Using biosolids as a water source in non-catalytic hydrolysis reactions for biofuel production

by

Lin Xia

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Department of Agricultural, Food and Nutritional Science
University of Alberta

Abstract

Biosolids are residues produced from the treatment of municipal sludge and are rich in organic materials. The growing volume of biosolids and concerns over microbial safety highlight the difficulties associated with biosolids disposal. Canada generates about 660,000 dry tons of biosolids annually,[1, 2] which is becoming an environmental issue. The biosolids used in this study are semisolids containing mostly water. Biosolids have difficulty settling, and the microbes and heavy metal content adds complexity to biosolids disposal. Furthermore, the cost of biosolids management accounts for more than half of the total operating cost of a wastewater treatment facility. Currently, solutions for safe disposal and utilization of biosolids have become ever more diverse, especially its application in energy recovery and biofuel production.

A two-step lipid pyrolysis approach has been designed to be a sustainable biofuel technology that can widely use renewable lipid feedstocks. Thermal hydrolysis will convert lipids into protonated fatty acids, which later can be used as feedstock for pyrolysis to produce hydrocarbon-based drop-in fuels (Indistinguishable from petroleum-based hydrocarbons). Also, the hydrolysis process promotes the settling of biosolids. Several studies have investigated the thermal hydrolysis of brown grease with water, but nothing has been reported on using biosolids as the water source in the hydrolysis of brown grease. The primary goal of this study was to investigate the possibility of utilizing water and organic materials in biosolids for the hydrolysis process.

The first objective of this research was to characterize the biosolids (water concentration >96%) and investigate the hydrolysis of unamended biosolids alone. The free fatty acids(FFA) in the hydrolysate were solvent extracted and analyzed to determine the impact of the hydrolysis on FFA %. The results showed that the quantity of lipid materials in biosolids following hydrolysis was too small but still can contribute for substantial hydrocarbon production. And the settling performance of the hydrolysates was excellent.

In a second study, a renewable lipid feedstock, brown grease, was blended with biosolids to explore the hydrolysis performance of using biosolids as a substitute for water to hydrolyze brown grease, with regards to FFA% in the recovered lipid phase of hydrolysate and the FFA conversion. Different pH, reaction time, and temperature for hydrolysis were studied. The results showed the performance of the biosolids was similar to distilled water in terms of phase separation, FFA% and FFA conversion.

The third research objective focused on the quality of the biosolids-hydrolyzed brown grease lipid phase influenced by the temperature. The hydrolysis was conducted at three different temperatures, starting from 280°C, and at a water-to-oil ratio of 5:1. Sulfur, nitrogen and other compounds were analyzed. Biosolids performed similarly to water in terms of free fatty acid conversion, but had slightly elevated sulfur and nitrogen content in the product.

Preface

This thesis is an original work by Lin Xia. No part of this thesis has been previously published. Dr. Michael Chae contributed to manuscript edits. The experiments were conducted in Professor David C. Bressler's lab at the University of Alberta. The CHNS analysis and the elemental analysis in Chapter 3 were performed by the Analytical and Instrumentation Laboratory of the Chemistry Department and Laboratory in the Department of Earth and Atmospheric Science, respectively. The sulfur content was analyzed at the Natural Resources Analytical Laboratory. The nitrogen content was analyzed with the help of Dr. Kelvin Lien in the analytical laboratory in the Department of Agricultural, Food and Nutritional Science. The LC-MS was performed in the Lipid Chemistry Lab (South Academic Building 5-52) in the Department of Agricultural, Food and Nutritional Science. All the laboratories are located at the University of Alberta.

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List of abbreviations

ASTM	- American Society for Testing and Materials
DG	- diglyceride
FAME	- fatty acid methyl ester
FFA	- free fatty acid
FID	- flame ionization detector
GC	- gas chromatography
HPLC	- high performance liquid chromatography
MG	- monoglyceride
MS	- mass spectrometer
TG	- triglyceride
FAAES	- fatty acid alkyl esters

1 Introduction

1.1 Project Background

Because the issues surrounding energy independence and environmental pollution are increasing, which is aggravated by lack of innovation and inefficient usage of resources,[3] the exploration for safe and clean substitutes for traditional fossil energy has been intense. Renewable fuels are one of the alternatives that can extract energy from waste materials. Even though biofuels derived from renewable resources may not dominate over future energy sources such as solar energy and nuclear power, the generation and accumulation of common waste materials is ongoing. Also, the interest in developing a more efficient treatment method for waste materials will never fade.

Water is a key resource for human existence, and the quantity consumed on a daily basis is tremendous; about 10 billion tons globally.[4] Correspondingly, the waste water produced every day imposes a heavy strain on wastewater treatment, including the accumulation of biosolids and sewage sludge, the primary organically treated wastewater residue after wastewater treatment.[2] The wide variety of components such as heavy metals and microbes contained in biosolids derived from household, industrial, and commercial wastewater, pose a threat to human and environmental health and require safe disposal or utilization. The difficulty of biosolids settling also creates issues in storage, transportation, and processing. In Edmonton, the disposal of biosolids cannot keep pace with the accumulation rates, and the lagoons used for biosolids storage are nearly full.[1] The disposal of biosolids by landfilling or combustion has been proven to

be inefficient[5], and other energy extracting methods such as composting, gasification, or anaerobic decomposition have issues with energy loss.[6]

Biosolids are classified into different types that vary based on their composition. Primary biosolids contain 6-30% (dry weight) fat and grease, which is a great source for biofuel production.[7] Technologies that have been developed recently to convert organic-rich biosolids into biofuels include acid/base-catalyzed transesterification for biodiesel production, directly pyrolysis to produce bio-oil, fermentation for bioethanol production and other technologies.[8-10] Those technologies show the possibility of effectively utilizing the organic matter in biosolids.

Lipid pyrolysis, which is a non-catalytic thermal conversion technology, has numerous benefits such as a relatively low reaction temperature (compared to other thermal treatments like gasification), no catalyst requirement, and rapid reaction rates compared with other technologies. The conditions utilized for this technology are sufficient to produce subcritical water and could provide a more ionized environment without adding base or acid catalyst.[11] Which means that without catalysts, the subcritical water could still react with fat and oil and achieve a more than 97% conversion (the subcritical water was reviewed in section 2.3). [12] The dependence of some biofuel conversion technologies on the use of catalyst of has always been problematic due to the high cost of catalyst, the catalyst poisoning and recovery. [13] Also, the tolerance of such technologies to lower grade feedstock reduces the cost of biofuel production. Several renewable feedstocks such as used cooking oil, beef tallow, and brown and yellow greases have already been utilized in lipid pyrolysis and have

been successfully converted into hydrocarbon-based biofuels. Hydrolysis is the first step to converting fat into protonated fatty acids and consumes substancial quantities of water. The debate surrounding water consumption in biofuel production is on the rise and raises concerns about the sustainability of biofuels. Currently, there has been no research examining biosolids as a substitute for water in lipid pyrolysis.

The key difficulty in the settling of biosolids arises because of the presence of capillary water, bound water, as well as other organics especially ECP (extracellular polymer).[14] And only thermal treatment or mechanical dehydration can realize the water removal of biosolids.[15] Thus, in addition to providing a water replacement, acceleration of dewatering and sterilization become an extra benefit of incorporating biosolids into lipid pyrolysis.

Based on the background described above, it was hypothesized that biosolids could substitute for water in the hydrolysis process of lipid pyrolysis, resulting in the production of drop-in hydrocarbon fuels that are indistinguishable from those obtained using traditional water sources for hydrolysis. In this study, biosolids were used as both a source of water and lipids (albeit at low levels for the latter) in blending with brown grease for the hydrolysis reaction.

1.2 Thesis Objectives

The overall objective of this research was to investigate the possibility of using biosolids as both a source of lipids and water in hydrolysis reactions in thermal non-catalytic lipid pyrolysis, without impacting quality and quantity of the final fuel product. The specific objectives were to:

1. Characterize the composition of biosolids and explore the free fatty acid (FFA) conversion of using biosolids as the only feedstock in the hydrolysis reaction, and how the biosolids settling performance will be affected by hydrolysis and acidification. (Section 4.1)
2. Investigate the hydrolysis performance (FFA conversion and FFA% in the recovered lipid phase in hydrolysate) of using biosolids as a substitute for water for hydrolyzing lipid feedstocks at different reaction time, pH and temperature. (Section 4.2)
3. Determine the effect of reaction temperature on hydrolysis performed with biosolids and compare the hydrolysis performance, FFA % in the recovered lipid phase, and product quality (especially sulfur content in the lipid phase of hydrolysatet). (Section 4.3)

2 Literature Review

2.1 Overview of Biosolids

2.1.1 Introduction

Broadly speaking, biosolids are the residues remaining after a series of processes in wastewater treatment that reduce the concentrations of materials that can be readily removed and easily decomposed. The highly polluted wastewater that enters a wastewater treatment facility usually undergoes three processes for safe recycling as shown in **Figure 2-1**. First, there is a physical settling or screening step to remove the undissolved sediment; the liquid product remaining after this process is referred to as primary sludge and usually contains 93%-99.5% water and a high ratio of organic matter.[16] A secondary step is a biological process, which will be applied to convert dissolved biological matter into a solid material through cultivation. The solids residue from this step is called activated sludge and the total solids concentration ranges from 0.8% to 1.2%. The type of biological method used can affect the solid content. Finally, the tertiary step is a chemical and/or biological process to remove nutrients that might cause eutrophication of water, such as nitrogen and phosphorous, so that the effluent may be safely disposed of. [16]

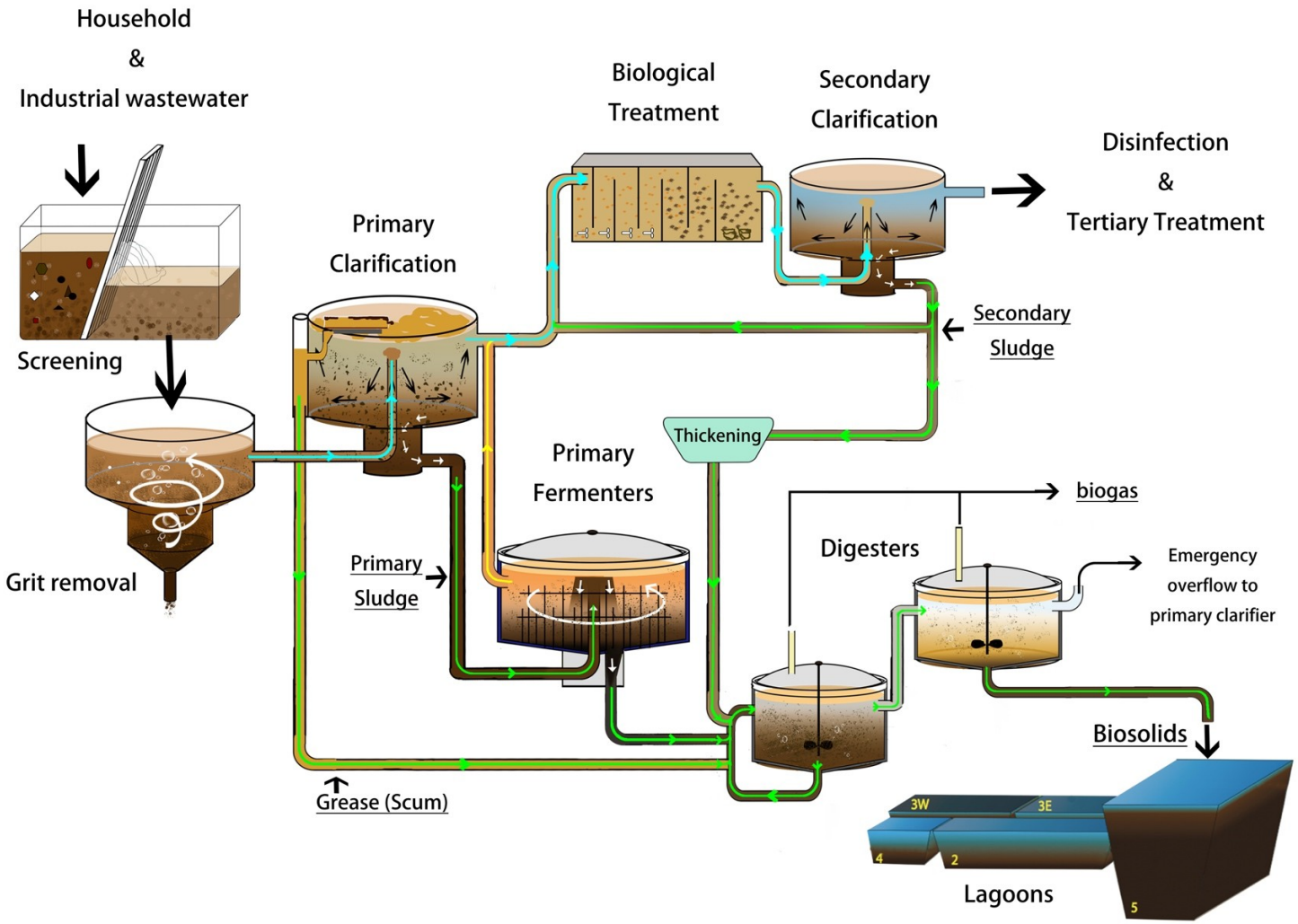


Figure 2-1 A diagram of the conventional wastewater treatment process This figure was drawn by the author according to the Gold Bar wastewater process described on https://www.epcor.com treatment The images of the lagoons were sourced from SMA Consulting [1]

The residue produced after the three steps discussed above is referred to as biosolids, which consists of 59-88% (dry weight %) organic compounds.[17] These molecules are decomposable, the source of an offensive odor, and facilitate pathogen growth. Thus, biosolids require several ongoing treatments including digestion and alkaline stabilization for safe utilization.[18] The organic portion contains 50-55% carbon, 25-30% oxygen, 10-15% nitrogen, 6-10% hydrogen, 1-3% phosphorus and 0.5-1.5% sulfur.[19] The inorganic solids in biosolids are minerals such as quartz, calcite or microline, which are rich in Fe, Ca, K, and Mg, and heavy metals such as Cr, Ni, Cu, Zn, Pb, and Hg.[20]

The amount of dry biosolids that are produced daily per person is estimated to be nearly 30 g; this number will be substantial when applied to a city, a country, and the whole world.[21] In Canada, the estimated biosolids production is 27.7 kg (dry weight) per capita annually, which means that more than 4,000 sewage treatment facilities across Canada are handling more than 860,000 tons of dry biosolids annually.[22] In China, wastewater treatment facilities produce 1.3 million tons of untreated biosolids (on dry basis) with an annually increasing rate of more than 10%.[23]

The different regulatory standards among provinces in Canada exacerbate the difficulties of biosolids management. The cost of biosolids treatment varies from 18% to 50% of the total operating costs of the wastewater treatment plant in different provinces in Canada. As for the USA, this ratio reaches as high as 57%.[24]

Generally, biosolids contain microorganisms and the biological and inorganic substances absorbed or metabolized by microbial species.[25] Other harmful toxins may

also contaminate the biosolids such as detergents, various salts toxins, and pesticides.[26] Thus, the disease and environmental pollution caused by inappropriate treatment or disposal of biosolids have caused a rising concern of utilizing and treating biosolids sustainably.

The forms of water that exist in biosolids can be classified into 2 types: one is free water which is easy to remove by settling, while another is bound water that includes interstitial water, hygroscopic water, and intracellular water. The bound water is held with capillary or adhesive forces, and will affect the release of free water and dewaterability of biosolids and is the biggest obstacles for water removal.[27] The removal of bound water will be extremely slow without human intervention, but the bound water removal will dramatically reduce the volume of biosolids as indicated by Wang *et al.* (2013). When the water content of the biosolids is decreased from 99% to 96%, the volume of biosolids will be reduced to 1/4 of the original volume.[15]

Some wastewater solids management systems rely on lagoon treatment and/or storage, which is relatively less expensive. This includes the Edmonton biosolids treatment plant. Biosolids used in our studies were from the biosolids settling lagoons located in Edmonton as shown in **Figure 2-2**. The lagoons consist of 5 active above-ground cells that allow the biosolids go through for settlement and thickening. Biosolids from Gold Bar wastewater treatment plant or surrounding wastewater treatment plants are pumped into Cell 5 (the largest and deepest lagoon) and Cell 3, respectively. Cells 5 and 3E store condensed biosolids that have already reached their maximum self-settling ability[1]. Thus, to accelerate the dewatering of the biosolids in

Cell 5, the biosolids are dewatered by polymer conditioning and centrifugation. After some settling, the supernatants are then move to Cell 2. After additional settling, the supernatant from Cell 2 goes to Cell 4. Cell 4 is the final and cleanest lagoon and the supernatant will be recycled from Cell 4 and returned to the Gold-bar wastewater treatment plant. As reported by SMA Consulting, there is currently no extra capacity for the accumulating biosolids, and the main biosolids treatment method is still composting. Thus, there is a compelling need to embrace more efficient and beneficial utilization of biosolids.

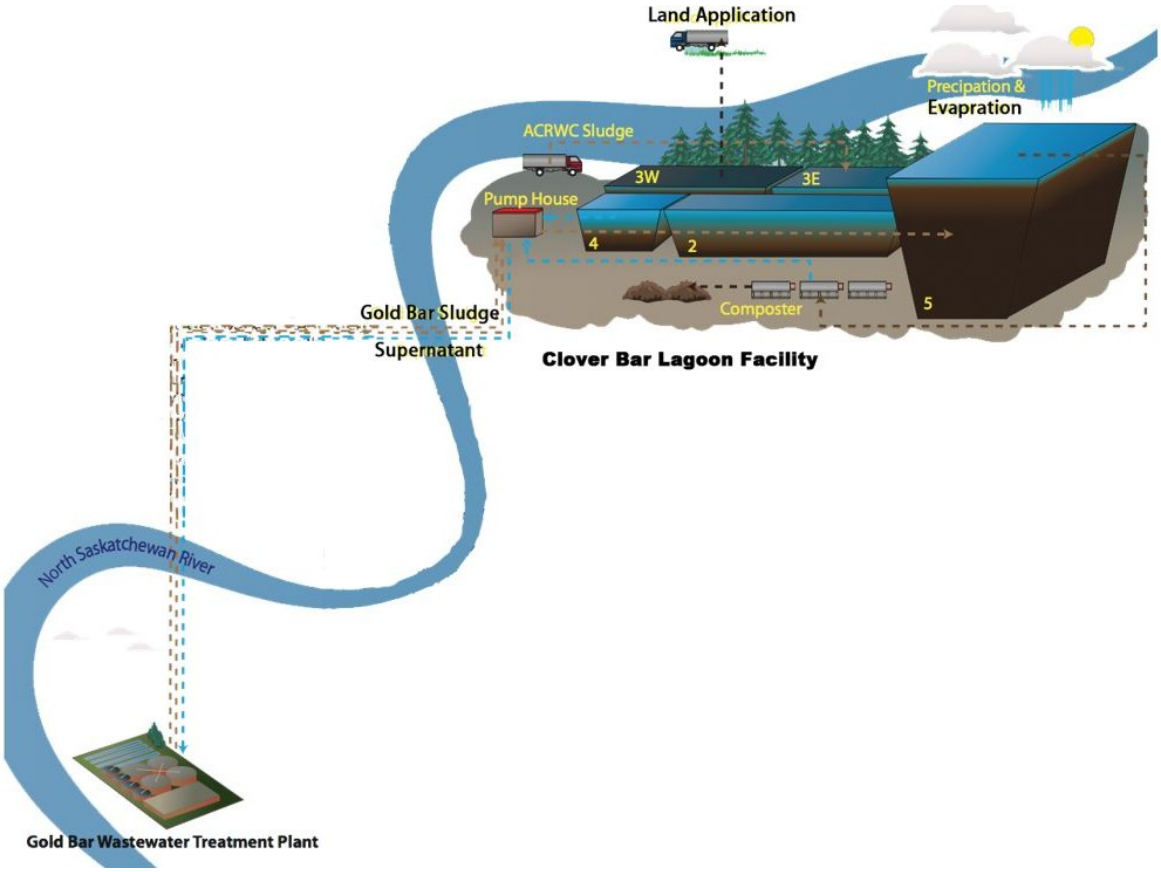


Figure 2-2 Edmonton Biosolids treatment. Reused with permission from SMA Consulting.[1]

2.1.2 Progress and Issues of Biosolids Processing and Management

Biosolids management is increasingly expensive and the utilization of biosolids is challenged by several factors such as the expanded view of beneficial use, the complexity of the regulatory and policy landscape, as well as public perception (e.g. the odor control).[28] Large quantities of municipal biosolids are still disposed of rather than being used beneficially; this includes both developed and undeveloped areas. In Canada, 13.2 million wet tons of sewage sludge (assuming 5% total solids content) need to be managed annually and this number keeps rising.[2]

Most lagoons are partly or mostly anaerobic, and methane emissions can occur. Nitrous oxide emissions may also occur.[29] The IPCC (Intergovernmental Panel on Climate Change who provides the world with an objective, scientific view of climate change and its political and economic impacts) reported that methane emissions from anaerobic digestion of wastewater represented 8-11% of the global anthropogenic methane emissions, which are estimated to range from 30-40 tetragrams per year (Tg/year).[30]

2.1.2.1 Dumping and Landfilling

The sea dumping of sewage sludge has already been forbidden recently on a global scale due to the drastic consequences of the hazards (such as microbes) and heavy metals in sewage sludge. Landfilling, which seems to be the least expensive option, requires management due to the environmental and public health impacts caused by disposal of untreated biosolids. The hazards/heavy metals in landfilled biosolids might have a chance to contaminate the underground water environment via rainfall in

the landfilling area. Globally, regulations require that the biosolids must be dried to at least 15-20% solids for landfilling.[31, 32] Biosolids usually contain more than 94% water and the dewatering process is formidable and usually requires mechanical or thermal dehydration. Also, similar to solid waste landfilling, methane, a powerful greenhouse gas, is generated during active landfilling or even in bioreactor landfilling and is hard to be captured.

2.1.2.2 Incineration

Incineration, which completely oxidizes organic compounds at high temperature, is also one of the common choices that can significantly decrease the volume of biosolids with or without energy capture. However, the massive infrastructure investment and the demand for energy (usually fossil energy for burning), have made this treatment not cost-effective. Even worse, incineration forms mainly water and carbon dioxide (a major GHG), but can also generate additional air pollutants. The heavy metals in biosolids will diffuse with the soot and dust during incineration, which would also cause heavy metal accumulation in the environment. Developing beneficial uses for biosolids can potentially sequester the GHG emissions and atmospheric carbon.[24] Disadvantages aside, the sludge ash after incineration could have considerable potential for use in various forms in concrete and contributes to achieving the target of zero waste for construction industry.[33], [34] Incineration may need to be reluctantly accepted by some undeveloped areas, but the reality is it has become standard practice in some technologically advanced countries such as Japan and

the Netherlands with incineration rates achieved at 70% and 58%, respectively. As for Canada, 1/3 of the biosolids are processed by incineration.[35] Thus, technology and research have progressively enhanced the energy recovery efficiency from incineration in the form of heat or electricity. Some incineration facilities in the USA are designed to implement power recovery and the energy generated can substitute 20% to 40% of the facilities energy demand.[36] Co-incineration is also a good alternative method with a higher energy recovery efficiency, but it requires dry biosolids, and thus the cost is highly dependent on the dewatering method.[37]

2.1.2.3 Composting

Amid the search for a better treatment method that can realize a more efficient energy recovery, composting of biosolids has become popular in recent years. The high levels of nitrogen and phosphorus restrict the discharge of biosolids to the river and sea, but biosolids can be a good nutrient source for fertilization purposes. During composting, the organic matter in biosolids is biologically decomposed in a closed tunnel, with addition of an amendment, for at least two weeks. Substantial amounts of heat is released and can be used as energy. Also, the composting end product contains sufficient organic material for further application such as use as a soil amendment or a conditioner for fertilizers.[38, 39] A study using biosolids compost as a soil amendment for the growth of terfgrass showed that the biosolids compost performed well and provided a long term supply of plant nutrients.[40] The biogas production during composting is another benefit; Zsirai *et al.* (2011) estimated that it could theoretically

give 121 TWh/year electrical energy, assuming a global sludge volume of 50M T DS/year.[41] Some research has pointed out that the cell wall of the microorganisms will inhibit the hydrolysis of intracellular compounds towards production of methane, resulting in a longer digestion time.[23] Apart from being time-consuming, the secondly biggest problem surrounding composting is that it requires the moisture of materials to be less than 60%(wet weight basis).[42] Other drawbacks include the land requirements, cost of amendment, as well as volume increases issues due to the addition of the amendment.

2.1.2.4 Thermal Hydrolysis

Thermal hydrolysis has already been applied in biosolids treatment with a temperature ranging from 130-220°C and a 30-60 min holding time.[43] The main purpose of thermal hydrolysis is to make materials in biosolids more biodegradable for the subsequent anaerobic digestion. The addition of thermal hydrolysis before digestion could decrease the digester heating requirements and produce less odorous compounds compared the digestion of untreated biosolids.[44] The dewaterability of sludge will also be significantly improved. Ødeby *et al.* (1996) showed an increase of dewaterability between 60-80% when the thermal hydrolysis pretreatment was combined with anaerobic digestion.[45] Hydrolyzing biosolids (5-6% dry solids) with acid at 80-155°C was studied by Neyens *et al.* (2003) and the research showed after two different filtration method, a 70% reduction of solids was found in acid hydrolyzed thickened sludge compared with the untreated sludge.[14] The biosolids that will be

used in lipid pyrolysis currently has poor dewaterability and low lipids content, and is the residue after digestion without a pretreatment method. Lipid pyrolysis has the potential to utilize the water and the lipids contained in biosolids for biofuel production.

2.1.3 Application of Biosolids for Biofuel Production

Climate change and its impacts, and the fluctuating and uncertain energy price continue to drive the already emergent interest in green technologies. Biosolids are defined as "free" resources, and even carry negative value in economic models due to charges associated with treating and disposing of biosolids. In Canada, charges for the treatment of biosolids is about $\$0.6/m^3$ [b]. The current policies and funding are moving towards more beneficial utilization, promoting the exploration of using biosolids for biofuel production. Biosolids can be directly or indirectly used for producing biofuel. Biosolids can be directly used as a feedstock through extraction of its energy value. The Netherlands and Germany are the pioneers of using composted biosolids directly as biofuel, and in 2004, a biosolids composting product (moisture content about 30%) was used either as an additive or as a stand-alone biofuel in a power station.[46] Biosolids could also be used for biofuel production through indirect application as a kind of nutrient source for growing feedstocks for biofuel production.

2.1.3.1 Indirect Application of Biosolids for Biofuel Production

Biosolids contain microbes and heavy metals that may contaminate the soil environment, which may have long-term effects. Thus, the land application of biosolids

is restricted by policies and regulations in order to avoid the risks of contamination of food products and the environment. However, using biosolids and its derivatives to substitute the mineral fertilizers to fertilize energy crops does not involve any direct risk since energy crops are not intended for the food industry. Moreover, most of the energy crops are grown in abandoned agricultural fields. The USA is a leader in the crop-to-biodiesel space, and biosolids have already been used to grow fuel crops. Research from Spain also showed the utilization of biosolids as fertilizer increased the energy crop production (*Cynara cardunculus* L.) and oilseeds up to 40% to 68%, respectively.[47] Honda *et al.* (2011) studied the potential of greenhouse gas reduction through sewage sludge cultivated fuel crops in Japan; results showed the usage of sewage sludge for soybean cultivation not only increased the crop productivity, but also corresponded to a 4.0% net reduction of GHG emitted from wastewater treatment plants in Japan.[48] Liu *et al.* (2015) applied biosolids in a sustainable bioenergy cropping system for switchgrass and found that biosolids can be implemented as an alternative nitrogen source for the growth of switchgrass biomass. However, results also showed the application of biosolids cannot be large and frequent, as this may negatively affect the feedstock quality and curb the biomass-to-biofuel efficiency. [49]

2.1.3.2 Fermentation

Different technologies have been developed for the direct use of biosolids for biofuel production. Hydrogen-based energy has been under hot debate recently as it is one of the most promising clean fuels for the future; this will be largely due to the fact

that it can be directly used in fuel cells to provide electricity and with a clean residue of water. The large quantities of carbohydrate and proteins that are contained in biosolids are a good source to produce methane or hydrogen gas. The organic acids generated from organic matter in biosolids in the first step of anaerobic digestion can be used for hydrogen gas production by using bacteria such as *Escherichia coli* or *Aerobacter* in the fermentation step.[50] Dark fermentation was one of the sustainable methods to produce hydrogen, through anaerobic degradation of organic substrates by heterotrophic microorganisms in the absence of light. A high hydrogen production was achieved by Yilmazel *et al.* (2015), by growing *Caldicellulosiruptor bescii* on biosolids as the only carbon source at 2.5 g volatile solids/L or lower to achieved an H_2 yield of 85.8 mL per gram of dry biosolids.[51]

2.1.3.3 Catalytic Hydrolysis for Biodiesel Production

The biodiesel produced from dewatered biosolids results from a similar process as transesterification of edible or inedible oil, which will be reviewed in 2.3.2.1. It makes use of the lipid fraction, which consists of oils, greases, fats, and long chain fatty acids originating from phospholipids in the cell membranes of microbes, its metabolites and by-products, as well as from the lipids from domestic and industrial sludge in municipal biosolids.[52] Application of this technology usually requires the biosolids to be dewatered and filtered and sometimes an additional lipid extraction method is needed to extract the lipids out as the raw materials. This process is time-consuming due to the further cleaning process of extracted biodiesel, including centrifugation, settling and

filtration by filter membranes containing anhydrous sodium sulfate, and also has a scale up problem to maintain the process conditions. The process design is given in the **Figure 2-3**.[53]

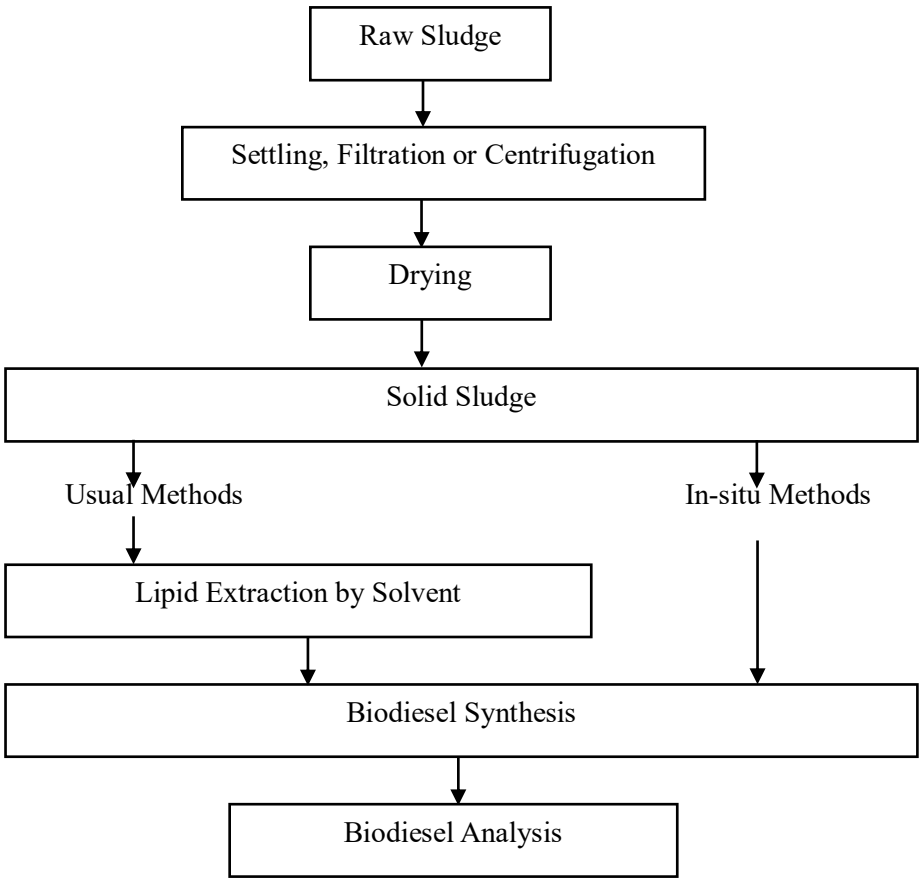


Figure 2-3 Overall Biodiesel production scheme

Mandala *et al.* (2009) researched the biodiesel production by *in situ* transesterification of municipal primary and secondary sewage sludge, and showed that the fatty acid methyl ester (FAME) yield of acid/base catalyst hydrolysis varies from 2%-14% (wt. % of dry biosolids). The FAME yield will be dependent on the different

types of biosolids, with primary biosolids producing more FAME because there are more lipids before biological treatment.[54] A Korean group (Eilhann, 2012) improved the transesterification by using heat instead of catalyst, which solved the issues relating to poisoning of the catalyst by impurities in biosolids lipids. In Eilhann's method, extracted biosolids lipids were thermally treated with methanol at 380°C in a reactor containing porous materials (non-catalytic) such as activated alumina. Also, carbon dioxide was added to the reactor to improve the reaction yield. The results showed that the biodiesel conversion can achieve 98% and biosolids produced 2,200 times more lipids/g than soybeans and with a much lower cost by using the same method.[13]

2.1.3.4 Directly Pyrolysis

Pyrolysis is a thermochemical reaction carried out at an elevated temperature (500-1000°C) with or without catalyst. This reaction leads to decomposition of materials in the absence of oxygen, producing condensable and non-condensable gases, bio-oils, and char as a solid product. Lipid materials and lignocellulosic biomass are typically used as raw materials for bio-oil production. Pyrolysis oil product or biodiesel product from catalytic hydrolysis can be easily stored and transported compared with other products obtained from biosolids treatment such as composting.[55] Tukey has applied biosolids for refining alcohols and other fuels by pyrolysis and gasification.[56]

Most of the pyrolysis methods demand a dehydration pretreatment to enhance the organic content in biosolids. Kim's (2012) research showed that pyrolysis of dewatered sludge at 500°C would generate about 28-42% oil yield, which has a real

economic value ranging between 5.6-9.9 ¢ /kg dry solid.[57] Similar results have been found in Shen's (2005) studies: pyrolysis of dry biosolids at 525°C can achieve a maximum 30% (wt% dry basis of sludge) oil yield. This bio-oil is made up of a group of aromatic clusters with one to three aromatic rings connected by long straight chain hydro-carbons with hydroxyl groups.[58] For a faster dewatering, flocculants (polymers) are sometimes added during centrifugation, which positively enhanced the caloric value of pyrolysis oil by producing hydrocarbon during the pyrolysis.[59] Even pyrolysis oil has potential to substitute for the conventional petroleum-based fuels, but there are also many undesirable qualities of the pyrolysis oil that need further upgrading for use in transport application.

In summary, treatments of biosolids consume huge financial and energy resources as a result of the need to mitigate the negative influence of these materials on human health and the environment. But the root obstacle for beneficial utilization of biosolids is the current requirement of a low water concentration. A rapid method that can break the emulsions formed by strongly bounded water is through thermal treatment. Most of the existing thermal technologies for biosolids application still require a high solids content of biosolids to ensure adequate product yield. Thus, a thermal technology that can take advantage of the high water content of biosolids, will be ideal for the biosolids management.

2.2 Biofuel from Renewable Lipid-Rich Materials

2.2.1 Trend of the Future Energy

The search for renewable sources of diesel and gasoline seems to be an irreversible trend. The cleanest-burning fossil fuel is natural gas, which is one of the most promising alternatives for petrol/gasoline and diesel, but it faces several problems to be the mainstream fuel for the future. In particular, the limited range for natural gas vehicles, the high cost for conversion and the lack of infrastructure to support them are the biggest hindrances.[60]

Currently, the two most common biofuels are bioethanol from renewable crops, or biodiesel produced from clean oil source such as palm oil and vegetable oil. The increasing food-scarcity problem has let to challenges for traditional biofuel technologies that may compete with food crops. A world bank report that published in the Guardian in 2008 pointed out that the plant-derived biofuels have forced global food prices up by 75%.[61] Also, more evidence is showing that for biofuels, especially first generation biofuels, the GHG emissions and the impact on food prices, resources and biodiversity may be worse than first thought. The GHG saving estimates for most of the first generation biofuels were positive without including the emissions caused by land use changes. Several studies have shown that the increase of GHG emissions due to intensification and deforestation couldn't be offset by the GHG reduced by using these fuels.[62, 63] The drawbacks associated with producing biofuel have stimulated more research on novel energy-production technologies for the future, such as hydrogen fuel.

Hydrogen energy is seen as a very attractive future fuel as its use is not associated with CO_2 emissions, which appeases the rising consideration of climate change and fuel sustainability for the long term. Tarpenning, an executive of Tesla has been quoted as saying "There's a saying in the auto industry: Hydrogen is the future of transportation and always will be".[64] He also pointed out that there are too many advances that need to be made for this technology to solve the problems regarding storage and high production costs.[64] The main competitor to the hydrogen fuel cell is electricity, which is also a clean energy, but currently, much cheaper than hydrogen fuel. Although these two technologies do not lead to emissions while running, the conventional means through which they are produced will create pollution.[65]

The application of waste materials and/or generation of co-products will greatly improve the economics of producing renewable biofuels. An undisputed fact is that there will always be thousands of tons of waste materials produced every day that need to be utilized properly. Rather than disposal of these waste materials without energy recovery and with the risk of creating pollution, highly-efficient utilization is imperative. There will always be a requirement for better technologies to produce energy in an environmentally friendly and totally sustainable manner.

2.2.2 Current State and Issues of Renewable Biofuel

Utilizing wastes for biofuel production seems to be the best option for waste treatment and is an almost obligatory need. There is an increasingly large amount of

research trying to explore better technologies and better types of biofuel. This section reviews the most promising fossil fuel substitutes.

2.2.2.1 Biodiesel

Biodiesel is a liquid fuel produced from lipid sources that contain either triglycerides or fatty acids, and is produced through transesterification. Biodiesel is renewable, biodegradable, has excellent lubricity, and has a similar energy density to diesel.[66] It is comprised of fatty acid alkyl esters (FAAES) such as fatty acid methyl ester (FAME) via base- or acid-catalyzed transesterification of lipids using alcohol such as methanol as shown in **Figure 2-4**.[67]

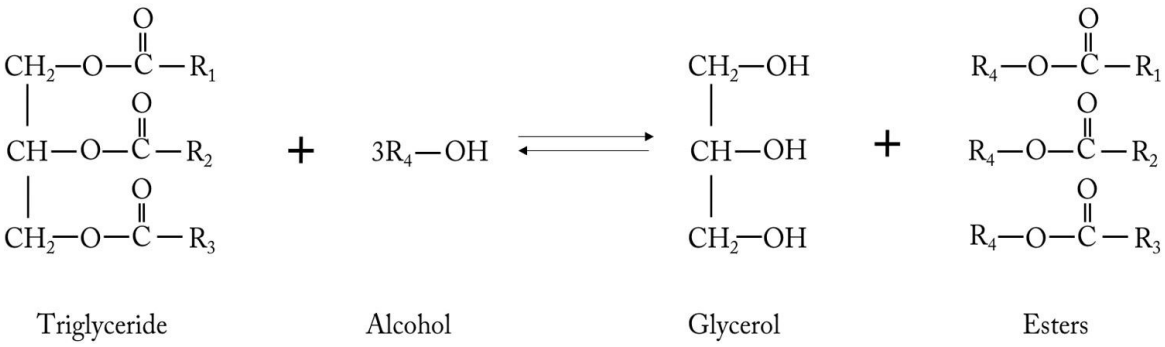


Figure 2-4 Transesterification of triglycerides to alkyl esters (biodiesel)

The production of biodiesel dates back to the 1930s and has already been deeply researched and commercialized. Compared with other types of emerging biofuel, biodiesel has a relatively mature and integrated production and processing system. The

issues surrounding biodiesel are intolerance to low temperatures, engine incompatibility, and the feedstock. The current base-catalyzed hydrolysis can only have excellent performance by using feedstock that contains a low concentration of free fatty acids and water, such as pure vegetable oil, or waste cooking oil. Also, the homogeneous acid catalyst, which is more tolerant to FFA, is also challenged by corrosion concerns, recovery issues, and an increasing sulfur level in the product due to the sulfate ion involved in side reactions when using sulfuric acid as the catalyst.[68]

Several studies have developed different technologies to enhance the compatibility of the process with highly contaminated feedstocks and to decrease the cost of the catalyst. Navajas *et al.* (2013) successfully converted waste cooking oil to biodiesel by using cheap egg shells as the base solid catalyst with a high production. Moreover, as is the case with homogeneous base catalysts, an FFA elimination process by esterification is needed before the base catalyzed transesterification.[69] The heterogeneous acid catalyst aroused some researchers' interest as it is insensitive to FFA and can be recovered more easily compared with the homogeneous acid catalyst. Compared with the enzyme catalysts, the solids acid catalyst can be derived from really cheap materials such as starch or cellulose(Lou, 2008).[70] Other methods that have compatibility with high FFA feedstocks also included enzyme catalysis and non-catalyst thermal transesterification. All of these technologies provide great opportunities for highly beneficially utilization of lipid materials that contain high FFA.

2.2.2.2 Hydrocarbon-based Biofuels

Renewable hydrocarbon biofuels, also called biohydrocarbons or drop-in fuels, are clean liquid fuels produced from renewable biomass sources through a variety of biological or thermochemical processes include hydro-treating, gasification, pyrolysis, etc.[71] Unlike biodiesel, which is exclusively sourced from lipids, especially oils with low FFA, the technology of producing hydrocarbon fuels are more tolerant to a variety of feedstocks such as lipids and waste oils, woody biomass, switchgrass, and even algae. Also, the chemically similar characteristics with petroleum fuel and the reduced levels of oxygen-based molecules make hydrocarbon fuels a better drop-in replacement for FAMEs, and without any need for engine modification. Compared with ethanol fuel, the higher energy density of hydrocarbon fuel facilitates 30% more gas mileage.[72]

Table 2-1 lists different technologies for producing hydrocarbon biofuels using various types of feedstocks; most of the technologies demand catalyst and/or hydrogenation. Only a few types of research focus on non-catalytic processes. The hydrocarbon fuel converted from biomass is one group of the non-conventional hydrocarbons and is usually paraffinic fuel or super cetane fuel (C17 and C18 *n*-alkanes), and produced through a catalytic hydroprocessing process using lipid biomass as feedstock. Usually, the hydrocarbon fuel produced through hydrogenolysis contains less sulfur and nitrogen content because the reaction results in the cleavage of C-S, C-N or C-O bond, which is also a common desulfurization process.[73] Most of the hydrogenation methods need H_2 to be present in the reaction, but Fu *et al.* (2011) found that water can also act as H_2 for the hydrogenation reaction for hydrocarbon production

with the help of catalyst at 330°C. However, the hydrocarbon yield of this reaction was not good: less than 20%, with around 70% unreacted FFA left that then needed further steps to remove.[74] Other than hydrogenation, pyrolysis with catalyst such as Pd/C, Pt/C, Ru/C or other catalyst was also used for hydrocarbon fuel production using lipid biomass as feedstock.

Table 2-1 Technologies for hydrocarbon biofuels production by using various types of feedstocks

	Feedstocks	Catalyst	Techniques & condition	Product	References
Catalytic					
Lipid	Brown Grease	Pd/C	Catalytic hydropretreating under 100°C and decarboxylation under 300°C	C17 hydrocarbon	[75]
	Soybean oil	Na2co3	Pyrolysis at 350-400°C	C10-C18 hydrocarbons	[76]
	FFA with water	Pt/c	Hydrolysis at 330 °C without H_2 added	Ketones, C14-C16 alkanes	[74]
	Canola oil	Pd/c	Hydrolysis at 250°C and catalytic decarboxylation at 300°C	C15-C21 alkanes	[77]
	Plant oil, animal fats	Metal catalyst	Decarboxylation or hydrodeoxygenation with H_2 and catalyst	Hydrocarbon diesel and jet fuel	[73]
	Vacuum distillate contains mostly FFA	Ni-Mo	Hydrocracking at 400-420°C	Saturated hydrocarbons	[78]
	Seed oil or plant fruits	Pd/C	Pyrolysis at 370°C	Terpenoid-based fuel	[79]
Cellulosic	γ-valerolactone derived from cellulosic biomass	Ru/C	Oligomerization and hydrogenation	C9-C18 alkanes	[80]
	Cyclopentanone derived from pyrolyzed woody biomass	Solid base catalyst	hydrodeoxygenation	High-density hydrocarbon fuels	[81, 82]
Fermentation					
	Cyanobacteria	enzyme	Fermentation: Expression of cyanobacteria pathway in *E.coli*	C13-C17 hydrocarbons	[83]
	Algae: *Botryococcus braunii*	Na_2Co_3	Thermal chemical liquefaction at 300°C	C17-C22 hydrocarbon	[84]
Non-catalytic					
	Lipid biomass	none	Hydrolysis at 250-350°C and pyrolysis at 350-450°C	Hydrocarbons and Chemicals	[85]

Usually, the products produced through fermentation were lipids or fatty alcohols, which demand an additional hydro treatment to get the final hydrocarbon product. Recently, some researchers discovered a pathway that allows for the direct production of C_{13}~C_{17} alkanes in *E. coli.*[83] Compared with catalytic and fermentation processes, thermal conversion technologies that are free of catalyst and enzyme will provide more benefits since there will always be catalyst and enzyme related issues such as catalyst poisoning, recovering and cost issues. Also, the troublesome problems that occur with hydrocarbon fermentations such as limited solubility of the substrate and the greater oxygen demand in the fermentation, mean additional expense and equipment considerations.[86]

2.2.3 Challenges for Biofuel Production

2.2.3.1 Lipid Source Challenge

Most of the lipid-to-biofuel conversion technologies have a requirement for a highly pure lipid feedstock, and thus, vegetable oil has been more popular as a feedstock for biofuel production. Impurities could introduce uncertainty to any step in the production chain. Thus, the high purity of the vegetable oil could reduce the difficulty of the manufacturing process. But there are still several challenges to using food based lipid materials. First generation biofuels create controversy regarding food security as they use crops that are traditionally used for food. And even second generation biofuel derived from non-food based crops, have the potential to compete for land and water that could be used to grow food.[87] The choice of waste lipid materials could

circumvent the issues on food security and reduce the cost of feedstock, but as discussed above, impurities might create problems to any of the steps and thus increase the process cost. Brown grease, which is a highly contaminated grease, could perfectly explain the double-edged sword of utilizing waste oil such as used cooking oils, animal fats and waste trap greases.

The corrosion and pipe blockage problems that arise during sewage transport of lipid material in urban wastewater (fat, oil, and grease) increase management costs. In fact, the disposal of the urban wastewater lipids may account for 10 % of the total cost of sludge disposal.[88] Brown grease is one of the waste trap greases separated during waste water treatment. The brown grease tends to solidify at 5-10°C, which makes the recovery of brown grease from trap waste not efficient or effective since the grease tends to clog the pipelines during recovery.[89] In the USA, 1.84 million tons of brown grease are produced annually. The costs associated with landfill disposal of brown grease was 110 US dollar per metric ton in 2002.[90] Thus, compared to disposal of brown grease without any energy recovery, use of brown grease to produce biofuel seems to be a better choice. Not only will this solve the disposal issues, but it will also cut the cost of biofuel production.

The application of brown grease in biofuel technologies was limited for biodiesel application due to the high concentration of FFA (15% to almost 100%). The high concentration of FFA that are produced inside a grease trap tank due to hydrolysis of triglyceride molecules would make the transesterification reaction difficult due to the formation of soap with the alkaline catalyst.[89] Also water and impurities in brown

grease such as some metals, nitrogen compounds, and sulfur compounds, could also destroy or deactivate various metal or metal oxide catalysts.[91] Sari *et al.* (2013) showed that the unidentified impurities in brown grease poisoned the catalyst for batch thermal decarboxylation of brown grease for biodiesel production, and that the FFA conversion can only achieve 37.9 % compared with a 99.4% FFA conversion of oleic acid at the same condition. Nowadays, more technologies are being developed that are not sensitive to the FFA concentration. Lipid pyrolysis is one such process that could use feedstocks containing any concentration of FFA and is in fact better suited for those with higher FFA concentrations.[92]

Microalgae and cyanobacteria have great potential to substitute for terrestrial plants due to their high oil production and sustainable. However, aside from the high-cost issues, there are still some challenges for some types of microalgae, which have been shown to rapidly accumulate toxins such as heavy metals.[93]

2.2.3.2 Water Source Challenge

In addition to food security, water consumption issues are another concern for biofuel production. Currently, irrigation is needed for growing feedstocks, especially corn or soybean, for which water consumption is quite high. As shown by Pate (2007), growing soybeans is the most water-intensive process. The water consumption for soy irrigation is about 7000 gal water consumed per gal fuel (gal/gal)[94]; the total water consumption for microalgae is comparatively less, but still needs 200~2000 gal/gal(Tu, 2016).[95]

For those technologies that do not require crop-based feedstocks, the manufacturing process also demands significant water, especially for second generation bioethanol production (about 4 gal/gal). The water consumption for biodiesel refining is up to 3 gal/gal, and thermochemical conversion for biofuel production requires the least amount of water of all technologies examined (2 gal/gal).[96] The idea of introducing wastewater to substitute for the water source for biofuel production could eliminate water shortage issues and could also provide a better way for wastewater treatment. Nutrients within, such as oil and grease, could also contribute the biofuel production. However, as is the case with using waste lipid materials, the downside of using wastewater is the presence of impurities such as metals, and the high concentration of phosphorus, sulfur, and nitrogen, along with pH related concerns.

2.3 Non-catalytic Fat-Splitting Technology

Hydrolysis of fat and oil is a procedure that is wildly used in saponification for soap production, and is also a key reaction for some of the technologies that produce biofuel. This reaction is conducted at 200-300°C under sub- or supercritical conditions, or through contact with superheated steam without the help of a catalyst. Through hydrolysis, fatty acids can be liberated from the glycerol backbone in triglycerides. Addition of an acid or base catalyst could contribute to reducing the reaction temperature to ambient condition, but brings about several problems such as catalyst poisoning and separation.[97] Enzymatic hydrolysis is also an option, but the cost of enzyme is an issue. Nevertheless, the use of lipase for hydrolysis could lower the

reaction pressure to atmospheric pressure, and the reaction could even take place at room temperature depending on the different types of lipase and oil used.[98]

2.3.1 Mechanisms

Water is supercritical or near supercritical (subcritical) above its critical points (> 647.096K, >22.064 MPa). The unusual properties of sub- and supercritical water allow it to act as both a solvent and a reagent in this non-catalytic reaction. Bandura *et al.* (2006) investigated the ionization constant of water as a function of pressure and temperature.[99] There are more ions produced under subcritical conditions, which means there could be a more ionic environment without adding base or acid catalyst (**Figure 2-5**). Because of this, a high-efficiency of non-catalytic hydrolysis of fats is possible with subcritical water. Also, the solubility of fat in water is hindered through hydrolysis at low temperature; water must have contact with lipids to function as a reactant. Under subcritical conditions, water performs like an organic solvent and thus, non-polar compounds are highly soluble in subcritical water.[100]

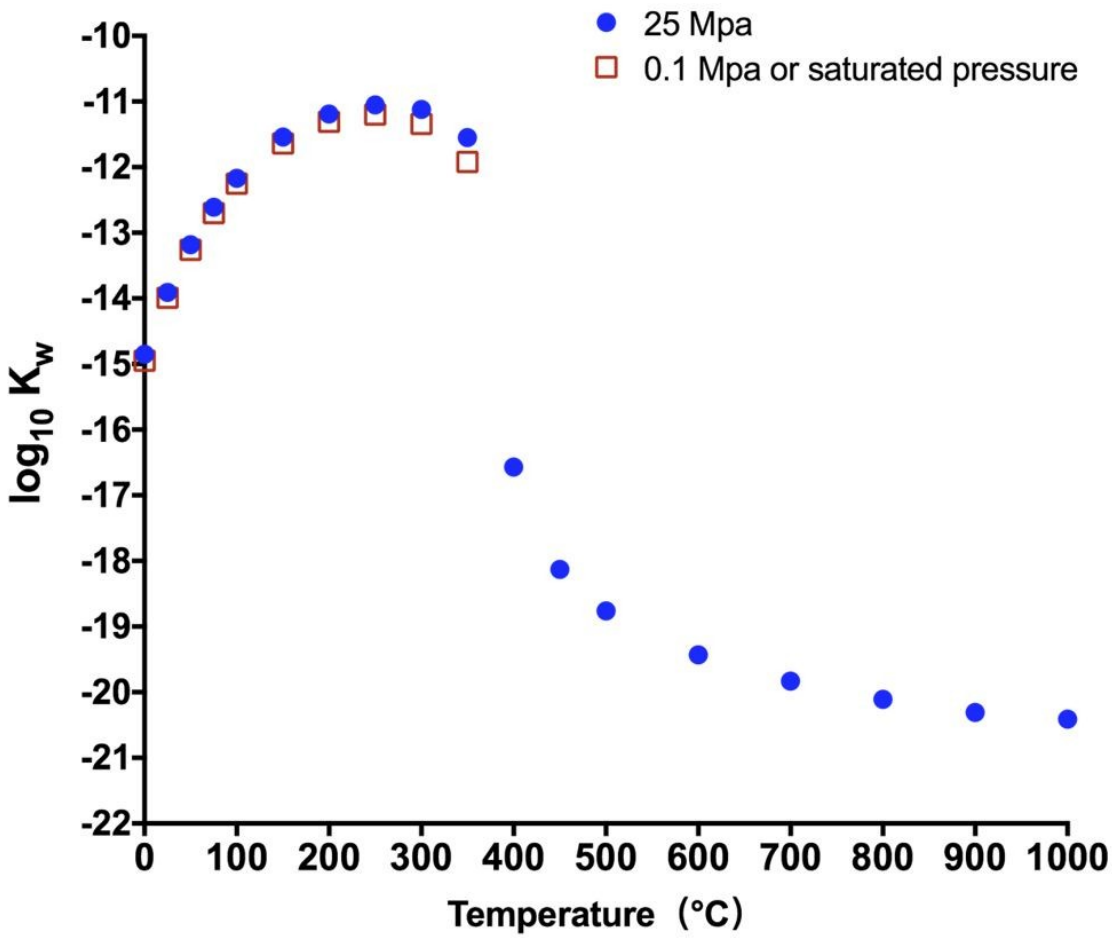


Figure 2-5 Ion dissociation constant (Kw) of water as a function of temperature. Graph was drawn according to the data presented by Bandura *et al.* (2006) [99]

The mechanism of fat hydrolysis is illustrated by Mills and McClain (1949) and consists of 3 reversible reactions.[101] As showed in Equations 2-1 to 2-3, one mol of TAG is firstly hydrolyzed to one mol of DG, and the DG is then hydrolyzed to one mol of MG, and the MG is further hydrolyzed to one mol of glycerol. One mole of FFA is generated with each step and three mol in total after three reactions. The release of a

FFA chain requires consumption of one mol of water. The backward reactions indicate that there is a demand for excess water to force the reaction from right to left.

Equation 2-1

$$C_3H_5(OOCR)_3 + H_2O \underset{k'_1}{\overset{k_1}{\rightleftarrows}} C_3H_5(OH)\cdot(OOCR)_2 + RCOOH$$

Triglyceride Water Diglyceride Fatty acid

Equation 2-2

$$C_3H_5(OH)\cdot(OOCR)_2 + H_2O \underset{k'_2}{\overset{k_2}{\rightleftarrows}} C_3H_5(OH)_2\cdot(OOCR) + RCOOH$$

Diglyceride Water Monoglyceride Fatty acid

Equation 2-3

$$C_3H_5(OH)_2\cdot(OOCR) + H_2O \underset{k'_3}{\overset{k_3}{\rightleftarrows}} C_3H_5(OH)_3 + RCOOH$$

Monoglyceride Water Glycerol Fatty acid

The mechanism of non-catalytic hydrolysis in sub- and supercritical water is the same as acid catalyzed hydrolysis of triglycerides, where water is a weak nucleophile and attacks the ester bond.[102] Therefore, the fatty acid produced during hydrolysis can also act as an acid catalyst to promote the reaction. As showed in Equation 2-4, the proton released from FFA dissociation protonates the carbonyl oxygen of TG, promoting subsequent nucleophilic attack by water. This reaction is also referred to as an autocatalytic reaction. Increasing temperature can accelerate the reaction rate, but

when the water to oil ratio was not high enough (i.e. 1:1 (v/v)), studies showed that equilibrium would always be reached at around 90 % conversion no matter how high the temperature is due to the reverse reaction.[103]

Equation 2-4

$$FFA \rightleftharpoons FFA^- + H^+ \quad \text{(Dissociation of FFA)}$$

$$TG + H^+ \rightleftharpoons TG^+ \quad \text{(Protonation of TG)}$$

$$TG^+ + H_2O \rightleftharpoons DG + FFA^+ \quad \text{(Hydrolysis of TG)}$$

$$FFA^+ \rightleftharpoons FFA + H^+ \quad \text{(Deprotonation)}$$

2.3.2 Types of Thermal Hydrolysis

The earliest hydrolysis process was the Twitchell process, which used a base catalyst at low temperature. However, the saponification of lipids turned the researchers' interests to thermal non-catalytic hydrolysis processes.[104] Nowadays, a continuous hydrolysis process called the Colgate-Emery process is widely used at industry scale, whereas batch hydrolysis, which takes longer to heat up and cool down, is usually used at a lab scale. [97]

2.3.3 Research and Achievements for Lipid Hydrolysis

Several studies focused on the optimization of hydrolysis conditions by using different lipid feedstocks as listed in **Table 2-2**. The fats and oils, especially waste lipid materials, are complicated feedstocks and thus the optimized condition for different types of lipids feedstock varies. All the experiments with higher water to oil ratios (at

least 2:1(v/v)) resulted in a FFA conversion of at least 95%. Temperature is a major factor and could affect the hydrolysis velocity before the reaction achieved equilibrium. However, after reaching equilibrium, the temperature could barely affect the hydrolysis yield.[11] Increasing the temperature to 340°C could significantly shorten the reaction time, which will be more helpful for the continuous and batch system.

The saturation of the lipid feedstock will also affect the process. As illustrated by Barnebey *et al.* (1949), fish oil, which was reported to have a high degree of unsaturation, could not be satisfactorily split using the Colgate-Emery process and the unsaturated fatty acids significantly increased after hydrolysis (iodine value dropped from 60 to 40).[105] The solubility of fatty acids in water will also affect the contact of fatty acid with water during the reaction. Khuwijitjaru *et al.* (2007) also proved that FFA with a smaller carbon number have a higher solubility in water. In another work, he pointed out that oleic acid and linoleic acid had a similar solubility in water at temperatures above 200°C, which were higher than those of saturated fatty acid with the same carbon number and even C16:0 fatty acids.[106, 107]

Table 2-2 Conditions and FFA conversion for hydrolysis of fats and oils in subcritical water

Type	T (°C)	Reaction time(min)	Water/Oil ratio	Pressure (Mpa)	Conversion (%)	Reference
Refined edible rapeseed oil	340	12	2:1 (v/v)	>12	>95%	[11]
Corn oil	280	40	20:3 (m/m)	>2	100%	[108]
Sunflower oil	350	15	50/50 (v/v)	20	92.80%	[109]
Soybean oil	270	20	25:4 (v/v)	Density of water is 0.7 g/mL	>97%	[12]
Hydrogenated soybean oil	280	15				
Linseed oil	280	20				
	260	69				
Coconut oil	270	15				
Vegetable oil	260	120-180	2:1 (m/m)	4.83	98%-99%	[110]
Waste frying oil	200	60	1:1 (m/m)	22.1	>75%	[111]
	250				>90%	
Soybean oil	338	7.8	1:2.5 (v/v)	13.6	90.40%	[112]
		9.9	1:5 (v/v)	13.1	100%	
		14.8			99%	

2.4 Non-catalytic Lipid Pyrolysis

The technology used in this thesis is lipid pyrolysis, which employs hydrolysis to generate fatty acids that can subsequently be pyrolyzed to produce hydrocarbon-based drop-in fuels. The thermal cracking of triglyceride-rich oil has been studied for a long time, and the liquid fuel produced has great potential to be a substitute for petroleum gasoline, diesel or jet fuel.[113] Lipid pyrolysis can be further developed to improve the product quality by adding a hydrolysis step prior to pyrolysis. This approach has a high tolerance to various types of lipid feedstock. The main advantages of this approach are that it does not require a catalyst, and the required temperature is relatively low (less than 400°C for both hydrolysis and pyrolysis process) compared with traditional pyrolysis method. Production of biofuel could be achieved by direct pyrolysis, but there will be more unwanted compounds, such as oxygenated compounds, in the final product. The addition of hydrolysis as the first step helps free the carboxylic chain from the glyceride backbone and removes water-soluble molecules and solids prior to pyrolysis. Also, glycerol could be recovered through this process, which can then be recycled for other applications. The product produced by pyrolysis of free fatty acids includes a gas, liquid, and solid (coke) product. Asomaning *et al.* (2014) applied lipid pyrolysis to inedible lipid feedstocks such as beef tallow and greases and generated renewable drop-in diesel. The conversion of those low-grade lipids to free fatty acids by hydrolysis with water were all successful and the subsequent pyrolysis could yield about 80% organic liquid product, which has comparable quality with gasoline and diesel.

Even more remarkable, the product from beef tallow, brown and yellow greases had higher cetane indices than diesel fuel.[85]

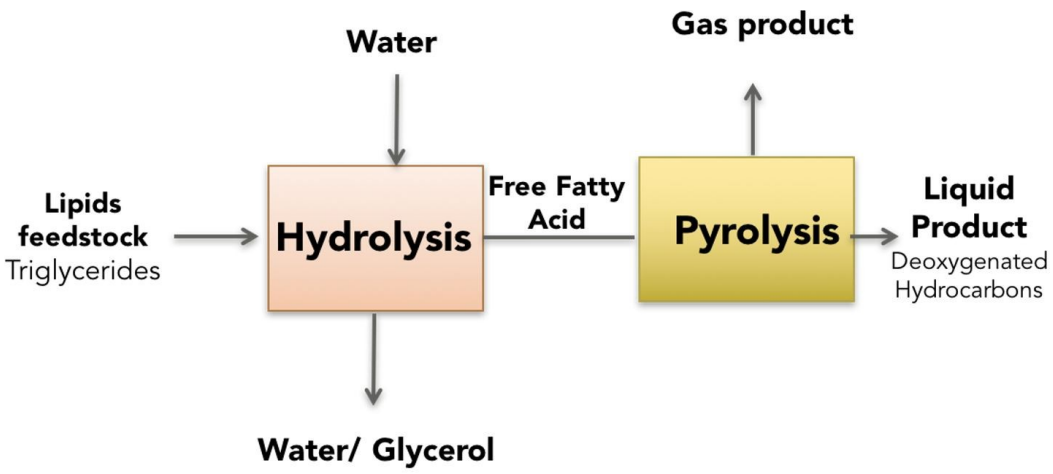


Figure 2-6 Two-step lipid pyrolysis. Reused with permission from Asomaning.[85]

The work presented in this thesis examines the incorporation of biosolids into the hydrolysis step as a replacement for water and as a potential source of small amounts of lipid material that can be processed to biofuels. A series of experiments were conducted to test the possibility of using biosolids in hydrolysis for fatty acids production and the effect of biosolids on the quality of lipid product. Further exploration of reducing the impact of using biosolids in hydrolysis reaction on lipid product quality through alterations to the temperature was also attempted.

3 Materials and Method

3.1 Feedstock

The biosolids used in this study was residuals from wastewater treatment produced at the Gold Bar Wastewater Treatment Plant (GBWWTP) in Edmonton and stored at the Clover Bar Biosolids Lagoons for further thickening and settlement. Thus, the biosolids sample received was the residue after clarification, digestion and stabilization and other primary and secondary treatment steps employed by the wastewater treatment facility.

Biosolids samples were obtained from the Clover Bar Wastewater Treatment Plant Lagoon cell five as shown in **Figure 3-1**.[1] The sample was collected before centrifugation in the co-composting facility and contained roughly 3.5% total solids, according to the treatment plant. Brown grease samples were donated by Rothsay.

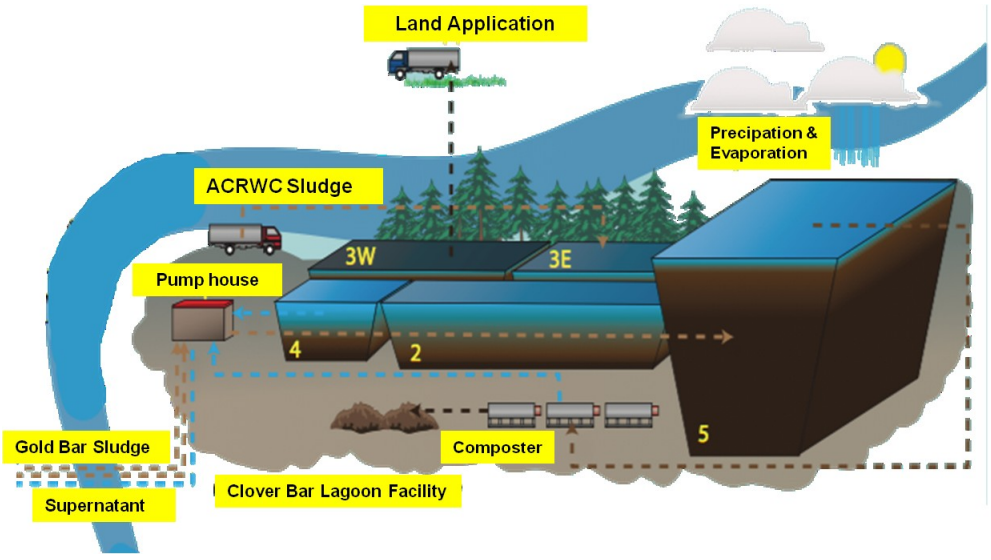


Figure 3-1 Clover Bar lagoon facility

(Reused with permission from SMA consulting)

3.2 Solvents and Analytical Standards

Oleic acid (≥99%; the internal standard for Gas Chromatography (GC) analysis of the organic phase), nonadecanoic acid methyl ester (99%; the standard for High-Performance Liquid Chromatography (HPLC) analysis of the organic phase), glyceryl trioleate (≥99%), dioleoylglycerol (≥99%), and 1-oleoyl-rac-glycerol (≥99%), and oleic acid (≥99%), were purchased from Sigma-Aldrich (St. Louis, MO, USA). The solvents used for analysis, acetyl chloride (HPLC grade, >99.9%), methanol (HPLC grade, >99.9%), toluene (HPLC grade, >99.9%), hexane (HPLC grade, >99.9%), pentane (HPLC grade, >99.9%), acetone (HPLC grade, >99.7%), acetic acid (>99.85%), and the *o*-phosphoric acid (85%) for acidification, were all obtained from Fisher Scientific (Fairlawn, NJ). Nitrogen gas (99.998%) was obtained from Praxair (Mississauga, ON).

For thin layer chromatography, iodine (≥ 99.99%) and diethyl ether (≥98%), were purchased from Sigma-Aldrich (St. Louis, MO, USA). For derivatization of aliphatics, acetyl chloride (99%) and a Diazald® kit used to prepare diazomethane for derivatization of fatty acids were purchased from Sigma-Aldrich (St. Louis, MO, USA). Chloroform (HPLC grade, >99.9%), hexane (HPLC grade, >99.9%), and methanol (HPLC grade, >99.9%) were obtained from Fisher Scientific (Fairlawn, NJ). Samples and chemicals were used as received.

3.3 Sterilization

Biosolids are classified as biohazardous materials. Thus, biosolids must be autoclaved prior to any operation, except the thermal hydrolysis process. The conditions employed for the thermal hydrolysis process (described in section 3.5) far exceed the minimum requirements for sterilization via autoclaving. For autoclaving, samples were sterilized at 121°C and 15 psi for 1 hour.

3.4 Analytical Methods for Biosolids Composition

The moisture content of biosolids was determined gravimetrically by drying samples in a freeze dryer (VirTis Ultra 35L, SP Scientific, Stone Ridge, NY, USA). Homogenized biosolids were loaded to pre-weighed 50 mL plastic conical centrifuge tubes (Fisher Scientific, Fairlawn, NJ), frozen at -80°C, and then freeze-dried for 72 hours. The samples were weighed and masses were recorded. Then, they were redried for an additional 2 hours. The weight check procedure was performed several times until no further weight changes were observed. Water content was calculated by the mass difference between samples before and after dehydrating with a freeze dryer.

The ash content was determined following ASTM D5347-95 (2012). The ash content was determined gravimetrically by burning the sample in a muffle furnace at 550°C for 1 hour, followed by cooling down in desiccator. The weight was then recorded and the sample was reheated in the muffle oven at 550°C for another 1 hour. The cool down and weight check procedures were repeated several times until there was no change of weight.

3.5 Hydrolysis in 5.5L Batch Reactor

Hydrolysis of biosolids was conducted in a batch 5.5 L reactor (Model 4580, Parr Instrument Company, Moline, IL, USA) at 280°C or 300°C for 60 min. The initial pressure was 100 psi (689.5 kPa), with the pressure stabilizing at 1200-1300 psi when the set temperature was reached. The reaction began when the set temperature was reached that would give biosolids a subcritical or supercritical condition as discussed in section 2.3. When the reaction finished, the reactor heater was turned off and the reactor was cooled down to room temperature by an external cooling system (VWR, Radnor, PA, USA) set to -20°C.

Since the lipid content in biosolids is small and hard to separate directly by separation funnel as specified for the method of hydrolyzing brown grease under subcritical condition [114], a further extraction and centrifugation of the hydrolysis product was required as described in 3.1.7.

3.6 The Effect of Hydrolysis on Settling Performance

The effects of acidifying biosolids before or after hydrolysis, and after autoclaving on settling performance were also studied. Because biosolids are classified as a biohazard, the original biosolids were autoclaved at 121°C for 1 hour to address safety concerns prior to further experimentation. The hydrolysis condition of all of the experiment should be high enough to make distilled water or biosolids subcritical or supercritical. For these experiments, biosolids were hydrolyzed with or without acidification in the 5.5 L reactor at 280°C for 1 hour. The biosolids were acidified with

phosphoric acid to a pH that is lower than the pKa of FFAs (4.7) in order to eliminate deprotonated FFAs, which can be found in the aqueous phase. These experiments also explored the effect of adding acid to biosolids before or after hydrolysis on settling rates.

The treated biosolids were homogenized and loaded into a 1 L measuring cylinder. The biosolids were allowed to settle for 2 hours, and the settled solids volumes were recorded every 1 min for the first hour and then every 3 min for another 1 hour. All the experiments were performed in triplicate.

3.7 Solvent Extraction

In this study, hexane was used as the solvent to extract the organic substances such as free fatty acids, triglycerides, monoglycerides, and diglycerides, as well as other hexane soluble compounds from biosolids. Compounds extracted with hexane without hexane removal are referred to as hexane extracts; compounds extracted with hexane, but subjected to subsequent hexane removal, are referred to as hexane extractables. The original biosolids were acidified after autoclaving in order to protonate the FFAs present in the aqueous phase, which is necessary for hexane extraction of FFAs. The extraction method used for hydrolyzed and unhydrolyzed biosolids was the same.

Specifically, the sample was homogenized and then extracted with hexane at a ratio of 1:1 (v/v) three times. After extraction, the hexane layer was filtered through #1 Whatman filter paper (Maidstone, Kent, UK) to remove traces of cell debris and small solid particles suspended in the hexane extracts. A Buchi R-205 rotary evaporator (Brinkmann Instruments, Inc, Westbury, NY, USA) was then used to remove most of the

hexane at 40°C under 325 mmHg of vacuum, and extracts were concentrated down to about 10 mL in the evaporator. The concentrated lipids-rich layer was transferred and diluted with *n*-hexane to 25 mL in a volumetric flask. 10 mL was then transferred out into a pre-weighed glass vial, and the solvent was removed at 25°C under 20 psi stream of N_2 using an analytical evaporator system (Glas-col, Terre Haute, IN, USA). The recovered extract was weighed for calculations. The extracts and the rest of solution were stored at 4°C until further analysis

3.8 Hydrolysis in Microreactors

Hydrolysis of brown grease with distilled water or biosolids were conducted in lab-scale 15 mL batch microreactors constructed with stainless steel Swagelok fittings and tubing (0.75 inches) and heated in a Techne SBS-4 fluidized bed sand bath with a TC-D8 controller (Burlington, NJ, USA). The vessel had an internal volume of 15 mL and a pressure rating of 10,000 psi. Samples were loaded into a clean and dry microreactor; nitrogen was purged into the reactor after closing the reactor to provide an initial pressure of 500 psi. The reactor was put into the sand bath preheated to the desired temperature. The reaction was conducted with agitation and reacted for a specific amount of time. The vessel was then removed from the sand bath and immediately cooled with water to room temperature.

Three temperatures, 280°C, 310°C, and 340°C, were examined for the brown grease hydrolysis. A 5:1 w/w ratio was adopted to ensure a better hydrolysis conversion according to other researchers listed in **Table 2-2**. The reaction time was 1 hour, and

the starting pressure of each experiment was 500 psi for all the batches. Lipid, sulfur, and nitrogen content, along with the gas portion, were analyzed using different analytical methods.

For studies examining the possibility of using biosolids as a substitute for water to hydrolyze into FFA, all hydrolysis reactions were conducted in microreactors. In this experiment, biosolids provided a source of water for hydrolysis of brown grease at 280°C. Each of the biosolids batches had a control consisting of distilled water hydrolyzed brown grease sample treated under the same experimental conditions. The liquid to lipid ratio used in this experiment was 1:1 according to the method described by Asomaning *et al.* (Asomaning et al., 2014b).

In contrast to distilled water (pH=6.8), the biosolids were slightly alkaline (pH=9), which might have an influence on the hydrolysis reaction rate. However, acidification of biosolids to a pH similar to that of distilled water required an addition of acid, and under the thermal conditions, acid can cause corrosion problems and make reactor maintenance cumbersome. Thus, an experiment was done to investigate whether the high pH of biosolids would affect the FFA conversion. Biosolids were acidified to 2 different pHs: one was around 3, which is lower than the pKa of FFA (around 4.5), promoting the protonation of FFA in the aqueous phase, while another was adjusted to 6.8, which was the same as distilled water. Hydrolysis of all samples were conducted under the same conditions (in triplicate): a temperature of 280°C, liquid to lipid ratio of 1:1 (m/m) for 1 hour. Samples were subjected to phase separation and analyzed by HPLC-ELSD and GC for FFA conversion.

The collection steps for the hydrolysis products consisted of pouring the vessel contents into a centrifuge tube, adding a small volume of phosphoric acid to the aqueous phase, and transferring out the oil layer after phase separation. In this experiment, to facilitate separation, the samples were centrifuged at 8648 x *g* for 5 min prior to removal of the oil layer. The oil phase was then subjected to HPLC-ELSD and GC analysis to determine the degree of hydrolysis. GC-MS and GC-FID analyses were then utilized to identify and quantify the free fatty acids composition.

3.9 Product Identification and Quantification

3.9.1 Qualitative Analysis by Thin Layer Chromatography

Thin layer chromatography (TLC) was performed for the qualitative analysis of lipid classes. A TLC Whatman aluminum silica plate (Maidstone, Kent, UK) was used with standards of glyceryl trioleate (≥99%), dioleoylglycerol (≥99%), 1-oleoyl-rac-glycerol (≥99%) and oleic acid (≥99%). Hexane:diethyl ether:acetic acid (80:20:1) was used as the mobile phase. Two μL of 6 mg/mL samples were spotted on the marked line of the plate and developed in a closed TLC chamber with the sample spot well above the level of the mobile phase. The monoglyceride, diglyceride, triglyceride and free fatty acids were separated according to their polarity; the least polar compounds will travel further up the plate than the less polar compounds. The visualization of the spot was done in an iodine chamber.

3.9.2 Lipid Fraction Analysis by HPLC-ELSD

The quality of biosolids lipids was studied using HPLC coupled with an evaporative light scattering detector (ELSD). In this experiment, a 100 Å Phenogel column((300 mm × 7.8 mm internal diameter 5 m) protected with a SecurityGuard C18 Guard cartridge system (Phenomenex, Terrence, CA, USA), was used to analyze the triacylglycerol (TG), diacylglycerol (DG), monoacylglycerol (MG) and free fatty acid (FFA) composition of the feeds. A high-performance size-exclusion chromatography system consisting of an Agilent 1200 series binary pump, a high-performance autosampler, and an evaporative light scattering detector (Agilent Technologies, Santa Clara, CA, USA) was employed. The detector temperature was set at 40°C, and N_2 gas was set as 3.5 bars. Toluene containing 0.25% acetic acid was chosen as the mobile phase as it allows for better resolution of the lipid classes as described by Kittirattanapiboon *et al.* (2008).[115] The flow rate of the mobile phase was 1.0 mL/min, the concentration of samples was 3.5 mg/mL, and all the standards and samples were prepared by dissolving in toluene (>99.9%).

3.9.3 Characterization of Fatty Acids by Gas Chromatography

Detailed profiles of FFAs, hydrocarbons, and other organic compounds were identified by GC combined with mass spectrometry and quantified by GC with a flame ionization detector. The calculation of FFA% in the recovered lipid phase and FFA conversion were based on gas chromatography. Diazomethane was used as an esterification reagent to methylate free fatty acids since this method was quick and

simple and can selectively derivatize fatty acids.[116] The analysis of methyl ester derivatives was conducted on an Agilent 6890N gas chromatograph equipped with a flame-ionization detector (FID) and an HP 7683 autosampler. Helium was the carrier gas, at a constant flow rate of 1 mL/min. Separation of components was performed on a 30 m × 0.32 mm (internal diameter) HP-5ms capillary column with a 0.25 μm film thickness (Agilent Technologies, Santa Clara, CA, USA). The injector and detector temperature were set at 300°C and 350°C, respectively. The following oven temperature program was used: 0.1 min hold at 35°C, then ramp to 280°C at 10°C per minute and retained for an additional 5.4 min for a total run time of 30 min. A 1:40 split injection ratio was used, and the injection volume was 1 μL.

GC with mass spectrometric detection analysis (GC-MS) was also employed using a similar column and conditions as GC-FID (described above) and performed on an Agilent GC 6890N coupled to an Agilent 5975B EI/CI MS instrument operated in electron ionization (EI) mode. The temperature of the GC-MS interface was kept constant at 320°C.

The quantification of fatty acid was based on the formula as follows:

Equation 3-1:

$$\textbf{Free fatty acids, \%} = (\sum W_{FAMEi} \times f_{Fai} / W_{test\ portion}) \times 100\%$$

Where:

W_{FAMEi} = weight of individual FAME in the test portion;

f_{Fai} = conversion factor for conversion of FAMEs to their corresponding fatty acids (according to AOAC Official Method 996.06);[117]

$W_{test\ portion}$ = weight of test portion, g

Because diazomethane can derivatize FFA but not glycerides, samples from the same sample solution used for diazomethane derivatization were also derivatized separately with 10% acetyl chloride in methanol at 80°C for 2 hours using a method described elsewhere.[118] This approach could derivatize both fatty acids and the acylglycerols for GC analysis.

Fatty acids conversion was calculated by the following equation:

Equation 3-2:

$$\textbf{Fatty acid conversion} = \frac{W_{FFAd}}{W_{FFAa}} \times 100\%$$

Where:

W_{FFAd} = weight of fatty acid in sample derivatized with diazomethane;

W_{FFAa} = weight of fatty acid in sample derivatized with acetyl chloride.

The fatty acids conversion was also calculated by using the results from HPLC-ELSD as follows:

Equation 3-3:

$$\textbf{Fatty acid conversion} = \frac{W_{FFA}}{W_{Total\ Lipids}} \times 100\%$$

Where:

W_{FFA} = weight of FFA in sample detected by HPLC-ELSD;

$W_{Total\ Lipids}$ = Sum of the weight of TG, DG, MG, and FFA in the original sample or in the hydrolyzed lipid detected by HPLC-ELSD

3.9.4 Elemental Analysis

The elemental analysis was performed using different technologies, according to the element of interest. The CHNS analysis of the hydrolyzed biosolids lipids phase (hexane extractables) was conducted by the Department of Chemistry, University of Alberta using a CHNS analyzer. The metal content in lipids was analyzed by ICP-MS (Department of Earth Science and Atmospheric, University of Alberta). The sulfur content was analyzed by ICP-OES and performed by the Natural Resources Analytical Laboratory, University of Alberta. Moreover, the nitrogen content was analyzed using the FLASH 2000 combustion unit in the analytical laboratory in the Department of Agricultural, Food and Nutritional Science, University of Alberta.

3.10 Statistical analysis

All of the experiments were done in triplicate. The statistical analysis of data was done using one way ANOVA with the mean comparison by Tukey test or two way ANOVA with mean comparison by Sidak multiple comparisons test (GraphPad Prism 6 software, La Jolla, CA) based on a confidence level of 95%.

4 Results and Discussion

4.1 Hydrolysis and Characterization of Biosolids

Substances that biosolids will bring into the reaction might positively or negatively affect the formation and the quality of the final product of lipid pyrolysis. Biosolids are sourced from a variety of wastes that undergo the wastewater treatment and are a complex heterogeneous mixture of microorganisms, undigested organics, inorganics, and water. The undigested organic materials contain a highly complex mixture of molecules including lipids, proteins, peptides, polysaccharides, plant macromolecules with phenolic structures (e.g. lignin or tannins) or aliphatic structures (e.g. cutin or suberin).[119] They can also carry organic micro-pollutants such as polycyclic aromatic hydrocarbons (PAH) or dibenzofurans.[119] The wastewater treatment processes are not standardized in different treatment plants. For this reason, the composition of the biosolids researched in various studies may vary significantly. Especially the lipid concentration, that would increase the FFA concentration in the product, and other elements that might have effect on the quality of the final product.

The most abundant compound in biosolids is water, which is hard to separate without any physical or chemical treatment. Compared with distilled water that is often used in lab scale hydrolysis, there may be a lot of undesirable compounds in biosolids such as sulfur, nitrogen, as well as some metals. Those compounds not only create issues for biosolids disposal but also have a big chance to affect the product quality if used for the hydrolysis step of lipid pyrolysis. However, because of its high water content (>95%), biosolids could serve as a replacement for water during the hydrolysis

step in lipid pyrolysis. This thermal treatment could also destroy microorganisms and molecular structures within biosolids, which could serve as a sterilization mechanism and improve the settleability of biosolids. Proximate analysis, lipid analysis, and elemental analysis of biosolids and hydrolyzed biosolids were performed to explore the effects of hydrolysis, and to identify possible constituents that biosolids might bring into the final product.

To determine how thermal hydrolysis impacts the composition and quality of biosolids, compositional analyses were performed on autoclaved biosolids compared with biosolids subjected to thermal hydrolysis. Autoclaving was done to sterilize the biosolids such that they could be used for further experimentation but using much milder conditions that would not be anticipated to substantially alter the composition of the material.

In the first experiment of our study, biosolids were hydrolyzed with the objective of exploring FFA % in the recovered lipid phase and product quality of the resulting hydrolysates. This experiment was designed to acquire a better knowledge of the specific biosolids source that will be used to substitute for water in the hydrolysis reaction of lipid pyrolysis and to study the behavior of the materials in biosolids alone as a baseline for other studies. Biosolids were hydrolyzed independently (in triplicate) using the method described in section 3.5 Due to the alkaline condition of biosolids, another experimental sample set was developed by adjusting the pH of biosolids to 3 with phosphoric acid before hydrolysis, which could result in deprotonation of the fatty acids in biosolids. Moisture and ash content were performed on both treated and

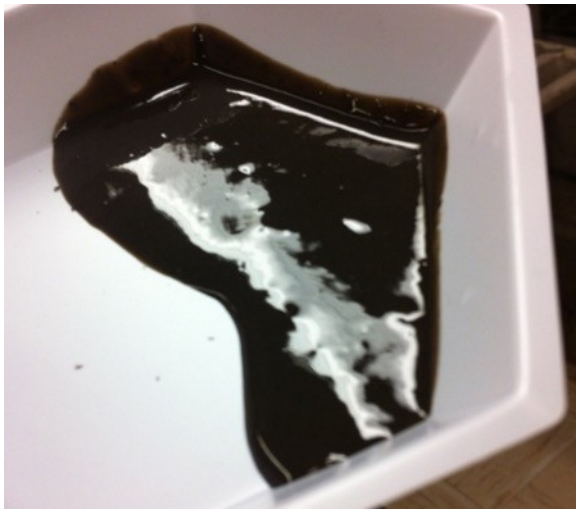
untreated biosolids by the procedure described in section 3.4. Also, lipids from the untreated and treated biosolids were extracted by using the method described in section 3.7. Metal content and CHNS were also explored. Thin layer chromatography was also performed to determine in detail the biosolids' lipids composition，

4.1.1 Proximate Analysis of Biosolids

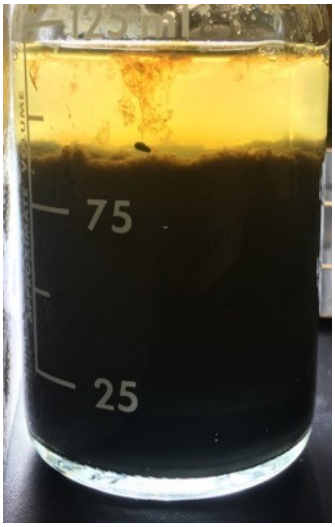
The biosolids were received as a thick liquid as shown in

Figure 4-1 (A), and the settling performance was poor. As seen in

Figure 4-1 (B), after storage of biosolids at room temperature for four months, only about one-quarter had settled. Proximate analysis of biosolids (autoclaved or hydrolyzed) was performed, and the results are shown in **Table 4-1**. The pH of biosolids was around 9 due to the lime stabilization process, which was quite high compared with distilled water. The most abundant compounds in the received biosolids was water as shown in **Table 4-1**, which accounted for more than ~96% (wt. %) and this result confirmed the analysis done by the wastewater treatment plant (~96.5%). The inorganic components (ash content) represented about ~1 % of the total biosolids, and the remaining ~2% were organic compounds.



(A)



(B)

Figure 4-1 Biosolids received from Cell 5 in Clover Bar lagoon. (A)Homogenized biosolids; (B) Biosolids stored at room temperature for 4 months. All the images of unhydrolyzed biosolids in this thesis were taken after autoclaving to destroy all microorganisms that may be present. This was done to address safety issues.

Table 4-1 Proximate analysis of biosolids

	Water, %	Ash, %	FFA, %
Original Biosolids (Autoclaved)	96.8 ± 0.1^{a}	1.0 ± 0.1^{a} (31 ± 3% dry weight)	0.008 ± 0.001^{a} (0.2 ± 0.1% dry weight)
Hydrolyzed Biosolids	96.5 ± 0.1^{b}	1.2 ± 0.2^{a} (34 ± 5% dry weight)	0.03 ± 0.01^{b} (0.8 ± 0.2% dry weight)

(All analysis were conducted triplicated. Within a given column, means that do not share the same letter are statistically different. The statistical analysis was done using one-way ANOVA with mean comparison by Tukey test based on a 95% confidence level.)

Bridle *et al.* (1990) indicated that the metals such as Cu, Cr, Ni, Na, K and Ca in the ash of biosolids were known to catalyse the cracking of hydrocarbons and reduce the viscosity of final production by increasing the proportion of straight chain alkanes during pyrolysis.[120] The ash content was not changed significantly before (31% ± 3%) and after (34 ± 5%) hydrolysis on a dry weight basis, but was higher than biosolids used in other studies. The ash content of the biosolids in a study of pyrolysis of biosolids for fuel production was 15% (Shen et al. 2005) and 21.32% in the biosolids used by Revellame (2012) who was using activated sludge for biodiesel production. [121] [122] The higher ash content could be explained by the fact that the biosolids used in our study have been through all the digestion treatment, and Otero *et al.* (2002) illustrated that the ash content would increase with a more completed digestion process.[123]

4.1.2 Hydrolysis Performance

For integration into lipid pyrolysis, a better of understanding of the hydrolysis of the lipids in biosolids is required. To this end, thin layer chromatography (TLC) was employed to rapidly separate different fractions in biosolids lipids based on polarity. For these experiments, the standards used were pure model triacylglyceides, diacylglycerides, monoacylglycerides, and fatty acids. The fractions of biosolids lipids were distributed across the whole column (**Figure 4-2**) and indicated the complexity of biosolids lipids. However, it could still be clearly observed that after hydrolysis, almost all triglycerides were converted to free fatty acids. HPLC-ELSD was also employed to separate and quantify the lipid fractions, but broad peaks and overlap among different

fractions were observed using these methods (**Figure 4-3**). Thus, the FFA conversion was calculated by using two types of derivatization methods (as described in 3.1.9.3) and the calculation was done according to **Equation 3-1**. The FFA conversion (FFA conversion) of hydrolyzing using biosolids was 88% ± 1%; there were approximately 12% lipids that were not converted, which were probably DG and MG according to the developed spots on the TLC plate. The results of FFA% in biosolids and the composition of original and hydrolyzed biosolids lipids were illustrated in next section, that could help with more understanding of the effect of hydrolysis on biosolids lipid composition.

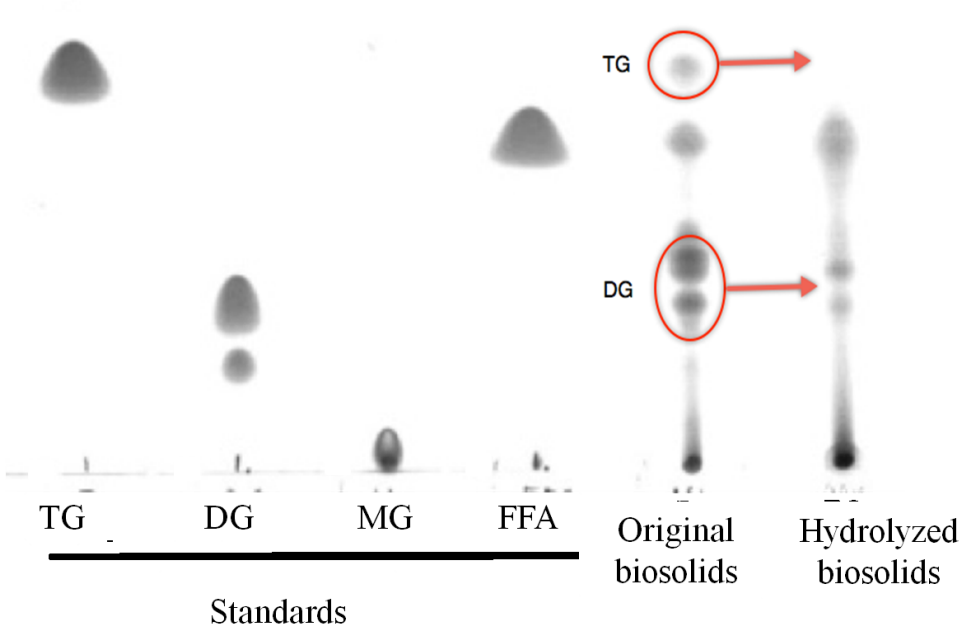


Figure 4-2 Thin layer chromatography of lipids from hydrolyzed and unhydrolyzed biosolids.

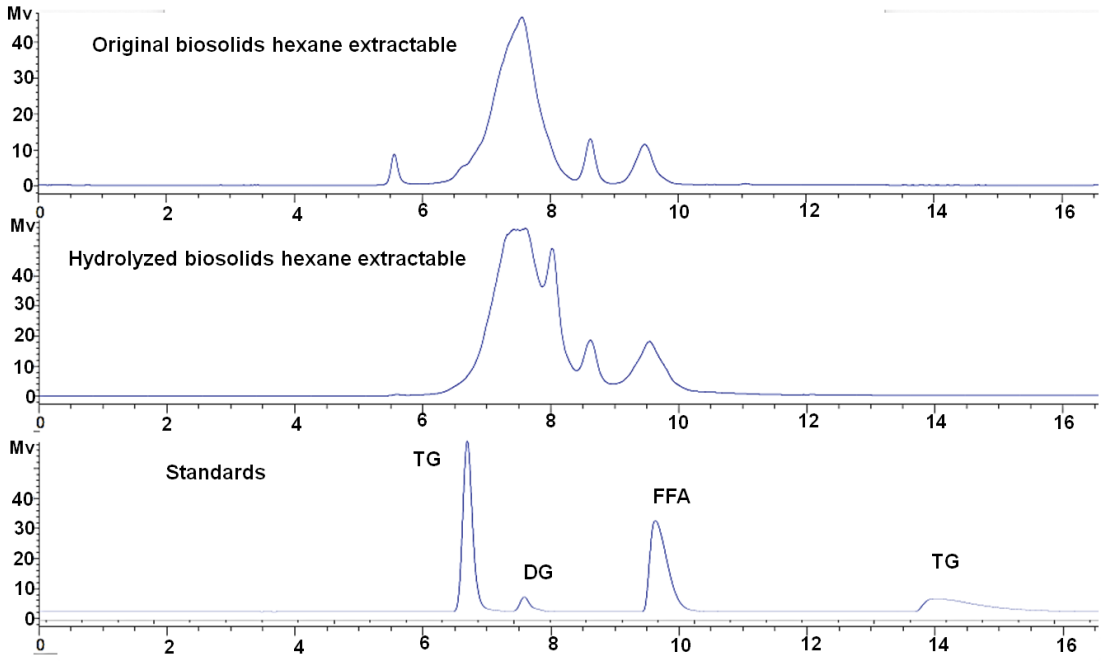


Figure 4-3 HPLC-ELSD analysis of lipids classes in the hexane extractables obtained from biosolids hydrolyzed at 280°C

4.1.3 Lipid Composition in Original and Hydrolyzed Biosolids

In previous attempts at lipid pyrolysis, the lipid material was of such great quantity that the lipid and aqueous phases generated following hydrolysis could be easily separated using a separatory funnel (Asomaning *et al.* 2014).[85] However, the amount of lipids in biosolids is not sufficient to allow for phase separation. Therefore, an extraction method was applied. *n*-hexane is the most common solvent used in lipid extraction in industrial applications due to its non-polarity, low toxicity, and easy removal. [124, 125] Also, because it possesses a better lipid accumulating property, hexane is widely used for extracting lipids content in biosolids.[126]

Lipids in biosolids, especially FFA, are a key component for biofuel production. Moreover, the concentration and distribution of FFA will affect the quality and types of product generated through lipid pyrolysis. The color of the two hexane extracts (200 mg/mL dissolved in hexane) was different as shown in **Figure 4-4**. After hydrolysis, the color of the hexane extract was dark brown compared with the orange color of the hexane extract from unhydrolyzed biosolids. There was also an unpleasant and strong burnt smell coming from the hydrolyzed sample. Biosolids contain various components such as carbohydrates and proteins, and compounds produced through chemical reactions at high-temperatures, such as the Maillard reaction and the formation of polycyclic aromatic hydrocarbons (PAHs), would likely be responsible for the smell and the dark color.[127]

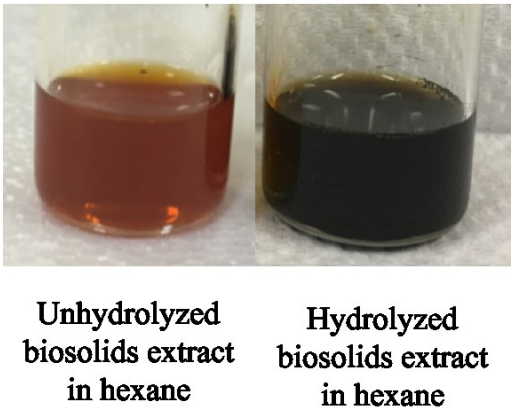


Figure 4-4 Picture of biosolids hexane extracts.

Figure 4-5 shows the distribution of organic components in hexane-extractables from hydrolyzed and unhydrolyzed biosolids. FFA concentration (based on dry weight) in biosolids samples before and after hydrolysis (in hexane extractables)

were analyzed by GC-FID/MS. The results showed that the FFA extracted from the unhydrolyzed sample only accounted for 0.2% based on dry weight of biosolids. After hydrolysis, the FFA concentration increased to 0.8% due to the releasing of FFA from the hydrolysis of fatty glycerides. The results of the FFA% in hydrolyzed biosolids (0.8 % ± 0.2% dry weight basis) were comparable with the research done by Revellame *et al.* (2012). Revellame showed that biodiesel convertible compounds (TG, DG, MG and FFA) corresponded to approximately 1.20-3.50% (weight) FAMEs yield based on dried activated sludge.[122] However, in this study, without a dewatering, the FFA% in hydrolyzed biosolids would be extremely low since the biofuel convertible compounds in received biosolids contain very few lipids, even on a dry weight basis.

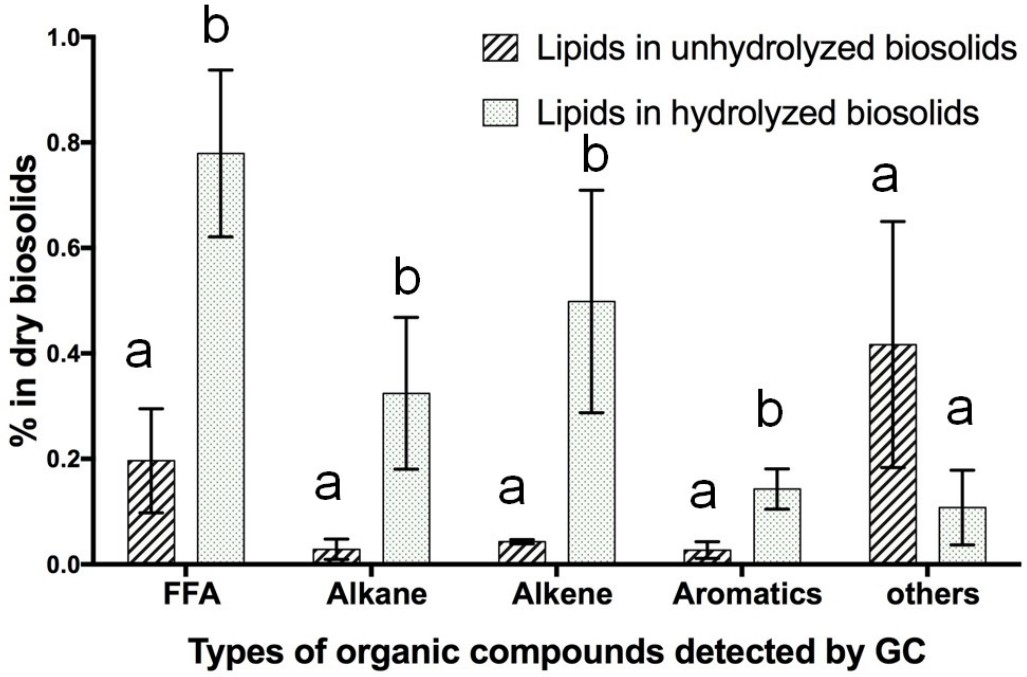


Figure 4-5 Organic components in biosolids before and after 280°C hydrolysis for 1 hour in a 5.5L reactor. All of the hydrolysis reactions were triplicated. The statistical analysis was done using one-way ANOVA with mean comparison by Tukey test based on a 95% confidence level.

FFA, aromatics, alkene, alkane, and ketones were detected in both samples before and after hydrolysis samples. Moreover, after hydrolysis, alkane, aromatics, alkenes, and FFA were significantly increased. The hydrolysis would convert triglycerides into FFA, which explained the increase of FFA. The reason for the increase of alkanes and alkenes was that under the hydrolysis temperature, there was some thermal cracking of FFA, which could convert FFA to hydrocarbons. The increasing of

aromatics may occur because of the existing protein, lignin and cellulose in the biosolids and the thermal condition could result in the formation of aromatics.[128]

The distribution of fatty acids in hydrolyzed biosolids is shown in

Figure 4-6. The fatty acids detected ranged from C6 to C26 with different level of unsaturation. The most abundant fatty acid in hydrolyzed biosolids was monounsaturated C16:1, which accounted for 34.5% of the total fatty acids. Conversely, the amount of C16:1 fatty acids in the lipid phase of hydrolyzed brown grease (

Figure 4-6) was only around 1~2%. A similar result was described in the study by Aosmaning *et al.* (2014) in their study on the hydrolysis of brown grease with distilled water. The fatty acids in hydrolyzed biosolids were predominantly monounsaturated (58% ± 5% of the fatty acids) with C16:1 (34% ± 5 %) and C18:1 (23% ± 1%) being the top two abundant fatty acids. Saturated fatty acids ranged in carbon number from C6 to C26 and accounted for 41% ± 6%. Another major difference among biosolids lipids compared with other types of lipids was that there was a significantly higher amount of long chain fatty acids from C20:0 to C26:0. Bozaghian *et al.* (2014) also observed the presence of long chain fatty acids (C20~C24) in dried sludge extracted lipids, which were used for the synthesis of biodiesel.[129] Also, the presence of odd carbon number fatty acids—C15:0 (11% ± 4%) made the fatty acids composition of biosolids lipids unique. A study of lipid extracted from wastewater activated sludge biosolids conducted by Garcia Becerra *et al.* (2010) indicated that the biosolids lipid were enriched with C15-C17 fatty acids fractions, which is consistent with the fact that microbial cell membranes are typically enriched with palmitic (C16) fatty acids.[130]

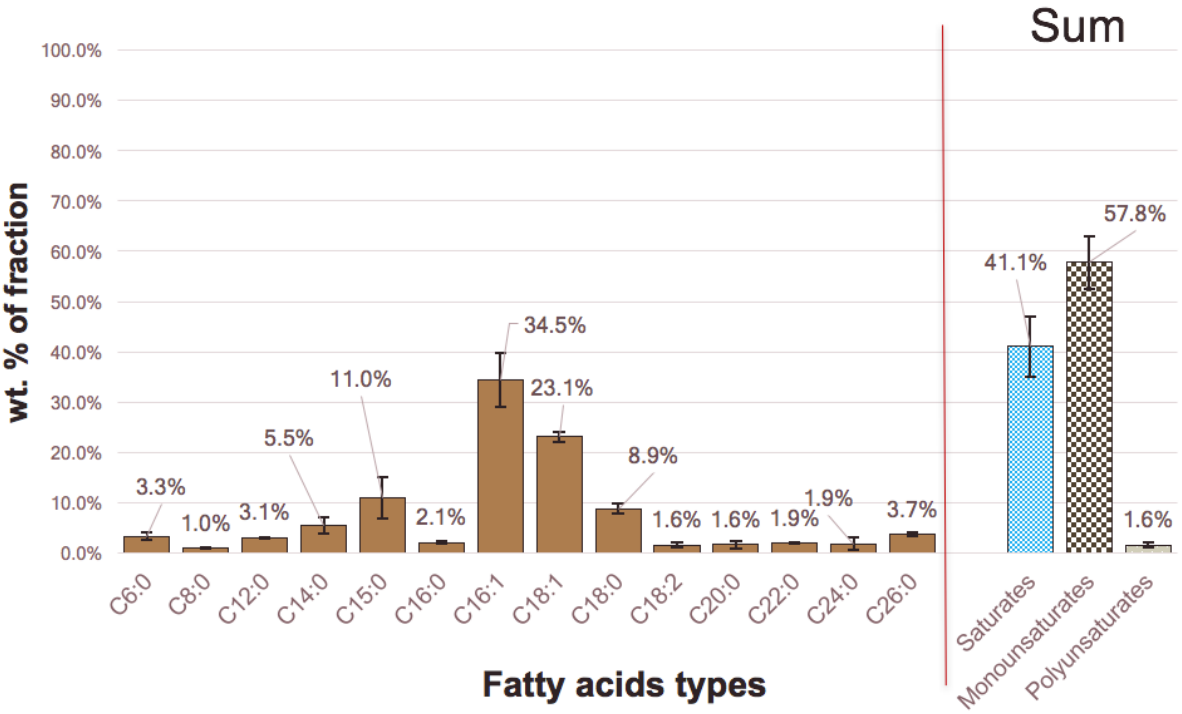


Figure 4-6 FFA distribution in hydrolyzed biosolids analyzed by GC-MS/FID.

CHNS analysis of lipids in hydrolyzed biosolids

Biosolids contain sulfur and metals that might affect the quality of the final product. Several studies showed that super and subcritical water could help break C-S bonds and convert non-aromatic sulfur to H_2S and reduce the chance of sulfur going into the end product.[131] Metal ions such as Al^{3+}, Ni^{2+}, and Cu^{2+} that exist in biosolids will also promote the decomposition of some sulfur compounds at 240°C.[132] Thus elemental analysis was important for the study of product quality.

Table 4-2 shows the CHNS analysis of hexane extractables from hydrolyzed biosolids. Sulfur and nitrogen were also detected in lipids in hydrolyzed biosolids. The sulfur content of renewable fuels is restricted by ASTM standards. According to the in-

use ASTM diesel sulfur specification (<500 ppm; ASTM D975), S15 fuel (sulfur content less than 15 ppm) always performs better than S500 and S5000 fuel in terms of thermal stability.[133] Environmental regulations did not specify concentration limits for nitrogen in transportation fuels, but a high nitrogen content surely would prompt serious issues of NOx emission in future fuel application.[134]

Table 4-2 CHNS analysis of hydrolyzed biosolids lipids

	C	H	N	S	O
	%	%	%	%	%
wt.% of biosolids lipid	78.6 ± 1.6	11.0 ± 0.2	1.2 ± 0.5	1.9 ± 0.6	6.1 ± 0.9

Metal Analysis

There were several metals detected in biosolids lipids by ICP-MS as shown in **Table 4-3**. The most abundant metals in both before and after hydrolysis of biosolids lipids were Fe, Ca, Mg, and Al. After hydrolysis metals such as Mg, Ca, Cr, Mn, Se, Sn, Pb significantly decreased; only As increased after hydrolysis. Heavy metals in the fuel product have been proven to be harmful to cars' engine and emission system. Because the brown grease hydrolyzed product would be used for producing biofuel, a regulation for the heavy metals in gasoline was found and compared. This regulation specified the lead and manganese in clean air limits specified by the EPA (Environmental Protection Agency, US): A lead concentration in gasoline of no more than 5 ppm and a Mn concentration of no more than 2 ppm (2014).[135] After hydrolysis the concentration of Mn was significantly decreased From 5.7 ± 0.8 to 1.1 ± 0.4, which is lower than the

EPA regulation. Wang *et al.* (2013) indicated in their experiment of the pyrolysis of sewage sludge for pyrolysis oil that Si, Al, Mg, Ti, Mn were easy to be enriched in the solid phase under supercritical condition. This explained the transfer of those metals from the lipid phase into other phases. The metal content of the aqueous and solids phases was not detected in this experiment, but the increase of some metals in the other phases would have some influences on downstream applications, such as the application of aqueous phase (e.g. for using as a growth medium) and solid phase (e.g. for metal extraction).[15]

Table 4-3 Elemental analysis of biosolids lipids by ICP-MS

Metal (ppm)	Biosolids lipids hexane extractables	
	Unhydrolyzed	Hydrolyzed
B	15 ± 5	36 ± 18
Na	65 ± 15	59 ± 32
Mg*	118 ± 21	32 ± 13
Al	113 ± 3	73 ± 31
K	BDL	44 ± 26
Ca*	580 ± 114	54 ± 3
Ti	9 ± 5	13 ± 8
V	4 ± 2	1.7 ± 0.4
Cr*	12 ± 1	3 ± 1
Fe	185 ± 111	169 ± 32
Mn*	5.7 ± 0.8	1.1 ± 0.4
Co	0.9 ± 0.5	0.4 ± 0.1
Ni	5 ± 2	12 ± 3
Cu	20 ± 4	23 ± 2
Zn	9 ± 5	10 ± 6
As*	1.2 ± 0.1	6 ± 2
Se*	9 ± 2	1.1 ± 0.1
Sr	5 ± 2	0.9 ± 0.4
Y	0.1 ± 0.01	BDL
Zr	33 ± 12	2.4 ± 0.9
Nb	0.3 ± 0.1	0.3 ± 0.1
Mo	4 ± 2	5 ± 3
Ag	1 ± 0.2	1.2 ± 0.5
Cd	0.12 ± 0.01	BDL
Sn*	6.1 ± 0.9	0.6 ± 0.1
Sb	0.12 ± 0.03	0.7 ± 0.2
Ba	3.9 ± 0.4	1.9 ± 0.8
Hf	2.3 ± 0.3	3 ± 2
Ta	0.12 ± 0.01	0.4 ± 0.2
W	2 ± 1	1.5 ± 0.1
Os	0.2 ± 0.1	0.5 ± 0.1
Ir	0.2 ± 0.1	2 ± 1
Pb*	4 ± 1	1.3 ± 0.4

The statistical analysis was done using one-way ANOVA with mean comparison by Tukey comparisons test based on a 95% confidence level. Within the same row, an asterisk indicates that the 2 numbers are significantly different ($p<0.05$). BDL- Below detection limits (K: 0.006, Y: 0.00002, Cd: 0.00006)

It should be noted that heavy metals such as nickel have been shown to have a positive effect on oil cracking or subsequent pyrolysis as illustrated by Churin *et al.* (1991).[136] Also some metal oxides such as KOH and ZnO were also functioned as a

catalyst and promoted the thermal conversion of oil into the organic liquid product.[137] These data highlight the necessity to conduct metal analyses to monitor levels in materials that are used for lipid pyrolysis as the conditions used for thermal hydrolysis in these studies can significantly impact the distribution of metals into the different phases.

4.1.4 Settling Performance of Hydrolyzed Biosolids

Biosolids received from wastewater management were a smelly and viscous biohazard liquid, of which dewaterability was poor. To improve dewatering performance, the undigested biosolids usually undergo several disintegration processes that include physical, biochemical, and thermal treatments (such as hydrolysis) that range from 40-180°C.[27] The settling performance of biosolids is one of the primary concerns when dealing with biosolids management. A series of settling performance experiments were performed to study the influence of thermal treatment on the settling performance. Research on the thermal treatment of biosolids has focused on lower temperatures in order to reduce operational costs. However, if the biosolids could be incorporated into another process that employs high-temperature conditions, then the costs of thermal treatment would already be accounted for in the process economics. The hydrolysis temperature in the lipid pyrolysis process ranges from 260-340°C.

In the modified lipid pyrolysis process, the thermal hydrolysates need to be acidified to a pH that is lower than the pKa of FFAs, to facilitate maximal recovery of the deprotonated FFA from the aqueous phase to the lipid phase. Thus, our experiments

also explored the effect of adding acid to biosolids (before or after hydrolysis) on settling performance. Neyens *et al.* (2003) showed that hydrolyzing biosolids (5-6% dry solids) with acid at 80-155°C could give a significant reduction of solids in biosolids.[14] Our study was an extension of their research, but the hydrolysis was conducted at higher temperature (280°C), and natural settling rates were chosen to illustrate the settling performance without any filtration or mechanical dewatering method applied. For these experiments, biosolids were hydrolyzed with or without acidification in a 5.5 L lab scale reactor at 280°C for 1 hour. Since biosolids are classified as a biohazard, the unhydrolyzed biosolids were autoclaved at 121°C for 1 hour to address safety concerns.

Figure 4-7 shows the settling rate of solids. Regardless of whether acidification was applied, the 121°C autoclave treatment did not have a significant effect on the settling performance since there was no settling observed within 2 hours. This is consistent with our previous data that showed that autoclaved biosolids demonstrated minimal settling even after a 4-month period (

Figure 4-1 (B)). The settling performances of the crude biosolids hydrolysates without acidification and crude biosolids acidified after hydrolysis were not significantly different from each other. In both systems, after around 70 min the setting became slow and the settled solids volume stabilized. Conversely, biosolids that were acidified before hydrolysis settled to a stable solid volume within 15 min and the settled solids volume percentage (11.3% ± 0.3%) was lower (about half) than that of the

other 2 hydrolyzed sample after 2 hours settling (Crude biosolids hydrolysates without acidification: 21.8% ± 0.8%; crude biosolids acidified after hydrolysis: 24.3% ± 2.3%). Neyens *et al.* (2003) indicated that the present of extracellular polymer (ECP) was one of the reason for the difficulty in biosolids dewatering, and the acid treatment can cause ECP to leave the biosolids surface and makes it easy to pack the sludge aggregates and for a easier reduction of water content in biosolids.[14]

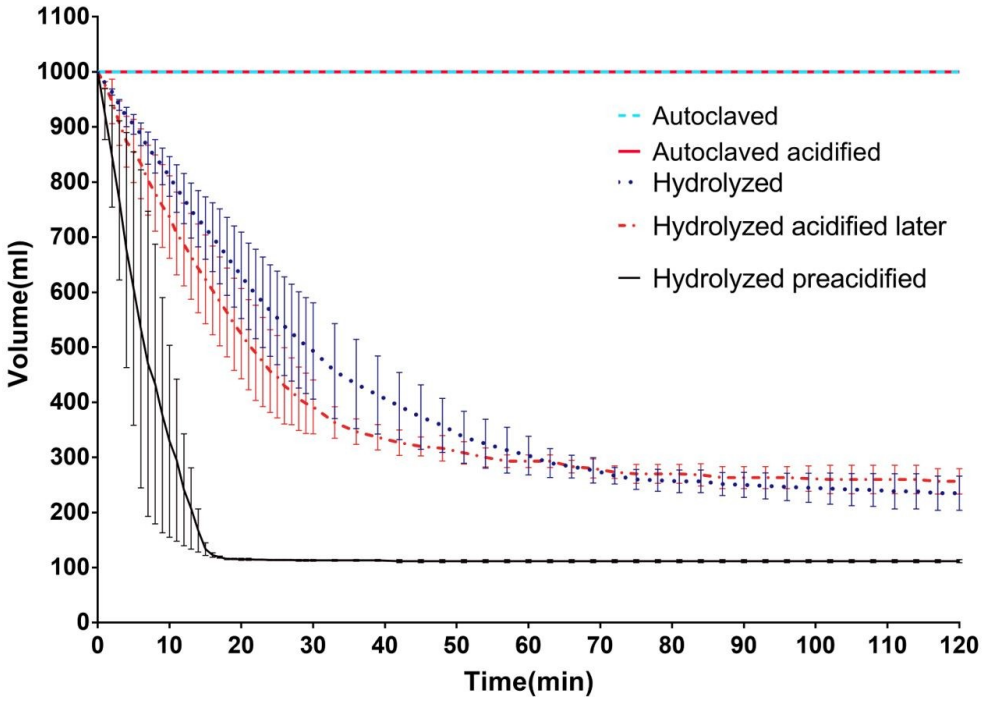


Figure 4-7 Plot of settled solids volume against the time of biosolids with 5 different treatments in a measuring cylinder. All experiments were triplicated. The statistical analysis was done using one-way ANOVA with mean comparison by Tukey multiple comparisons test based on a 95% confidence level.

Figure 4-8 and **Figure 4-9** were photos taken at 15 min and 60 min, respectively. The photos demonstrate that hydrolyzed biosolids performed much better than the autoclaved samples. The high temperature and acid condition would help break down complex structure and cell structure in biosolids.[138] And Feng *et al.* (2014) showed that 1-hour thermal treatment at 170°C could help denature and precipitate the organic materials in biosolids.[139] Neyens *et al.* (2003) also indicated that the acid in thermal hydrolysis could result in a better accessibility of cell content due to the lysis of microbes in biosolids.[14] One issue that should be considered is that although acid addition improved settling, acidic conditions can cause corrosion issues and lead to increased maintenance of the reactor. Thus, despite having a lower effect on settling rates, it may make sense economically to add acid after hydrolysis as this system still dramatically outperformed the control.

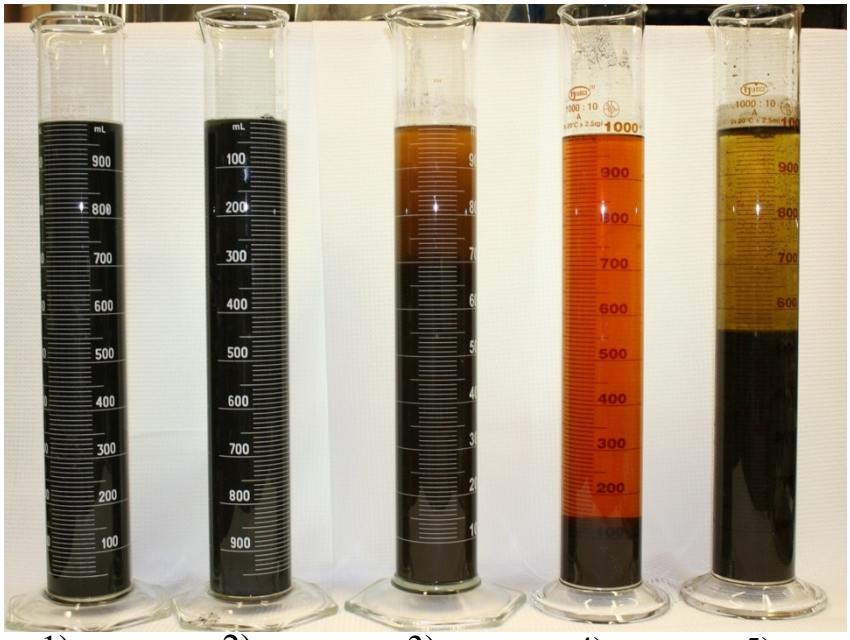
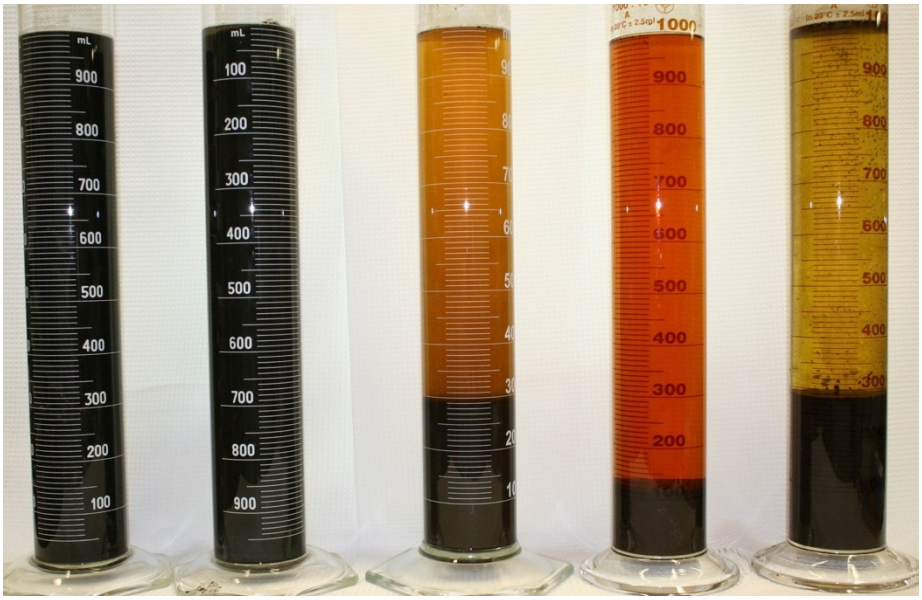


Figure 4-8 Picture of biosolids settling experiment at 15 min. All of the experiments were triplicated. Sample from left to right were 1) Autoclaved biosolids without acidification; 2) Autoclaved biosolids acidified to pH=3; 3) Biosolids hydrolyzed without any acidification; 4) Biosolids acidified to pH=3 and then hydrolyzed; 5) Biosolids hydrolyzed and acidified to pH=3 after hydrolysis. Hydrolysis was performed at 280°C for 1 hour. All of the hydrolysis experiments performed in triplicate.



1) 2) 3) 4) 5)

Figure 4-9 Picture of biosolids settling experiment at 60 min. All of the experiments were triplicated.Sample from left to right were 1) Autoclaved biosolids without acidification; 2) Autoclaved biosolids acidified to pH=3; 3) Biosolids hydrolyzed under 280°C without any acidification; 4) Biosolids acidified to pH=3 and then hydrolyzed; 5) Biosolids hydrolyzed and acidified to pH=3 after hydrolysis. Hydrolysis was performed at 280°C for 1 hour. All of the hydrolysis experiments performed in triplicate.

4.1.5 Conclusions

Even though the lipids concentration in received biosolids was less than 1% (on a dry weight basis), the data from hydrolysis of the materials in biosolids was as expected: the triglyceride was successfully converted to FFA. Due to the tiny amount of lipids in biosolids, the biosolids would have to be blended with a lipid source to increase lipid loading to make the lipid pyrolysis process more cost effective. Hydrolysis is a thermal process that requires high temperature; if biosolids alone were used in the hydrolysis step of lipid pyrolysis, the cost of energy required would be far

more than the energy recovered. Analysis of lipids from hydrolyzed biosolids indicated relatively high levels of sulfur and nitrogen, which must be taken into account for fuel applications. Biosolids are a source of a low amount of organic materials, much of which are unknown because of the complex mixture of the original source material. Those unknown organics also need to be considered when looking into subsequent fuel applications. The heavy metal content in biosolids was diverse, but the concentrations were relatively low as well. This experiment studied the behavior of the materials in biosolids alone as a baseline for other studies, and the organics that can be extracted by hexane were possible compounds that will go into the pyrolysis process as a part of feedstock. However, the total amount of these organics is very small and may not have a huge influence on the final product. This study also showed the benefit of hydrolysis at 280°C, especially when acid was applied. The hydrolysis at 280°C could significantly improve settling compared with the unhydrolyzed biosolids. And a better settling of biosolids could make the reuse or disposal of both solids and aqueous easier. Further experimentation, especially experimentation on pyrolysis of lipids obtained through hydrolysis of biosolids, is ongoing but is outside the scope of this project.

4.2 Performance of Hydrolyzing Biosolids with Brown Grease

Compared with relatively pure lipid feedstocks (e.g. vegetable oil), which are currently the major feedstocks for non-ethanol liquid biofuel production, lower grade

lipids such as waste cooking oil and trap grease have potential for improving the economics of production-oriented approaches.[140] The brown grease usually contains more than 15% FFAs, which can cause problems for currently commercialized base catalyzed biodiesel manufacturing. On the other hand, lipid pyrolysis has been studied recently, and the performance of converting low-grade lipid feedstock into biofuels was promising (as reviewed in 2.5). Thus, this study tried to use both low-grade lipid feedstock and water source in the hydrolysis step.

4.2.1 Brown Grease Characterization

The results of the preliminary experiments concerning the thermal hydrolysis of biosolids indicated that the small amount of FFA and lipids (including TG, DG, MG) in biosolids from the Clover Bar lagoon could serve as feedstock for lipid pyrolysis if biosolids were used as a water source. Waste lipids and oils such as yellow grease, brown grease, and beef tallow have been successfully applied in the hydrolysis process to produce protonated FFA for biofuel production as demonstrated by Asomaning *et al.* (2014).[85] To investigate the possibility of using the water in biosolids instead of the distilled water that is usually sourced, experiments on hydrolyzing lipid feedstocks with biosolids as a substitute for water were performed.

The waste lipid feedstock used in this experiment was brown grease and the moisture, ash, and hexane insoluble components were analyzed to profile the received brown grease. The composition of brown grease is shown in **Table 4-4.** The total lipids content was calculated according to HPLC-ELSD results. The brown grease contains

about 87% total lipids (FFA = 42% ± 1%, TG = 26.2% ± 0.1%, DG = 16.5%± 0.3%, and MG = 3.2% ± 0.1%). The moisture content was around 0.4 ± 0.1%, and the ash content for this brown grease was about 0.06 ± 0.01%. Therefore, besides moisture, ash, and lipids, 12% of the material was not detected through HPLC-ELSD. This corresponded to the results of Kim *et al.* (2011) for the characterization of brown grease; there observed about 10-20% of GC undetectable compounds.[141]

Table 4-4 Brown grease characterization and composition

Composition	Wt. % of sample loaded
Moisture	0.4 ± 0.1
Ash	0.06 ± 0.01
Hexane insoluble components	0.9 ± 0.1
Lipids (TG+DG+MG+FFA)	87 ± 1
TG	26.2 ± 0.1
DG	16.5 ± 0.3
MG	3.2 ± 0.1
FFA	42 ± 1

Lipids fractions were identified and quantified by HPLC-ELSD

4.2.2 The Effect of pH on Hydrolyzing Biosolids with Brown grease

The pH of received biosolids was about 9, making it a slightly alkaline mixture. The mechanisms for hydrolysis of lipids in acidic and alkaline environments are different. The acid-catalyzed hydrolysis was an equilibrium, and the equilibrium needs to be disturbed by the use of excess reagent (water), where in the base catalyzed hydrolysis, the basic leaving group ionized the product and disturbed the equilibrium. Thus the base catalyzed hydrolysis was easier to go to a completion.[142] On the other hand, the acid condition could also cause corrosion issues to the reactor, and the

choice of acid would be restricted if acidification was necessary before hydrolysis. Thus, it is important to study whether the pH is a significant factor that would influence the hydrolysis. Different pH conditions of hydrolysis were applied: pH=3.3 (biosolids acidified with phosphoric acid to 3.3, which is lower than the pKa of fatty acids), pH = 6.2 (biosolids were acidified with phosphoric acids to 6.2, which is same as distilled water), or pH = 8.9 (original biosolids with no acidification). The FFA conversion and phase separation were studied for all three conditions.

After hydrolysis, the pH of the aqueous phase for all hydrolyzed samples except the acidified biosolids significantly decreased as shown in **Table 4-5**, likely through the generation of FFAs from lipids, which were deprotonated in the aqueous phase. This is an important observation as recovery of FFAs from the hydrolysates requires that they are in a protonated form prior to extraction. Since the pH in all systems is acidic following hydrolysis, little (if any) pH adjustment would be required before extraction of FFA from the hydrolysates. This could result in cost-savings through reduced acid use during lipid pyrolysis, and/or reduced reactor corrosion.

Table 4-5 Change of pH of aqueous phase after hydrolysis

Sample	pH of hydrolyzed aqueous phase			
	Distilled water	Acidified biosolids	biosolids acidified to pH 6.2	Original biosolids

Unhydrolyzed	6.4 ± 0.3	3.3 ± 0.1	6.2 ±0.1	8.9 ± 0.1
Hydrolyzed	4.0 ± 0.2	3.1 ± 0.1	4.9 ±0.3	5.3 ± 0.2

Data in each column showed the changes of pH between before and after hydrolysis of different biosolids. All of the experiments were triplicated

As for the phase separation performance, after hydrolysis, all samples where biosolids were used for hydrolysis of brown grease had a solids phase (bottom layer) that was not observed in the sample where distilled water was employed for hydrolysis (**Figure 4-10).** Biosolids contain about 4% solids, and thus the extra solid phase in the biosolids/brown grease hydrolysates was expected. The quick settling and clear layering dismissed the concern of difficulties in settling caused by emulsions.

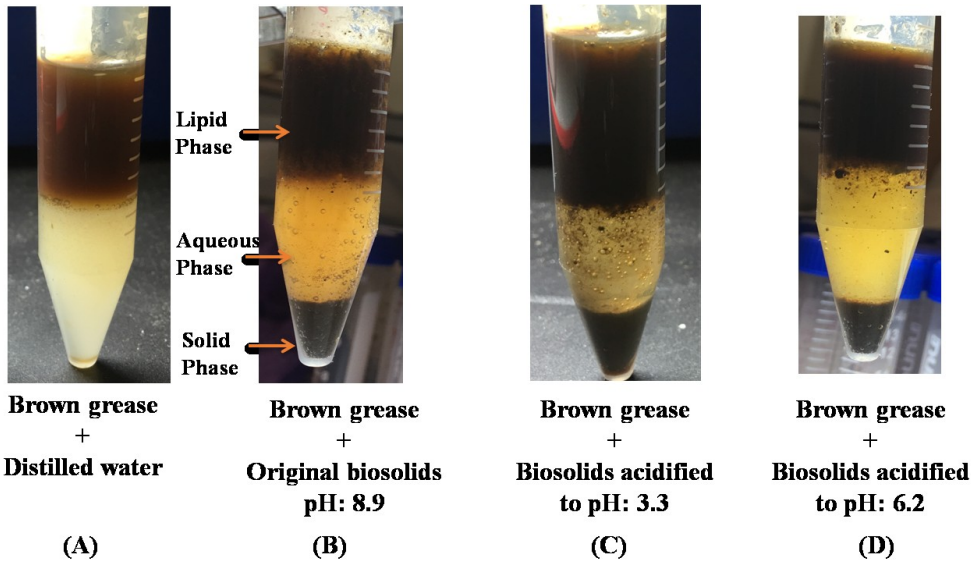


Figure 4-10 Hydrolysates from hydrolysis of brown grease with distilled water (A) or biosolids (B-D). For the samples incorporating biosolids, the sample was adjusted to pH 3.3 (B) or 6.2 (C) prior to hydrolysis, or not adjusted at all. All hydrolysis was performed at 280°C for 1 hour at liquid to lipid ratio of 1:1 and triplicated.

The performance of the hydrolysis reactions was assessed through determination of FFA% in the recovered lipid phase and FFA conversion as shown in **Figure 4-11** and **Table 4-6.** No significant difference was observed between any of the samples although the starting pH was different in all systems. At the temperature and pressure used for hydrolysis, the reaction of lipids with subcritical water and the catalytic property of the acid or base did not appear to have a significant effect on the hydrolysis. All hydrolysis runs converted all the TG and most of the DG into FFA; only the MG had no significant changes before and after hydrolysis. The difficulties associated with the conversion of MG to FFA could be explained by the kinetic model derived from the consecutive reaction mechanism proposed by Diasakou *et al.* (1998), where the conversion of MG to glycerol was much slower than TG and DG and results in the hard removal of MG from the product.[143] The **Equation 2-3** showed in 2.3.1 was reversible and this reverse reaction has a larger K-value, and without the intermediates (MG), there was not enough water would have been emulsified into the oil mixture to push reactions of **Equation 2-3** forward.[144] A similar conclusion could be drawn from the FFA conversions shown in **Table 4-6;** the FFA conversions of all runs were not significantly different from each other, ranging from 90.6 ± 0.4 to 91.5 ± 0.4.

Table 4-6 FFA conversions of hydrolyzing brown grease with distilled water or biosolids at different pH

	Brown grease hydrolyzed with distilled water	Brown grease hydrolyzed with acidified biosolids	Brown grease hydrolyzed with biosolids that acidified to pH 6.2	Brown grease hydrolyzed with original biosolids
FFA conversion	91.5 ± 0.4% [a]	90.6 ± 0.4% [a]	91.0 ± 0.2% [a]	91.3 ± 0.4% [a]

All of the experiments were triplicated. Means that do not share the same letter are statistically different. The statistical analysis was done using one-way ANOVA with mean comparison by Tukey multiple comparisons test based on a 95% confidence level.

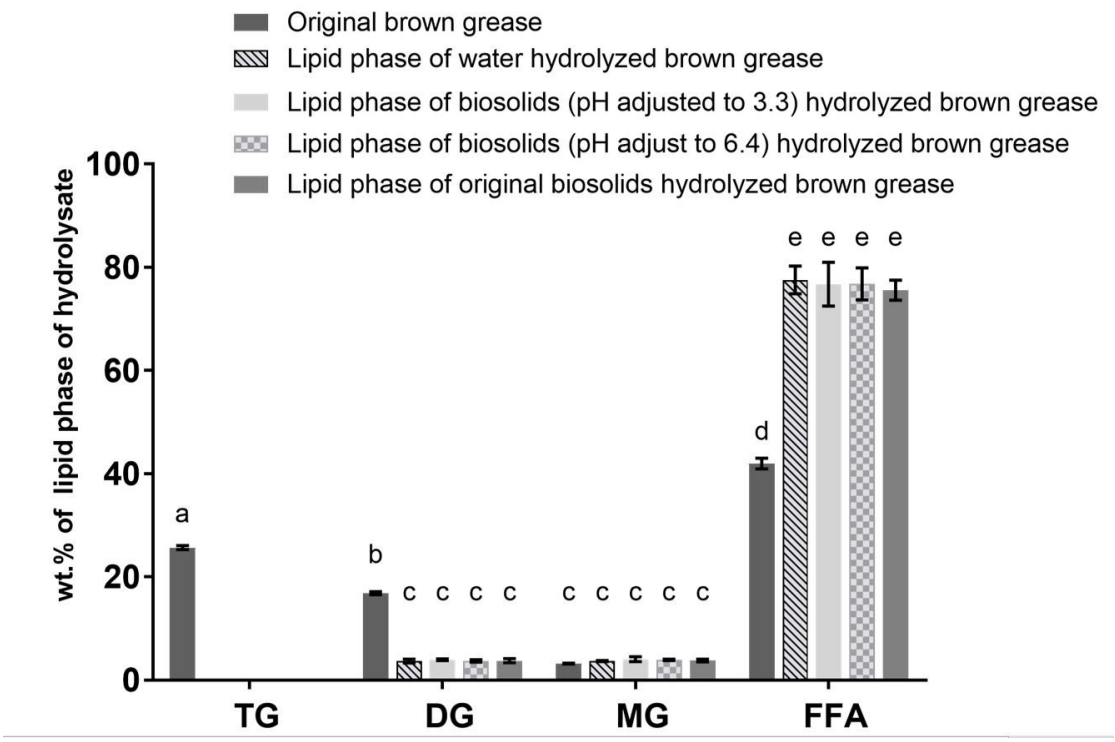


Figure 4-11 Lipid composition of the lipid phase of hydrolyzed brown grease. The Effect of Reaction Time and Temperature. Hydrolysis of the various systems was conducted at 280°C for 1 hour with a distilled water (or biosolids) to lipid ratio of 1:1 and triplicated. With each lipid type, means that do not share the same letter are statistically different. The statistical analysis was done using one-way ANOVA with mean comparison by Tukey multiple comparisons test based on a 95% confidence level.

4.2.3 The Effect of Reaction Time and Temperature on Hydrolyzing Biosolids with Brown Grease

As discussed in 4.2.2, during hydrolysis at 280°C, with a liquid to lipid ratio of 1:1 and a 1-hour reaction time, the biosolids performs in a similar manner to distilled water with regards to hydrolyzing brown grease, even when the pH of the system was different. Further exploration was done by changing the hydrolysis temperature and

reaction time to investigate how hydrolysis performed with shorter or longer reaction time and how biosolids performed differently with distilled water at 260°C compared with 280°C. Hydrolysis reaction time was examined as hydrolyzing lipids with distilled water for 0.75 hours, 1 hour, and 2 hours, as demonstrated in **Figure 4-12**, showed the extension of reaction time did not affect the FFA conversion and FFA%, but shorter reaction time did affect the hydrolysis performance in the recovered lipid phase.

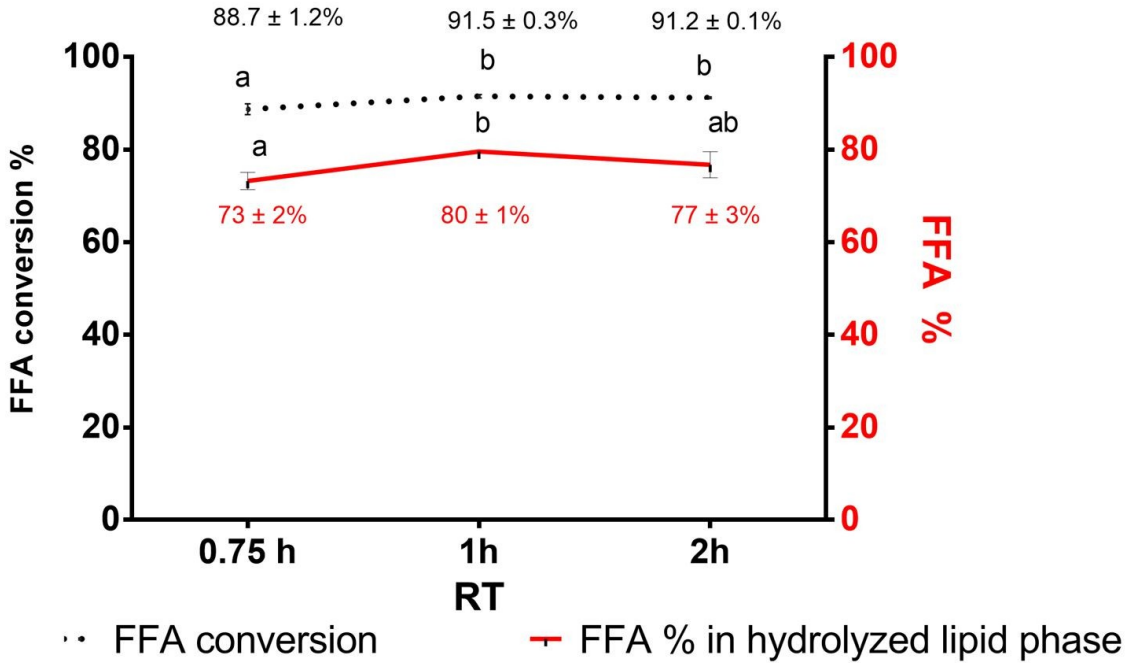


Figure 4-12 Performance of brown grease hydrolyzed with distilled water under 280°C for 0.75, 1, and 2 hours (liquid to lipid ratio 1:1) by using time as a variable. All of the experiment were conducted triplicated. Means that do not share the same letter are statistically different. The statistical analysis was done using one-way ANOVA with mean comparison by Tukey multiple comparisons test based on a 95% confidence level.

Table 4-7 FFA conversion and FFA % of the lipid phase of distilled water or biosolids hydrolyzed brown grease at 280°C and 260°C for 1 hour at liquid to lipid ratio 1:1 and triplicated,

Conditions(hydrolyzed with brown grease)	260°C distilled water	260°C biosolids	280°C distilled water	280°C biosolids
FFA % in the recovered lipid phase	78 ± 0^{a}	77 ± 1^{a}	78 ± 3^{ab}	80 ± 0^{bc}
FFA conversion (%)	91.2 ± 0.1^{a}	90.6 ± 0.2^{b}	91.5 ± 0.4^{a}	91.3 ± 0.4^{a}

All samples analyzed were the lipid phase of hydrolysate. Means that do not share the same letter are statistically different. The statistical analysis was done using one-way ANOVA with mean comparison by Tukey comparisons test based on a 95% confidence level.

The metals in biosolids could potential perform as catalysts and lower the required reaction energy (i.e. temperature). Thus, an examination of thermal reactions at a lower temperature 260°C was further studied and discussed. When changing the temperature with a hydrolysis ratio at 1:1 (w/w) as shown in **Table 4-7**, the FFA conversion for 260°C biosolids hydrolyzed brown grease was significantly lower (90.6 ± 0.2) than the other 3 conditions, which were not significantly different from each other, ranging from 91.2 ± 0.1 % to 91.5 ± 0.4%. Looking strictly at FFA%, at a given temperature, distilled water performed similarly with biosolids. Increasing the temperature to 280°C did not significantly impact the FFA % in the recovered lipid phase of hydrolyzed brown grease by distilled water, but there was a significant increase in FFA% when the temperature was increased from 260°C to 280°C using biosolids for hydrolysis (78% ± 1 % at 260°C vs. 80% ± 0% at 280°C). Thus, the metals in biosolids did not perform as catalysts, thereby reducing the reaction temperature. In fact, both

FFA conversion and FFA% in the recovered lipid phase indicated that biosolids performed worse than distilled water at lower temperature.

The comparison of FFA types before and after hydrolysis of brown grease is shown in **Figure 4-13**. Each of the percentages were based on the wt. % of sample weighed for analysis. Except for C18:2, there was no significant difference between the lipid phase of distilled water or biosolids hydrolyzed brown grease for all the FFA types, where oleic acid and linoleic acid were the two most abundant FFAs (~40% and ~17%, respectively). Palmitic acid and stearic acid were the most abundant saturated FFAs, accounting for ~12% and ~5%, respectively, in all samples.

The unhydrolyzed lipids in the lipid phase of hydrolyzed brown grease (LP-Water/BG D or LP-Biosolids/BG D in **Figure 4-13**) had a similar distribution of the main FFA types, but all of those FFA were about half of the hydrolyzed sample, which corresponded to the lipid fraction analysis where total FFA was half of the total lipids. More C16:1 and C18:3 was found in hydrolyzed samples than in the unhydrolyzed samples, which means that there were C16:1 and C18:3 fatty acids chains in the unhydrolyzed triglycerides in the original brown grease, and after hydrolysis those unsaturated fatty acids had been released from the glycerol backbone.

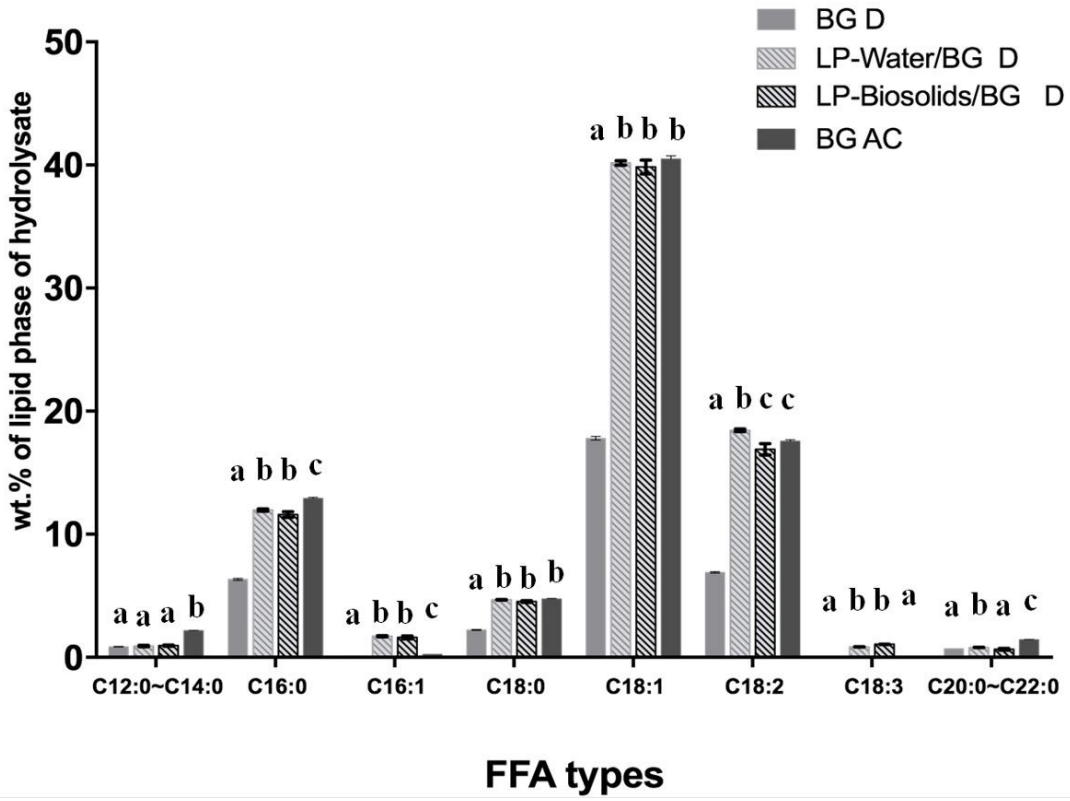


Figure 4-13 FFA distribution of the lipid phase of hydrolyzed brown grease at 280°C for 1 hour at liquid to lipid ratio 1:1. All the sample analyzed were the lipid phase of hydrolysate and all of the experiments were triplicated. BG D: Original brown grease derivatized by diazomethane; BG AC: Original brown grease derivatized by acetyl chloride; LP-Water/BG D: Lipid phase of distilled water hydrolyzed brown grease derivatized by diazomethane; LP-Biosolids/BG D: Lipid phase of biosolids hydrolyzed brown grease derivatized by diazomethane. Within FFA type, means that do not share the same letter are statistically different. The statistical analysis was done using one-way ANOVA with mean comparison by Tukey multiple comparisons test based on a 95% confidence level.

4.2.4 Conclusions

Even though the pH and general contents of biosolids were different from that of distilled water, the biosolids performed similarly to distilled water regarding the FFA conversion and FFA types generated. Furthermore, there were almost no emulsions observed during the phase separation step when biosolids were used for hydrolysis. There was an extra solids phase observed in the hydrolysates from biosolids hydrolyzed brown grease due to the solids content in biosolids, compared to samples obtained from the use of distilled water. The solid phase of biosolids hydrolyzed brown grease was easy to be separated from aqueous phase for disposal or other application, and the aqueous phase in the waste stream that contains glycerol could also be recovered and reused. Biosolids has significant amount of sulfur, nitrogen content if compared with distilled water. And those compounds could possibly be intruded to product. The next experiment was mainly focus on improving the quality of biosolids hydrolyzed brown grease product by increasing temperature.

4.3 The Effect of Temperature on The Quality of Product Producing after Hydrolyzing Brown Grease with Biosolids

The experiments described in section 4.2 showed the similar hydrolysis performance between distilled water and original biosolids when hydrolyzing brown grease. The experimental conditions, especially the liquid to lipid ratio (1:1), was based on an optimized condition of hydrolyzing yellow grease illustrated by Asomaning *et al.* (2014).[145] According to the results presented above, the liquid to lipid ratio 1:1 did not result in complete fatty acid conversion (only ~91% conversion was achieved when the reaction condition was 280°C, with a liquid to lipid ratio of 1:1 and 1 hour hydrolysis time). Thus, a higher liquid to lipid ratio was applied in the following experiments to ensure the highest FFA conversion. More participation of biosolids might probably introduce more contaminates to the FFA product, but could give us a better observation of how the contaminates in biosolids will affect the product quality.

The experiments described below investigate the effect on hydrolysis product quality when increasing the temperature of hydrolysis of biosolids with brown grease, especially the composition of the product and the influence on sulfur and nitrogen content in hydrolysis product.

4.3.1 FFA Conversion and Unhydrolyzed Lipid in The Recovered Lipid Phase

The hydrolysis of triglycerides is a reversible reaction, and thus, according to Le Chatelier's Principle, we can shift the reaction towards product formation by

increasing the amount of reactant.[146] Based on this, a higher liquid to lipid ratio would increase the conversion of glyceride to glycerol and FFA compared with the same hydrolysis performed at lower liquid to lipid ratios.

Based on the HPLC-ELSD results, there was no TG left in any of the hydrolyzed samples when the liquid-to-lipid ratio was 5:1 (m/m). **Figure 4-14** shows a chromatogram generated using HPLC-ELSD on the lipid fraction. Since the disappearance of the TG peak was similar for all other conditions, only one chromatogram is presented. The brown grease hydrolyzed with distilled water (liquid to lipid ratio 5:1 m/m) at 280°C could achieve about 98.0% ± 0.3% conversion (**Figure 4-15**), which was significantly higher than the hydrolysis under the same conditions, but using a liquid to lipid ratio of 1:1 (91.2 ± 0.1%). This result was comparable with Alenezi's research of hydrolysis of different types of vegetable oil at 280°C, which achieved more than 97% conversion.[109]

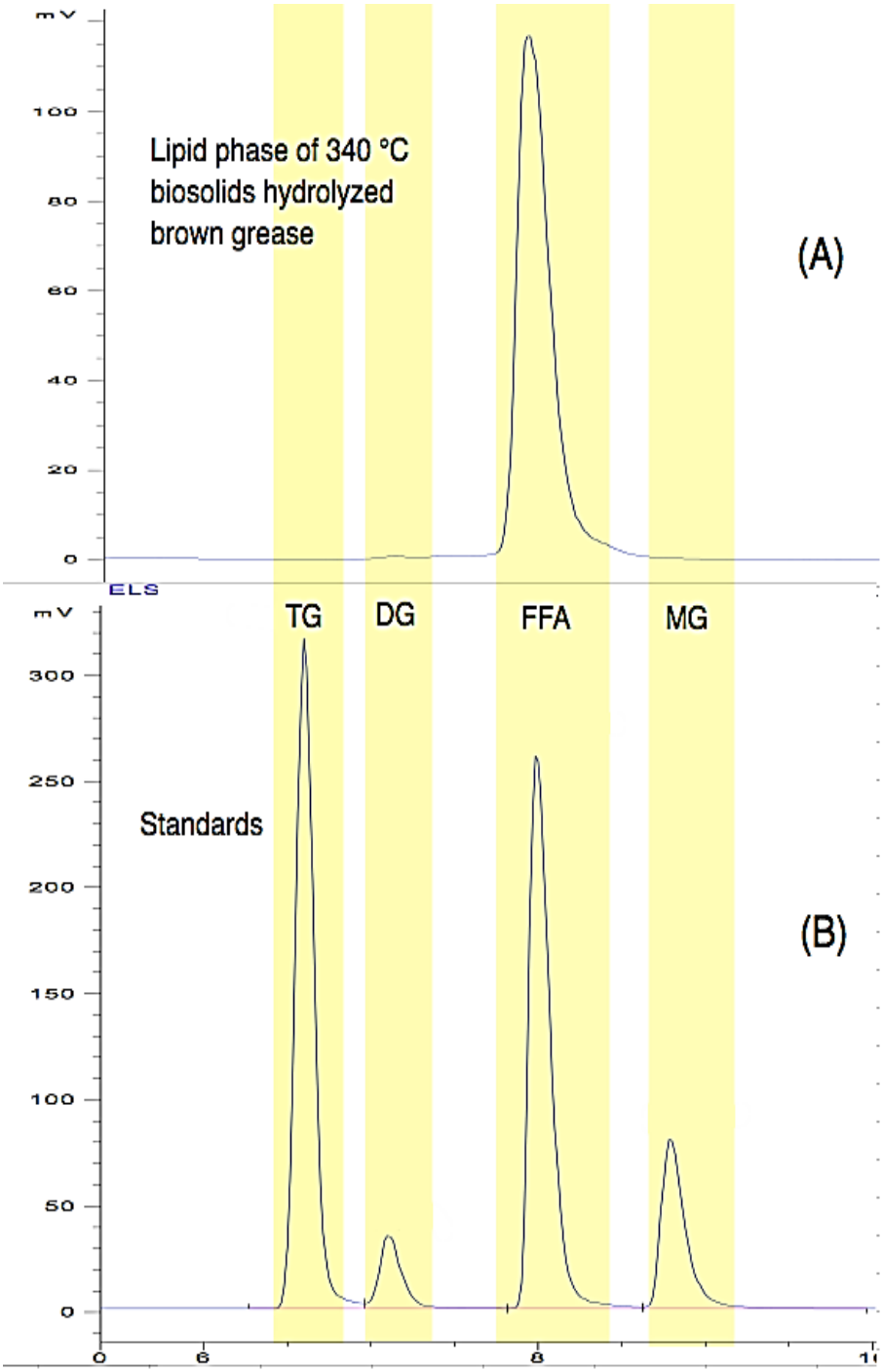


Figure 4-14 Chromatograms from HPLC-ELSD analyses of the lipid phase from brown grease hydrolyzed with biosolids at 340°C (A) and lipid standards (B).

An increase in temperature above 280°C did not significantly improve the conversion in hydrolysis using biosolids or distilled water when the liquid to lipid ratio was 5:1 (**Figure 4-15**). Only at 310°C was the FFA conversion of hydrolysis with biosolids significantly lower than the hydrolysis with distilled water (95.7% ± 0.8 versus 98.5% ± 0.7%), but the reason behind this was not clear. The FFA conversion could also be confirmed through quantification of the residual unhydrolyzed lipids using HPLC-ELSD. The unhydrolyzed lipids in the distilled water or biosolids hydrolyzed samples were only about 3% and no significant difference was observed at different temperature (**Table 4-8**).

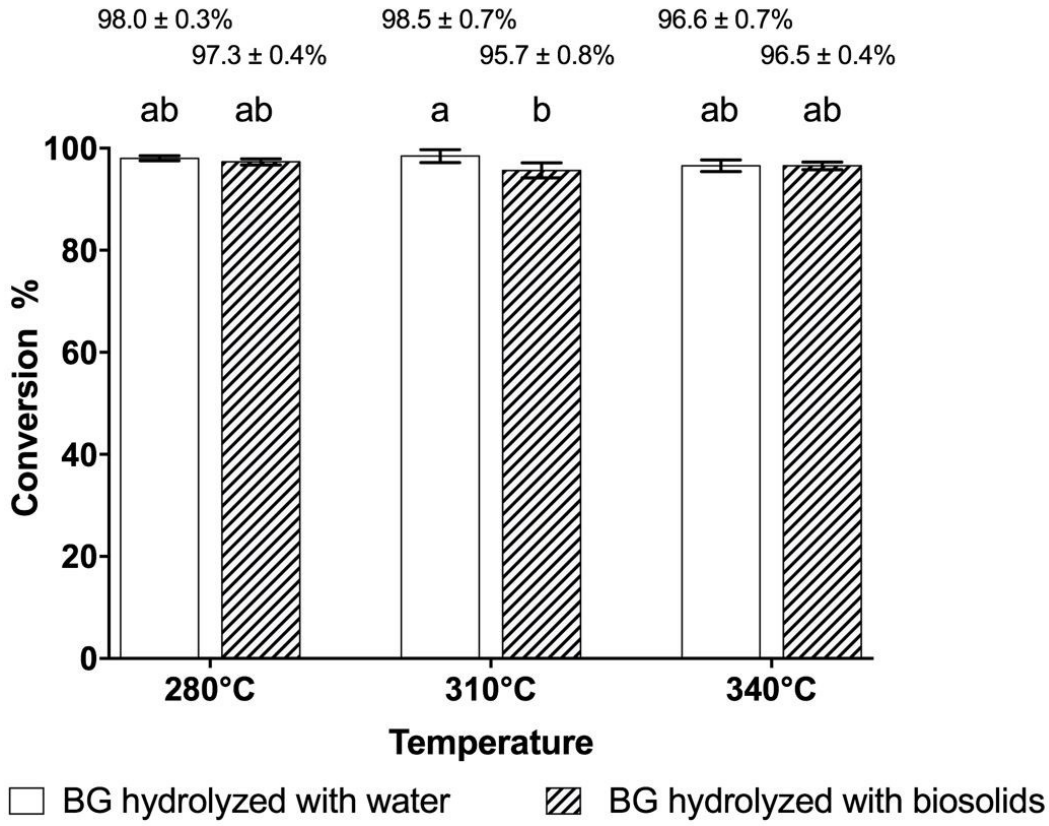


Figure 4-15 Comparison of FFA conversion of brown grease between distilled water and biosolids at different temperatures. Brown grease (BG) was hydrolyzed with distilled water or biosolids at a liquid to lipid ratio of 5:1 for 1-hour at the temperatures indicated and triplicated. Means that do not share the same letter are statistically different. The statistical analysis was done using two-way ANOVA with mean comparison by Sidak multiple comparisons test based on a 95% confidence level.

Table 4-8 Unhydrolyzed lipids in the lipid phase of hydrolyzed brown grease (liquid to lipid ratio of 5:1) at different temperatures as detected by HPLC-ELSD.

Sample Type	Temperature		
	280°C	310°C	340°C
Distilled water hydrolyzed brown grease(w.t.% of sample loaded)	3.4 ± 0.4% [a]	3.3 ± 0.4% [a]	3.3 ± 0.8% [a]
Biosolids hydrolyzed brown grease(w.t.% of sample loaded)	3.2 ± 0.3% [a]	3.6 ± 0.1% [a]	2.7 ± 0.2% [a]

All of the experiments were triplicated. Means that do not share the same letter are statistically different. The statistical analysis was done using two-way ANOVA with mean comparison by Sidak multiple comparisons test based on a 95% confidence level.

4.3.2 FFA % in Recovered Lipid Phase

In the modified lipid pyrolysis approach, hydrolysis should ideally achieve maximal conversion of lipids to FFA. Thus, the FFA % in the lipid phase of hydrolyzed brown grease was an essential index to measure the performance or degree of hydrolysis. Ideally, all the glycerides should be converted to free fatty acid. At a given condition, the FFA % in the lipid phase isolated from hydrolysates derived from biosolids and brown grease was similar to that obtained when hydrolysis of brown grease was performed with distilled water. In both systems, the FFA% dropped significantly as the temperature increased from 280°C to 340°C as shown in **Figure 4-16**.

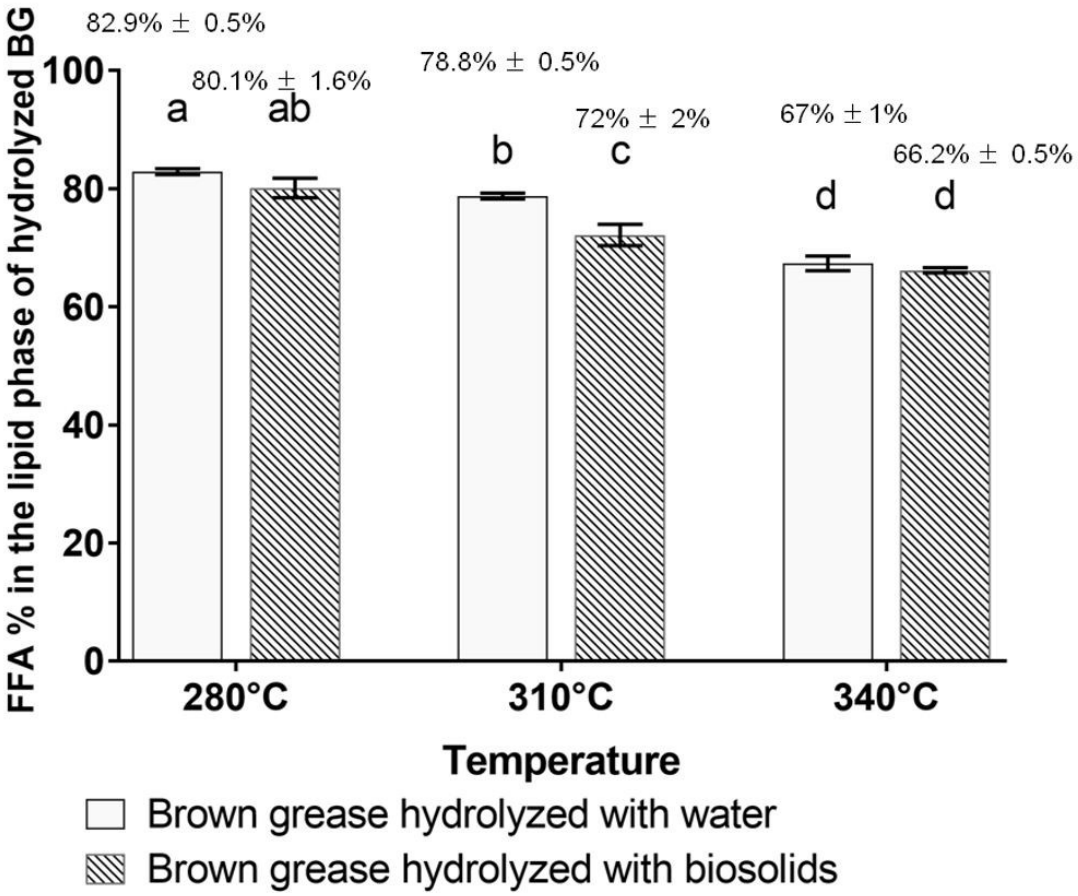


Figure 4-16 FFA % in the lipid phase extracted from hydrolysates derived from hydrolysis of brown grease with biosolids or distilled water at different temperatures. Brown grease (BG) was hydrolyzed with distilled water or biosolids at a liquid to lipid ratio of 5:1 for 1-hour at the temperatures indicated and triplicated. FFA % was determined by GC analysis. Means that do not share the same letter are statistically different. The statistical analysis was done using two-way ANOVA with mean comparison by Sidak multiple comparisons test based on a 95% confidence level.

Also, the FFA% was not significantly different between the systems employing distilled water and biosolids at 280°C and 340°C, but at 310°C, the biosolids (FFA%: 72% ± 2%) performed worse than distilled water (FFA%: 78.8% ± 0.5%). The undetectable

compounds observed during GC analysis showed an increase in both the lipid phases from distilled water or biosolids hydrolyzed brown grease with increasing temperature, especially the lipid phase of biosolids hydrolyzed brown grease. The GC undetectable compounds were possibly fatty acids dimer (as discussed in section 3.3.3.2). The significant difference between FFA% in the lipid phase from brown grease hydrolyzed with distilled water and biosolids could be explained by the complexity of biosolids.

Brown grease and the lipid phase of distilled water or biosolids hydrolyzed brown grease contain compounds that our GC methods could not detect. As illustrated in **Figure 4-17,** if there were only TG, DG, MG, and FFA in the sample, the GC detectable compounds of sample derivatized by acetyl chloride should be closed to 100%, but this was not the case. Confirmed the mass loss of the same sample that derivatized by diazomethane were not issued form the unhydrolyzed TG, DG, and MG. The trend of FFA% reduction was a little different between the 2 systems of hydrolysis: The lipid phase obtained after hydrolysis of brown grease with biosolids displayed a reduction of ~7% FFA for every 30°C increase in temperature (from 80.1% ± 1.6% to 72% ± 2% to 66.2% ± 0.5%). However, the FFA % in the recovered lipid phase of distilled water hydrolyzed brown grease was significantly reduced from 280°C to 310°C (from 82.9% ± 0.5% to 78.8% ± 0.5%), then sharply decreased by ~11% to 67% ± 1% at 340°C.

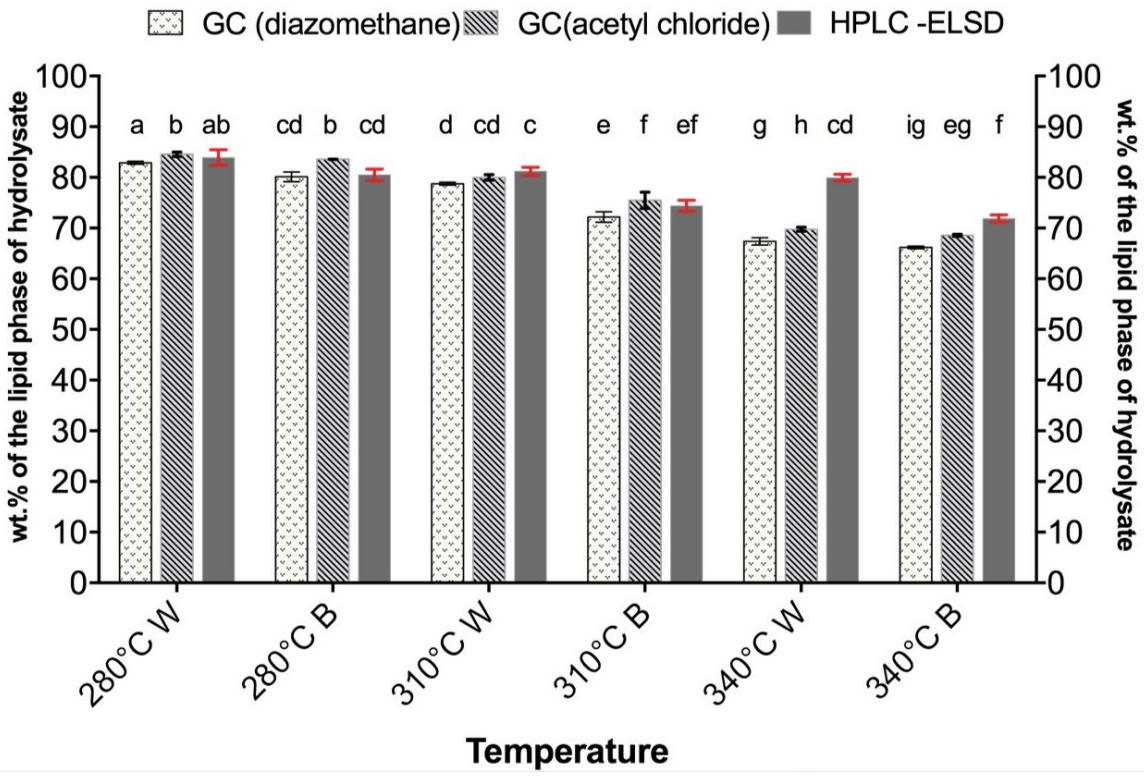


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Figure 4-17 Comparison of the FFA% in the lipid phase of distilled water (W) or biosolids (B) hydrolyzed brown grease at 3 different temperatures analyzed by HPLC-ELSD or derivatized by diazomethane or acetyl chloride and analyzed by GC. Means that do not share the same letter are statistically different. All the experiments were triplicated. The statistical analysis was done using two-way ANOVA with mean comparison by Sidak multiple comparisons test based on a 95% confidence level

The lipid fractions were also analyzed by HPLC-ELSD. **Figure 4-17** illustrates that the GC results (amount of fatty acids, based on % of sample loaded) using 2 derivatization methods, as well as HPLC-ELSD results. The GC results of samples

derivatized using diazomethane had a high agreement with those analyzed by HPLC, where there was no significant difference in FFA% between the two methods (95% confidence interval). However, at 340°C, the HPLC-ELSD results presented a significantly higher FFA% in both distilled water (HPLC-ELSD: 79.9% ± 0.7% versus GC: 67.4% ± 0.7%) and biosolids (HPLC-ELSD: 71.9% ± 0.7% versus GC: 66.2% ± 0.3%) hydrolyzed brown grease. At a higher temperature, the hydrolyzed brown grease lipid phase might contain some types of compounds that can only be analyzed by HPLC-ELSD. An HPLC-MS allowed for better identification of the HPLC-ELSD undetectable compounds and will be explained in more detail in section 3.2.3.2.

The distribution of different types of FFA in the lipid phase of hydrolyzed brown grease is shown in **Figure 4-18**. Alteration of the hydrolysis temperature slightly changed the saturation of the FFA in the lipid phase of hydrolyzed brown grease. The amount of C18:2 decreased dramatically for the lipid phase of both distilled water and biosolids-hydrolyzed brown grease when increasing the temperature from 280°C to 340°C.

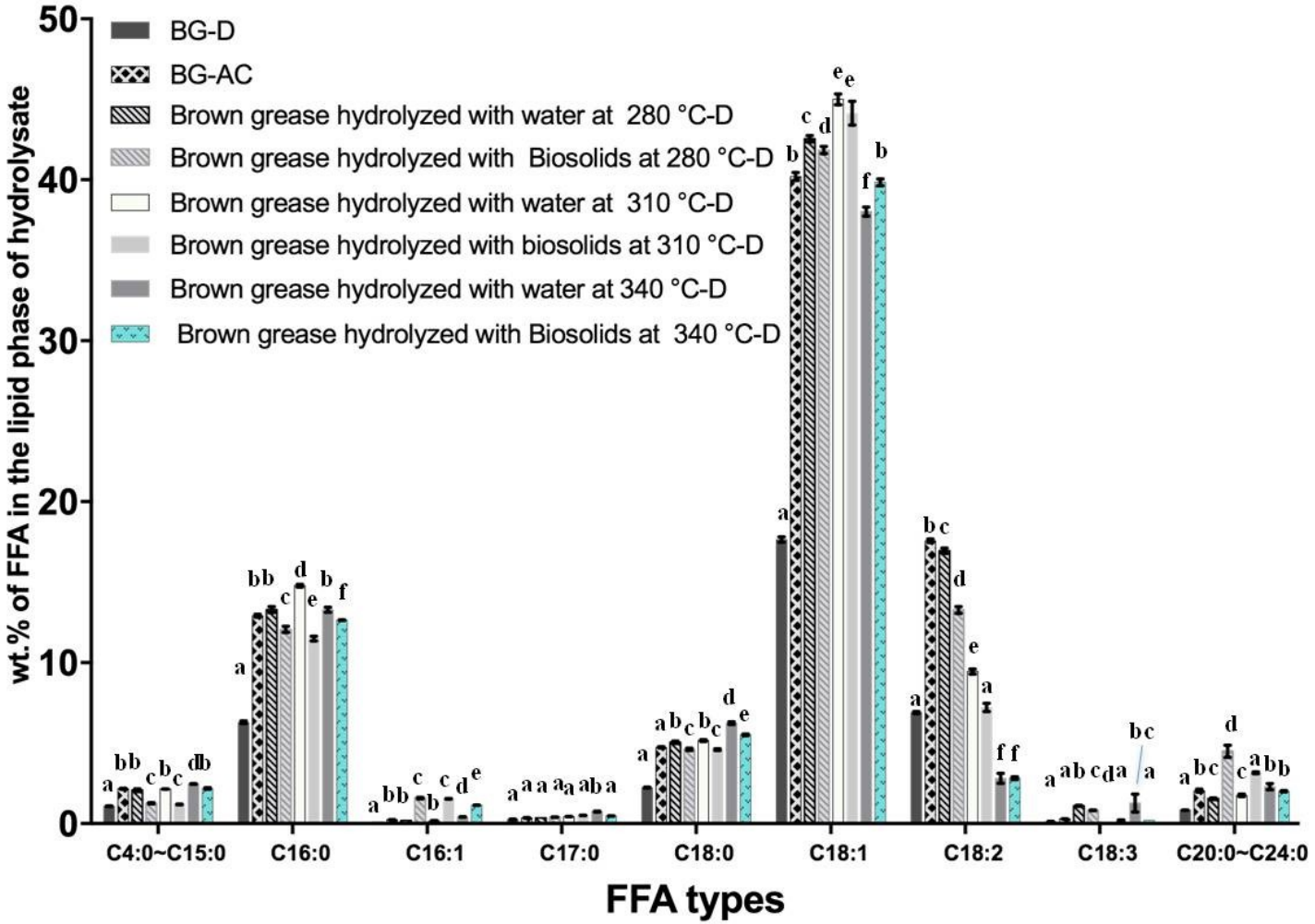

BG-D
BG-AC
Brown grease hydrolyzed with water at 280 °C-D
Brown grease hydrolyzed with Biosolids at 280 °C-D
Brown grease hydrolyzed with water at 310 °C-D
Brown grease hydrolyzed with biosolids at 310 °C-D
Brown grease hydrolyzed with water at 340 °C-D
Brown grease hydrolyzed with Biosolids at 340 °C-D
wt.% of FFA in the lipid phase of hydrolysate
0
10
20
30
40
50
C4:0~C15:0
C16:0
C16:1
C17:0
C18:0
C18:1
C18:2
C18:3
C20:0~C24:0
FFA types

Figure 4-18 The distribution of different types of FFA in the lipid phase from distilled water or biosolids hydrolyzed brown grease at 3 different temperatures for 1-hour reaction time and triplicated. BG-D: Original brown grease derivatized by diazomethane; BG-AC: Original brown grease derivatized by acetyl chloride. The remaining samples with –D were derivatized using diazomethane. Within FFA type, means that do not share the same letter are statistically different. The statistical analysis was done using one-way ANOVA with mean comparison by Tukey multiple comparisons test based on a 95% confidence level.

The C18:1 in the lipid phase of distilled water as well as biosolids hydrolyzed brown grease were significantly increased when the temperature was raised from 280°C to 310°C. At 340°C, the C18:1 in the lipid phase of both biosolids and distilled water hydrolyzed brown grease was significantly reduced by about 5% compared with the number at 310°C. The C18:0 of distilled water hydrolyzed brown grease at each temperature were significantly higher than biosolids hydrolyzed brown grease, and at 340°C, the C18:0 FFA significantly increased about 1 % in both the distilled water or biosolids hydrolyzed brown grease compared to 310°C. This trend was not observed for C16 FFA. For the lipid phase of distilled water hydrolyzed brown grease, the C16:0 increased significantly when at 310°C and then dropped back at 340°C to the same level compared to 280°C hydrolysis. For the biosolids hydrolyzed brown grease, there were more C16:1 for all 3 temperatures when compared to the lipid phase of distilled water hydrolyzed brown grease and the original brown grease, which shows the effect of biosolids on FFA distribution. Moreover, a slight reduction of C16:1 could be observed when increasing the temperature from 310°C to 340°C, while the C16:0 had a reversed trend. The loss of unsaturated FFA and increasing of FFA saturation at the higher temperature may be explained by the occurrence (or increased occurrence) of other types of reactions other than hydrolysis such as hydrogenation, which could possibly account the formation of GC undetectable compounds. The slight increase of C17:0 and short chain FFA at a higher temperature also showed the cracking of FFA at a higher temperature.

For the polyunsaturated FFA C18:3, there was a small amount in the original brown grease, and hydrolysis at 280°C promoted the production of C18:3. For the lipid phase of biosolids hydrolyzed brown grease, the amount of C18:3 was reduced with increasing temperature from 280°C to 310°C: 0.83% ± 0.02% at 280°C, 0.20 % ±0.03% at 310°C, but when increased from 310°C to 340°C, a significant change was not observed relative to the value at 310°C. For the distilled water hydrolyzed samples, the C18:3 was decreased from 1.11% ± 0.03% to 0.087% ± 0.003% from 280°C to 310°C, but was increased to 1.8% ± 0.3% at 340°C. The polyunsaturated FFA in the hydrolysis reaction would have coupling reactions and form some compounds that could not elute from GC and HPLC-ELSD as illustrated by Fu *et al.* (2011).[74] This may explain the decrease of the C18:3 in the lipid phase of distilled water or biosolids hydrolyzed samples from 280°C to 310°C. But the increase of C18:3 in the lipid phase of distilled water hydrolyzed brown grease when increasing the temperature to 340°C was not clear.

4.3.3 The GC and HPLC-ELSD Undetectable Compounds

GC and HPLC-ELSD undetectable compounds in untreated brown grease

The original brown grease contains a significant amount of material that could not be identified through GC and HPLC-ELSD, approximately 10-20%, which was in line with what was observed by Kim *et al.* (2011). Kim *et al.* investigated the esterification of brown grease to fatty acid methyl ester (FAME) and showed that brown grease would separate into 2 layers when stored at 80°C for 3 days. They found that the FAME% of the esterification of the bottom phase was less than 40%, whereas the

FAME% of the esterification of the top phase was 81%. They concluded that there were some compounds that could not be converted to FAME and cannot be analyzed by GC. This group also suggested that this material represented sulfur and sulfur-containing components.[141] The phase separation of brown grease was also observed in the sample used in this experiment (**Figure 4-19**). A similar conclusion was drawn by Sari (2013) that about 25% of the brown grease was undetectable through GC analysis.[92]

Figure 4-19 Separated layers of brown grease

Undetectable Compounds in the Lipid Phases

The compounds that could not be detected through HPLC-ELSD, especially in biosolids hydrolyzed samples, could also be explained by the mechanism of oxidative crosslinking of unsaturated FFA. The unsaturated FFA could be oxidized, and polymerization could occur to form macromolecules through oxidative crosslinking and network formation as illustrated by Mulzebelt *et al.* (1996).[147] The oxidized compounds that contain an epoxy group (m/z = 299.27) detected by the LC-MS had a high probability of being polymerized and form large molecular compounds. The formation of large polymers from fatty acids with epoxy functional groups was confirmed by Samuelsson *et al.* (2004).[148] Also, the guard column connected to the gel permeation chromatography column would filter out any large size compounds.

HPLC-ELSD Detectable but GC Undetectable Compounds

Even though the lipid phase of both biosolids and distilled water hydrolyzed brown grease had a similar decreasing trend of FFA % with temperature as analyzed by GC, the reason for GC undetectable compounds was a little bit different from that of HPLC-ELSD. At 340°C, the HPLC-ELSD analysis of FFA in the lipid phase of distilled water hydrolyzed brown grease was significantly higher than the lipid phase of biosolids hydrolyzed brown grease, while the FFA calculated by GC-FID of both of them were similar (**Figure 4-17**). LC-MS was used to analyze the lipids in the lipid phase of 340°C distilled water hydrolyzed brown grease (**Figure 4-20**). Using LC-MS, 3 peaks were shown on the chromatogram in contrast to the 1 FFA peak in HPLC-ELSD. The first

peak in **Figure 4-21** likely represented compounds with a dimer structure, according to MS/MS analysis of the fragment ion of the heavy compounds (**Figure 4-22**), and the third peak was likely fatty acid oxygenized compounds. Identification of the exact structures of those compounds were not performed in this study, so the reason of formation of the oxygenized compounds was not clear. The dimer and oxidized FFA could be detected by HPLC-ELSD, but the signal was too small accurately measure. Also, the dimer structure compounds could not be detected by the described GC method since there no peak was identified as dimers by GC-MS; a higher running temperature and another type of GC column and derivatization method was needed for dimer identification.[149] One possible reason for the discrepancy between HPLC-ELSD and GC results at 340°C could be that there were compounds that could not be detected by GC but could be detected by HPLC-ELSD at the same retention time with FFA, which would affect the overall calculations. The MS spectral characteristics confirmed this assumption where the information of the FFA peak of 340°C the lipid phase of biosolids or distilled water hydrolyzed brown grease at the same retention time was different as shown in **Figure 4-20**. Only the distilled water hydrolyzed sample contains other compounds with the same retention time as FFA, which showed an m/z at 335. It is possible that the overlapping compound at m/z 335 might be the adduct FFA peroxide $[M+Na]^+$, but this was not confirmed. Kuwajima *et al.* (1972) reported that α-metallated carboxylic acids might be oxidized with oxygen alone to the a-hydroxy-acids, and with oxygen in the presence of copper(II) salts to the corresponding dimers.[150]

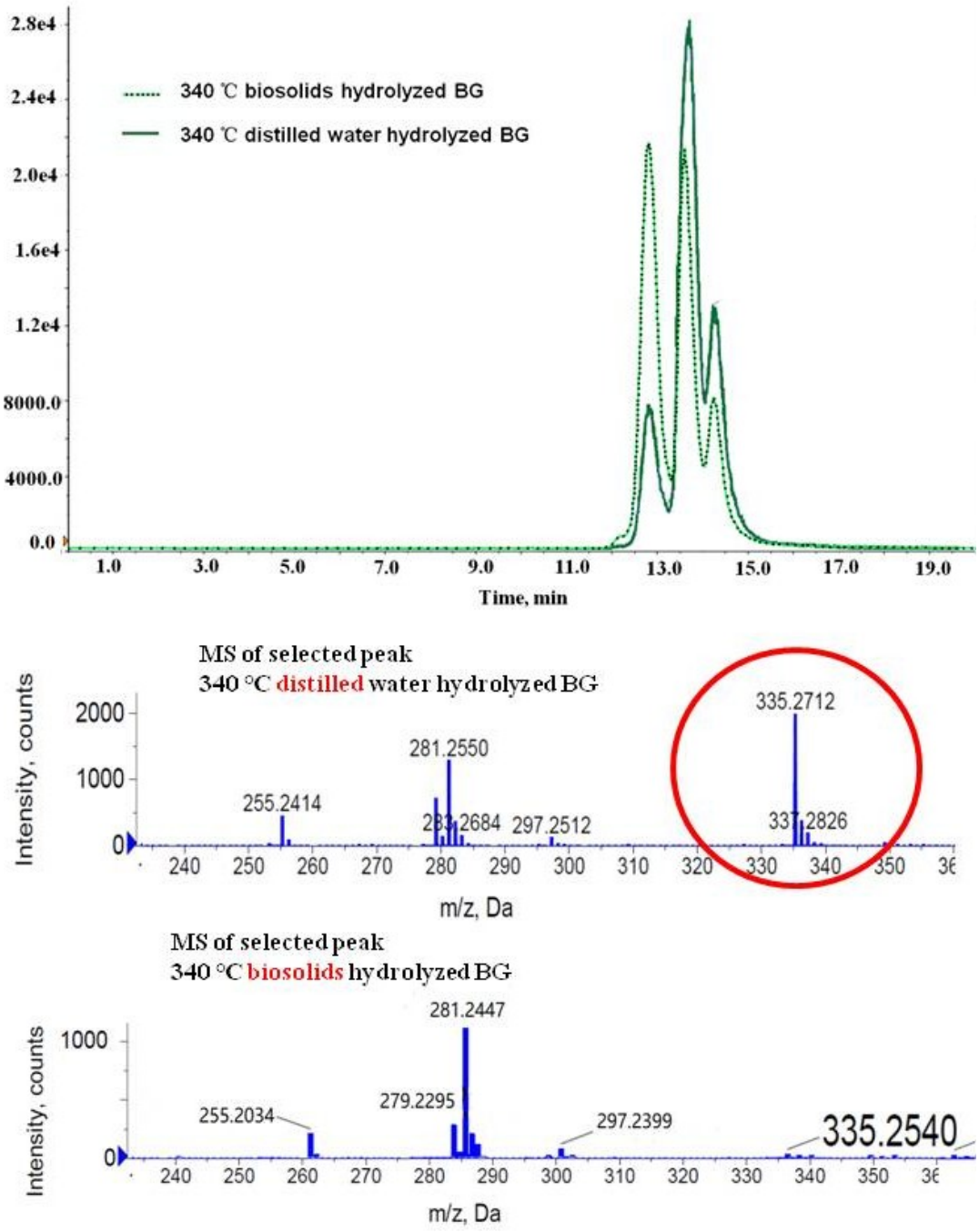


Figure 4-20 Comparison of LC-MS of the FFA contained peak of 340°C the lipid phase of distilled water or biosolids hydrolyzed brown grease

Goebel *et al.* (1947) studied the polymerization of unsaturated fatty acids and illustrated that at a temperature range from 330°C to 360°C, under 85~400 psi pressure, and in the presence of water, unsaturated fatty acids can be partly converted to dibasic fatty acids or higher polybasic fatty acids. Moreover, Goebel *et al.* pointed out when water was present; the heat-induced polymerization was much more likely to occur rather than decarboxylation and decomposition.[151] Sari (2013) also indicated that even without catalysts, the hydrolysis of oleic acid at 400°C would produce 4% of heavy molecules (c>25). Even though the temperature (400°C) used by Sari was higher than the condition used in our studies (340°C), Sari's results still showed that other compounds could be produced non-catalytically in the thermal hydrolysis of lipids. Also, the prevalence of heavy molecules would increase if there were the metal catalysts present, such as Ni, NiMo, Ru, Pd, PdPt, Pt, Ir, Os, Rh or in alkali condition.[92]

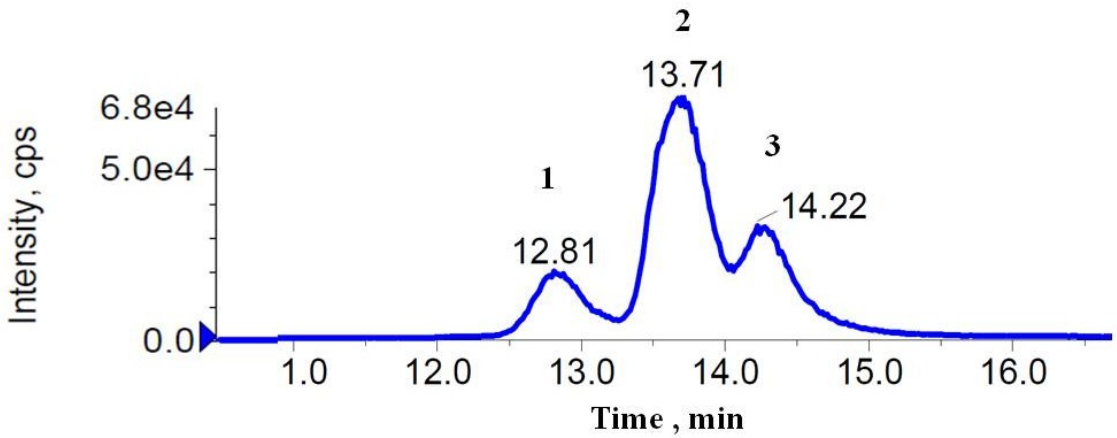
As mentioned above, certain substances, such as metal in biosolids and brown grease, might also perform as a catalyst and promote some chemical reactions that would convert the glyceride or FFA into GC undetectable compounds. The production of higher-molecular-weight material was also found in a couple of studies of metal catalytic hydrolysis of lipid at 300-360°C. Snåre *et al.* (2008) indicated that there are heavier components that cannot elute from GC columns when analyzing the hydrolysis product, which were produced during a hydrolysis reaction where coupling reactions would occur with unsaturated compounds. [74, 152] The high level of unsaturation in brown grease was a reason for the formation of heavier products (C> 25) in the hydrogenation reaction at 300°C in the research of Sari (2013).[153] The sharp

decrease of C18:2 fatty acids and C18:1 fatty acids in the GC chromatogram confirmed the loss of unsaturated fatty acid as shown in **Figure 4-18**. Also, there was a possibility of formation of fatty acid dimers via a Diels-Alder mechanism.[154] Fu *et al.* (2011) confirmed the loss of total mass calculated by GC, where the mass balance for acid hydrolyzed linoleic acid was only 75%. They claimed that the coupling reactions can occur with this polyunsaturated compound to produce higher molecular compounds that could not elute from GC and thus may be responsible for the compounds not detected via GC.[74] Watanable *et al.* (2006) showed that a bimolecular decarboxylation would happen in hot pressed water (400°C) between two fatty acid molecules, producing heavy molecule compounds (e.g. two C17 acids will be decarboxylated and form a C35 ketone).[155] **Table 4-9** reviews the studies that reported the heavy compounds produced through thermal hydrolysis or hydrogenation of lipids.

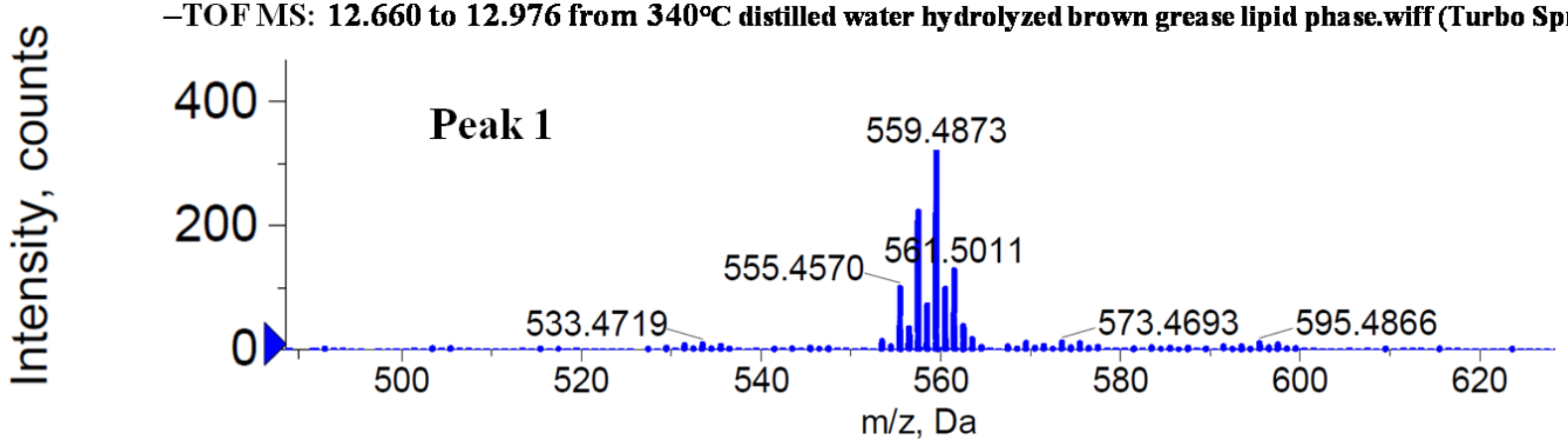
Temperature	Feedstock	Water	H_2	Catalyst	Analysis Method	Result	Reference
300°C	Oleic Acid	Super critical water	no	no	GC with Rxt-65 TG column without derivatization	4% C25+heavy compounds	[92]
	Brown Grease			Pd/C		• 30% C25+heavy compounds 1-hour reaction • 25% macromolecular that can be filtered out	
254°C	Oleic Acid	5%	no	clay	Waters HPLC column	• Water will promote the formation of dimers and trimers	[156]
300°C	Linoleic Acid	no	1%	Pd/C	SEC with 3 different columns	• 4% dimers and trimers were produced	[157]
330°C	Linoleic Acid	95% water	no	Pt/C	Agilent 6890 GC Hp-5 column and Nukol capillary column	• 25% GC undetected compounds • Higher-molecular weight materials have been produced through coupling reactions	[74]
	Oleic Acid					• 7% mass loss and incorrect calculation of uncertain compounds	
300-360°C	Oleic Acid	no	5% Ar and H_2	Pd/C	GC (DB-5) Sample was silylated before analysis	• Heavy compounds molecular weight >400	[152]
340°C	Triolein	liquid to lipid ratio 5:1 (m/m)	no	no	GC-MS/FID HPLC-ELSD LC-MS described in 3.2.8.2	• Dimer and oxygenated compounds were detected • HPLC-ELSD: 85% FFA (12% undetected) • GC-Diazomethane FFA: 80.0% • GC acetyl: Total: 81.2%	This study

Table 4-9 Studies where production of high molecular weight compounds was thought to impact detectability during GC.

TIC of –TOF MS from 340°C distilled water hydrolyzed brown grease lipid phase.(Turbo Spray)



–TOF MS: 12.660 to 12.976 from 340°C distilled water hydrolyzed brown grease lipid phase.wiff (Turbo Spray)



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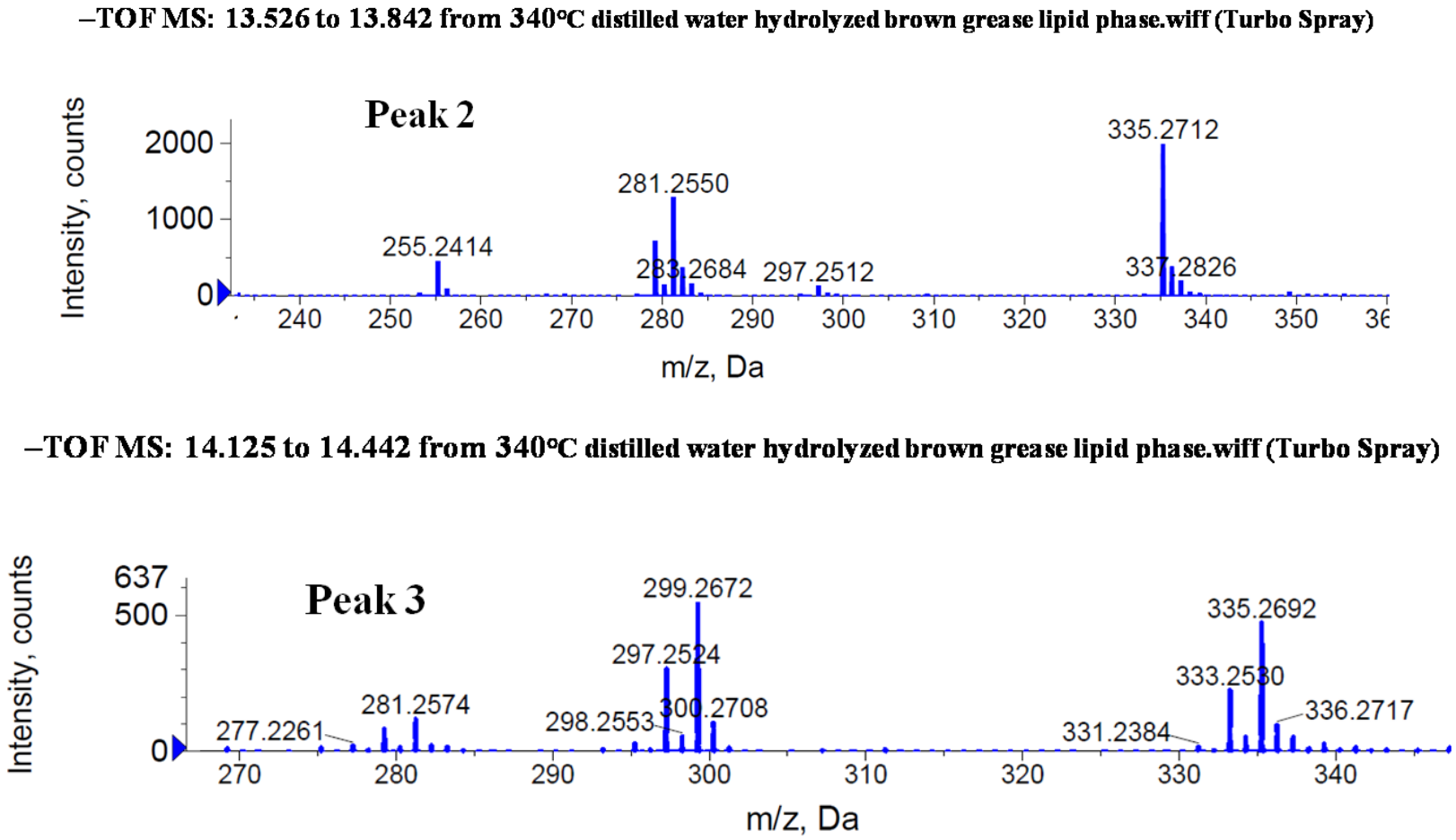

Figure 4-21 The chromatogram for LC-MS analysis of 340°C distilled water hydrolyzed BG at liquid to lipid ratio 5:1 for 1-hour reaction time

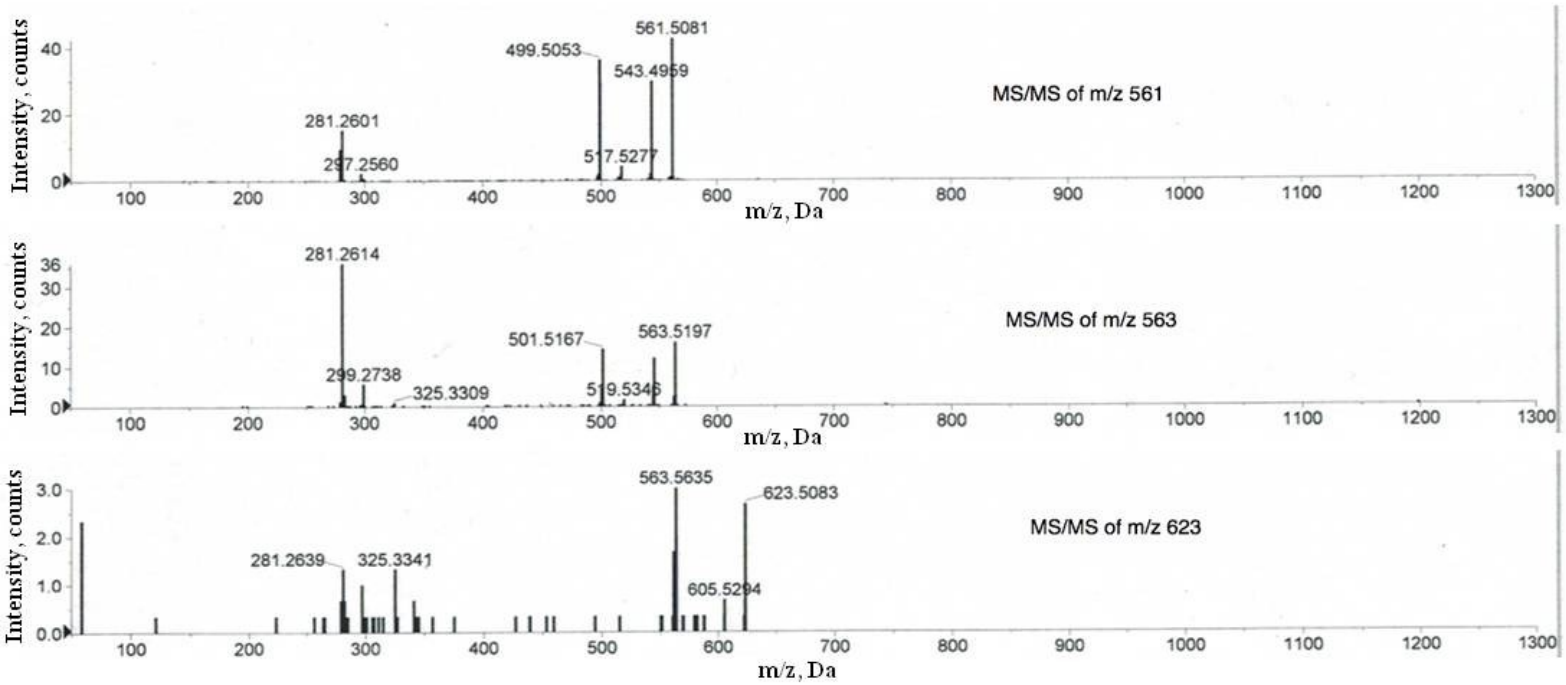

Figure 4-22 MS/MS analysis of peak 1. MS/MS analysis product ion spectra of 1) m/z 561, 2) m/z 563, 3) m/z 623 in peak 1 (retention time 12.660 to 12. 976 shown in Figure 3-22)

The increase in heavy molecular compounds when increasing the temperature might also be caused by the presence of 30% FFA in brown grease. Myllyoja (2011) did an experiment involving catalytic hydrotreating of a lipid feedstock at 305°C for hydrocarbon production. They demonstrated that when the feedstock contained 10% FFA and 90% triglyceride, there were heavy molecules produced that were comparable with the reaction of pure triglyceride.[158]

4.3.4 Sulfur Content Produced During Hydrolysis

Any sulfur existing in brown grease and biosolids could influence the final quality of the fuel. Thus, it was imperative that we examine the amount of S in the lipid phase generated after hydrolysis of brown grease to determine whether it is impacted by the incorporation of biosolids into the hydrolysis procedure.

CHNS analysis

Table 4-10 shows the CHNS analysis of original brown grease and the lipid phase of hydrolyzed brown grease. Unfortunately, the S contents in original brown grease and distilled water hydrolyzed brown grease were below the detection limits of the CHNS analyzer, so a better method for sulfur content measurement was needed.

Table 4-10 CHNS analysis of original brown grease and hydrolyzed brown grease

wt.% of the sample	C	H	N	S	O
	%	%	%	%	%
Brown grease	75.3 ± 0.1	11.6 ±0.1	<0.1%	<0.1%	11.6 ±0.2
The lipid phase of distilled water-hydrolyzed brown grease	76.4 ± 0.1	11.9 ± 0.1	<0.1%	<0.1%	10.7 ±1.2
The lipid phase of biosolids-hydrolyzed brown grease	76.6 ±0.1	12.0 ± 0.0	0.4 ± 0.1	<0.1%	11.7 ±0.1

The detection limit of the analysis was 0.1% for C, H, N, S.

Since the levels of sulphur in brown grease as well as in the lipid fractions obtained through hydrolysis of brown grease with distilled water or biosolids were too low to measure through elemental analysis, sulfur analysis was performed by ICP-AES. The results from this analysis showed that the concentration of sulfur in biosolids and brown grease were 474 ± 20 ppm and 162 ± 16 ppm, respectively **Table 4-11**). Thus, the sulfur content of the hydrolysis product could also be affected by using biosolids as distilled water source with brown grease.

Table 4-11 Sulfur content in the lipid phase of hydrolyzed brown grease at 3 different temperatures.

Sulfur content (ppm)	Hydrolysis temperature			Original
	280°C	310°C	340°C	
Original Biosolids				474 ± 20^{d}
Original brown grease				162 ± 16^{e}
The lipid phase of distilled water hydrolyzed brown grease	145 ± 8^{c}	140 ± 5^{c}	132 ± 2^{c}	
The lipid phase of biosolids hydrolyzed brown grease	825 ± 33^{a}	860 ± 3^{a}	776 ± 14^{b}	

Brown grease was hydrolyzed with distilled water or biosolids at a liquid to lipids ratio 5:1 for 1-hour reaction time. All of the hydrolysis reactions were experimentally triplicated. Means that do not share the same letter are statistically different. The statistical analysis was done using two-way ANOVA with mean comparison by Sidak multiple comparisons test based on a 95% confidence level.

Several studies showed that thermal treatment could help for desulfurization of organic compounds.[131, 159] Metal ions such as Al^{3+}, Ni^{2+}, and Cu^{2+} that exist in biosolids may also promote the decomposition of some sulfur compounds at 240°C.[132] Higher temperatures can promote the decomposition of sulfur contained compounds, but at the same time, there might be unfavorable reactions as well that could bring the sulfur content from the biosolids to the final product. One study showed that high temperatures might lead to accumulation of sulfur in hydrolyzed oil when the temperature exceeds 375°C[160].

As demonstrated in **Table 4-11**, the sulfur concentration of the original brown grease was around 162 ppm ± 16 ppm, and after hydrolyzing with distilled water, the sulfur concentration of lipid phase of hydrolyzed brown grease was decreased to 132 ± 2 (310°C)and then to 145 ± 8 ppm (340°C). Some of the sulfur bonded in lipids might be released to the water phase during the cracking of lipids. This result could be confirmed according to the research of Javadli (2012) and Vogelaar (1999); super- and subcritical water hydrolysis can break C-S bonds and less stable non-aromatics sulfur compounds can be desulfurized.[131, 159] However, with increased temperature, the decrease in the sulfur concentration was not significantly from 145 ppm ± 8 ppm to 132 ppm ± 2 ppm.

The performance of desulfurization of biosolids through hydrolysis was entirely different from hydrolysis with distilled water as shown in **Table 4-11**. The high sulfur content in biosolids could potentially affect the partitioning of sulfur between the various phases and be introduced into the lipid phase during hydrolysis and dramatically increased the sulfur content of the lipid phase of biosolids hydrolyzed brown grease compared with distilled water hydrolyzed one. Also, the sulfur concentration reduction showed a different trend compared with distilled water hydrolysis as demonstrated in **Table 4-11**. The sulfur content of biosolids hydrolyzed brown grease was stable when increasing the temperature from 280°C to 310°C, but dropped at 340°C. The presence of sulfur and metals favor reactions with organic compound, particularly free fatty acids, which is the product of hydrolysis.[68] This explained the reason why the sulfur content was not dropped when increase the temperature from 280°C to 310°C in the first place.

As showed in **Table 4-11** after hydrolyzed with biosolids, the lipid phase of hydrolysis product contained significantly higher sulfur content than in the lipid phase in distilled water hydrolyzed brown grease for all the 3 temperatures. Moreover, when the reactant (biosolids) contains a significant amount of sulfur, increasing the temperature from 280°C to 310°C promoted the transfer of sulfur into the lipid phase rather than promoting desulfurization, but when the temperature rose to 340°C, the significant decrease of sulfur content in the lipids phase of biosolids hydrolyzed brown grease showed that the high temperature was functioned for desulfurization. The high sulfur content will cause problems with emissions of SO_X pollution during pyrolysis, which is the next thermal step in lipid pyrolysis process. Also, the sulfur brought into the final product could potentially increase SOx emissions during fuel combustion. SOx in the atmosphere reacts with water forming a sulfurous acid/sulfuric acid droplet, which are toxic. According to the Canadian General Standards Board (CGSB) Standards for Sulphur Content in Diesel Fuel, sulfur is limited to 15 ppm (CGSB-3.522-2010). Thus, the high sulfur content (800 ppm) of the lipid phase of biosolids hydrolyzed brown grease (feedstock for producing hydrocarbon fuels) may bring high levels of sulfur into the final biofuel product, which would necessitate further refining.

4.3.5 Nitrogen Content

Similar to sulfur content, the nitrogen content is also an environmental concern regarding greenhouse gas emissions. NO and NO_2 formed from the liberation of nitrogen contained in the fuel is one of the main NO_X sources.[161] As showed in

Figure 4-23 there was no significant changes in nitrogen content in distilled water hydrolyzed brown grease when increased the temperature from 280°C to 340°C. The high nitrogen content of biosolids brought more nitrogen into the lipid phase of hydrolyzed brown grease, and increased first at 310°C (5790 ppm ± 92 ppm) and decreased to 4874 ppm ± 230 ppm at 340°C which was at the same level with the nitrogen content at 280°C. A similar explanation with the change the sulfur content could be addressed since the organic compounds would favor the reaction with nitrogen and when there was no more nitrogen that could be brought into the lipid phase, the decomposition of nitrogen contained compounds started to significantly affect the trend of nitrogen concentration in the lipid phase of biosolids hydrolyzed brown grease. The nitrogen concentration of the lipid phase of biosolids hydrolyzed brown grease was significantly higher than the allowable nitrogen content in diesel fuel as proposed by the Pembina Institute, which is 10 ppm for diesel fuel and 500 ppm for alternative diesel.[162] The nitrogen content might have a chance to be decomposed and decreased during the pyrolysis and distillation process.

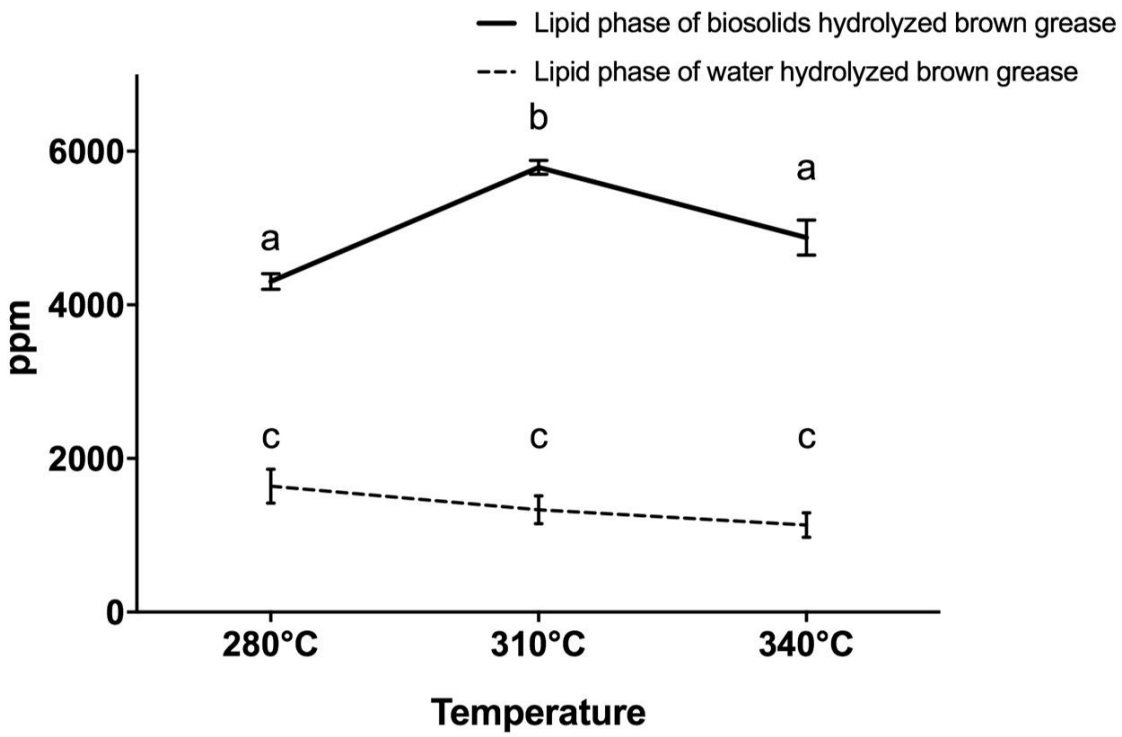

Figure 4-23 The nitrogen content in the lipid phase of distilled water or biosolids hydrolyzed brown grease at different temperatures. Brown grease was hydrolyzed with distilled water or biosolids at a liquid to lipids ratio 5:1 for 1 hour and experimentally triplicated. The sulfur content was analyzed using a flash 2000 combustion unit. Means that do not share the same letter are statistically different. The statistical analysis was done using two-way ANOVA with mean comparison by Sidak multiple comparisons test based on a 95% confidence level.

4.3.6 Hydrocarbons

The lipid phase of hydrolyzed brown grease also contained a few hydrocarbons analyzed by GC-MS/FID as shown in **Figure 4-24**. Alkane and alkane were both observed in the unhydrolyzed brown grease. There were no significantly more alkanes produced under the temperatures of 280°C and 310°C compared with the unhydrolyzed

brown grease, but there was a significant increase when the hydrolysis temperature was raised to 340°C, which means more cracking of the fatty acid occurred at 340°C. The difference between alkanes in distilled water and biosolids hydrolyzed brown grease were not significant, so it is likely that the metals and/or other compounds in biosolids did not affect the cracking of FFA at 340°C. The alkenes in distilled water hydrolyzed brown grease under all 3 temperatures were all significantly higher than the biosolids hydrolyzed brown grease. At 340°C, the alkenes in distilled water hydrolyzed brown grease significantly increased. Aromatics were present in all samples but at levels less than 0.1%.

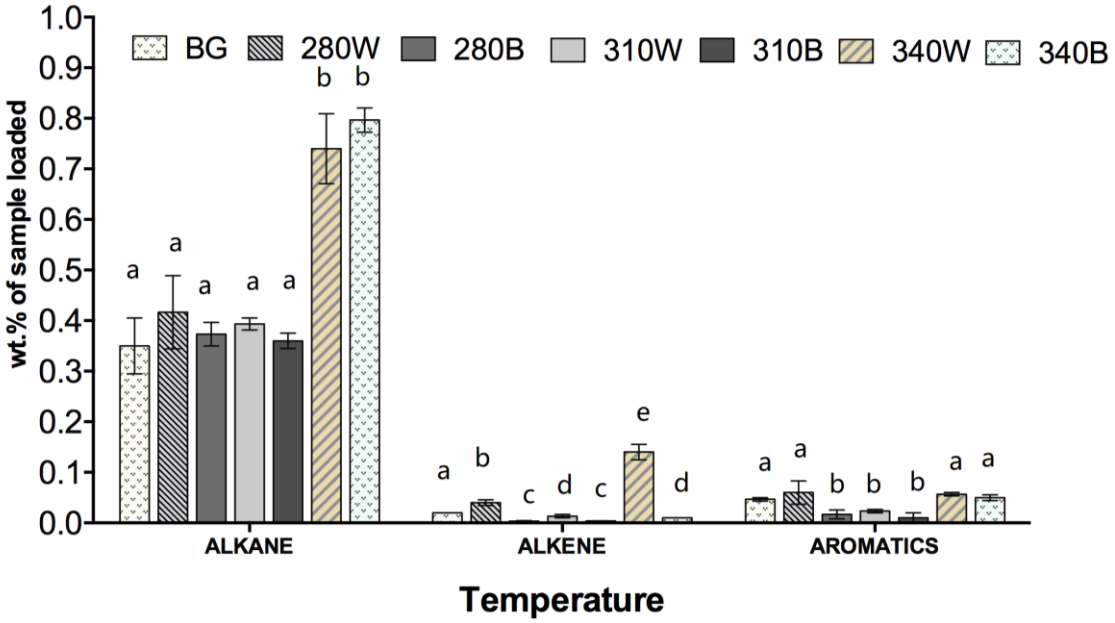

Figure 4-24 Comparison of hydrocarbons in the lipid phase of hydrolyzed brown grease. The hydrocarbon compounds were analyzed by GC-MS/FID. W: lipid phase of brown grease hydrolyzed with distilled water; B: Lipid phase of brown grease hydrolyzed with biosolids. In each hydrocarbon type, means that do not share the same letter are statistically different. The statistical analysis was done using one-way ANOVA with mean comparison by Tukey multiple comparisons test based on a 95% confidence level.

4.3.7 Conclusions

Biosolids performed equally well as distilled water at 280°C with regards to the hydrolysis performance (FFA% in the lipid phase of hydrolyzed sample and the FFA conversion). With increasing temperature, more undesired compounds were produced, especially in biosolids hydrolyzed sample, and the FFA % in the recovered lipid phase was significantly decreased by moving to a higher temperature. Thus, for the brown grease, increasing temperature to 310°C or 340°C would have a negative affect on the

FFA% in the recovered lipid phase of hydrolyzed brown grease. When biosolids were used for hydrolysis, increasing temperature from 280°C to 310°C introduced more nitrogen into the lipid phase of the hydrolyzed brown grease, but the sulfur and nitrogen content were significantly reduced when temperature was 340°C. Increasing the temperature to a certain degree did help with the reduction of sulfur and nitrogen, but the reduction of sulfur and nitrogen at a higher temperature may not offset the drawback of the decrease of FFA % in the recovered lipid phase at a higher temperature.

5 General Discussion, Conclusion, and Recommendations

Biosolids samples collected from Lagoon Cell 5 contain mostly water, and the amount of lipids present alone would not facilitate a cost effective and energy saving biofuel production process. However, research on the hydrolysis of biosolids itself provided valuable information for the future application of biosolids. The reaction temperature range of hydrolysis is much higher than the autoclave condition and can lead to sterilization of biosolids, and the waste stream could be safely handled since it is no longer biohazardous. The successful conversion of TG to FFA in biosolids also means that it could also contribute to fuel production, albeit at low levels that are dependent on the lipid concentration in biosolids. Thus, the biosolids that contain more lipids and with fewer treatment processes could be considered as a better water substitute than the received biosolids in future studies. This could help to cut the cost of some of the biosolids treatment.

The biosolids contain various metals mostly of trace concentration, and the Al, Mg, Cd, Ni and some of other metals might have a chance to act as catalysts and trigger or accelerate several chemical reactions. However, from the result of the application of biosolids in the hydrolysis of brown grease, the biosolids did not function any differently than distilled water for the hydrolysis in terms of FFA% in the recovered lipid phase. Moreover, a possible downside of biosolids was the high sulfur and nitrogen content present in biosolids hydrolyzed brown grease. However, determination of whether the sulfur and nitrogen components would affect the quality of pyrolysis product still needs to be examined.

The thermal hydrolysis dramatically changed the performance of biosolids settling. The received biosolids could barely be settled by gravity even after several months, and the dewatering performance was poor as well. The hydrolyzed biosolids settled quickly within 2 hours, especially the sample hydrolyzed with an addition of acids. The organic phase, aqueous phase and solids phase could be easily separated by gravity, and it was useful for the continuous application or disposal of the aqueous phase.

The performance of biosolids in hydrolyzing brown grease was similar with distilled water for the aspects of FFA conversion, FFA % in the recovered lipid phase, fatty acid distribution, and the phase separation (lipid phase, aqueous phase, and solid phase). In this case, the high concentration of sulfur and nitrogen became the biggest weakness of using biosolids as the substitute of water. However, it worth mentioning that for a larger scale application, the distilled water/biosolids to oil ratio may not be that high and the reduced amount of biosolids in the reaction will positively affect the sulfur content that could be brought into the organic phase. Also, increasing the reaction temperature led to a decrease in sulfur concentration in hydrolyzed brown grease. When the temperature rose to 310°C or higher, more other types of organic compounds that could not be detected and quantified by current analysis methods were formed rather than FFA. Even though those compounds could contribute to the future biofuel production, it would be harder for quality control because some of the compounds were difficult to analyze. It is worth mentioning that all hydrolysis reactions were conducted with a 1-hour reaction duration; at a higher temperature, a shorter

reaction time (e.g. 9 to15 min at 340°C) could be achieved, and with less reaction time, there will be less chance of other types of reactions participated.

This study shows the possibility of applying biosolids as a substitute for water in a lipid pyrolysis technology. The benefit for wastewater treatment will be tremendous due to the excellent settling performance and sterilization of biosolids after hydrolysis. Even though the sulfur and nitrogen content of hydrolysis product were slightly high, the hydrolysis conversion was not affected by the complexity of biosolids. The biosolids also achieved better settling after thermal hydrolysis. This study also indicates that increasing temperature to 340°C could significantly decrease sulfur content but also significantly decrease the FFA%. In the future, more studies should focus on the sulfur and nitrogen removal. Also, a shorter reaction time could be investigated to limit the interaction of other compounds in biosolids with lipids during the hydrolysis. With further development, the application of biosolids into lipid pyrolysis could prove to be a win-win for both wastewater treatment and biofuel production processes.

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