Supercritical Fluids*. 13(1-3): 261-268.

Sato, N., A. T. Quitain, K. Kang, H. Daimon and K. Fujie. 2004. Reaction kinetics of amino acid decomposition in high-temperature and high-pressure water. *Industrial & Engineering Chemistry Research*. 43(13): 3217-3222.

Schwald, W. and O. Bobleter. 1989. Hydrothermolysis of cellulose under static and dynamic conditions at high-temperatures. *Journal of Carbohydrate Chemistry*. 8(4): 565-578.

Shock, E. L. 1993. Hydrothermal dehydration of aqueous organic compounds. *Geochimica et Cosmochimica Acta*. 57(14): 3341-3349.

Srokol, Z., A. G. Bouche, A. Van Estrik, R. C. J. Strik, T. Maschmeyer and J. A. Peters. 2004. Hydrothermal upgrading of biomass to biofuel; studies on some monosaccharide model compounds. *Carbohydrate Research*. 339(10): 1717-1726.

Suyama, K. and S. Adachi. 1980. Origin of alkyl-substituted pyridines in food flavor - formation of the pyridines from the reaction of alkanals with amino-acids. *Journal of Agricultural and Food Chemistry*. 28(3): 546-549.

Suzuki, A., T. Nakamura, S. Yokoyama, T. Ogi and K. Koguchi. 1988. Conversion of sewage sludge to heavy oil by direct thermochemical liquefaction. *Journal of Chemical Engineering of Japan.* 21(3): 288-293.

USDA. 2006. Agricultural research service, FY-2005 annual report manure and byproduct utilization (Accessed November 2, 2010).

USDOE. 2002. The roadmap for biomass technologies in the U.S. Biomass R&D Technical Advisory Committee, U.S. Department of Energy, Accession No. ADA 436527.

USDOE. 2009a. Renewable energy consumption and electricity preliminary statistics http://www.Eia.Doe.Gov/cneaf/alternate/page/renew_energy_consump/rea_prereport.Html Accessed on Feb. 25, 2010.

USDOE. 2009b. Annual energy review 2008. Energy information administration, office of energy markets and end use, U.S. Department of Energy. DOE/EIA-0384(2008).

USEPA. 2008. Municipal solid waste in the United States: 2007 facts and figures. EPA530-R-08-010. EPA Office of Solid Waste. Washington D.C.

Watanabe, M., T. Iida and H. Inomata. 2006. Decomposition of a long chain saturated fatty acid with some additives in hot compressed water. *Energy Conversion and Management*. 47(18-19): 3344-3350.

Watanabe, M., Y. Aizawa, T. Iida, C. Levy, T. M. Aida and H. Inomata. 2005. Glucose reactions within the heating period and the effect of heating rate on the reactions in hot compressed water. *Carbohydrate Research*. 340(12): 1931-1939.

Williams, P. T. and J. Onwudili. 2005. Composition of products from the supercritical water gasification of glucose: A model biomass compound. *Industrial & Engineering Chemistry Research*. 44(23): 8739-8749.

Wood, T. M. and S. I. McCrae. 1986. The effect of acetyl groups on the hydrolysis of ryegrass cell-walls by xylanase and cellulase from trichoderma-koningii. *Phytochemistry*. 25(5): 1053-1055.

Yanik, J., S. Ebale, A. Kruse, M. Saglam and M. YuÌˆksel. 2007. Biomass gasification in supercritical water: Part 1. Effect of the nature of biomass. *Fuel*. 86(15): 2410-2415.

Yaylayan, V. and N. G. Forage. 1991. Determination of the kinetics and mechanism of decomposition of tryptophan amadori rearrangement product by RP-HPLC analysis. *Journal of Agricultural and Food Chemistry*. 39(2): 364-369.

Yaylayan, V. A. and A. Keyhani. 2001. Carbohydrate and amino acid degradation pathways in l-methionine/d-[c-13] glucose model systems. *Journal of Agricultural and Food Chemistry*. 49(2): 800-803.

Yokoyama, S.-Y., T. Ogi and K. Koguchi. 1985. Direct liquefaction of wood by catalyst and water: Effects of reaction parameters on the yield of heavy oil, 31-32.

Yu, Y. and H. Wu. 2010. Significant differences in the hydrolysis behavior of amorphous and crystalline portions within microcrystalline cellulose in hot-compressed water. *Industrial & Engineering Chemistry Research*. 49(8): 3902-3909.

Yuan, X. Z., J. Y. Tong, G. M. Zeng, H. Li and W. Xie. 2009. Comparative studies of products obtained at different temperatures during straw liquefaction by hot compressed water. *Energy and Fuels*. 23(6): 3262-3267.

AUTHOR'S BIOGRAPHY

Zhichao Wang was born on August 15, 1981 in Fushun, Liaoning, China. He graduated from Tsinghua University, China in 2002 with a Bachelor of Science degree in Environmental Science and Engineering. He completed a Master of Science degree in Environmental Engineering from Tsinghua University in 2005. He was a graduate research assistant during his Master's study and participated in various research projects. In 2006, Zhichao joined the Ph.D program in the Department of Agricultural and Biological Engineering at the University of Illinois at Urbana-Champaign. As a graduate research assistant, he had the opportunity to work in several research projects under the supervision of Professor Yuanhui Zhang. He also served as a teaching assistant to several courses including Engine and Tractor Power, Grain Drying and Storage Systems, and Renewable Energy Systems. He was awarded the Graduate Student Achievement Fellowship in 2008. Zhichao is affiliated with the American Society of Agricultural and Biological Engineers (ASABE).