Bucknell University
Bucknell Digital Commons

Honors Theses | Student Theses

Spring 2020

Aqueous and Oil Products from Hydrothermal Liquefaction of Manure Digestate

Kenzie Burns
Bucknell University, mmb025@bucknell.edu

Follow this and additional works at: https://digitalcommons.bucknell.edu/honors_theses

Part of the Catalysis and Reaction Engineering Commons, and the Environmental Engineering Commons

Recommended Citation

Burns, Kenzie, "Aqueous and Oil Products from Hydrothermal Liquefaction of Manure Digestate" (2020). *Honors Theses*. 523.
https://digitalcommons.bucknell.edu/honors_theses/523

This Honors Thesis is brought to you for free and open access by the Student Theses at Bucknell Digital Commons. It has been accepted for inclusion in Honors Theses by an authorized administrator of Bucknell Digital Commons. For more information, please contact dcadmin@bucknell.edu.

THIS PAGE IS INTENTIONALLY LEFT BLANK.

AQUEOUS AND OIL PRODUCTS FROM HYDROTHERMAL LIQUEFACTION OF MANURE DIGESTATE

by

McKenzie M. Burns

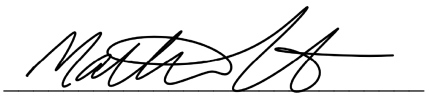
A Thesis Submitted to the Honors Council

For Honors in Environmental Engineering at Bucknell University

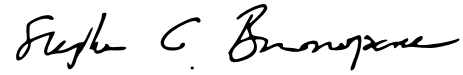
May 2020

Approved by:

Adviser: Dr. Deborah L. Sills
Department of Civil and Environmental Engineering



Secondary Adviser: Dr. Matthew J. Higgins
Department of Civil and Environmental Engineering



Department Chair: Dr. Stephen Buonopane
Department of Civil and Environmental Engineering

ACKNOWLEDGEMENT

I would like to acknowledge everyone who has made this research a possibility. First and foremost, I would like to express my deepest gratitude to my research adviser and mentor, Dr. Deborah L. Sills, for the opportunities she has presented to me through this research, and for her unwavering support and encouragement over the past four years. Without her patience and guidance, this work would not have been possible. I also extend thanks to Dr. Matthew J. Higgins for sharing knowledge on foundational topics of environmental chemistry and for always expressing an interest in my lab work.

Special thanks to Dr. Roy Posmanik and Dr. Borja Cantero-Tubilla of Cornell University for performing the HTL reactions, and for sharing their knowledge and expertise on the intricacies of HTL. Additional thanks to Dr. Jeff Tester, Cornell University, for lending laboratory equipment and manpower that made this work possible.

I cannot begin to express my thanks to Monica Hoover for her support and assistance with laboratory instrumentation, experimental methodology, and for always being there to talk through research problems with me. I also want to thank Steve Beightol for assistance in the laboratory, Alexa Ghiz for her work performing GC-MS analysis of the oil samples, and Dalton Stewart for his assistance in measuring ammonia in the aqueous samples.

My sincere gratitude to Wendelin Wright, Karen Castle, and the Clare Boothe Luce Foundation for Undergraduate Research, Carl Kirby and the Katherine Mabis McKenna Foundation, and Thomas DiStefano and the Chiloro Fund for awarding the financial means to conduct this research. Sincerest thanks to Sabrina Kirby for her help in the technicalities of writing, and finally, I would like to thank my mother, father, and stepmother for their support and encouragement throughout my Bucknell career; I wouldn’t be here without y’all.

TABLE OF CONTENTS

ACKNOWLEDGEMENT ii
LIST OF TABLES v
LIST OF FIGURES vi
ABSTRACT viii
CHAPTER 1: INTRODUCTION 1
1.1. Largescale Waste Production 1
1.2. Energy Demand 3
1.3. Project Objectives and Scope 4
1.4. Organization 5
CHAPTER 2: BACKGROUND AND LITERATURE REVIEW 6
2.1. Anaerobic Digestion (AD) 6
2.2. Overview of Hydrothermal Treatment Processes 8
2.2.1. Types of Hydrothermal Processes 8
2.2.2. Hydrothermal Liquefaction (HTL) 10
2.3. Previous Work on HTL 13
CHAPTER 3: METHOD DEVELOPMENT FOR GAS CHROMOTOGRAPHY MASS SPECTROMETRY OF HTL PRODUCTS 20
3.1. Introduction 20
3.2. GC-MS Instrumentation and Operation 21
3.3. Internal Standard Selection and Use 23
3.3.1. Use of an Internal Standard 23
3.3.2. Selection of an Internal Standard 24
3.3.3. Compound Classification for oil phase samples 26
3.4. Triple Liquid Extraction for Aqueous Product Analysis 28
3.4.1. Procedure Development 28
3.4.2. Internal Standard Use 30
3.4.3. Compound Classification 31
3.5. Conclusion 32
CHAPTER 4: EFFECT OF ACID CATALYST AND HYDRAULIC RETENTION TIME ON AQUEOUS AND OIL PRODUCTS FROM HTL 34
4.1. Background and Motivation 34
4.2. Methods 36

4.2.1. Experimental HTL 36
4.2.2. Analytical Materials and Methods 38
4.3. Results and Discussion 42
4.3.1. Aqueous Product Analysis 42
4.3.2. Oil Product Analysis 48
CHAPTER 5: CONCLUSION 52
BIBLIOGRAPHY 55
APPENDIX ..... A 1

LIST OF TABLES

Table 2.1 Summary of reaction parameters for two main types of hydrothermal processing 9

Table 4.1 Summary of compound groups present in HTL aqueous product. 47

Table 4.2 Relative area percentages for representative compound groupings in bio oil produced from HTL reactions of manure digestate with no catalyst and acid catalyst at varying retention times 49

LIST OF FIGURES

Figure 1.1 U.S. primary energy consumption by source. Obtained from eia.gov. 4

Figure 1.2 Schematic describing the role WtE technologies play in reducing harmful waste flows to the environment. 4

Figure 2.1 Reaction pathways occurring in an anaerobic digester. 7

Figure 2.2 Phase diagram for water. 11

Figure 3.1 Gas chromatograph mass spectrometer schematic. 22

Figure 3.2 A sample chromatogram from GC-MS of an HTL-produced oil product. 23

Figure 3.3 Example chromatograms used to illustrate the need for an internal standard 24

Figure 3.4 GC-MS chromatograms from 860 ppm and 21 ppm toluene in DCM.....26

Figure 3.5 Procedure for aqueous phase triple extraction.....29

Figure 3.6 Chromatograms produced from the GC-MS analysis of the same aqueous triple extraction sample with and without a 5 ppm toluene internal standard. 32

Figure 4.1 Phase diagram for water 35

Figure 4.2 Process flow schematic for the hydrothermal liquefaction of manure digestate and subsequent phase separation. 37

Figure 4.3 HTL batch reactor and schematic, with the heat exchange loop indicated in red in both the image and the schematic 38

Figure 4.4 Carbon recovery and nitrogen recovery and speciation in the aqueous product from HTL of dairy manure digestate under differing catalytic conditions.....44

Figure 4.5 Speciation of organic carbon in the aqueous product under differing catalytic conditions. 44

Figure 4.6 HTL reaction pathway for complex carbohydrates remaining in dairy manure digestate. 46

Figure 4.7 Higher heating value (HHV) of bio oils under catalyzed and non-catalyzed conditions for differing retention times. 50

ABSTRACT

Hydrothermal liquefaction is a promising thermochemical waste-to-energy (WtE) technology for conversion of wet biomasses, such as manure and digested manure, into value-added products, namely energy-rich biofuel and a nutrient-rich aqueous product. WtE technologies can address environmental issues relating to overproduction of organic wastes and increasing dependence on fossil fuels, which leads to climate change. HTL has not been implemented on a commercial scale, in part due to the uncertainty regarding specific reaction pathways for different feedstocks and ideal conditions for production of the most valuable products.

This research investigates the compositions of the aqueous and oil products from HTL of dairy manure digestate under phosphoric acid catalyzed and non-acid catalyzed conditions at differing retention times. The reactants are placed in a 300 mL batch reactor, heated to 300°C, and run for retention times from 5 to 40 minutes, after which the liquid product is collected and separated into an aqueous and an oil product. Our work aims to characterize the chemical compounds in the oil and aqueous products through gas chromatography mass spectroscopy (GC-MS) analysis, as well as elucidate the partitioning of carbon and the partitioning and speciation of nitrogen into the oil and the aqueous products.

This work included development of methods for GC-MS analysis of both the aqueous and oil products, and illustrates the importance of an internal standard in GC-MS analysis. Results from this work show that presence of an acid catalyst leads to an increased amount of nitrogen in the aqueous product, but has minimal effect on carbon partitioning between the aqueous and oil products. Additionally, we show that

increasing retention time leads to decreased recovery of carbon and nitrogen in the aqueous product regardless of catalytic conditions. This means that there is a tradeoff between carbon and nitrogen recoveries in the aqueous phase, as it is desirable to maximize nitrogen and minimize carbon in the aqueous phase. Phenols, alkanes, and sulfurous acids were consistently present in the aqueous product regardless of catalytic condition or retention time, indicating efficient lignin degradation in a range of hydrothermal reaction conditions.

Analysis of the oil product revealed that the energy content of the bio oil was unaffected by the addition of an acid catalyst. Additionally, the decreased amount of fatty acids in the oil product for acid-catalyzed samples suggests enhancement of the decarboxylation reaction that transforms fatty acids to hydrocarbons in the presence of the acid catalyst. Increased presence of phenol in the acid-catalyzed oil samples compared to the non-catalyzed oil samples indicates an enhanced lignin degradation pathway in the presence of the acid catalyst. More research needs to be done into the quantification of compounds in the aqueous product before any definitive conclusions can be drawn in relation to optimal reaction conditions and retention time for HTL of manure digestate.

While most HTL work remains at the laboratory and pilot scale, the expanding interest in WtE technologies as way to address pressing environmental challenges makes HTL a promising candidate for research and development to industrial and commercial scale. The work presented in this thesis contributes to the growing body of research relating to HTL as a viable process for simultaneous waste remediation and energy source production, and lays groundwork for important future work.

CHAPTER 1: INTRODUCTION

While global environmental issues are numerous, two stand paramount: overproduction of wastes and increasing dependence on non-renewable primary energy sources, namely fossil fuels. These problems impact every aspect of society, from the economy to the environment. Recovering energy from wet organic wastes, such as manure digestate, can offer solutions to both of these challenges. The work presented throughout this thesis contributes to alleviating both of these problems at once, joining the growing body of science working to develop waste-to-energy (WtE) technologies.

In 2009, Rockström et al. published the revolutionary paper *A safe operating space for humanity*, which lays out a framework of nine "planetary boundaries" within which global systems are to operate in order for the planet to remain habitable for the foreseeable future. At the time of publication, the proposed boundaries for three of the nine control variables had already been transgressed, one of which was the climate change variable quantified by atmospheric CO_2 concentration. In the climate's current state, there is no room for the continuous depletion of fossil fuels at the rapid rate demanded by U.S. energy infrastructure. Current renewable energy infrastructure, such as that for wind and solar power, has its place in the nation's energy economy, but liquid biofuels can supplement these intermittent renewable energy technologies. Waste biomass, such as animal manure, is a promising feedstock for liquid fuel.

1.1. Largescale Waste Production

WtE technologies aim to alleviate stresses created by overproduction of organic wastes; in the United States alone, nearly 77 MT of organic wastes are produced each year (Skaggs et al., 2018). Of the four main types of organic wastes produced in the

U.S. (animal wastes, food wastes, wastewater sludge and fats/oils/greases), animal wastes from largescale livestock operations are of chief concern. On a nationwide scale, the agricultural industry produces nearly 250 million tons of manure (dry weight basis) annually (Xiu et al., 2010).

In recent years, the American livestock industry has seen a shift towards a smaller number of highly concentrated animal feeding operations, called CAFOs (Skaggs et al., 2018). A CAFO is classified as an animal feeding operation with more than 1,000 active animal equivalent units, or AEUs (1 AEU = 1,000 lbs. live animal). In Pennsylvania alone, there are 289 active dairy CAFOs, with a minimum of 289,000 pounds of cattle creating enormous quantities of wastes (~8% of body weight excreted per animal per day) (*Concentrated Animal Feeding Operations (CAFOs)*, n.d.; Theegala & Midgett, 2012). The concentrated nature of CAFOs leads to exacerbated environmental impacts of manure production, compared to less concentrated farming operations.

Animal wastes are not only an environmental liability; they are also a vastly undervalued resource, as they contain large amounts of untapped energy and nutrients. The traditional approaches to manure management involve either direct land application as fertilizer, which harnesses some of the nutrient potential and beneficially recycles carbon to the soil, or anaerobic digestion for production of biogas, which taps into the energy potential of manure. However, each of these management techniques has disadvantages and limitations. Direct land application of manure can lead to the spread of pathogens, as well as leave large quantities of valuable nutrients (nitrogen and phosphorus) open to runoff (Skaggs et al., 2018). Excess nutrient loading due to

runoff from CAFOs and croplands is one of the main cause of eutrophication, or the creation of aquatic "dead zones," which disrupts aquatic ecosystems and threatens major aspects of the food chain and fishing economy. Conversely, anaerobic digestion, which relies on microbial growth for conversion of manure to biogas, is limited due to its low conversion of organic material to biogas (between 40 and 50%) and production of large volumes of liquid effluent, called digestate, still rich in energy and nutrients (Posmanik et al., 2018). There is yet to be a commercial treatment process that successfully extracts most of the energy and nutrient potential in animal manure while also minimizing the volume of waste.

1.2. Energy Demand

Energy demands throughout the United States are increasing. Under the nation's current energy infrastructure, the majority of this demand is met via burning of non-renewable primary energy sources, e.g. fossil fuels (Figure 1.1). (*U.S. Energy Facts Explained - Consumption and Production - U.S. Energy Information Administration (EIA)*, n.d.). The burning of fossil fuels leads to emission of greenhouse gases and therefore contributes to increasing climate change (Yu et al., 2011). Liquid biofuels produced from waste biomasses (i.e. WtE) offer an attractive, renewable alternative to fossil fuels. Hydrothermal liquefaction (HTL) is one of these WtE pathways, and is illustrated in Figure 1.2 below. Using the digestate waste from anaerobic digestion as an HTL feedstock will reduce the release of nutrients and unused energy into the environment while providing a biofuel to supplement nonrenewable liquid fuels (such as fossil fuels) and therefore also decrease greenhouse gas emissions and climate change effects.

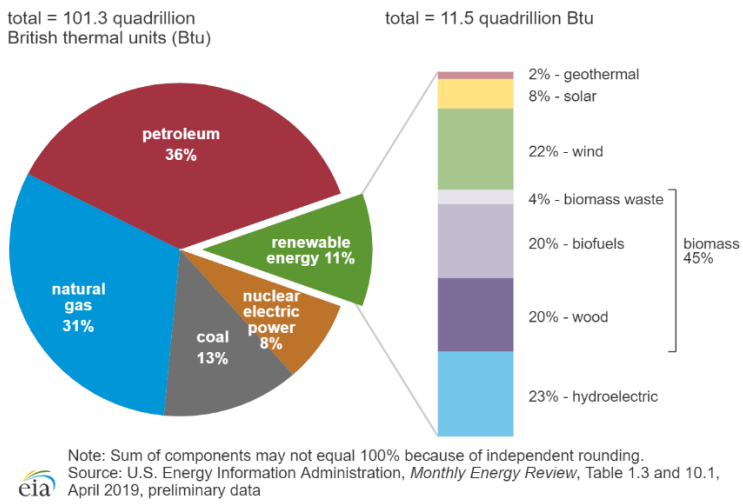


Figure 1.1 U.S. primary energy consumption by source. Obtained from eia.gov.

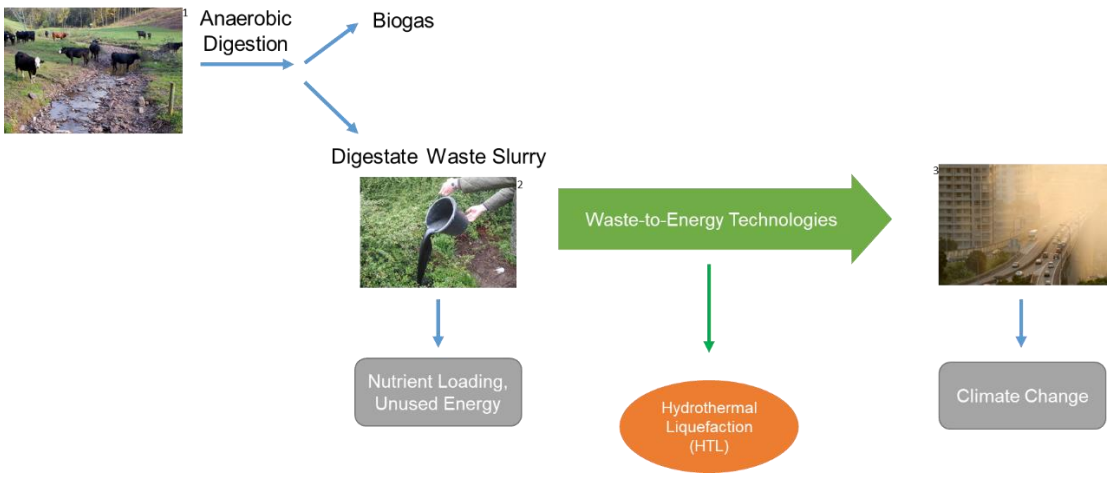


Figure 1.2 Schematic describing the role WtE technologies, such as HTL, play in reducing harmful waste flows (here nutrient loading and carbon emissions/climate change) to the environment.

1.3.Project Objectives and Scope

This work investigates the compositions of the aqueous and oil products from hydrothermal liquefaction (HTL) of dairy manure digestate.

The objectives of this research are two-fold:

1. To develop gas chromatography mass spectrometry (GC-MS) methods for the analysis of aqueous and oil products from HTL of manure digestate.
2. To measure the effect of hydraulic retention time on aqueous and oil products from HTL of manure digestate, performed in triplicate, with and without an acid catalyst.

1.4. Organization

Chapter 2 provides an overview of the relevant processes and literature investigated throughout this work. Chapter 3 addresses Objective 1, and describes the processes followed to develop methods to analyze aqueous and oil HTL products using GC-MS technology. Chapter 4 presents the results from analysis of the aqueous and oil products from HTL of manure digestate and elucidates the effects of retention time and catalytic conditions on each of these products. Chapter 5 provides a summary of the work, its engineering relevance, and future work needed on this topic. Appendices are included at the end of this thesis and contain relevant data and additional information for aid in comprehension of the ideas presented in this work.

CHAPTER 2: BACKGROUND AND LITERATURE REVIEW

This chapter provides background information on the technologies relevant to this research. Such technologies include anaerobic digestion (AD), which produces the digestate used as a feedstock for HTL, and hydrothermal liquefaction (HTL), on which this thesis focuses. The bulk of information presented throughout Chapters 3 and 4 of this thesis describes experimental analysis of the aqueous and oil products from HTL of manure digestate; therefore, it is important to first gain an understanding of the processes and technologies that produced the feedstock and the aqueous and oil products we analyzed.

First, we will briefly discuss AD, reviewing its mechanisms, products, and relation to the HTL reactions performed for this research. Next, there will be a discussion of generalized hydrothermal technologies and HTL specifically, which will reveal how the reactions work, the advantages and disadvantages of HTL as compared to other hydrothermal technologies, and a review of relevant HTL studies. Finally we discuss the research which immediately proceeded this work and how it paved the way for our work.

2.1. Anaerobic Digestion (AD)

AD is an established method for management of wet organic wastes that is widely used across the globe. AD is well suited for treatment of animal manure, which typically contains 4-10% solids by weight (Lorimore et al., 2004). As of January 2015, the United States Environmental Protection Agency (US EPA) estimated that there were nearly 250 anaerobic digesters operating on commercial livestock farms throughout the nation (Costa et al., 2015). AD capitalizes on the naturally occurring

microbial reactions within multiple groups of microorganisms that use organic carbon as fuel. Four main microbial reactions occur throughout the various stages of anaerobic digestion: hydrolysis, fermentation, acetogenesis, and methanogenesis. The progression of these reactions is as follows:

Hydrolysis describes the conversion of complex organic matter into simpler material that is readily biodegradable, called soluble microbial product, or SMP. The majority of the carbon (and therefore the energy) found in manure is too complex for microbes to biodegrade outright, and so hydrolysis is a necessary predecessor to the chain of biodegradation reactions occurring in a digester. Following hydrolysis is fermentation, also called acidogenesis, during which the microbes further degrade SMPs into short chain volatile fatty acids, or VFAs. VFAs are then used to make acetic acid in the acetogenesis reaction. Finally, the methanogens convert acetic acid into methane through a process called methanogenesis. Methane is the main product of value produced during anaerobic digestion. (Costa et al., 2015). Figure 2.1 illustrates the chemical reaction pathways occurring during anaerobic digestion.

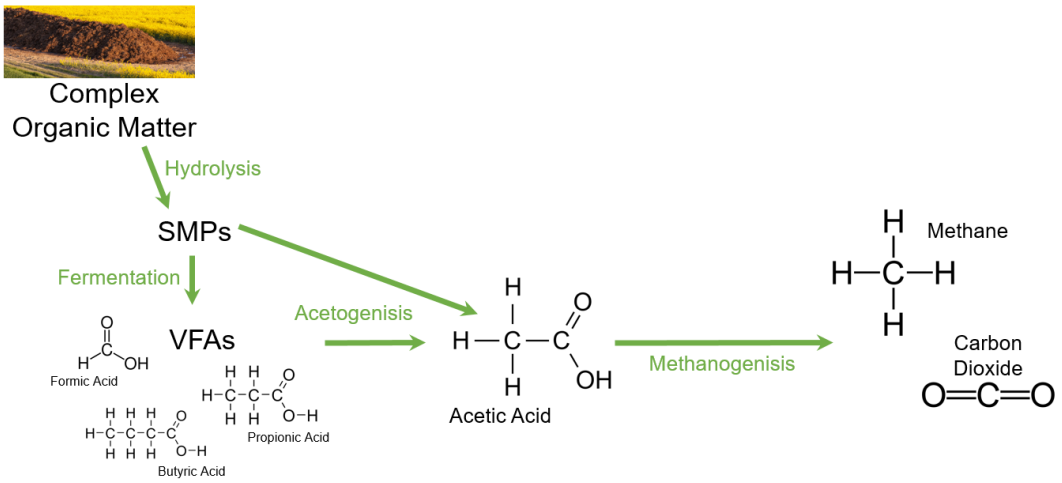


Figure 2.1 Reaction pathways occurring in an anaerobic digester.

Anaerobic digestion produces two main products: biogas and a liquid waste slurry, called digestate. The biogas produced is typically 60-70% methane and 20-30% carbon dioxide, with trace amounts of other gases like hydrogen sulfide. This biogas can be burned directly as a heat source, as is often the case in developing countries where anaerobic digester technology exists, or used in conjunction with a generator in order to produce power (Costa et al., 2015). The digestate contains undigested organic material in solid and liquid form, and is particularly rich in nitrogen and phosphorus (Costa et al., 2015). AD digests only about half of the organic carbon in the feedstock to biogas, so this digestate is also a rich carbon source. Current digestate management strategies typically involve direct land application; however, this practice can lead to the spread of pathogens and excess nutrient loading on nearby aquatic ecosystems. There is a growing body of research for alternative uses and management practices for manure digestate. One such approach is to further extract the remaining energy and nutrients from the digestate via thermochemical treatment processes, such as HTL.

2.2. Overview of Hydrothermal Treatment Processes

2.2.1. Types of Hydrothermal Processes

Two main types of hydrothermal processes are used to convert biomasses to fuels: hydrothermal gasification (HTG) and hydrothermal liquefaction (HTL). Hydrothermal technologies are defined as those physical and/or chemical processes performed at temperatures between 200°C and 600°C and at pressures between 5 MPa and 40 MPa (Peterson et al., 2008). Hydrothermal gasification is ideal for production of gaseous fuels, whereas liquefaction is geared towards production of liquid fuels, similar to crude oil. The difference in products is accounted for by the various

conditions of liquefaction versus gasification (Table 2.1). HTG is a versatile process that, depending on the conditions of reaction, can produce several different products. Researchers typically classify HTG into 3 categories based on the end product: (1) gas rich in hydrogen formed via high temperatures (T > 500°C) either without catalysts or with non-mental catalysts; (2) gas rich in methane formed at near-critical temperatures in the presence of catalysts; and (3) gaseous product formed via subcritical catalytic processing (Peterson et al., 2008). HTG is typically performed at high pressures, although research has shown that changes in temperature have a much stronger effect on the product type and quality from HTG than variation of reaction pressure (Peterson et al., 2008). The specific mechanisms of HTG reactions are complex and highly dependent on reaction temperature, feedstock, and the presence of catalysts; these details are outside the scope of this research.

Table 2.1 Summary of reaction parameters for two main types of hydrothermal processing. Adapted from Peterson et al. (2008).

Reaction Condition	**HTL**	**HTG**
Temperature (°C)	200-400	450+
Pressure (MPa)	5-20	20-50
Target Product	Biocrude oil	Energy-rich gas
Critical Condition	Subcritical	Supercritical
Mechanisms of Reaction	Solvolysis, decarboxylation, dehydration, hydrogenation of functional groups, etc.	Highly dependent on reaction conditions.

2.2.2. Hydrothermal Liquefaction (HTL)

This thesis investigates the products from HTL of manure digestate. Thus, it is important to understand the details of HTL reactions in order to fully comprehend the relevance of this work. While there is a growing body of research investigating the intricacies of HTL reaction pathways, there is general understanding of the mechanisms behind HTL reactions and the benefits of HTL as a WtE technology.

2.2.2.1. Mechanisms of Reaction

HTL is a thermochemical conversion process, meaning that the driving forces behind the reactions occurring in HTL are increased temperature and pressure. Specifically, HTL takes advantage of the unique properties of water near its critical point, around 374°C and 22 MPa. Figure 2.2 shows the phase diagram for water. The green-shaded area in the figure indicates the typical range of temperatures and pressures in most of the current HTL work (Alimoradi et al., 2020; Karagöz et al., 2004; Kruse & Gawlik, 2003; Peterson et al., 2008; R. Posmanik et al., 2017; Tekin et al., 2014; Xiu et al., 2010; Yin et al., 2010). HTL is performed at pressures greater than the critical pressure of water, in order to prevent water from undergoing specific phase change. When pressure is below critical pressure, water will undergo phase change to the vapor state as temperature increases above 200°C (Figure 2.2). At pressures greater than critical pressure, water will remain in a “critical” state (either sub- or super-) as temperature increases. Avoiding a specific phase change maintains the water-rich reaction medium required for HTL.

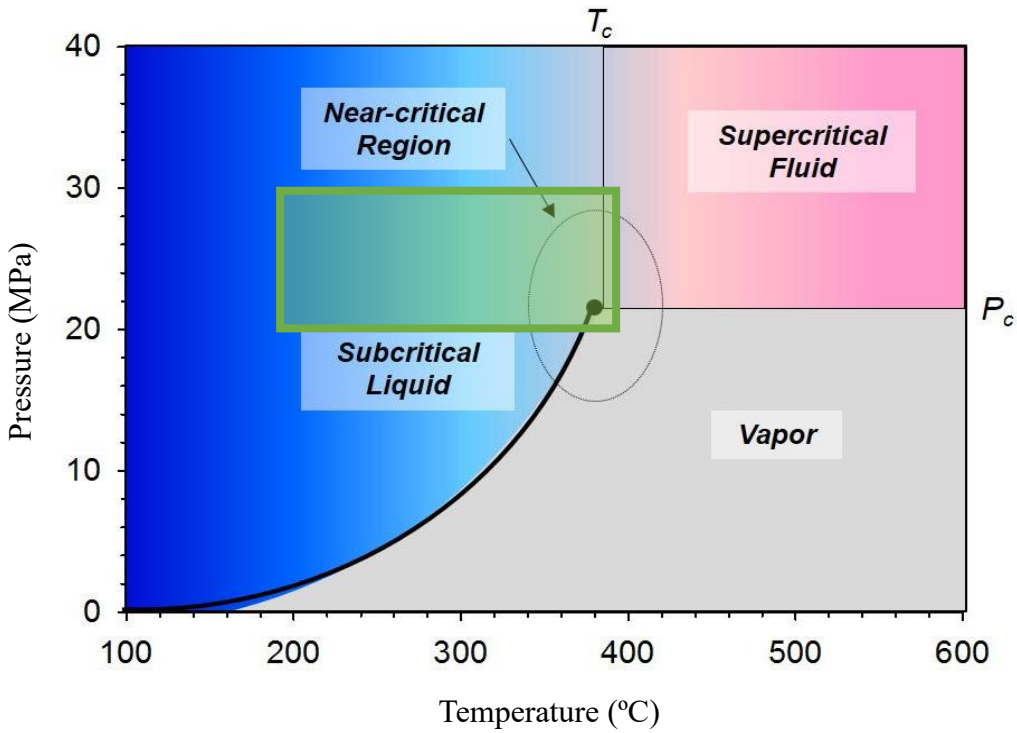


Figure 2.2 Phase diagram for water, with typical temperature and pressure range for hydrothermal liquefaction shaded in green.

As a near-critical liquid, water takes on several interesting properties that make it an ideal medium for conversion of biomasses to liquid and gaseous fuels. For example, as water progresses through the sub- and supercritical liquid phases, the dielectric constant drops significantly (from about 80 at 20°C to under 2 at 450°C) (Peterson et al., 2008). With a low dielectric constant, hydrogen bonding behavior typically characteristic of liquid water ceases and we see a behavior similar to that of a polar solvent, such as hexane (Peterson et al., 2008). This change in behavior is what gives the HTL process the alternate name of "solvolysis." Additionally, water in the subcritical liquid phase exhibits a heightened self-ionization constant, K_W, which describes the ability of water to self-ionize into H^+ and OH^- ions. An increased K_w indicates that near-critical water is able to ionize more than liquid water, leading to in

increased concentration of ions in solution. This increased ionization allows for the near-critical water media to catalyze both acidic and basic reactions with relative ease (Yin et al., 2010). Such enhanced properties of near-critical water media are the mechanisms which allow for the complex series of reactions, including solvolysis, dehydration, decarboxylation, hydrogenation of functional groups, etc., which occur during HTL to happen.

2.2.2.2. Advantages and Disadvantages of HTL

Hydrothermal liquefaction has both advantages and disadvantages relating to its application as a WtE technology. For example, HTL is compatible with wet feedstocks, such as animal manures, food wastes, and liquid digestates. Compatibility with wet feedstocks removes the energy- and time-intensive drying step required by other WtE technologies and can be beneficial on both the economic and environmental fronts. Additionally, HTL is a rather simple process from an operation standpoint, as the only reactants are organic wastes and water (although catalysts can be used) (Peterson et al., 2008). HTL is also considered to be very efficient from a chemistry standpoint, with high conversion yields (anywhere from 5 to 30%) for biomass to bio-oil and minimal waste stream (Skaggs et al., 2018; Vardon et al., 2011). Furthermore, the high temperatures and pressures of HTL make it insensitive to feedstock particle size, therefore eliminating any need for mechanical or chemical pretreatment (Akhtar & Amin, 2011 in Skaggs et al., 2018). The main disadvantages of HTL are its current scale and the required high temperatures/pressures. Temperatures and pressures as high as those required for HTL are both difficult to achieve and costly to maintain for long periods, requiring large energy inputs. This, however, can be alleviated through heat

recovery. Additionally, HTL for waste treatment is currently only available at lab and pilot scales, as there is still much unexplored territory in relation to HTL and its processes. Industry is not yet convinced that HTL can perform adequately or compete with established technologies. However, the WtE characteristic of HTL, along with the other advantages listed above, make it an ideal candidate for research.

2.3. Previous Work on HTL

Wastewater sludge, anaerobic digestate, animal manures and food wastes are among the main complex waste biomasses used as feedstocks for HTL (W.-T. Chen, Zhang, Zhang, Schideman, et al., 2014; Mau et al., 2016; Munir et al., 2017; R. Posmanik, Martinez, Cantero-Tubilla, et al., 2018; Skaggs et al., 2018; Theegala & Midgett, 2012; Vardon et al., 2011; Xiu et al., 2010; Yin et al., 2010). There is also significant research into HTL with various algal species as a feedstock (W.-T. Chen et al., 2017; W.-T. Chen, Zhang, Zhang, Yu, Schideman, et al., 2014; Y. Chen et al., 2017; Pham et al., 2013; Vardon et al., 2011; Yu et al., 2011). Since the details of the chemical reactions occurring throughout HTL processes are still widely unknown, there is also a large body of work that uses model compounds as feedstocks (Karagöz et al., 2004; Posmanik et al., 2017; Tekin & Karagöz, 2013; Wang et al., [in review]; Wahyudiono et al., 2009; Yin & Tan, 2012).

Model compounds (e.g., carbohydrates, proteins, and lipids) are typically good starting points for research into complex processes, as they can help researchers elucidate reaction mechanisms in more complex “real” feedstocks. Model compounds can also be selected to represent certain specific complex feedstocks; such is the case investigated by Posmanik et al. (2017). These researchers used three model compounds

(potato starch, bovine serum albumin, and linoleic acid) to simulate three main components of food wastes: carbohydrates, proteins, and lipids. In studying the effects of various combinations of these three model compounds, the researchers found that production of value-added HTL products can be maximized by selection of appropriate proportions of each substrate in the reaction feedstock, as well as discovered an advantageous reaction pathway involving lipids and polysaccharides which enhances the quality of the bio-oil produced (R. Posmanik et al., 2017).

Celluose, a major component of cow manure and fibrous plant materials, is often used as a model compound for HTL studies. Yin and Tan (2012) investigated the effects of varying pH (3-14) and temperature (275-320°C) on the products from HTL of cellulose. They found that the chemical compositions of the bio-oils produced varied depending on the pH conditions of the HTL reactions, and that higher temperatures and retention times (greater than 300°C and 10 minutes, respectively) yielded less bio-oil regardless of pH conditions. Their study also revealed that the reaction mechanisms differed with changes in pH, as was evident by the differing yields of other HTL products, namely solids and gases. Similarly, Kruse and Gawlik (2003) selected biomass degradation intermediates to study, such as phenols, furfurals, short-chain organic acids, and aldehydes. These chemicals are all intermediate products in the decomposition of lignocellulose, or plant cell walls (Kruse & Gawlik, 2003). The researchers found that reaction temperatures above 374°C favored cellulose to glucose/fructose to acid/aldehyde decomposition pathways, whereas reaction temperatures below 374°C favored cellulose to glucose/fructose to furfural to phenols/acids/aldehydes decomposition pathways (Kruse & Gawlik, 2003). This study helped explain the reaction pathways of these intermediates at sub- and supercritical

conditions, which gives indication of the reaction pathways for more complex feedstocks at similar conditions.

Algae is a promising feedstock for HTL because of its versatility; it can grow in most water sources (including saltwater and wastewater) and on marginal lands, and it has a rapid growth rate (W.-T. Chen et al., 2014). Unlike terrestrial biomasses used for bioenergy in the U.S. (mainly corn and soy), algae does not compete with food crops for arable land. Additionally, algae has a high water content, making it a good candidate for reaction in aqueous media. One of the main variables researchers are investigating in relation to HTL of algal feedstocks is the lipid content of the algal feedstock. Conventional algae-to-biodiesel approaches focus on cultivation of pure, high-lipid strains of algae, due to the energy lost during extraction and transesterification to biodiesel; however, high-lipid algae has a lower biomass productivity than low-lipid algae and is very sensitive to stressful conditions (W.-T. Chen, Zhang, Zhang, Schideman, et al., 2014; Y. Chen et al., 2017). The HTL process is able to accommodate for algae with lower lipid content without sacrificing energy or oil yield as the reaction converts carbohydrates and proteins into bio oil. In addition, HTL takes place in an aqueous medium and thus extensive drying is not necessary (W.-T. Chen, Zhang, Zhang, Schideman, et al., 2014; Y. Chen et al., 2017). Yu et. al. (2011) studied the distributions of key nutrients in the products from HTL of low-lipid microalgae, finding that carbon, nitrogen, and energy recovery into the bio-oil product increased with increasing temperature and retention time. They also found that nitrogen recovery into the aqueous product increased with increasing temperature and retention time, suggesting that more of the feedstock was degraded as time and temperature increased (Yu et al., 2011). This study on algae relates to the experimental work presented in this

thesis, as we measured the effect of retention time on carbon and nitrogen recovery into the HTL aqueous product.

While the previous study specifically investigated the partitioning of several key elements in the products from HTL of algae, Y. Chen et al. (2017) investigated the products from HTL of algae more broadly, looking to complete an analysis of the organic compounds present in the products. The researchers paid particular attention to the identification of compounds in the aqueous product with two differing pretreatment methods, namely dried aqueous extracts (DAE) and solid-phase micro-extraction (SPME). The study found that DAE was ideal for identifying strong polar compounds in the aqueous product, while SPME excelled at identifying weaker polar compounds. The researchers also investigated the compositions of the bio oil and gaseous products from HTL of microalgae, finding that carbon dioxide and carbon monoxide were the main components of the gaseous product, while the bio oil contained phenols, ketones, alcohols, hydrocarbons and N-containing compounds (Y. Chen et al., 2017). This research relates to our work in that our work also aims to compile a complete characterization of the aqueous and oil HTL products.

Using waste biomasses as HTL feedstocks is advantageous compared to the use of dedicated bioenergy crops such as algae, as doing so simultaneously eliminates a waste and creates a value-added product. Most research involving waste biomasses as feedstocks aims to investigate the effects of reaction conditions, such as pH, temperature, retention time, etc., on the composition, quantity, and quality of the bio oil produced. For example, Yin et al. (2010) investigated the effects of various conversion parameters on the elemental composition, higher heating value (HHV; a

measure of the energy potential in oil), and quantitative yield of the bio oil product from HTL of cattle manure. Their study found that oil yield depended mainly on conversion temperature and process gas, but that increased initial pressure, longer retention times, and increased mass ratio of cattle manure to water had negative impacts on the bio oil yield. In addition, the researchers reported that the major non-polar components of the bio oil were the BTEX chemicals (benzene, toluene, ethyl benzene, and xylene), which are also the major components of crude oils, gasoline, and diesel (Yin et al., 2010). Similarly, Xiu et al. (2010) conducted research on the effects of operating parameters on the product yield and characterization of bio oil from HTL of swine manure, and reported very similar results to those of Yin et al. (2010).These studies did not analyze the composition of the aqueous phase product from HTL, which we elected to study.

Theegala and Midgett (2012) focused their efforts on the HTL of dairy manure in the presence of a sodium carbonate catalyst. The results showed that maximum oil production could be achieved at a temperature of 350°C with 1 g of catalyst (300 mL reactor with 20% total solids, 85% of which were volatile solids), with approximately 68% of the energy potential in the raw manure recovered into the oil. The authors also found that the bio oil produced was high in phenolic compounds, and had an average HHV of 32 MJ/kg, a decent value for bio oils (Theegala & Midgett, 2012). Vardon et al. (2011) explored the effects on the chemical properties of the bio oil HTL product from three different complex feedstocks: algae, swine manure, and anaerobic digestate. The research reported the highest oil yield with the algae feedstock and the lowest oil yield with the digestate feedstock, although the bio oils produced had similar HHVs. The chemistry of the oil produced varied with feedstock composition, with heavier molecules found in the digestate-produced oil versus lighter molecules in the oil from

algae, and a similar trend in the boiling point distributions of the bio oils produced. The researchers assert that the feedstock composition remains of key importance in the HTL process, even when complex and more representative feedstocks are used (Vardon et al., 2011). Again, these researchers did not investigate the composition of the aqueous products, on which our research focused.

A 2018 study by Posmanik et al. is the immediate predecessor of the work presented in this thesis. Their study investigated the effect of acid and alkali addition to HTL of both manure digestate and carbohydrate-rich food waste. Three HTL products were analyzed for quantity and characterized for their quality: the bio oil product, the aqueous product, and the solid (hydro-char) product. The study focused on the effects of varying pH on the HTL reaction for each feedstock. For both feedstocks, the researchers found a wide range of carbon recoveries in all three products investigated: between 26 and 61 wt % for the bio oils, between 9 and 49 wt % for the aqueous products, and between 1 and 36 wt % for the hydro-char (Posmanik et al., 2018). Additionally, acid catalyst addition had a greater effect on the HTL reactions performed with digested manure than those performed with food waste. Across both feedstocks, addition of acid to the HTL reaction led to a decrease in recovery of short-chain organic acids, such as acetic, formic, and lactic acids, in the aqueous product, but increased recovery of 5-hydroxymethylfurfural (HMF), an important biofuel precursor.

Posmanik et al. (2018) also examined the composition of the oil product. They reported decreased oxygen to carbon (O/C) ratios in the oil for both feedstock conditions, and decreased hydrogen to carbon (H/C) ratio in the oil for the food waste feedstock condition in comparison to the elemental ratios of the food waste feedstock

(Posmanik et al., 2018). These observations indicate that the mechanism for oxygen removal in HTL of manure digestate is decarboxylation, where as in HTL of food waste both decarboxylation and dehydration played a role in oxygen removal. Enhanced decarboxylation with minimal dehydration yields higher quality oil in need of less upgrading before use as fuel; therefore, the oil produced from HTL of manure digestate has an advantage compared to oil produced from food waste. Qualitative characterization of the oils produced revealed that, with manure digestate as the feedstock, addition of acid and alkali catalysts favored production of long-chain fatty acids, whereas no additive HTL of manure digestate yielded bio oil with strong presence of cyclic hydrocarbons. For food waste feedstock, unmodified HTL (i.e. no added catalyst) favored production of cyclic hydrocarbons and long-chain fatty acids equally. Acid addition to food waste HTL favored furan production indicating an enhanced dehydration process, whereas alkali addition favored phenol and fatty acid production (Posmanik et al., 2018). The results reported in Posmanik et al. (2018), which did not measure nitrogen in the aqueous product and included a fairly limited characterization of the oil and aqueous phase products, led to the work presented in this thesis.

CHAPTER 3: METHOD DEVELOPMENT FOR GAS CHROMOTOGRAPHY MASS SPECTROMETRY OF HTL PRODUCTS

3.1. Introduction

Using GC-MS to analyze oil and aqueous products from HTL requires development of methods specific to the reaction feedstock, conditions, and goals of product characterization. One goal of this work was to develop methods for GC-MS analysis of oil and aqueous products produced from hydrothermal liquefaction of manure digestate. Developing the GC-MS methodology consists of multiple steps, including devising sample preparation procedures, choosing of an internal standard, creating a data analysis method within the GC-MS software, and interpreting the experimental data. Each step in the method development requires experimental design, testing, and evaluation. For example, in the sample preparation portion alone, changes in sample concentration, choice and concentration of internal standard, type of solvent, mixing time with the solvent, temperature, etc., all can lead to changes in the data output.

This chapter will detail the methods developed for GC-MS analysis of the oil and aqueous products from HTL of manure digestate. We begin with a brief description of the GC-MS instrument, its operation, and a brief overview of how the data reported from the analysis is read and used. We then discuss the importance of using an internal standard for GC-MS and the selection of an internal standard compound for the oil product. A brief description of the procedures for the analysis of the data reported by the GC-MS follows. Then the methods for analysis of the aqueous product from HTL via GC-MS are discussed, including the modifications made to the triple liquid

extraction procedure used by Alimoradi et al. (2020). Finally, a brief description of the procedure for analyzing the data reported by the GC-MS for the aqueous product is included.

3.2. GC-MS Instrumentation and Operation

Figure 3.1 shows a schematic of a typical GC-MS instrument. The GC-MS has two main components: the gas chromatograph and the mass spectrometer. The gas chromatograph consists of an inlet, the column (held in a heated oven), and an outlet. The sample is injected in the inlet, where (if it is liquid) it is immediately vaporized. The sample is then carried through the column with an inert gas (helium). The column separates compounds in the sample based on their molecular structure and interactions with the column packing material (*Gas Chromatography Mass Spectrometry (GC-MS) Information - US*, n.d.). After eluting from the column, the substances are transported into the mass spectrometer. The mass spectrometer consists of an ionization source, a mass filter and a detector housed in an evacuated chamber. The ionization source ionizes the compounds before they are sent through a mass filter, which fragments the compounds based on their mass-to-charge ratio (Mellon, 2003). Finally, the abundance of each detected fragment's mass-to-charge ratio is measured as an electrical signal intensity by the detector and sent to the computer ("How Does a GC-MS Work," n.d.). The resulting mass spectrum, which represents the mass-to-charge ratio and relative abundance of each compound, is compared to a database of known compound spectra to identify compounds. The quality of the compound's identification is reported and used as a measure of the likelihood that the compound identified is the compound

reported. The GC-MS reports the collective spectra of the sample in a chromatogram, similar to the one shown in Figure 3.2 below.

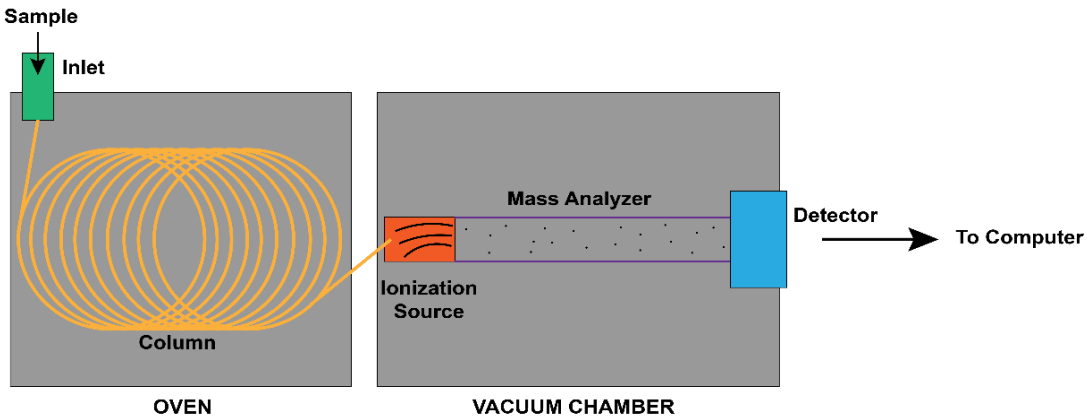


Figure 3.1 Gas chromatograph mass spectrometer schematic.

Each peak in Figure 3.2 represents a different detected compound, identified by its corresponding spectra in comparison to the library of spectra installed on the computer. The area of the peak for each compound is related to the abundance of the compound in the sample. However without running a known compound (referred to as an internal standard) for comparison, the area of a peak itself cannot provide quantitative information regarding concentration. Peaks within a sample can be compared and conclusions can be drawn about the abundance of one compound with respect to another within the same sample (with a larger peak area corresponding to more compound present), but this does not transfer to comparing across samples. In order to do so, an internal standard is required (W.-T. Chen, Zhang, Zhang, Yu, Schiedman, et al., 2014).

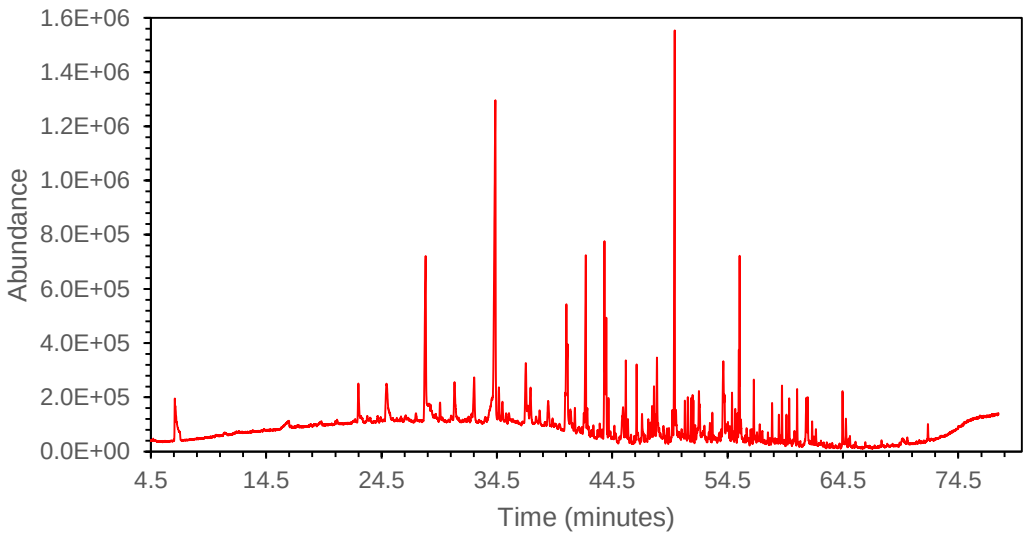


Figure 3.2 A sample chromatogram from GC-MS of an HTL-produced oil product.

3.3. Internal Standard Selection and Use

3.3.1. Use of an Internal Standard

When an internal standard is used, we can compare relative abundances of compounds across samples. To aid in explanation of this concept, Figure 3.3 presents four fabricated example chromatograms. In each chromatogram, there is a peak labeled for compound X. In Figures 3.3(a) and 3.3(b), samples P and Q were run alone, whereas in Figures 3.3(c) and 3.3(d) the samples were run with an internal standard of known concentration. Comparing the relative amounts of compound X in samples P and Q based on the peak areas in chromatograms 3.3(a) and 3.3(b) is invalid and would lead to the conclusion that sample P contains a higher concentration of compound X than sample Q. Adding an internal standard of known concentration allows us to calculate the relative peak area percent for compound X within each sample via Equation 3.1 below. (Note that percent of total peak area is commonly used in place of actual

numbers corresponding to peak area, as percentages are easier to work with than abundance units.) In comparing the relative area percent of compound X in sample P to the relative area percent of compound X in sample Q, we see that sample Q contains a higher amount of compound X than sample P does (Figures 3.3(c) and 3.3(d)).

$$realtive\ area\ \% = \frac{\%\ total\ area\ of\ compound\ X}{\%\ total\ area\ of\ internal\ standard} \tag{3.1}$$

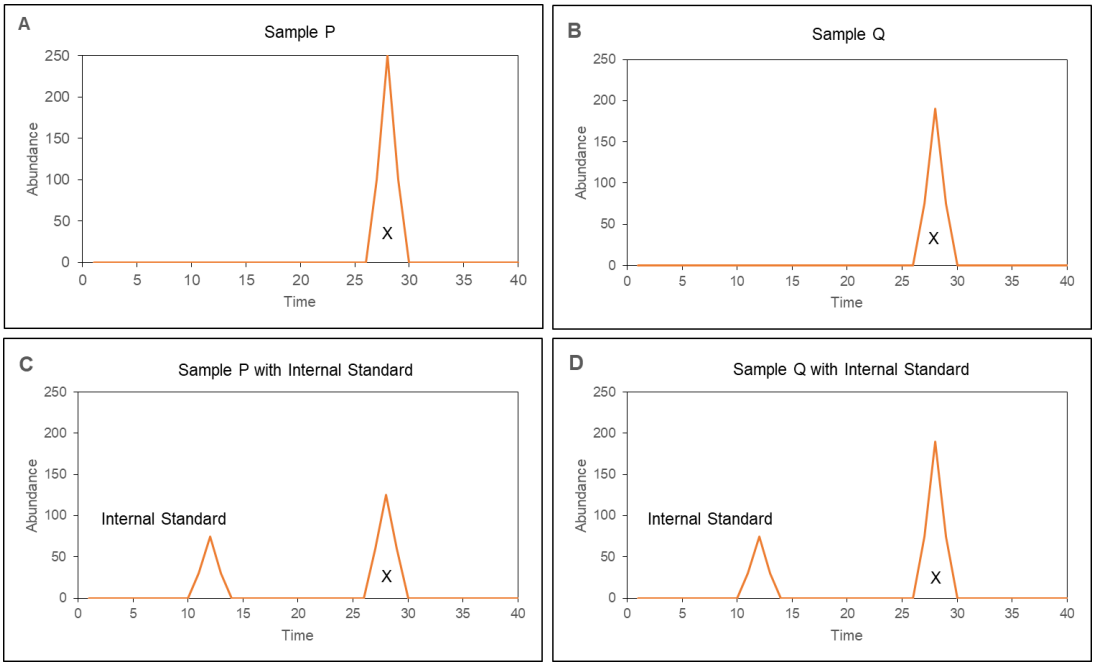


Figure 3.3 Example chromatograms used to illustrate the need for an internal standard to compare compound concentrations across samples.

3.3.2. Selection of an Internal Standard

Internal standards for use in GC-MS analysis must meet two main criteria. The selected internal standard (1) cannot be present in the sample itself, and (2) it must not interfere with the peaks of compounds present in the sample. In this research, the following compounds were investigated as potential internal standards for analysis of the oil product via GC-MS: pentadecanoic acid methyl ester, phenol, and toluene.

Pentadecanoic acid methyl ester was found to interfere with peaks of compounds in the oils. Therefore, pentadecanoic acid methyl ester could not be used as an internal standard for this work. The remaining two candidates were then tested for compatibility as an internal standard.

When phenol was used as an internal standard, the chromatograms exhibited very inconsistent phenol peak areas. This was due to the presence of phenol in the samples themselves, which led to a second phenol peak in the chromatogram at a later retention time. There is no way of distinguishing the added phenol from the phenol already present in the bio oil samples. Therefore, phenol could not be used as an internal standard. When toluene was used as an internal standard, the chromatograms produced showed that the toluene peak did not interfere with the peaks of sample compounds, and exhibited consistent toluene peak areas when added as the same concentration to several different oil samples. These observations led to the selection of toluene as the internal standard for use in this work. The next step was to pinpoint an appropriate concentration of toluene to add to each sample. Initial concentrations tested ranged from 860 to 1000 parts per million (ppm); however, these concentrations were deemed too high after investigation of the chromatograms produced when toluene was run in DCM, the solvent, alone (Figure 3.4).

Figure 3.4(a) shows a chromatogram produced from a toluene concentration of roughly 860 ppm. Notice the presence of a double peak, or a riding peak on the front end of the main toluene peak in the chromatogram. This undesired behavior is due to an overloading of the MS detector, meaning the compound in question is present at a concentration too high for the instrument to accurately measure. Figure 3.4(b) shows

the chromatogram produced from a toluene concentration of roughly 20 ppm, with no double peak. After confirming peak area consistency across samples, approximately 20 ppm was determined to be the appropriate concentration of toluene to be used as an internal standard in GC-MS analysis of HTL-produced oil.

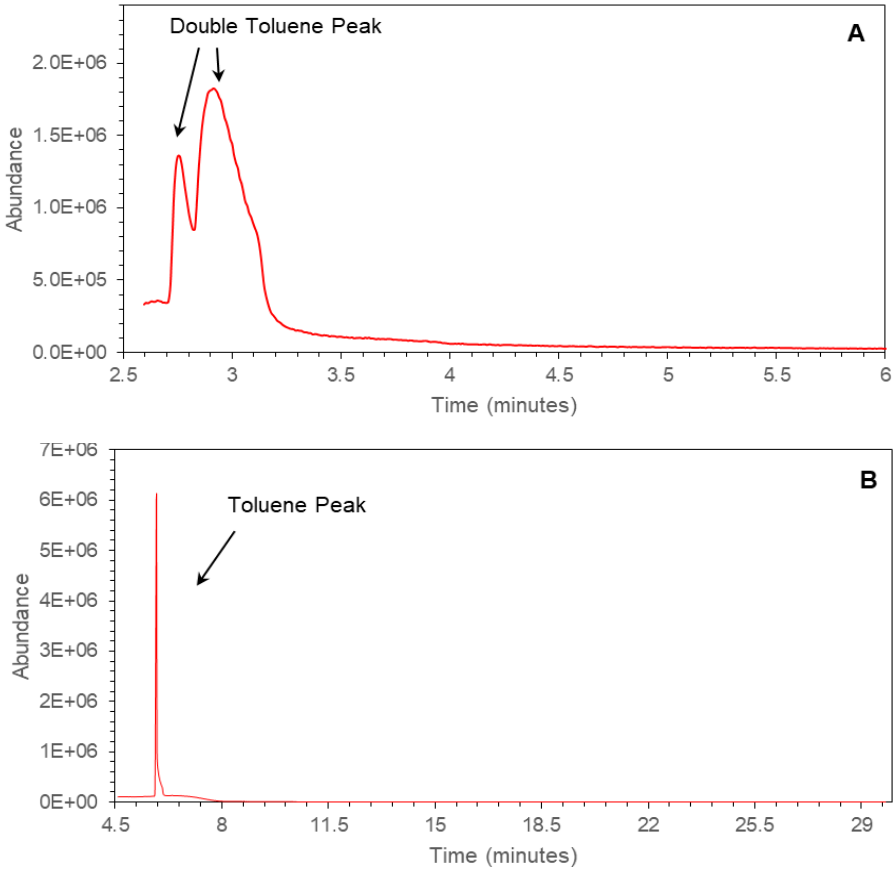


Figure 3.4 GC-MS chromatograms from 860 ppm (a) and 21 ppm (b) toluene in DCM. Note that the x-axis was adjusted to show an excerpt of the early chromatogram to clearly illustrate the riding peak.

3.3.3. Compound Classification for oil phase samples

After a GCMS run was completed, the corresponding data set, containing the chromatogram produced, the area percent report for each of the peaks in the

chromatogram, and the library search report naming the chemical compound corresponding to each chromatogram peak, was retrieved from the computer for analysis. The area percent report and the library search results were used in tandem to match each compound identified to the appropriate chromatogram peak and peak area. To simplify data and increase the robustness of the results, we used the following criteria for including compounds in our analysis: Only compounds identified with greater than 50% confidence (quality parameter > 50) and total area percent greater than 1% were considered. Due to limitations within the GCMS software itself, however, compounds and their corresponding peaks not meeting this criteria were still integrated and accounted for in the raw data reported. To aid in quick exclusion of these peaks, we created an external selection process in the form of a Macro program in Microsoft Excel (see Appendix). This program removed compound/peak data that did not meet the aforementioned criteria. The peaks for compounds that were determined to be of high enough quality and sufficient abundance were used to calculate the relative area percent for each compound using Equation 3.1. The compounds were then grouped according to the following categories: alkanes, alkenes, cyclic hydrocarbons, fatty acids, N-heterocyclic, O-heterocyclic, phenols, and others. The cumulative relative area percent was then reported for each compound grouping, and the final data (reported in Chapter 4 of this thesis) was taken to be the average cumulative relative area percent for each compound grouping across the triplicate reaction sets.

3.4. Triple Liquid Extraction for Aqueous Product Analysis

3.4.1. Procedure Development

Although produced from the same HTL reactions, the aqueous and oil products are quite different in composition and therefore require distinct methods of analysis. One goal of this research is to produce a complete characterization of the aqueous product, which included elemental quantification for carbon and nitrogen, as well as determining the speciation of said elements within the aqueous product. We began this work by using high performance liquid chromatography, or HPLC. The details of the results from HPLC analysis of the aqueous product are discussed in depth in Chapter 4 of this thesis; however, we briefly discuss the HPLC results here to provide motivation for GC-MS analysis of the aqueous phase. The aqueous product was tested for 9 different compounds via HPLC, but the chromatograms produced from this analysis showed several unidentified compound peaks. Chromatography itself, whether liquid or gaseous, cannot identify unknown compounds unless coupled with mass spectrometry. Therefore, to identify the detected unknown compounds in the aqueous samples, additional analysis was needed. GC-MS analysis is well-suited for identifying unknown compounds; however, it requires a volatile sample, meaning that the carrier solvent cannot be aqueous in nature. As such, GC-MS analysis of aqueous product samples requires extensive pretreatment, such as in the form of a triple liquid extraction procedure. The methods developed for the triple liquid extraction procedure used in this work were modified from those detailed in Alimoradi et al., 2020; Johansen et al., 1996 and Pham et al., 2013. The triple liquid extraction procedure is outlined in the schematic depicted by Figure 3.5 below, and is described in detail in the following paragraphs.

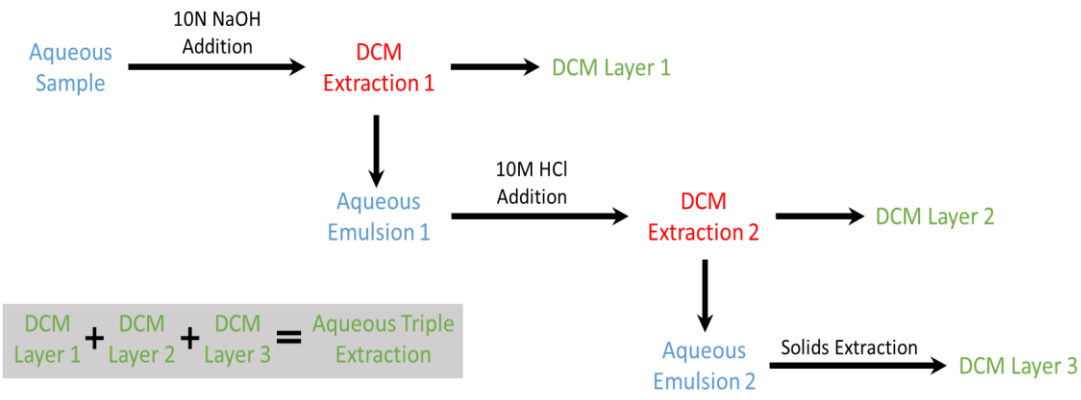


Figure 3.5 Procedure for aqueous phase triple extraction.

First, the aqueous sample is thawed and vortexed to ensure homogeneity, and a 3 mL aliquot is removed for the extraction. This aliquot is made alkaline (pH > 13) via addition of 10N NaOH (drop by drop through a transfer pipette). The alkaline aliquot is then placed in a separatory funnel and mixed vigorously with 50 mL of dichloromethane (DCM) for approximately 1 minute. The emulsion is left in the funnel to settle for approximately 5 minutes, after which the DCM layer is collected as DCM Layer 1. The remaining aqueous emulsion is recovered and acidified via the addition of 10 N HCl to a pH around 5. The acidified sample is again placed in the separatory funnel and mixed vigorously with 50 mL of DCM for 1 minute and left for 5 minutes to settle. This DCM layer is recovered as DCM Layer 2. The remaining supernatant is recovered and passed through a solids separation cartridge (Sep-Pak tC18 Plus Cartridge, Waters Associates, Milford, MA). The adsorbed sample is recovered from the cartridge using 10 mL of DCM to create DCM Layer 3. The three DCM Layers are combined to create the Aqueous Triple Extraction (ATE). The ATE is then flash mixed to ensure homogeneity, filtered through a 0.45 micron PTFE filter and analyzed on the

GC-MS. Originally, toluene was added to the ATE as an internal standard for aid in GC-MS analysis; however, the dilute nature of the ATE caused complications with use of an internal standard.

3.4.2. Internal Standard Use

The concentration of the relevant organic compounds ATE is much more dilute than their concentration in the oil product samples analyzed via GC-MS (due to the nature of both the aqueous product itself and the non-condensing triple liquid extraction procedure). Thus, adjustments to the concentration of toluene used as an internal standard in GC-MS analysis of the ATE were needed to avoid sample compound peak exclusion (Figure 3.6). A concentration of 10 ppm toluene was originally selected. A GC-MS heating ramp and program file suggested in Alimoradi et al. (2020) was used for GCMS analysis of the aqueous extractions; however, after the first few runs of samples using this program scheme, we noticed that the peak for the toluene internal standard was not appearing in the chromatograms. To fix this, the solvent delay time (a GC-MS method program parameter which delays the recording of compounds by the detector so as to not collect data on the solvent) was shortened from 6 minutes to 4.5 minutes. After this switch, the toluene compound peak and the associated data were collected and included in the data reports compiled by the GC-MS software. Although this fixed the issue of not detecting the toluene peak at all, further problems were discovered once the toluene peak was accounted for.

Figure 3.6 shows two chromatograms, both produced from GCMS runs of the same aqueous extraction sample, one with 5 ppm toluene internal standard added (Figure 3.6(a)) and one with no internal standard added (Figure 3.6(b)). Notice the

difference in size of the highlighted compound peaks in each chromatogram. In the case depicted by the chromatogram in Figure 3.6(a), the toluene peak is overshadowing the sample compound peaks to a point where the majority of sample compound peaks are unidentifiable in the chromatogram. This indicates that there is an overabundance of toluene in the sample compared to other compounds, which can affect the quality of analysis and the ability of the GC-MS software to detect all of the relevant compounds within the sample. Due to the timeline of this work, it was necessary to move on and complete the GC-MS analysis of the aqueous extractions without the addition of an internal standard. The GC-MS analysis of the aqueous extractions was continued as a qualitative analysis to contribute to the characterization of the aqueous products produced from HTL of manure digestate. This work leaves the door open for future research to quantitatively characterize the aqueous products from HTL reactions of this nature, perhaps via liquid chromatography mass spectrometry or refined GC-MS technologies.

3.4.3. Compound Classification

The final step in the aqueous product analysis, then, was to classify and group the compounds detected similarly to what was done in the final stages of the oil product data analysis from GC-MS. Again, the aqueous product GC-MS data was exported to Microsoft Excel, where a Macro was used to remove data falling outside the criteria for inclusion (quality parameter > 40%; percent total area > 0.5%) (see Appendix). Note that the criteria for inclusion of peaks was lowered for aqueous product analysis compared to oil product analysis in order to compensate for the dilute nature of the samples. The qualifying compounds were then classified into one of the following

compound groupings: phenols, alkanes, alkenes, fatty acids, sulfurous acids, and ketones.

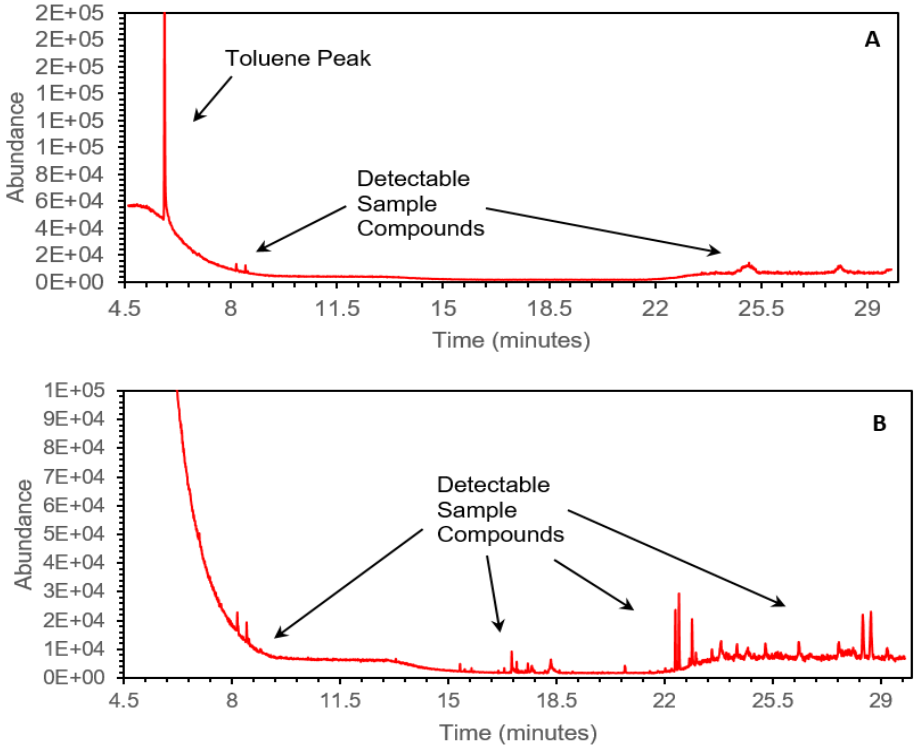


Figure 3.6 Chromatograms produced from the GC-MS analysis of the same aqueous triple extraction sample with (A) and without (B) a 5 ppm toluene internal standard.

3.5. Conclusion

The goal of this work was to develop a GC-MS method that characterizes oil and aqueous products from HTL of manure digestate, a representative wet organic waste. Investigating the compositions of these products at varying catalytic conditions and retention times provides insight into the HTL reaction pathway, as well into the optimal retention time for production of carbon-dense oil and nutrient-rich aqueous products. For the oil product, an internal standard was used to quantify relative concentrations of compounds of interest and enabled us to compare concentrations across different oil samples. To prepare aqueous products for GC-MS analysis, a triple

extraction procedure was developed by modifying a method from the literature. The internal standard methodology for aqueous product preparation was not completed, but is the subject of future work in the Sills Lab. The methods developed here allowed us to measure the effect of hydraulic retention time and acid catalyst on HTL of manure digestate, described in Chapter 4.

CHAPTER 4: EFFECT OF ACID CATALYST AND HYDRAULIC RETENTION TIME ON AQUEOUS AND OIL PRODUCTS FROM HTL

4.1. Background and Motivation

The American dairy industry produces roughly 20 MT (megatons) of waste each year (Skaggs et al., 2018). This accounts for nearly 30% of the wet organic waste produced in the four main waste categories of wastewater sludge, animal wastes, food waste, and fats/oils/greases (Skaggs et al., 2018). A large portion of organic waste is left untreated, and has the potential to wash into bodies of water and create aquatic "dead zones." In addition to the waste problem, American society has seen an increased dependence on fossil fuels, a trend that is expected to continue (Xiu et al., 2010). Animal manure is often treated with anaerobic digestion, a process where microbes break down organics in the absence of oxygen, producing energy-dense biogas and a waste sludge, called digestate. Hydrothermal liquefaction (HTL) represents a promising process of producing bio oil from wet organic wastes (Karagöz et al., 2004; Posmanik et al., 2017; Tekin et al., 2014; Theegala & Midgett, 2012; Wahyudiono et al., 2009; Xiu et al., 2010; Yin et al., 2010), such as dairy manure digestate. Anaerobic digestion and HTL have the potential to simultaneously reduce both pollution from organic waste streams and our dependence on fossil fuels. This research investigates the compositions of the aqueous and oil products from HTL of dairy manure digestate. Such investigation is needed to further understand the effect of reaction conditions (such as retention time and catalytic conditions) on the HTL reaction. Understanding the effect of reaction conditions on HTL products can inform the design of a commercial HTL process and HTL-produced oil refining techniques.

HTL takes advantage of the altered physicochemical properties of water when it is heated and subjected to near-critical temperatures and pressures (Figure 4.1) (Tekin et al., 2014). Under such conditions, water becomes a good solvent for non-polar substances, such as organic matter (Tekin et al., 2014). HTL is similar to the geologic processes that produce fossil fuels, except HTL timelines are measured in much shorter time increments, typically minutes. Under hydrothermal conditions, organic substances depolymerize into smaller, unstable, and reactive pieces. Because of their instability and reactivity, some of the fragments are almost immediately repolymerized into oily and tar-like compounds, referred to as bio oils (Xiu & Shahbazi, 2012). To design a process which can be implemented on a larger scale, it is important to understand the effect of different reaction conditions, such as retention time and catalytic condition, on the products of HTL.

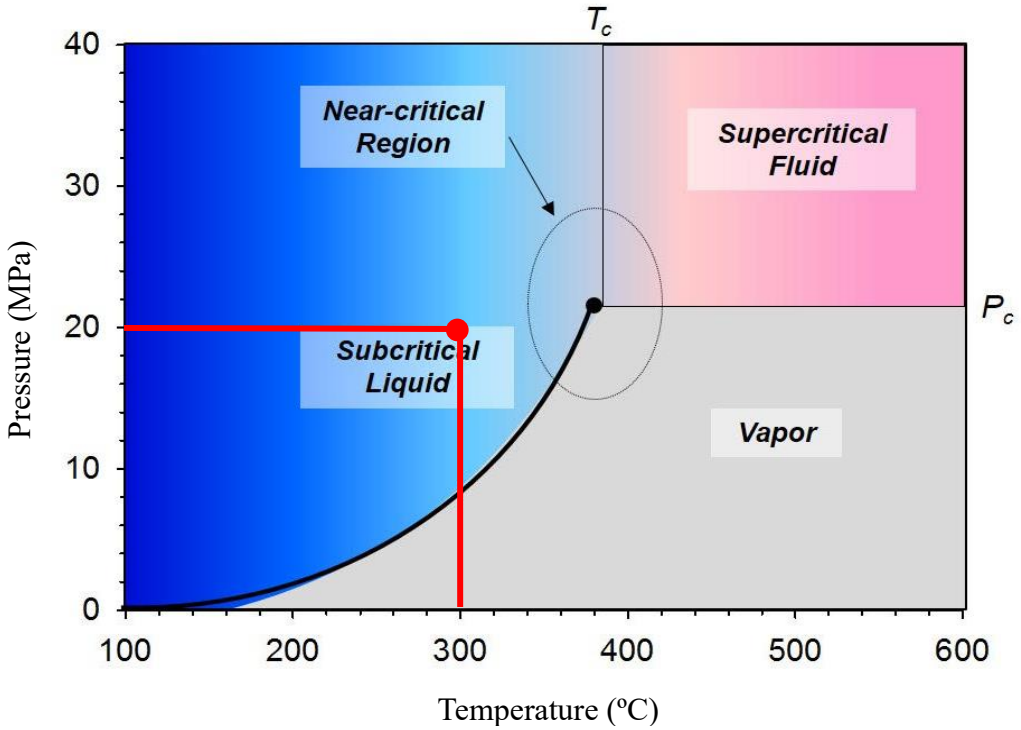


Figure 4.1 Phase diagram for water. Red lines indicate the reaction conditions used in this research. Modified from (Posmanik et al., 2017).

4.2. Methods

4.2.1. Experimental HTL

HTL reactions were run in triplicate using a method modified from the method described in Posmanik et al. (2017), and were conducted at Cornell University. Briefly, approximately 200 mL of digested dairy cow manure was placed in a well-mixed batch reactor and heated to 300°C and 20 MPa for specified retention times (5, 10, 20, 30, and 40 minutes). For the acid catalyzed reactions, 15 mL of 5 M phosphoric acid were added to the slurry. A heat exchanger, placed at the exit of the reactor, allowed for samples to be removed from the reactor immediately after reaching the desired retention time (Figure 4.3). The importance of the heat exchanger is discussed below. After removal from the reactor, samples were separated into the oil, aqueous, and solid products via a multi-stage phase extraction as published in Posmanik et al., 2020: (1) separation of the polar and non-polar liquids using a solid-phase extraction (SPE) system equipped with reversed phase sorbent tubes (Starta SDB-L, Phenomenex, Torrance, CA); (2) elution of the non-polar phase with dichloromethane (DCM, CH_2Cl_2) (Sigma Aldrich); (3) filtration of the polar phase aqueous product via 0.45 μm membrane filter; and (4) removing the DCM from the non-polar phase by drying at room temperature for 48–72 h for the bio oil product. Figure 4.2 outlines the HTL experiment in a schematic.

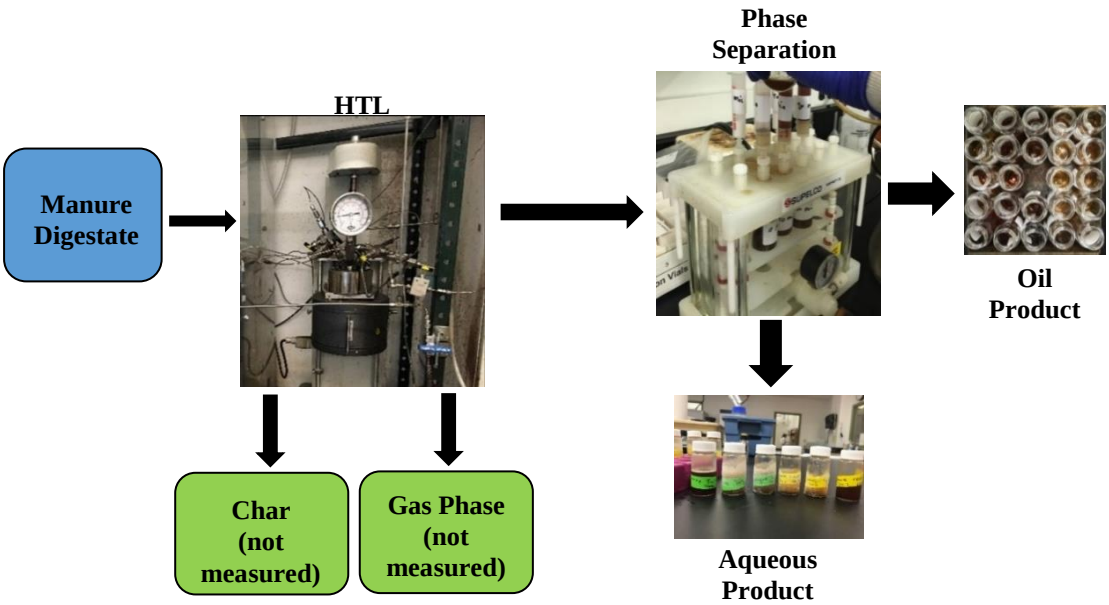


Figure 4.2 Process flow schematic for the hydrothermal liquefaction of manure digestate and subsequent phase separation.

The heat exchanger loop incorporated into reactor design eliminates any constituents of the products formed from residual reactions that can occur within the HTL reactor during the required cooling period in reactors without a heat exchanger loop. Thus, this research provides a more accurate characterization of the compositions of the aqueous and oil products from HTL of manure digestate as a function of retention time compared to research conducted in systems without this setup. This use of a heat exchanger helps to paint a clearer picture of the overall sequencing of HTL reactions by investigating differences in liquid product compositions at different retention times and under varying catalytic conditions.

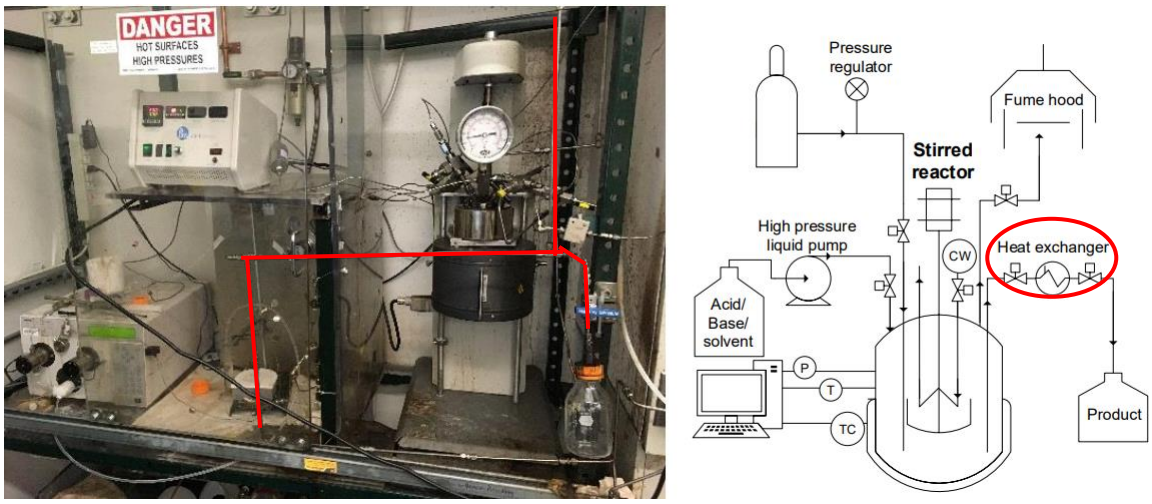


Figure 4.3 HTL batch reactor and schematic, with the heat exchange loop indicated in red in both the image and the schematic. Schematic notation is as follows: P, pressure reading; T, temperature reading; TC, temperature control; CW, cooling water. Figure modified from (Posmanik et al., 2018), supporting information.

4.2.2. Analytical Materials and Methods

To characterize the products from HTL of dairy manure digestate, several analytical procedures were completed in the Environmental Engineering and Science laboratory at Bucknell University. For each product phase, the following analytical procedures were performed: *for the oil product:* gas chromatography mass spectrometry (GC-MS); *for the aqueous product:* high performance liquid chromatography (HPLC), total organic carbon analysis (TOC), and nitrogen speciation analysis via colorimetric methods. CHN analysis was also performed on the dairy manure digestate used as a feedstock in this work so that percent recoveries of key nutrients could be calculated.

4.2.2.1. Feedstock and Chemicals

Dairy manure digestate was used as the feedstock for HTL reactions. The digested manure was obtained from an anaerobic digester on Sunnyside dairy farm, located in Scipio Center, NY. The average solids content was 10 wt %, and Milli-Q water was used as the reaction medium. Phosphoric acid was selected as the acid catalyst. Dichloromethane (DCM) 99.5% stabilized ACS (BDH Chemical) was used as the solvent for GC-MS analysis. Anhydrous toluene 99.8% (Sigma-Aldrich Corporation) served as the internal standard in GC-MS analysis of the oil product. LECO EDTA (p/n/ 502-092) was used to standardize the CHN instrument. Nine separate standards were run at representative concentrations to create standard curves for HPLC analysis of the aqueous product: glucose (Fisher Scientific), xylose (TCI America), arabinose (Sigma-Aldrich Corporation), fructose (Sigma-Aldrich Corporation), succinic acid ACS (Alfa Aesar by Thermo Fisher Scientific), lactic acid (L-(+)-lactic acid, >98%, Sigma-Aldrich Corporation), acetic acid (glacial, BDH Chemical), formic acid (99+% LC/MS grade, Fisher Scientific) and 5-hydroxymethylfurfurol (HMF, 99+%, Sigma-Aldrich Corporation). Sulfuric acid (HPLC grade, Fisher Scientific) was used to prepare the HPLC eluent.

4.2.2.2. CHN Analysis

The carbon and hydrogen content of the manure digestate used as the HTL feedstock was measured to calculate the percent yield for each of these elements in the oil and aqueous products. CHN analysis was performed using a CE440 elemental analyzer (Exter Analytical, North Chelmsford, MA). 5 replicates of 30, 40, 50, and 60 mg EDTA standard (LECO Corporation) were run to create the standard curve for the

instrument operation. For analysis, approximately 2 mg of the dried manure digestate was placed in the instrument. The sample was then volatized at 950°C and the vapor was passed through a porous crucible, scrubbed for impurities, and sent to the detector to determine the carbon and hydrogen on a mass basis. The HACH colorimetric test was used to measure Kjeldahl nitrogen in the feedstock (see section 4.2.2.6). These analyses revealed that carbon and nitrogen content of the feedstock were 43 wt % (dry mass basis) and 3 wt % (dry mass basis), respectively.

4.2.2.3. GC-MS Analysis

GC-MS analysis was conducted on a 7890A GC system and a 5975 C VL MSD (Agilent Technologies), with an HP5MS column (Agilent Technologies, P/N 19091S-433, 30m x 250um x 0.25um). Dichloromethane (DCM) was used as the solvent with both oil and aqueous products. Toluene at 21 ppm (used as an internal standard) was mixed with 10 mL DCM and the dried oil samples for approximately ten minutes. The aqueous product samples underwent a triple extraction procedure in preparation for GC-MS analysis, as described in Chapter 2. No internal standard was added to aqueous phase samples. Once prepared, the samples for analysis were filtered through a 0.45 μm filter into a 2 mL sealed vial, sealed, and injected to the GC-MS with an auto sampler. Samples not run directly after preparation were stored at 4°C until just prior to instrumental analysis, to avoid volatilization of the solvent and internal standard.

4.2.2.4. HPLC Analysis

HPLC analysis was performed using the Dionex UltiMate 3000 Liquid Chromatograph (ThermFischer Scientific) with the Aminex HPX-87H Column, 300 x 7.8 mm (BioRAD Technologies) installed. The device was equipped with a

refractive index detector (RefractoMax 521, ERC Inc.) and an ultraviolet wavelength detector (RS Variable Wavelength Detector, ThermoFischer Scientific) to identify compounds. Following the approach of Posmanik et al. (2018), 9 different compounds were selected for analysis: glucose, xylose, arabinose, fructose, succinic acid, acetic acid, lactic acid, formic acid, and hydroxymethylfurfural (HMF). Standard curves for each chemical compound were prepared in deionized water. A 0.005 M sulfuric acid solution was used as the eluent to run standards and samples through the column.

4.2.2.5. TOC Analysis

Total organic carbon content (TOC) of aqueous samples was measured with a Shimadzu Total Organic Carbon Analyzer (TOC-L CSH, Shimadzu Corporation) with the TNM-L unit addition (Shimadzu Corporation) and a non-dispersive infrared gas analyzer. The combustion chamber temperature was set to 680°C. The standard curve for TOC concentration calculation was calibrated using 100 ppm KHP in deionized water.

4.2.2.6. Nitrogen Speciation Analysis

Aqueous product samples were analyzed for total nitrogen (TN), total Kjeldahl nitrogen (TKN, ammonia + organic nitrogen), nitrate, nitrite, and ammonia concentrations using standard colorimetric methods (Hach, TNT 880). Organic nitrogen concentrations were calculated from the results of these tests.

4.3. Results and Discussion

4.3.1. Aqueous Product Analysis

4.3.1.1. Carbon and Nitrogen Distribution

Figure 4.4 shows the results from the analysis of carbon recovery and nitrogen recovery and speciation in the aqueous product from the HTL reactions (for calculations, see Appendix). Figures 4.4(a) and 4.4(b) indicate that there was no appreciable difference between the percent carbon recoveries in the aqueous product for catalyzed and non-catalyzed reactions; however, as retention time increased, carbon recovery in the aqueous product decreased. Previous work on HTL of manure digestate found that most of the carbon not recovered in the aqueous product is recovered in the oil product (Posmanik et al., 2017; Posmanik et al., 2018; Yu et al., 2011). Our results, in combination with these previous findings, suggest that a higher retention time would be appropriate to maximize the carbon recovered in the oil product and minimize the carbon recovered in the aqueous product.

In contrast, Figures 4.4(c) and 4.4(d) depict the nitrogen recovery into the aqueous product. The results of this analysis suggest that the presence of a phosphoric acid catalyst may increase nitrogen recovery into the aqueous product compared to reactions with no catalyst. Furthermore, the majority of nitrogen recovered in the aqueous product is in the form of mineralized nitrogen (i.e., ammonia and nitrate/nitrite) at all retention times and under both catalytic conditions. Recovery of nitrogen in the form of ammonia and nitrate/nitrite is desirable, as these nitrogen species are easily recovered from the aqueous phase product and readily usable as fertilizers and algal growth media (Alimoradi et al., 2020; Mau et al., 2016; Pham et al., 2013). Figures

4.4(c) and 4.4(d) also show that nitrogen recovery in the aqueous product decreased as retention time increased, suggesting that a lower retention time is optimal for maximizing the nitrogen recovery in the aqueous product and therefore maximizing its value as a marketable product. Unfortunately, longer retentions times are preferable for minimizing carbon recovery in the aqueous product; therefore, to recommend an optimal retention time for HTL of manure digestate, further research and economic comparison must be done in order to weigh the benefits and costs of minimizing carbon and maximizing nitrogen recovered into the aqueous product.

4.3.1.2. HPLC Results

The aqueous product was also analyzed using HPLC to characterize the organic carbon recovered. Figure 4.5 shows the results of this analysis of the aqueous product formed in both non-catalyzed and acid-catalyzed HTL reactions. In the absence of the phosphoric-acid catalyst, organic acids dominated the detected organic carbon in the aqueous product (Figure 4.5(a)), whereas in the presence of the catalyst, monosaccharides dominated (Figure 4.5(b)). These results give clues as to how the acid catalyst affects the progression of reaction during HTL.

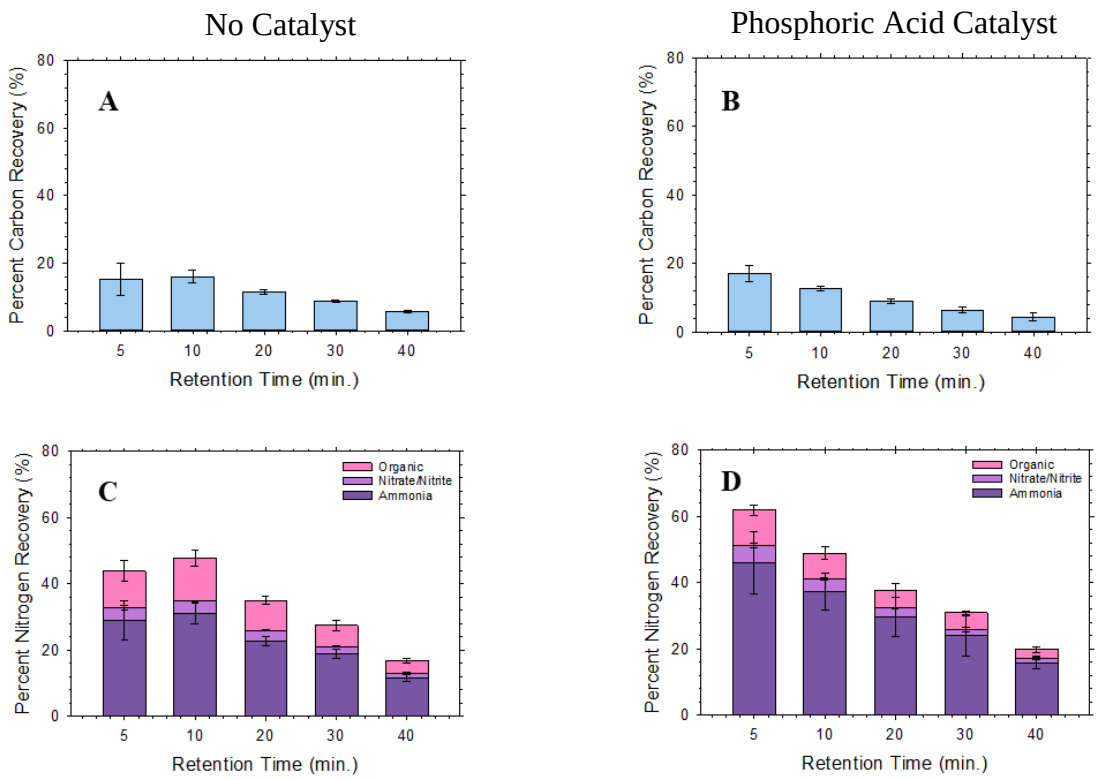


Figure 4.4 Carbon recovery and nitrogen recovery and speciation in the aqueous product from HTL of dairy manure digestate under differing catalytic conditions.

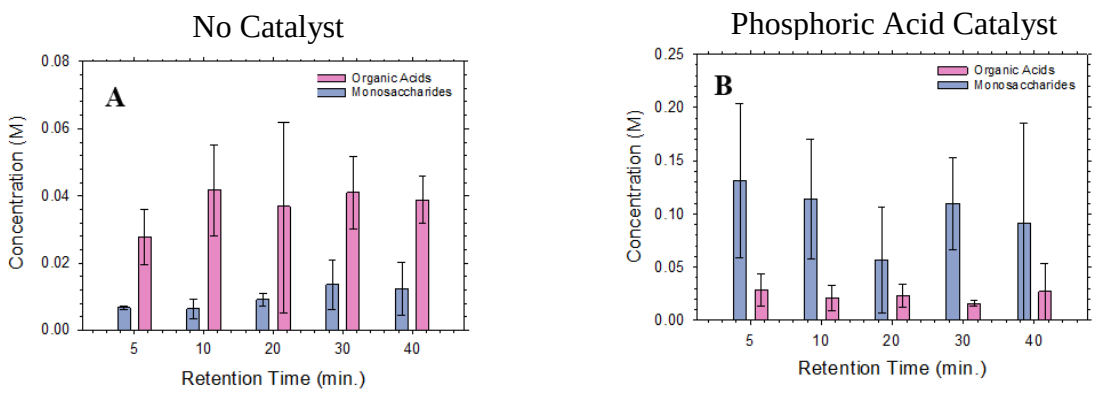


Figure 4.5 Speciation of organic carbon in the aqueous product under differing catalytic conditions.

Dairy manure digestate, the feedstock used, is rich in lignocellulose, the material that makes up plant cell walls. Lignocellulose consists of three main components: the complex carbohydrate polymers cellulose and hemicellulose, and the phenolic polymer lignin (Peterson et al., 2008; Sills & Gossett, 2012). Under hydrothermal conditions, cellulose and hemicellulose decompose into these simple sugars (such as glucose and xylose), which in turn are decomposed into compounds like aldehydes, furfurals, and organic acids (Peterson et al., 2008). A generalized reaction pathway is illustrated in Figure 4.6.

Figure 4.5 reveals that the concentrations of organic acids produced under both catalytic conditions are roughly equal, yet we see that the concentration of monosaccharides is much greater in the presence of the acid catalyst. This indicates that the acid catalyst may increase the rate of the degradation reaction from complex carbohydrates (cellulose, hemicellulose) to simple sugars; however, the catalyst does not appear to have an effect on the degradation of simple sugars to simple organic acids. It is unclear whether the excess monosaccharides present in the acid catalyzed samples are due to increased feedstock degradation (compared to the amount of degradation without the catalyst present) or if the abundance of monosaccharides is due to an inhibitory factor prohibiting the sugars from being converted into the oily compounds. Additional research is needed to elucidate the mechanism of increased presence of monosaccharides in the aqueous phase product from acid-catalyzed compared to non-catalyzed HTL reactions.

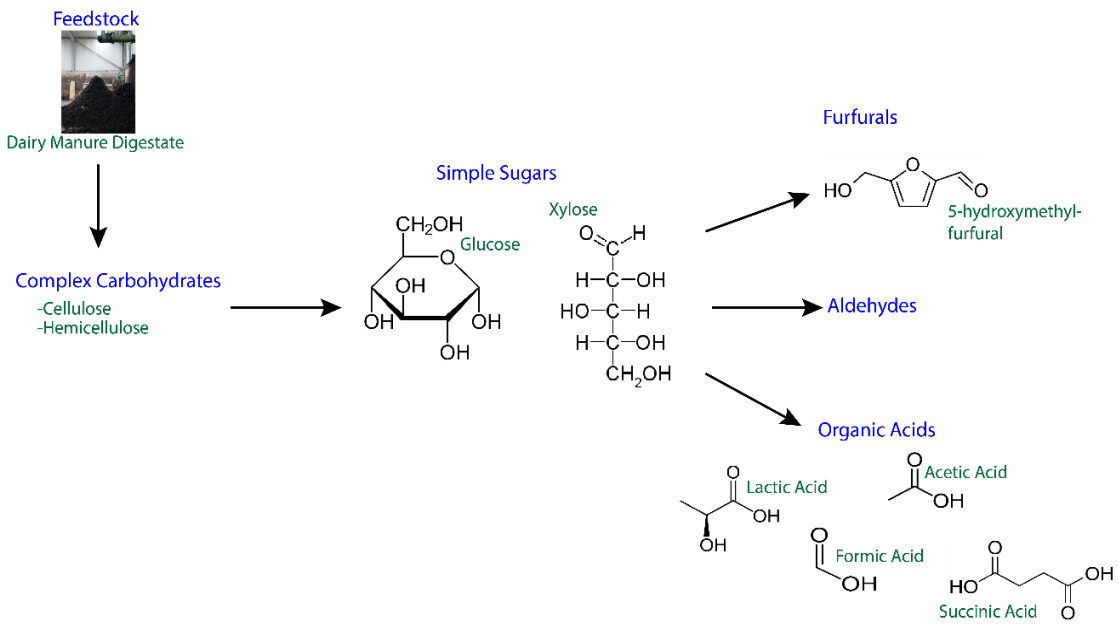


Figure 4.6 HTL reaction pathway for complex carbohydrates remaining in dairy manure digestate.

4.3.1.3. Compound Classifications

The final step in characterization of the aqueous product was GC-MS analysis to determine the unknown compounds found upon HPLC analysis of the samples. The development of the GC-MS methods used is discussed in Chapter 3 of this thesis. The aqueous product was analyzed in a qualitative manner for the presence of six types of compounds: phenols, alkanes, alkenes, sulfurous acids, fatty acids and ketones. Table 4.1 provides a summary of the compound groups present in the aqueous products at differing retention times and catalytic conditions. From the results, we can see that both phenols and alkanes are present in all samples, and sulfurous acids are present at all but one retention time/catalytic condition combination (no catalyst, 5 minutes). Lignin, a phenolic polymer, is a major component of manure digestate, and is most readily broken down into phenolic constituents, such as phenol and catechol (Wahyudiono et al.,

2009). The presence of phenols in the aqueous products regardless of catalytic conditions and retention time indicate that lignin is easily broken down by the hydrothermal liquefaction process, and also suggests that the unknown compounds detected during HPLC analysis may be phenolic compounds. The presence of alkanes in all aqueous samples is also important, as it indicates that none of the conditions investigated are completely efficient at diverting carbon into the oil product and supports the findings from HPLC analysis discussed above. To conduct a quantitative analysis that measures the relative amounts of compounds in each sample, the GC-MS method needs to be further developed to include use of an internal standard (see Chapter 3).

Table 4.1 Summary of compound groups present in HTL aqueous product after triple extraction.

Catalytic Condition	Retention Time (minutes)	Compound Groups Present					
		Phenols	**Alkanes**	**Alkenes**	**Sulfurous Acids**	**Fatty Acids**	**Ketones**
Catalyst	5	X	X	X	X		
	10	X	X		X	X	
	20	X	X		X	X	
	30	X	X		X	X	
	40	X	X		X	X	X
No Catalyst	5	X	X				
	10	X	X		X		
	20	X	X		X	X	
	30	X	X		X	X	
	40	X	X		X	X	X

The presence of sulfurous and fatty acids provides insight as to what some of the unknown compounds detected during HPLC analysis might be. As retention time

increases, fatty acids start to appear in the aqueous product, suggesting that either more sugar intermediates are being broken down (as per the reaction pathway demonstrated in Figure 4.6), or that more of the residual fats found in the manure digestate are being decomposed. In either case, longer retention times may lead to more complete degradation of the feedstock, though again, further research into inclusion of an internal standard with aqueous products analysis is required to quantify this increased degradation. Such quantitative data can inform design of commercial scale of HTL systems.

4.3.2. Oil Product Analysis

Oil product analysis was carried out in a similar manner to the aqueous product analysis via GC-MS, and a longer discussion of methods relating to this analysis is included in Chapter 3 of this thesis. The oil products were run on the GC-MS with the inclusion of a 21 ppm toluene internal standard to allow for quantification of the results. Table 4.2 below lists the relative area percentages, calculated via Equation 4.1, for each of the eight compound groups investigated (fatty acids, alkenes, alkanes, N-heterocyclic compounds, phenols, O-heterocyclic compounds, cyclic hydrocarbons, and others) for 3 representative retention times (5, 20, and 40 minutes) for both catalyzed and non-catalyzed reactions. The results of this analysis reveal several key pieces of information regarding the effects of both retention time and acid catalyst addition on the HTL reaction.

$$relative\ area\ \% = \frac{\%\ total\ area\ of\ compound\ X}{\%\ total\ area\ of\ internal\ standard} \tag{4.1}$$

Table 4.2 Relative area percentages for representative compound groupings in bio oil produced from HTL reactions of manure digestate with no catalyst and acid catalyst at varying retention times.

Compound Group	No Catalyst			Catalyst		
	HTL Retention Time (min.)					
	5	20	40	5	20	40
fatty acid	12	33	35	22	14	13
alkene	10	17	0	16	7	0
N-heterocyclic	21	0	0	0	10	0
phenol	85	64	243	75	79	427
alkane	15	7	5	11	19	0
O-heterocyclic	0	11	0	0	0	0
cyclic hydrocarbon	242	185	230	218	167	500
Other	0	17	0	25	0	0

For both reaction conditions, there is an observed decrease in alkane and alkene content as retention time increases; however, there is an increase in cyclic hydrocarbon content as retention time increases. Hydrocarbons, both chain and cyclic, are readily combustible carbon sources and increase the value of the oil (Posmanik et al., 2018). Higher heating value (HHV) is a measure of the energy content of the oil based on the relative amounts of carbon, hydrogen, and oxygen in the oil. HHV is calculated via Dulong's formula (Eq. 4.2):

$$HHV = 0.338 * C + 1.428 * \left(H - \frac{O}{8}\right) \tag{4.2}$$

where HHV is the higher heating value of the oil (MG/kg) and C, H and O are the mass percentages of carbon, hydrogen and oxygen in the dried sample, respectively (Posmanik et al., 2018). Note that the mass of carbon and nitrogen in the oil were measured at Cornell University, and the oxygen mass was calculated by subtraction.

Based on the data presented in Figure 4.7 below, the HHV of the oil product does not change significantly with varying retention time or catalytic conditions, supporting the idea that the decrease in chain hydrocarbon content with increasing retention time is compensated for by the increase in cyclic hydrocarbon content. Other research has shown that acid catalyst addition actually led to increased HHV for the oil (Posmanik et al., 2018). It is unclear why our work does not reflect the same trends as previous work, and this discrepancy is to be the subject of future analysis.

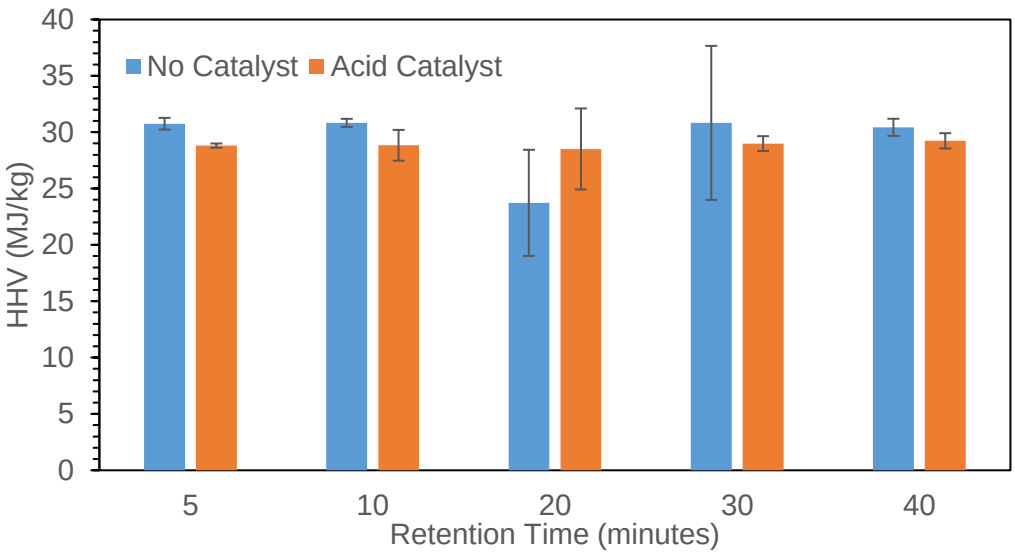


Figure 4.7 Higher heating value (HHV) of bio oils under catalyzed and non-catalyzed conditions for differing retention times.

Presence and amount of fatty acids in the oil product can provide information on the reactions occurring within HTL. A decrease in fatty acid content and an increase in hydrocarbon content suggests enhanced decarboxylation of fatty acids into hydrocarbons and carbon dioxide (Posmanik et al., 2018). The observed decrease in fatty acid content and increase in hydrocarbon content for the acid-catalyzed oil

samples (Table 4.2) is evidence that the decarboxylation pathway is, in fact, a primary reaction leading to oxygen removal from the oil product. Without the presence of an acid catalyst, there was an observed increase in fatty acid content while the hydrocarbon content increases only slightly. The increase in fatty acid content suggests that in the absence of a catalyst the decarboxylation reaction was disfavored. While these results provide valuable insight to the chemical reactions that occur during HTL, they are inconclusive regarding optimal reaction conditions (i.e. retention time and catalytic conditions). Further research into quantification of compounds in the aqueous product is necessary in order to more completely understand the effects of differing catalytic conditions or hydraulic retention time for HTL of manure digestate—under the presumption that compounds not in the oil product end up in the aqueous product and vice versa (Posmanik et al., 2017; Posmanik et al., 2018; Yu et al., 2011).

For both reactions conditions, the presence of phenolic compounds increased as retention time increased. The addition of acid catalyst, however, resulted in nearly twice the phenol content compared to non-acid catalyzed reactions. This implies that the phenolic lignin in the feedstock is more readily broken down under acidic conditions at longer retention times. Future research will need to quantify the phenol content in the aqueous products (discussed in section 4.3.1.3) to determine whether phenolic compounds are preferentially distributed to the oil or aqueous phase during HTL reactions. It is ideal to have phenol in the aqueous product as phenol must be removed from the bio oil before use, necessitating a more intensive upgrading procedure. Aqueous phase phenol, on the other hand, is easily recoverable and is of value in the chemical industry (Wahyudiono et al., 2009).

CHAPTER 5: CONCLUSION

This work focused on two aspects of hydrothermal liquefaction (HTL) applied to manure digestate: (1) method development for GC-MS analysis of the aqueous and oil products and (2) the effect of acid catalyst and hydraulic retention time on compositions of the aqueous and oil products from HTL.

Developing methods for GC-MS analysis was a crucial part of this work, as there are no previously developed methods that can characterize the aqueous and oil products from HTL of manure digestate in a both quantitative and qualitative manner. GC-MS was chosen as instrumentation because it can measure relative quantities of unknown compounds in a sample through the use of an internal standard. Our work shows that roughly 20 ppm toluene is an appropriate internal standard for use with the oil product. Concentration of toluene for use with the aqueous product was not determined due to the dilute nature of the triple-liquid extraction procedure that was developed to prepare aqueous samples for GC-MS analysis.

In the second part of this work, we found that acid catalytic conditions favor nitrogen yield in the aqueous product, but no link was established between catalytic conditions and carbon partitioning in the aqueous product. One of the major findings of this work is that the choice of retention time leads to a trade-off between partitioning of carbon and nitrogen into the oil product versus the aqueous product. Higher concentrations of both carbon and nitrogen in the aqueous product were observed at lower retention times. Minimizing carbon in the aqueous phase, which has been shown previously to maximize carbon recovery in the oil phase, occurs at higher retention times. On the other hand, maximizing nitrogen recovery as ammonia and nitrate/nitrite

in the aqueous phase, which occurs at lower retentions times, would improve resource recovery from HTL of manure digestate. These results can be used in future technoeconomic analyses and life cycle assessment models to assess the effect of reaction conditions, as well as carbon and nitrogen recoveries on the economic and environmental performances of HTL of manure digestate at different retention times.

GC-MS analysis of the oil product revealed that acid catalyzed reactions were favorable for both conversion of fatty acids to hydrocarbons and decomposition of lignin to phenolic intermediates. This again creates an interesting tradeoff, as hydrocarbons are favorable in the oil product, but phenolic compounds are not. Further experiments are required to determine the upgrading procedures needed to bring the oil quality to a usable standard Finally, GC-MS analysis of the aqueous product revealed the presence of phenols, chain hydrocarbons, sulfurous and fatty acids, and ketones.

The findings of this work leave interesting research questions open for further investigation. Further analytical work needs to be done regarding the quantification of the GC-MS analysis for the aqueous product, which could be done by including an appropriate internal standard. A more complete investigation would also include analysis of both the solid char and the gas product from HTL of manure digestate, with a focus on measuring carbon and nitrogen content in each. This information will allow researchers to complete the mass balance on these elements and further inform a conclusion regarding optimal conditions for HTL of manure digestate. Additionally, to draw definitive conclusions about the best catalytic conditions and retention times for HTL of manure digestate, researchers must investigate the tradeoff between carbon and

nitrogen partitioning in the oil and aqueous products and its impact on economic and environmental performance of HTL processing.

HTL of waste biomasses offers solutions to two of society's major problems: the overproduction of wastes and an increasing dependence on fossil fuels, which leads to climate change. While most HTL work remains at the laboratory and pilot scale, the expanding interest in waste to energy technologies as way to address pressing environmental challenges makes HTL a promising candidate for research and development to industrial and commercial scale. The work presented in this thesis contributes to the growing body of research relating to HTL as a viable process for simultaneous waste remediation and energy source production, and lays groundwork for important future work.

BIBLIOGRAPHY

Akhtar, J., & Amin, N. A. S. (2011). A review on process conditions for optimum bio-oil yield in hydrothermal liquefaction of biomass. *Renewable and Sustainable Energy Reviews*, *15*(3), 1615–1624. https://doi.org/10.1016/j.rser.2010.11.054

Alimoradi, S., Stohr, H., Stagg-Williams, S., & Sturm, B. (2020). Effect of temperature on toxicity and biodegradability of dissolved organic nitrogen formed during hydrothermal liquefaction of biomass. *Chemosphere*, *238*, 124573. https://doi.org/10.1016/j.chemosphere.2019.124573

Chen, W.-T., Qian, W., Zhang, Y., Mazur, Z., Kuo, C.-T., Scheppe, K., Schiedman, L. C., & Sharma, B. K. (2017). Effect of ash on hydrothermal liquefaction of high-ash content algal biomass. *Algal Research*, *25*, 297–306. http://dx.doi.org/10.1016/j.algal.2017.05.010

Chen, W.-T., Zhang, Y., Zhang, J., Schideman, L., Yu, G., Zhang, P., & Minarick, M. (2014). Co-liquefaction of swine manure and mixed-culture algal biomass froma wastewater treatment system to produce bio-crude oil. *Applied Energy*, *128*, 209–216.

Chen, W.-T., Zhang, Y., Zhang, J., Yu, G., Schideman, L. C., Zhang, P., & Minarick, M. (2014). Hydrothermal liquefaction of mixed-culture algal biomass from wastewater treatment system into bio-crude oil. *Bioresource Technology*, *152*, 130–139. https://doi.org/10.1016/j.biortech.2013.10.111

Chen, W.-T., Zhang, Y., Zhang, J., Yu, G., Schiedman, L. C., Zhang, P., & Minarick, M. (2014). Hydrothermal liquefaction of mixed-culture algal biomass from

wastewater treatment system into bio-crude oil. *Bioresource Technology*, *152*, 130–139. https://doi.org/10.1016/j.biortech.2013.10.111

Chen, Y., Zhao, N., Wu, Y., Wu, K., Wu, X., Liu, J., & Yang, M. (2017). Distributions of organic compounds to the products from hydrothermal liquefaction of microalgae. *Environmental Progress & Sustainable Energy*, *36*(1), 259–268. https://doi.org/10.1002/ep.12490

Concentrated Animal Feeding Operations (CAFOs). (n.d.). Department of Environmental Protection. Retrieved March 30, 2020, from https://www.dep.pa.gov:443/Business/Water/CleanWater/AgriculturalOperations/CAFOs/Pages/default.aspx

Costa, A., Ely, C., Pennington, M., Rock, S., Staniec, C., & Turgeon, J. (2015). *Anaerobic Digestion and its Applications*. 24.

Gas Chromatography Mass Spectrometry (GC-MS) Information—US. (n.d.). Retrieved February 18, 2020, from https://www.thermofisher.com/us/en/home/industrial/mass-spectrometry/mass-spectrometry-learning-center/gas-chromatography-mass-spectrometry-gc-ms-information.html

How Does a GC-MS Work. (n.d.). *EL Science*. Retrieved March 28, 2020, from https://elscience.co.uk/our-lab/gcms-work/

Johansen, S. S., Hansen, A. B., Mosbaek, H., & Arvin, E. (1996). Method development for trace analysis of heteroaromatic compounds in contaminated groundwater. *Journal of Chromatography A*, *738*(2), 295–304. https://doi.org/10.1016/0021-9673(96)00114-8

Karagöz, S., Bhaskar, T., Muto, A., Sakata, Y., & Uddin, Md. A. (2004). Low-Temperature Hydrothermal Treatment of Biomass: Effect of Reaction Parameters on Products and Boiling Point Distributions. *Energy & Fuels*, *18*(1), 234–241. https://doi.org/10.1021/ef030133g

Kruse, A., & Gawlik, A. (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. https://doi.org/10.1021/ie0202773

Lorimore, J., Powers, W., & Sutton, A. (2004). *Manure Characteristics*. *2nd Edition*.

Mau, V., Quance, J., Posmanik, R., & Gross, A. (2016). Phases' characteristics of poultry litter hydrothermal carbonization under a range of process parameters. *Bioresource Technology*, *219*, 632–642. https://doi.org/10.1016/j.biortech.2016.08.027

Mellon, F. A. (2003). Mass Spectrometry; Principles and Interpretations. In *Encyclopedia of food sciences and nutrition* (pp. 3739–3749). Academic Press.

Munir, M. T., Li, B., Boiarkina, I., Baroutian, S., Yu, W., & Young, B. R. (2017). Phosphate recovery from hydrothermally treated sewage sludge using struvite precipitation. *Bioresource Technology*, *239*, 171–179. https://doi.org/10.1016/j.biortech.2017.04.129

Peterson, A. A., Vogel, F., Lachance, R. P., Fröling, M., Michael J. Antal, J., & Tester, J. W. (2008). Thermochemical biofuel production in hydrothermal media: A review of sub- and supercritical water technologies. *Energy & Environmental Science*, *1*(1), 32–65. https://doi.org/10.1039/B810100K

Pham, M., Schideman, L., Scott, J., Rajagopalan, N., & Plewa, M. J. (2013). Chemical and Biological Characterization of Wastewater Generated from Hydrothermal Liquefaction of Spirulina. *Environmental Science & Technology*, *47*(4), 2131–2138. https://doi.org/10.1021/es304532c

Posmanik, R., Cantero, D. A., Malkani, A., Sills, D. L., & Tester, J. W. (2017). Biomass conversion to bio-oil using sub-critical water: Study of model compounds for food processing waste. *The Journal of Supercritical Fluids*, *119*, 26–35. https://doi.org/10.1016/j.supflu.2016.09.004

Posmanik, R., Martinez, C. M., Cantero-Tubiilla, B., Cantero, D. A., Sills, D. L., Cocero, M. J., & Tester, J. W. (2018). Acid and Alkali Catalyzed Hydrothermal Liquefaction of Dairy Manure Digestate and Food Waste. *ACS Sustainable Chemistry & Engineering*, *6*, 2724–2732. https://doi.org/10.1021/acssuschemeng.7b04359

Posmanik, R., Darzi, R., Glicksberg, R., Shabtay, A., & Cohen-Zinder, M. (2020). Hydrothermal conversion of beef cattle manure can enhance energy recovery in confined feedlots. *Environmental Science Water Research and Technology*, *6*, 1125–1138. https://doi.org/10.1039/c9ew00856j

Rockström, J., Steffen, W., Noone, K., Persson, Å., Chapin, F. S., Lambin, E. F., Lenton, T. M., Scheffer, M., Folke, C., Schellnhuber, H. J., Nykvist, B., de Wit, C. A., Hughes, T., van der Leeuw, S., Rodhe, H., Sörlin, S., Snyder, P. K., Costanza, R., Svedin, U., … Foley, J. A. (2009). A safe operating space for humanity. *Nature*, *461*(7263), 472–475. https://doi.org/10.1038/461472a

Sills, D. L., & Gossett, J. M. (2012). Using FTIR spectroscopy to model alkaline pretreatment and enzymatic saccharification of six lignocellulosic biomasses.

Biotechnology and Bioengineering, *109*(4), 894–903. https://doi.org/10.1002/bit.24376

Skaggs, R. L., Coleman, A. M., Seiple, T. E., & Milbrandt, A. R. (2018). Waste-to-Energy biofuel production potential for selected feedstocks in the conterminous United States. *Renewable and Sustainable Energy Reviews*, *82*, 2640–2651. https://doi.org/10.1016/j.rser.2017.09.107

Tekin, K., & Karagöz, S. (2013). Non-catalytic and catalytic hydrothermal liquefaction of biomass. *Research on Chemical Intermediates*, *39*(2), 485–498. https://doi.org/10.1007/s11164-012-0572-3

Tekin, K., Karagöz, S., & Bektaş, S. (2014). A review of hydrothermal biomass processing. *Renewable and Sustainable Energy Reviews*, *40*, 673–687. https://doi.org/10.1016/j.rser.2014.07.216

Theegala, C. S., & Midgett, J. S. (2012). Hydrothermal liquefaction of separated dairy manure for production of bio-oils with simultaneous waste treatment. *Bioresource Technology*, *107*, 456–463. https://doi.org/10.1016/j.biortech.2011.12.061

U.S. energy facts explained—Consumption and production—U.S. Energy Information Administration (EIA). (n.d.). Retrieved March 30, 2020, from https://www.eia.gov/energyexplained/us-energy-facts/

Vardon, D. R., Sharma, B. K., Scott, J., Yu, G., Wang, Z., Schideman, L., Zhang, Y., & Strathmann, T. J. (2011). Chemical properties of biocrude oil from the hydrothermal liquefaction of Spirulina algae, swine manure, and digested anaerobic sludge. *Bioresource Technology*, *102*(17), 8295–8303. https://doi.org/10.1016/j.biortech.2011.06.041

Wang, K., Ma, Q., Burns, M., Sudibyo, H., Sills, D., Goldfarb, J., and Tester, J. (2020). “Impact of Feed Injection and Batch Processing Methods in Hydrothermal Liquefaction”. *The Journal of Supercritical Fluids* [in review].

Wahyudiono, Sasaki, M., & Goto, M. (2009). Conversion of biomass model compound under hydrothermal conditions using batch reactor. *Fuel*, *88*(9), 1656–1664. https://doi.org/10.1016/j.fuel.2009.02.028

Xiu, S., & Shahbazi, A. (2012). Bio-oil production and upgrading research: A review. *Renewable and Sustainable Energy Reviews*, *16*(7), 4406–4414. https://doi.org/10.1016/j.rser.2012.04.028

Xiu, S., Shahbazi, A., Shirley, V., & Cheng, D. (2010). Hydrothermal pyrolysis of swine manure to bio-oil: Effects of operating parameters on products yield and characterization of bio-oil. *Journal of Analytical and Applied Pyrolysis*, *88*(1), 73–79. https://doi.org/10.1016/j.jaap.2010.02.011

Yin, S., Dolan, R., Harris, M., & Tan, Z. (2010). Subcritical hydrothermal liquefaction of cattle manure to bio-oil: Effects of conversion parameters on bio-oil yield and characterization of bio-oil. *Bioresource Technology*, *101*(10), 3657–3664. https://doi.org/10.1016/j.biortech.2009.12.058

Yin, S., & Tan, Z. (2012). Hydrothermal liquefaction of cellulose to bio-oil under acidic, neutral and alkaline conditions. *Applied Energy*, *92*, 234–239. https://doi.org/10.1016/j.apenergy.2011.10.041

Yu, G., Zhang, Y., Schideman, L., Funk, T., & Wang, Z. (2011). Distributions of carbon and nitrogen in the products from hydrothermal liquefaction of low-lipid microalgae. *Energy & Environmental Science*, *4*(11), 4587–4595. https://doi.org/10.1039/C1EE01541A

APPENDIX

TABLE OF CONTENTS

A.1. Excel Macro Code ..... A 1
A.1.1. Macro code used in analysis of oil samples. ..... A 1
A.1.2. Macro code used for analysis of aqueous samples. ..... A 2
A.2. GC-MS Procedures ..... A 2
A.2.1. Oil Sample Preparation ..... A 2
A.2.2. Triple Extraction Procedure ..... A 3
A.3. Nutrient Recovery Calculations ..... A 4
A.3.1. Carbon Recovery in the Aqueous Product ..... A 4
A.3.2. Nitrogen Recovery in the Aqueous Product ..... A 7

A.1. Excel Macro Code

A.1.1. Macro code used in analysis of oil samples.

```
Sub biooil_analysis_exclusions()
'
' analysis_test_relref Macro
'

'
    ActiveCell.FormulaR1C1 = "Test"
    ActiveCell.Offset(1, 0).Range("A1").Select
    ActiveCell.FormulaR1C1 = "=IF(OR(RC[-3]<1,RC[-1]<50),""x"",""""))"
    ActiveCell.Select
    ActiveCell.FormulaR1C1 = "=IF(OR(RC[-3]<1,RC[-1]<50),""X"",""""))"
    ActiveCell.Select
    Selection.AutoFill Destination:=ActiveCell.Range("A1:A2"), Type:= _
        xlFlashFill
    ActiveCell.Range("A1:A2").Select
    ActiveCell.Select
    ActiveCell.FormulaR1C1 = "=IF(OR(RC[-3]<1,RC[-1]<50),""X"",""""))"
    ActiveCell.Select
    Selection.AutoFill Destination:=ActiveCell.Range("A1:A75"), Type:= _
        xlFillDefault
    ActiveCell.Range("A1:A75").Select
    ActiveCell.Offset(-1, 0).Range("A1").Select
    Selection.AutoFilter
    ActiveSheet.Range("$A$1:$G$76").AutoFilter Field:=7, Criteria1:="<>"
    ActiveCell.Offset(2, 0).Range("A1:A74").Select
    Selection.EntireRow.Delete
    ActiveSheet.Range("$A$1:$G$22").AutoFilter Field:=7
    ActiveCell.Offset(4, 2).Range("A1").Select
End Sub
```

A.1.2. Macro code used for analysis of aqueous samples.

```
Sub aqueous_analysis_exclusion()
'
' analysis_test_relref Macro
'

'
    ActiveCell.FormulaR1C1 = "Test"
    ActiveCell.Offset(1, 0).Range("A1").Select
    ActiveCell.FormulaR1C1 = "=IF(OR(RC[-3]<1,RC[-1]<40),""x"",""""")"
    ActiveCell.Select
    ActiveCell.FormulaR1C1 = "=IF(OR(RC[-3]<1,RC[-1]<40),""X"",""""")"
    ActiveCell.Select
    Selection.AutoFill Destination:=ActiveCell.Range("A1:A2"), Type:= _
        xlFlashFill
    ActiveCell.Range("A1:A2").Select
    ActiveCell.Select
    ActiveCell.FormulaR1C1 = "=IF(OR(RC[-3]<1,RC[-1]<40),""X"",""""")"
    ActiveCell.Select
    Selection.AutoFill Destination:=ActiveCell.Range("A1:A75"), Type:= _
        xlFillDefault
    ActiveCell.Range("A1:A75").Select
    ActiveCell.Offset(-1, 0).Range("A1").Select
    Selection.AutoFilter
    ActiveSheet.Range("$A$1:$G$76").AutoFilter Field:=7, Criteria1:="<>"
    ActiveCell.Offset(2, 0).Range("A1:A74").Select
    Selection.EntireRow.Delete
    ActiveSheet.Range("$A$1:$G$22").AutoFilter Field:=7
    ActiveCell.Offset(4, 2).Range("A1").Select

End Sub
```

A.2. GC-MS Procedures

A.2.1. Oil Sample Preparation

1. Measure out dilution of DCM (5/10/20 mL) in graduated cylinder. (For small enough volumes, this can be done with a pipette and put directly into flask with sample.
2. Put in flask with sample.
3. Based on the volume of DCM you are adding, calculate the volume of toluene to add to the sample in order to achieve a toluene concentration of 21.5425 ppm.
4. Mix for ~10 minutes (may need longer time, until enough oil is dissolved in with the solvent).
5. Take up ~2mL of sample in syringe.
6. Using a .45um filter, filter sample into an LC vial, cap, and label.
7. Put remaining sample into scintillation vial, cap, and label.

A.2.2. Triple Extraction Procedure

Used to prepare aqueous samples for GC-MS analysis.

Extraction 1:

1. pH adjustment of aqueous phase sample (3 mL of sample in small beaker)
    a. pH > 13
    b. 10 M NaOH
    c. Acidic (pH ~ 2). Use pH strips to test solution pH, but be wary of discoloration due to sample color. Samples require ~ 25 µL (~5 drops from transfer pipette)
    d. Natural (pH ~7). Use pH strips to test solution pH, but be wary of discoloration due to sample color. Will require less than 25 µL
2. Create a solution of 2.5 mL of pH-adjusted sample with 50 mL of DCM directly in the seperatory funnel.
3. Shake gently for approximately 1 minute.
4. Let sit for 5 minutes, then collect the DCM layer in a 250 mL beaker and cover with parafilm and a watch glass. This is extract 1.

Extraction 2:

1. Recover emulsion from extract 1 into a 10 mL beaker
2. Emulsion pH adjustment
    a. pH ~ 5 (~3 drops from transfer pipette for acidic samples)
    b. 10N HCl
3. Solution from (2) into seperatory funnel with 50 mL DCM.
4. Shake gently for approximately 1 minute.
5. Let sit for 5 minutes, then collect the DCM layer into the 250 mL beaker with extract 1. Recover with parafilm and watch glass. This is extract 2.

Extraction 3/Combined Extract:

1. Recover the supernatant from extract 2 into a 10 mL beaker.
2. Using a 10 mL syringe, run the supernatant through a Sep-Pak tC18 cartridge for solid phase extraction into a scintillation vial.
3. With a fresh syringe, recover the absorbed sample from the Sep-Pak cartridge with 10 mL of DCM directly into the 250 mL beaker with extracts 1 and 2. This is extract 3/the combined extract.

Flash mix the combined extract for ~30s to ensure homogeneity. Using a 5 mL syringe, filter ~2 mL of the combined extract into an HPLC vial, cap, and label. Analyze on GC-MS immediately, or store at 4°C until analysis.
Pour an aliquot of the remaining UNFILTERED combined extract solution into a glass scintillation vial, cap, cover with parafilm, and label for storage. The remaining volume of combined extract solution can be emptied into a waste beaker and left in the hood to evaporate.

A.3. Nutrient Recovery Calculations

A.3.1. Carbon Recovery in the Aqueous Product

Carbon recovery into the aqueous product was calculated using the equations below:

$$M_{TS,feed} = 175\ g\ slurry * \%\ TS_{feed} \quad \text{(A.1)}$$

where $M_{TS,feed}$ is the mass of total solids in the feedstock, % TS_{feed} is the percent total solids of the feedstock, and the 175 g of slurry is the mass of digestate in the 350 mL total volume of reaction.

$$M_{C,feed} = M_{TS,feed} * \%\ C_{feed} \quad \text{(A.2)}$$

where $M_{C,feed}$ is the mass of carbon in the feedstock and % C_{feed} is the percent carbon in the feedstock.

$$\%\ Recovery = \frac{M_{C,aq}}{M_{C,feed}} * 100 \quad \text{(A.3)}$$

where % Recovery is the percent recovery of carbon into the aqueous product and $M_{C,aq}$ is the measured mass of carbon in the aqueous product.

Values used in calculations were the average of measurements of triplicate reactions.

Feedstock Information	
Feedstock	Manure Digestate
Fraction (% dry weight) C in manure feedstock	0.4349
Fraction Total Solids	0.1022
Mass in Reactor (g)	17.885
Mass C in Feedstock (g)	7.778

*for calc, see 8/28/18 in lab NB

Sample Number	TOC, diluted (mg/L)	TOC, undiluted (mg/L)	Reactor Vol. (L)	Mass C in aq. Phase (mg)	Mass C in aq. Phase (g)	Percent Recovery	Average Percent Recovery	St. Dev.
NA 1.0.1	41.1	4110	0.35	1438.5	1.4385	18.494028		
NA 2.0.1	39.78	3978	0.35	1392.3	1.3923	17.900059		
NA 3.0.1	66.6	6660	0.35	2331	2.331	29.968425	22.12084	6.802696
NA 1.5.1	50.28	5028	0.3	1508.4	1.5084	19.392695		
NA 2.5.1	26.31	2631	0.3	789.3	0.7893	10.14761		
NA 3.5.1	41.94	4194	0.3	1258.2	1.2582	16.176007	15.23877	4.693262
NA 1.10.1	43.14	4314	0.25	1078.5	1.0785	13.8657		
NA 2.10.2	51.82	5182	0.25	1295.5	1.2955	16.655553		
NA 3.10.1	54.68	5468	0.25	1367	1.367	17.574791	16.03201	1.931564
NA 1.20.1	46.56	4656	0.2	931.2	0.9312	11.971942		
NA 2.20.1	42.55	4255	0.2	851	0.851	10.940854		
NA 3.20.1	16.76	1676	0.2	335.2	0.3352	4.3094878	9.074095	4.158352
NA 1.30.1	45.04	4504	0.15	675.6	0.6756	8.6858293		
NA 2.30.1	47.14	4714	0.15	707.1	0.7071	9.090808		
NA 3.30.1	44.63	4463	0.15	669.45	0.66945	8.606762	8.794466	0.259666
NA 1.40.1	43.95	4395	0.1	439.5	0.4395	5.6504174		
NA 2.40.1	47.47	4747	0.1	474.7	0.4747	6.102965		
NA 3.40.1	42.95	4295	0.1	429.5	0.4295	5.5218527	5.758412	0.305238

Feedstock Information	
Feedstock	Manure Digestate
Fraction (% dry weight) C in manure feedstock	0.4349
Fraction Total Solids	0.1022
Mass in Reactor (g)	17.885
Mass C in Feeedstock (g)	7.778

*for calc, see 8/28/18 in lab NB

Sample Number	TOC, diluted (mg/L)	TOC, undiluted (mg/L)	Reactor Vol. (L)	Mass C in aq. Phase (mg)	Mass C in aq. Phase (g)	Percent Recovery	Average Percent Recovery	St. Dev.
A 1.0.1	41.05	4105	0.35	1436.75	1.43675	18.471529		
A 2.0.1	50.41	5041	0.35	1764.35	1.76435	22.683308		
A 3.0.1	25.63	2563	0.35	897.05	0.89705	11.532894	17.56258	5.630504
A 1.5.1	37.49	3749	0.3	1124.7	1.1247	14.459669		
A 2.5.1	48.57	4857	0.3	1457.1	1.4571	18.733158		
A 3.5.1	47.19	4719	0.3	1415.7	1.4157	18.200901	17.13124	2.328906
A 1.10.1	36.67	3667	0.25	916.75	0.91675	11.786166		
A 2.10.1	41.1	4110	0.25	1027.5	1.0275	13.21002		
A 3.10.1	40.71	4071	0.25	1017.75	1.01775	13.08467	12.69362	0.788372
A 1.20.1	33.28	3328	0.2	665.6	0.6656	8.5572646		
A 2.20.1	38.24	3824	0.2	764.8	0.7648	9.8326262		
A 3.20.1	34.39	3439	0.2	687.8	0.6878	8.8426782	9.077523	0.669329
A 1.30.1	38.79	3879	0.15	581.85	0.58185	7.4805355		
A 2.30.1	29.39	2939	0.15	440.85	0.44085	5.6677736		
A 3.30.1	31.39	3139	0.15	470.85	0.47085	6.0534676	6.400592	0.954934
A 1.40.1	35.96	3596	0.1	359.6	0.3596	4.6231856		
A 2.40.1	25.64	2564	0.1	256.4	0.2564	3.2963982		
A 3.40.1	41.73	4173	0.1	417.3	0.4173	5.3650038	4.428196	1.047997

A.3.2. Nitrogen Recovery in the Aqueous Product

Nitrogen recovery into the aqueous product was calculated using the equations below:

$$M_{TS,feed} = 175\ g\ slurry * \%\ TS_{feed} \quad \text{(A.1)}$$

where $M_{TS,feed}$ is the mass of total solids in the feedstock, % TS_{feed} is the percent total solids of the feedstock, and the 175 g of slurry is the mass of digestate in the 350 mL total volume of reaction.

$$M_{N,feed} = M_{TS,feed} * \%\ N_{feed} \quad \text{(A.4)}$$

where $M_{N,feed}$ is the mass of carbon in the feedstock and % N_{feed} is the percent nitrogen in the feedstock.

$$\%\ Recovery = \frac{M_{N,aq}}{M_{N,feed}} * 100 \quad \text{(A.5)}$$

where % Recovery is the percent recovery of nitrogen into the aqueous product and $M_{N,aq}$ is the measured mass of nitrogen in the aqueous product.

Eq. A.5 was used for recovery of the following species of N: TN, NO_2^-/NO_3^-, TKN, and NH_3. Organic nitrogen recovery was calculated as the difference between TKN recovery and NH3 recovery. Values used in calculations were the average of measurements of triplicate reactions.

Feedstock Information	
Feedstock	Manure Digestate
Fraction (% dry weight) N in manure feedstock	0.0325
Fraction Total Solids	0.1022
Mass in Reactor (g)	17.885
Mass N in Reactor (g)	0.5812625

*for calc, see 8/25/18 in lab NB

Sample Number	TN, diluted (mg/L)	TN, undiluted (mg/L)	Reactor Vol. (L)	Mass N in aq. Phase (mg)	Mass N in aq. Phase (g)	Percent Recovery	Average Percent Recovery	St. Dev.
NA 1.0.1	9.42	942	0.35	329.7	0.3297	56.72		
NA 2.0.1	9.89	989	0.35	346.15	0.34615	59.55		
NA 3.0.1	11.5	1150	0.35	402.5	0.4025	69.25	61.84	6.57
NA 1.5.1	10.1	1010	0.3	303	0.303	52.13		
NA 2.5.1	6.59	659	0.3	197.7	0.1977	34.01		
NA 3.5.1	8.27	827	0.3	248.1	0.2481	42.68	42.94	9.06
NA 1.10.1	10.4	1040	0.25	260	0.26	44.73		
NA 2.10.2	11.4	1140	0.25	285	0.285	49.03		
NA 3.10.1	10.8	1080	0.25	270	0.27	46.45	46.74	2.16
NA 1.20.1	9.9	990	0.2	198	0.198	34.06		
NA 2.20.1	10	1000	0.2	200	0.2	34.41		
NA 3.20.1	2.02	202	0.2	40.4	0.0404	6.95	34.24	15.75
NA 1.30.1	9.27	927	0.15	139.05	0.13905	23.92		
NA 2.30.1	10.6	1060	0.15	159	0.159	27.35		
NA 3.30.1	10.3	1030	0.15	154.5	0.1545	26.58	25.95	1.80
NA 1.40.1	9.31	931	0.1	93.1	0.0931	16.02		
NA 2.40.1	10.6	1060	0.1	106	0.106	18.24		
NA 3.40.1	8.88	888	0.1	88.8	0.0888	15.28	16.51	1.54

Feedstock Information	
Feedstock	Manure Digestate
Fraction (% dry weight) N in manure feedstock	0.0325
Fraction Total Solids	0.1022
Mass in Reactor (g)	17.885
Mass N in Reactor (g)	0.5812625

*for calc, see 8/25/18 in lab NB

Sample Number	NO2/NO3, diluted (mg/L)	NO2/NO3, undiluted (mg/L)	Reactor Vol. (L)	Mass N in aq. Phase (mg)	Mass N in aq. Phase (g)	Percent Recovery	Average Percent Recovery	St. Dev.
NA 1.0.1	0.823	82.3	0.35	28.805	0.028805	4.96		
NA 2.0.1	0.686	68.6	0.35	24.01	0.02401	4.13		
NA 3.0.1	0.917	91.7	0.35	32.095	0.032095	5.52	4.87	0.70
NA 1.5.1	0.825	82.5	0.3	24.75	0.02475	4.26		
NA 2.5.1	0.564	56.4	0.3	16.92	0.01692	2.91		
NA 3.5.1	0.813	81.3	0.3	24.39	0.02439	4.20	4.23	0.76
NA 1.10.1	0.83	83	0.25	20.75	0.02075	3.57		
NA 2.10.2	0.761	76.1	0.25	19.025	0.019025	3.27		
NA 3.10.1	0.889	88.9	0.25	22.225	0.022225	3.82	3.56	0.28
NA 1.20.1	0.929	92.9	0.2	18.58	0.01858	3.20		
NA 2.20.1	0.972	97.2	0.2	19.44	0.01944	3.34		
NA 3.20.1	0.513	51.3	0.2	10.26	0.01026	1.77	3.27	0.87
NA 1.30.1	0.751	75.1	0.15	11.265	0.011265	1.94		
NA 2.30.1	0.816	81.6	0.15	12.24	0.01224	2.11		
NA 3.30.1	0.817	81.7	0.15	12.255	0.012255	2.11	2.05	0.10
NA 1.40.1	0.91	91	0.1	9.1	0.0091	1.57		
NA 2.40.1	0.711	71.1	0.1	7.11	0.00711	1.22		
NA 3.40.1	0.795	79.5	0.1	7.95	0.00795	1.37	1.39	0.17

Feedstock Information	
Feedstock	Manure Digestate
Fraction (% dry weight) N in manure feedstock	0.0325
Fraction Total Solids	0.1022
Mass in Reactor (g)	17.885
Mass N in Reactor (g)	0.5812625

*for calc, see 8/25/18 in lab NB

Sample Number	TKN, diluted (mg/L)	TKN, undiluted (mg/L)	Reactor Vol. (L)	Mass N in aq. Phase (mg)	Mass N in aq. Phase (g)	Percent Recovery	Average Percent Recovery	St. Dev.
NA 1.0.1	8.59	859	0.35	300.65	0.30065	51.72		
NA 2.0.1	9.21	921	0.35	322.35	0.32235	55.46		
NA 3.0.1	10.6	1060	0.35	371	0.371	63.83	57.00	6.20
NA 1.5.1	9.25	925	0.3	277.5	0.2775	47.74		
NA 2.5.1	6.03	603	0.3	180.9	0.1809	31.12		
NA 3.5.1	7.46	746	0.3	223.8	0.2238	38.50	39.12	8.33
NA 1.10.1	9.56	956	0.25	239	0.239	41.12		
NA 2.10.2	10.7	1070	0.25	267.5	0.2675	46.02		
NA 3.10.1	9.87	987	0.25	246.75	0.24675	42.45	43.20	2.54
NA 1.20.1	8.97	897	0.2	179.4	0.1794	30.86		
NA 2.20.1	9.07	907	0.2	181.4	0.1814	31.21		
NA 3.20.1	2.3	230	0.2	46	0.046	7.91	31.04	13.35
NA 1.30.1	8.83	883	0.15	132.45	0.13245	22.79		
NA 2.30.1	10.6	1060	0.15	159	0.159	27.35		
NA 3.30.1	9.45	945	0.15	141.75	0.14175	24.39	24.84	2.32
NA 1.40.1	8.4	840	0.1	84	0.084	14.45		
NA 2.40.1	9.9	990	0.1	99	0.099	17.03		
NA 3.40.1	8.09	809	0.1	80.9	0.0809	13.92	15.13	1.67

Feedstock Information	
Feedstock	Manure Digestate
Fraction (% dry weight) N in manure feedstock	0.0325
Fraction Total Solids	0.1022
Mass in Reactor (g)	17.885
Mass N in Reactor (g)	0.5812625

*for calc, see 8/25/18 in lab NB

Sample Number	NH3, diluted (mg/L)	NH3, undiluted (mg/L)	Reactor Vol. (L)	Mass N in aq. Phase (mg)	Mass N in aq. Phase (g)	Percent Recovery	Average Percent Recovery	St. Dev.
NA 1.0.1	5.9	590	0.35	206.5	0.2065	35.53		
NA 2.0.1	7.41	741	0.35	259.35	0.25935	44.62		
NA 3.0.1	8.36	836	0.35	292.6	0.2926	50.34	47.48	7.47
NA 1.5.1	6.45	645	0.3	193.5	0.1935	33.29		
NA 2.5.1	4.24	424	0.3	127.2	0.1272	21.88		
NA 3.5.1	5.73	573	0.3	171.9	0.1719	29.57	31.43	5.82
NA 1.10.1	6.24	624	0.25	156	0.156	26.84		
NA 2.10.2	7.48	748	0.25	187	0.187	32.17		
NA 3.10.1	7.53	753	0.25	188.25	0.18825	32.39	30.47	3.14
NA 1.20.1	6.14	614	0.2	122.8	0.1228	21.13		
NA 2.20.1	6.74	674	0.2	134.8	0.1348	23.19		
NA 3.20.1	1.43	143	0.2	28.6	0.0286	4.92	22.16	10.01
NA 1.30.1	6.54	654	0.15	98.1	0.0981	16.88		
NA 2.30.1	7.54	754	0.15	113.1	0.1131	19.46		
NA 3.30.1	7.49	749	0.15	112.35	0.11235	19.33	18.55	1.45
NA 1.40.1	6.27	627	0.1	62.7	0.0627	10.79		
NA 2.40.1	7.24	724	0.1	72.4	0.0724	12.46		
NA 3.40.1	6.27	627	0.1	62.7	0.0627	10.79	11.34	0.96

Percent Recoveries

Sample Number	TN (%)	NO2/NO3 (%)	TKN (%)	NH3 (%)	Organic N	C (%)
NA 1.0.1	56.72	4.96	51.72	35.53	16.2	18.5
NA 2.0.1	59.55	4.13	55.46	44.62	10.8	17.9
NA 3.0.1	69.25	5.52	63.83	50.34	13.5	30.0
NA 1.5.1	52.13	4.26	47.74	33.29	14.5	19.4
NA 2.5.1	34.01	2.91	31.12	21.88	9.2	10.1
NA 3.5.1	42.68	4.20	38.50	29.57	8.9	16.2
NA 1.10.1	44.73	3.57	41.12	26.84	14.3	13.9
NA 2.10.2	49.03	3.27	46.02	32.17	13.8	16.7
NA 3.10.1	46.45	3.82	42.45	32.39	10.1	17.6
NA 1.20.1	34.06	3.20	30.86	21.13	9.7	12.0
NA 2.20.1	34.41	3.34	31.21	23.19	8.0	10.9
NA 3.20.1	6.95	1.77	7.91	4.92	3.0	4.3
NA 1.30.1	23.92	1.94	22.79	16.88	5.9	8.7
NA 2.30.1	27.35	2.11	27.35	19.46	7.9	9.1
NA 3.30.1	26.58	2.11	24.39	19.33	5.1	8.6
NA 1.40.1	16.02	1.57	14.45	10.79	3.7	5.7
NA 2.40.1	18.24	1.22	17.03	12.46	4.6	6.1
NA 3.40.1	15.28	1.37	13.92	10.79	3.1	5.5

Average Percent Recoveries

Retention Time (min)	TN (%)	NO2/NO3 (%)	TKN (%)	NH3 (%)	Organic N	C
0	61.84	4.87	57.00	43.49	13.51	22.12
5	42.94	3.79	39.12	28.25	10.87	15.24
10	46.74	3.56	43.20	30.47	12.73	16.03
20	34.24	3.27	31.04	22.16	8.88	11.46
30	25.95	2.05	24.84	18.55	6.29	8.79
40	16.51	1.39	15.13	11.34	3.79	5.76

Standard Deviation

Retention Time (min)	TN (%)	NO2/NO3 (%)	TKN (%)	NH3 (%)	Organic N	C
0	6.57	0.70	6.20	7.47	2.68	6.80
5	9.06	0.76	8.33	5.82	3.10	4.69
10	2.16	0.28	2.54	3.14	2.32	1.93
20	0.24	0.10	0.24	1.46	1.22	0.73
30	1.80	0.10	2.32	1.45	1.46	0.26
40	1.54	0.17	1.67	0.96	0.73	0.31

Feedstock Information	
Feedstock	Manure Digestate
Fraction (% dry weight) N in manure feedstock	0.0325
Fraction Total Solids	0.1022
Mass in Reactor (g)	17.885
Mass N in Reactor (g)	0.5812625

*for calc, see 8/25/18 in lab NB

Sample Number	TN, diluted (mg/L)	TN, undiluted (mg/L)	Reactor Vol. (L)	Mass N in aq. Phase (mg)	Mass N in aq. Phase (g)	Percent Recovery	Average Percent Recovery	St. Dev.
A 1.0.1	12.7	1270	0.35	444.5	0.4445	76.47		
A 2.0.1	13.8	1380	0.35	483	0.483	83.09		
A 3.0.1	6.41	641	0.35	224.35	0.22435	38.60	66.05	24.01
A 1.5.1	13.2	1320	0.3	396	0.396	68.13		
A 2.5.1	12.8	1280	0.3	384	0.384	66.06		
A 3.5.1	9.29	929	0.3	278.7	0.2787	47.95	60.71	11.10
A 1.10.1	13	1300	0.25	325	0.325	55.91		
A 2.10.2	10.9	1090	0.25	272.5	0.2725	46.88		
A 3.10.1	9.5	950	0.25	237.5	0.2375	40.86	47.88	7.58
A 1.20.1	10.6	1060	0.2	212	0.212	36.47		
A 2.20.1	12.5	1250	0.2	250	0.25	43.01		
A 3.20.1	8.95	895	0.2	179	0.179	30.80	36.76	6.11
A 1.30.1	12.7	1270	0.15	190.5	0.1905	32.77		
A 2.30.1	13.8	1380	0.15	207	0.207	35.61		
A 3.30.1	8.71	871	0.15	130.65	0.13065	22.48	30.29	6.91
A 1.40.1	13.1	1310	0.1	131	0.131	22.54		
A 2.40.1	9.91	991	0.1	99.1	0.0991	17.05		
A 3.40.1	11	1100	0.1	110	0.11	18.92	19.50	2.79

Feedstock Information	
Feedstock	Manure Digestate
Fraction (% dry weight) N in manure feedstock	0.0325
Fraction Total Solids	0.1022
Mass in Reactor (g)	17.885
Mass N in Reactor (g)	0.5812625

*for calc, see 8/25/18 in lab NB

Sample Number	NO2/NO3, diluted (mg/L)	NO2/NO3, undiluted (mg/L)	Reactor Vol. (L)	Mass N in aq. Phase (mg)	Mass N in aq. Phase (g)	Percent Recovery	Average Percent Recovery	St. Dev.
A 1.0.1	1.16	116	0.35	40.6	0.0406	6.98		
A 2.0.1	1.07	107	0.35	37.45	0.03745	6.44		
A 3.0.1	0.511	51.1	0.35	17.885	0.017885	3.08	5.50	2.12
A 1.5.1	0.996	99.6	0.3	29.88	0.02988	5.14		
A 2.5.1	1.17	117	0.3	35.1	0.0351	6.04		
A 3.5.1	0.906	90.6	0.3	27.18	0.02718	4.68	5.29	0.69
A 1.10.1	0.924	92.4	0.25	23.1	0.0231	3.97		
A 2.10.2	0.917	91.7	0.25	22.925	0.022925	3.94		
A 3.10.1	0.772	77.2	0.25	19.3	0.0193	3.32	3.75	0.37
A 1.20.1	0.809	80.9	0.2	16.18	0.01618	2.78		
A 2.20.1	0.713	71.3	0.2	14.26	0.01426	2.45		
A 3.20.1	0.683	68.3	0.2	13.66	0.01366	2.35	2.53	0.23
A 1.30.1	0.908	90.8	0.15	13.62	0.01362	2.34		
A 2.30.1	0.818	81.8	0.15	12.27	0.01227	2.11		
A 3.30.1	0.482	48.2	0.15	7.23	0.00723	1.24	1.90	0.58
A 1.40.1	0.838	83.8	0.1	8.38	0.00838	1.44		
A 2.40.1	0.544	54.4	0.1	5.44	0.00544	0.94		
A 3.40.1	0.718	71.8	0.1	7.18	0.00718	1.24	1.20	0.25

Feedstock Information	
Feedstock	Manure Digestate
Fraction (% dry weight) N in manure feedstock	0.0325
Fraction Total Solids	0.1022
Mass in Reactor (g)	17.885
Mass N in Reactor (g)	0.5812625

*for calc, see 8/25/18 in lab NB

Sample Number	TKN, diluted (mg/L)	TKN, undiluted (mg/L)	Reactor Vol. (L)	Mass N in aq. Phase (mg)	Mass N in aq. Phase (g)	Percent Recovery	Average Percent Recovery	St. Dev.
A 1.0.1	11.5	1150	0.35	402.5	0.4025	69.25		
A 2.0.1	12.7	1270	0.35	444.5	0.4445	76.47		
A 3.0.1	5.9	590	0.35	206.5	0.2065	35.53	60.41	21.85
A 1.5.1	12.2	1220	0.3	366	0.366	62.97		
A 2.5.1	11.6	1160	0.3	348	0.348	59.87		
A 3.5.1	8.39	839	0.3	251.7	0.2517	43.30	55.38	10.57
A 1.10.1	12.1	1210	0.25	302.5	0.3025	52.04		
A 2.10.2	9.98	998	0.25	249.5	0.2495	42.92		
A 3.10.1	8.73	873	0.25	218.25	0.21825	37.55	44.17	7.33
A 1.20.1	9.76	976	0.2	195.2	0.1952	33.58		
A 2.20.1	11.8	1180	0.2	236	0.236	40.60		
A 3.20.1	8.27	827	0.2	165.4	0.1654	28.46	34.21	6.10
A 1.30.1	11.8	1180	0.15	177	0.177	30.45		
A 2.30.1	13	1300	0.15	195	0.195	33.55		
A 3.30.1	8.23	823	0.15	123.45	0.12345	21.24	28.41	6.40
A 1.40.1	12.2	1220	0.1	122	0.122	20.99		
A 2.40.1	9.37	937	0.1	93.7	0.0937	16.12		
A 3.40.1	10.3	1030	0.1	103	0.103	17.72	18.28	2.48

Feedstock Information	
Feedstock	Manure Digestate
Fraction (% dry weight) N in manure feedstock	0.0325
Fraction Total Solids	0.1022
Mass in Reactor (g)	17.885
Mass N in Reactor (g)	0.5812625

*for calc, see 8/25/18 in lab NB

Sample Number	NH3, diluted (mg/L)	NH3, undiluted (mg/L)	Reactor Vol. (L)	Mass N in aq. Phase (mg)	Mass N in aq. Phase (g)	Percent Recovery	Average Percent Recovery	St. Dev.
A 1.0.1	9.37	937	0.35	327.95	0.32795	56.42		
A 2.0.1	10.3	1030	0.35	360.5	0.3605	62.02		
A 3.0.1	4.45	445	0.35	155.75	0.15575	26.80	48.41	18.93
A 1.5.1	10.1	1010	0.3	303	0.303	52.13		
A 2.5.1	9.34	934	0.3	280.2	0.2802	48.21		
A 3.5.1	6.71	671	0.3	201.3	0.2013	34.63	44.99	9.18
A 1.10.1	9.92	992	0.25	248	0.248	42.67		
A 2.10.2	8.21	821	0.25	205.25	0.20525	35.31		
A 3.10.1	7.37	737	0.25	184.25	0.18425	31.70	36.56	5.59
A 1.20.1	8.94	894	0.2	178.8	0.1788	30.76		
A 2.20.1	9.86	986	0.2	197.2	0.1972	33.93		
A 3.20.1	6.59	659	0.2	131.8	0.1318	22.67	29.12	5.80
A 1.30.1	9.84	984	0.15	147.6	0.1476	25.39		
A 2.30.1	10.9	1090	0.15	163.5	0.1635	28.13		
A 3.30.1	6.51	651	0.15	97.65	0.09765	16.80	23.44	5.91
A 1.40.1	10.2	1020	0.1	102	0.102	17.55		
A 2.40.1	8.34	834	0.1	83.4	0.0834	14.35		
A 3.40.1	8.62	862	0.1	86.2	0.0862	14.83	15.58	1.73

Percent Recoveries

Sample Number	TN (%)	NO2/NO3 (%)	TKN (%)	NH3 (%)	Organic N	C
NA 1.0.1	76.47	6.98	69.25	56.42	12.8	18.47
NA 2.0.1	83.09	6.44	76.47	62.02	14.5	22.68
NA 3.0.1	38.60	3.08	35.53	26.80	8.7	11.53
NA 1.5.1	68.13	5.14	62.97	52.13	10.8	14.46
NA 2.5.1	66.06	6.04	59.87	48.21	11.7	18.73
NA 3.5.1	47.95	4.68	43.30	34.63	8.7	18.20
NA 1.10.1	55.91	3.97	52.04	42.67	9.4	11.79
NA 2.10.2	46.88	3.94	42.92	35.31	7.6	13.21
NA 3.10.1	40.86	3.32	37.55	31.70	5.8	13.08
NA 1.20.1	36.47	2.78	33.58	30.76	2.8	8.56
NA 2.20.1	43.01	2.45	40.60	33.93	6.7	9.83
NA 3.20.1	30.80	2.35	28.46	22.67	5.8	8.84
NA 1.30.1	32.77	2.34	30.45	25.39	5.1	7.48
NA 2.30.1	35.61	2.11	33.55	28.13	5.4	5.67
NA 3.30.1	22.48	1.24	21.24	16.80	4.4	6.05
NA 1.40.1	22.54	1.44	20.99	17.55	3.4	4.62
NA 2.40.1	17.05	0.94	16.12	14.35	1.8	3.30
NA 3.40.1	18.92	1.24	17.72	14.83	2.9	5.37

Average Percent Recoveries

Retention Time (min)	TN (%)	NO2/NO3 (%)	TKN (%)	NH3 (%)	Organic N	C
0	66.05	5.50	60.41	48.41	12.00	17.56
5	60.71	5.29	55.38	44.99	10.39	17.13
10	47.88	3.75	44.17	36.56	7.61	12.69
20	36.76	2.53	34.21	29.12	5.09	9.08
30	30.29	1.90	28.41	23.44	4.97	6.40
40	19.50	1.20	18.28	15.58	2.70	4.43

Standard Deviation

Retention Time (min)	TN (%)	NO2/NO3 (%)	TKN (%)	NH3 (%)	Organic N	C
0	24.01	2.12	21.85	18.93	2.95	5.63
5	11.10	0.69	10.57	9.18	1.55	2.33
10	7.58	0.37	7.33	5.59	1.76	0.79
20	6.11	0.23	6.10	5.80	2.02	0.67
30	6.91	0.58	6.40	5.91	0.50	0.95
40	2.79	0.25	2.48	1.73	0.85	1.05