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The Esterification of Naphthenic Acids for Methyl Ester Production

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The Esterification of Naphthenic Acids for Methyl Ester Production

by

Zahra Dastjerdi

A thesis submitted to the Faculty of Graduate and Postdoctoral Studies in partial fulfillment of the requirements for the degree of

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In memory of my mother for her sincere love and her ever-present support of my personal endeavours towards learning

“***Wish You Were Here***”

Statement of Contributions of Collaborations

I hereby declare that I am the sole author of this thesis. I performed all the experimental designs, experiments, ^{1}H-NMR sample preparations and ^{1}H-NMR data analyses.

The scientific guidance throughout the project and editorial comments for the written work were provided by my supervisor Dr. Marc A. Dubé.

Zahra Dastjerdi

_______________ Date: ____________

Abstract

The remediation of tailings water, a by-product of bitumen extraction, is of utmost importance to the Athabasca oil sands industry due to its toxicity to aquatic environments. Naphthenic acids (NAs), natural components of bitumen, are the major contributor to the toxicity of tailings water. The use of algae for remediation of tailings water is a new approach to reduce the toxicity of tailings water. The combined extracted NAs and algal oils can potentially be used as a low cost feedstock for the production of biodiesel. In this study, the feasibility of using NAs for the production of biodiesel was investigated using mixtures of canola oil and commercial NAs as model compounds.

Since NAs are a mixture of cyclic and acyclic carboxylic acids, the acid-catalyzed esterification of NAs without canola oil was investigated. The acid-catalyzed esterification reaction was carried out with methanol, and the influence of variables affecting the esterification process was examined. The experimental results showed that increasing temperature and catalyst concentration increased the reaction rate and final conversion of NAs to their corresponding methyl esters. The catalyst study showed that sulfuric acid had a more positive effect on the esterification reaction compared to *p*-toluene sulfonic acid. Additionally, an excess methanol:oil ratio had a negative effect on the esterification reaction due to the solubility of NAs in methanol.

The acid-catalyzed transesterification/esterification of mixtures of NA and vegetable oil (canola oil along with 5% NAs) with methanol was investigated at different operating conditions. The outcomes of these experiments showed that increasing temperature, sulfuric acid concentration, and methanol content had positive effects on the rate of reaction and final ester products.

The general conclusion of the thesis is that the conversion of NAs to useful products is indeed feasible and can be part of the solution to tar sands tailing pond remediation.

Résumé

La rémédiation d'eau tailings, un dérivé d'extraction de bitume, est d'importance suprême à l'industrie de sables de pétrole de l'Athabasca en raison de sa toxicité aux environnements aquatiques. Les acides de naphthenic (NAs), la composante naturelle de bitume, sont la cause principale de la toxicité d'eau tailings. Jusqu'à présent, aucun processus de récupération n'a été éfficacement développé pour le traitement des grands volumes d'eau tailings. L'utilisation d'algues marines pour la rémédiation d'eau tailings est une nouvelle approche pour réduire la toxicité d'eau tailings. La combinaison des NAs extraits avec les huiles d'algues peuvent potentiellement être utilisés comme un feedstock à bas prix pour la production du biodiésel. Dans cette étude, la faisabilité d'utiliser les NAs pour la production du biodiésel a été enquêtée en utilisant des mélanges d'huile canola et de NAs commerciales comme modèles.

Puisque NA est un mélange de molécules cycliques et acycliques carboxyliques, une catalyse par acide pour une estérification de NAs sans huile canola a été enquêté. L'estérification catalysée par acide en présence du méthanol fut investiguée pour percevoir l'influence de plusieurs variables. Les résultats expérimentaux ont montré que l'augmentation de la température et de la concentration de catalyseur a augmenté le taux de réaction et la conversion finale de NAs. L'étude de catalyseur a montré que l'acide sulfurique avait un effet plus positif sur la réaction d'estérification comparée à l'acide p-toluène sulfonique. Supplémentairement, un excès de méthanol avait un effet négatif sur la réaction en raison de la solubilité de NAs dans le méthanol.

La transestérification/estérificat6ion catalysée par acide de mélanges de NA et d'huile végétale (canola avec NAs de 5%) avec le méthanol a été enquêté à de différentes conditions d'utilisation. Les résultats de cette expérience ont montré que l'augmentation de la concentration du catalyseur acide, de la température, et du pourcentage de méthanol avait des effets positifs sur le taux de réaction et de la conversion finale en produits d'ester.

La conclusion générale de la thèse était que la conversion de NAs aux produits utiles est effectivement réalisable et peut faire partie de la solution de goudronner de sables tailings pour la rémédiation d'étangs.

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My deepest gratitude goes to my family for their endless love and support throughout my life. I am indebted to my mother for her unconditional love and care. I would like to dedicate this work to my late mother, who passed away while I was far away from her busy with this work, may God bless her soul.

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Abbreviations and Nomenclature

FFA	free fatty acid
FAME	fatty acid methyl ester
GC	gas chromatography
HPLC	high performance liquid chromatography
^{1}H-NMR	proton nuclear magnetic resonance
IR	infrared spectroscopy
LC-GC	liquid chromatography with gas chromatography
MeOH	methanol
NAs	naphthenic acids
NAME	naphthenic acid methyl ester
p-TSA	*p*- toluene sulfonic acid
SA	sulfuric acid
TG	triglyceride
TLC	thin layer chromatography

Chapter 1

Introduction

1.1 Naphthenic acids

As a result of technology improvements, petroleum production in the Athabasca oil sands has increased enormously. Oil extraction and refinery plants release a large amount of process water as a by-product containing toxic contaminants, namely naphthenic acids (NAs) (Holowenko et al., 2002). NAs are complex mixtures of alkyl-substituted acyclic and cycloaliphatic carboxylic acids, with the general formula $C_nH_{2n+Z}O_2$, where n is the carbon number and Z describes the hydrogen deficiency resulting from cyclization. Z is zero or a negative integer, and the absolute value of Z divided by 2 gives the number of rings in the compounds (Brient et al., 1995). Typically, for a given Z number, more than one isomer exists in the mixture. Figure 1.1 shows examples of NA structures.

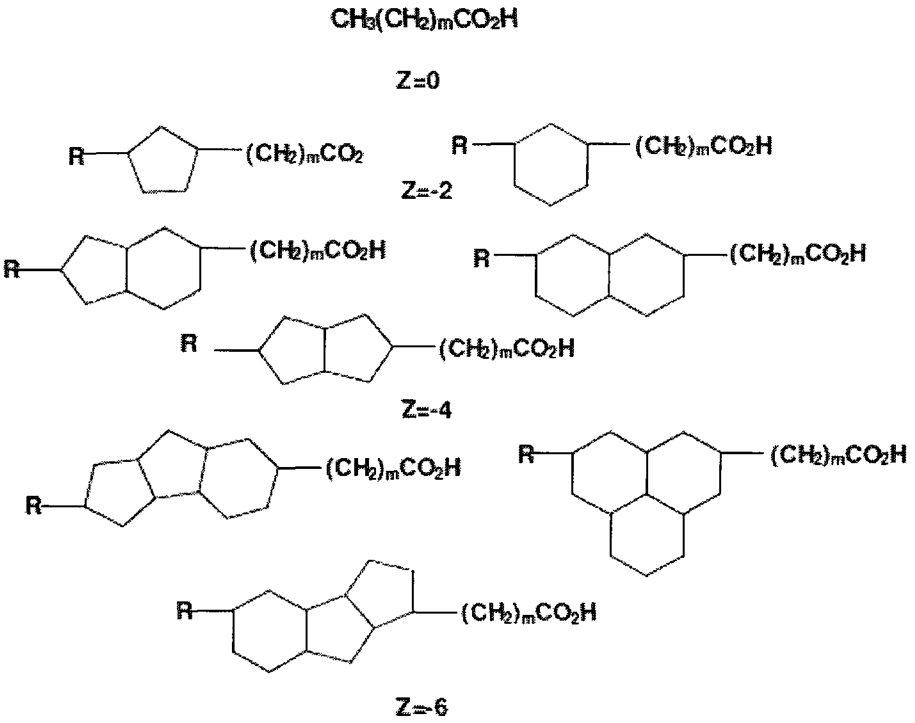

Figure 1.1 General chemical structures of naphthenic acid (Brient et al., 1995).

NAs, a major part of petroleum acids, are natural components of crude oils. The petroleum acid concentration varies from 0 to 3 wt.% in different oil fields (Brient et al., 1995). For instance, the petroleum acid concentration is approximately 2% in the Athabasca oil sands (Northeastern Alberta, Canada) (Conrad Environmental Aquatics Technical Advisory Group, 1998). It is said that NAs make up about 90% of the Athabasca petroleum acids (Quagraine et al., 2005). The Athabasca oil sands[1] deposit is one of the largest oil reserves in the world. It contains approximately 33.9 billion barrels of bitumen which is recoverable by surface mining (Alberta Government, 2007).

In the surface mining technique, the bitumen is extracted from mined oil sands by the Clark hot water method, in which oil sands ore is mixed with steam, warm water (40-80°C) and sodium hydroxide (50-200 g/t of oil sands) as a conditioner. The alkalinity of the mixture[2] helps NAs dissolve in the bitumen and releases them from the oily phase to the aqueous phase as naphthenate salts. The Clark hot water method extracts bitumen from oil sands deposits and removes NAs from bitumen simultaneously (FTFC, 1995a). NAs are corrosive to the oil processing equipment, particularly at the high temperatures encountered in the distillation units. NA corrosion may cause equipment failure resulting in critical safety and reliability issues (Elizabeth et al., 1993; Slavcheva et al., 1999). As mentioned above, the separation of NAs from the bitumen prior to the upgrading and refining stages is very important to oil-processing companies. Therefore, the oil operators in the Athabasca oil sands region extract bitumen by the Clark hot water procedure.

The extraction process requires a large volume of water. An average 3 m^3 of water is consumed to recover one m^3 of oil from mined oil sands. Although extraction

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[1] A mixture of sand, clay, water, and heavy black viscous oil called bitumen

[2] pH 8.5 - 10

water is pumped back to the extraction unit and reused in the extraction process many times, it cannot be reused indefinitely. Therefore, the NA rich extraction water is held in large settling ponds due to a "zero discharge policy". It was estimated that if oil processing continues at the existing rate, more than 1 billion m^3 of tailing ponds will be accumulated by 2025 (Herman et al., 1994; Rogers et al., 2002).

Some sample studies from tailing ponds have shown that NA concentrations range between 20-120 mg/L (Holowenko et al., 2001), which makes them the largest waste component in tailing ponds. Amongst numerous contaminants in tailing ponds, NAs have acute and chronic toxic effects on aquatic organisms (Clemente & Fedorak, 2005). An aquatic toxicity study showed that a 96 h exposure of freshwater fish to sodium naphthenate caused 50% lethality (LC_{50}) at 25, 50, and 75 mg/L for chum salmon, sturgeon, and roach, respectively (Dokholyan & Magomedov, 1983). Apart from the NA's lethality, some significant non-lethal effects such as leukocyte count, and muscle glycogen alteration were also observed in toxicity studies. An acute and chronic toxicity test on rats exposed to NAs revealed that liver was the potential target organ. The liver weight in the high-dose group was 35% higher than in the control group (Rogers et al., 2002).

The reclamation of highly toxic and concentrated NAs in the large settling ponds will be one of the main concerns for oil sands operations after stopping the surface mining projects; the NA concentrations in settling ponds should be reduced below toxic levels. Although some reclamation options have been considered, a definitive solution to the problem has yet to be put forth (FTFC, 1995b; Rogers et al., 2002).

In spite of the fact that photodegradation and biodegradation are known as primary degradative pathways of organic substances in the environment, NAs from tailing ponds are recognized to be recalcitrant to degradation. A photodegradation study indicated that NAs from tailing ponds were not completely degraded by natural sunlight and fluorescent radiation with or without TiO_2 (McMartin et al., 2004; Headley et al., 2009). Headley et al. (2009) pointed out that the degree of degradation of NA mixtures varies with the structure and size of the compounds. In a bioremediation process, the normal metabolic process of microorganisms alters the chemical structure of organic materials to compounds which are less toxic to humans or the environment. In general, bioremediation is carried out by manipulating microbiological cultures in ways that promote growth and metabolism of the microorganisms. Although NAs are known for their commercial use as antimicrobial agents such as wood preservatives, some studies have indicated that microbial activity in laboratory cultures decreases the NAs' toxicity (Herman et al., 1994). However, it is unclear whether or not NAs can be utterly degraded by microbes in the indigenous microbial community. Therefore, we have proposed the use of algae in the biodegradation of NAs from tailing ponds.

The application of algae for domestic and agricultural wastewater treatment has been investigated intensively (Oswald W.J, 1988). Algae have been used to remove heavy metals, nitrogen, and phosphorous from wastewaters (Wilde & Benemann, 1993; Weissman et al., 1998). Other studies reported that algae degraded phenol (Semple & Cataldo, 1996), naphthalene (Cerniglia et al., 1980), and crude oil (Raghukumar et al., 2001). Although little has been known about NA's toxicity to phytoplankton (a form of algae), it has been found that NA concentrations up to 45 mg/L had no significant effect on phytoplankton (Leung et al., 2003). A study revealed that Chlorophyta among six different algal divisions survived at higher NA concentrations (Leung et al., 2003). Another characteristic of algae is the ability of bioaccumulation. Bioaccumulation occurs when an organism absorbs a toxic substance at a rate greater than at which the substance is lost. Algae can possibly accumulate, adsorb NAs and degrade them to a lesser degree to metabolized NAs. Given the above-mentioned algae's characteristics, they might have the potential to be employed in the bioremediation of NA contaminated waters.

1.2 Biodiesel

In recent decades, population expansion and industrialization have increased the consumption of energy derived from the combustion of fossil fuels; mainly petroleum-based fuels. This ongoing trend has led to the depletion of the world's conventional oil reserves. Furthermore, petroleum combustion is known as a significant source of greenhouse gas, and air pollutants including NO_x, SO_x, CO, particulate matter and volatile organic compounds (Sheehan et al., 1998; Graboski & McCormick, 1998). As a result, other sustainable energy sources such as biofuels have become increasingly important for environmental, political and economic reasons.

Biodiesel, defined as fatty acid alkyl esters, is a, biodegradable, and environmentally friendly fuel which is generally produced from vegetable oils, animal fats, or waste greases. Biodiesel contains no sulphur, aromatic hydrocarbons, metals or crude oil residues. Moreover, given that the carbon in plant-derived oil or fat typically originates from CO_2 in the atmosphere through photosynthesis, the burning of biodiesel releases less net CO_2 into the air (Sheehan et al., 1998; Graboski & McCormick, 1998).

Biodiesel can be used in conventional engines without further engine modification. Due to biodiesel's oxygenated structure (ester-bonds), it prolongs engine life by playing the role of a lubricant (Anastopoulos et al., 2001). In addition, the characteristics of biodiesel are comparable with international fuel standards. All of the above-mentioned properties make biodiesel a promising alternative source of fuel. The main method to produce biodiesel is via transesterification.

Transesterification is an exchange reaction between an alcohol (e.g., methanol) and an ester group of triglyceride in the presence of a strong catalyst (usually base). Methanol has been used widely, due to its low price, in the transesterification process that produces biodiesel and glycerol as a main by-product. The complete transesterification reaction of TG molecules involves three consecutive reactions, as shown in Figure 1.2, where R_1, R_2, and R_3 represent typical fatty acid hydrocarbon chains (14-20 carbon atoms, 0-2 double bonds). In other words, the overall reaction produces one molecule of glycerol and three molecules of alkyl esters, as shown in Figure 1.3.

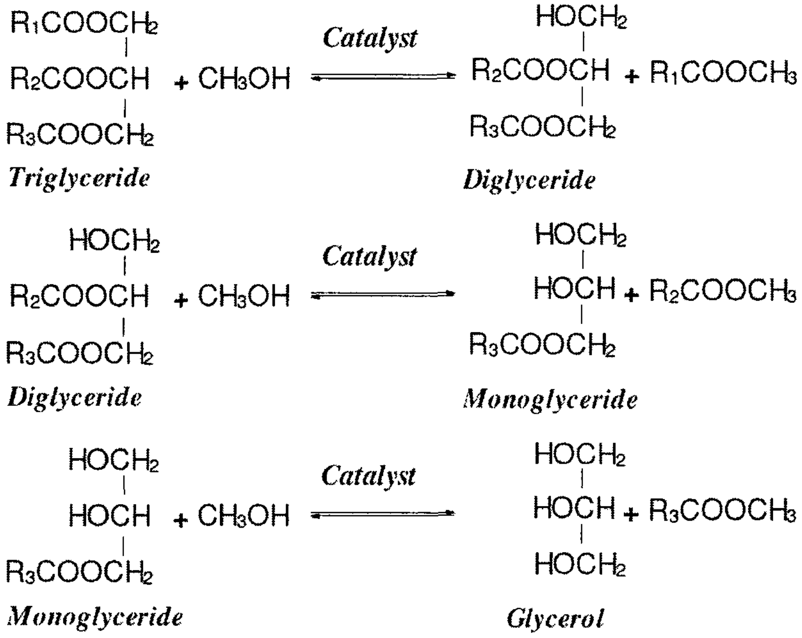


Figure 1.2 Three-step reaction of glycerides with methanol.

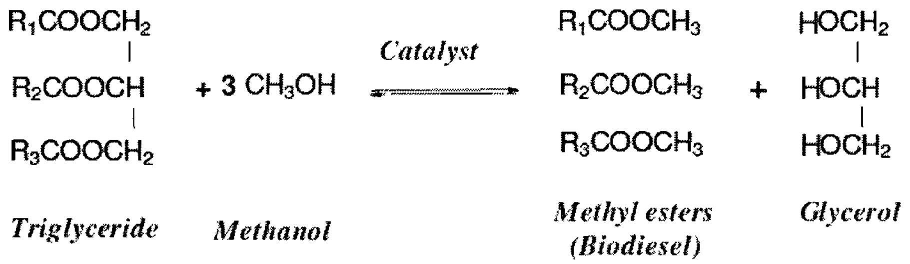
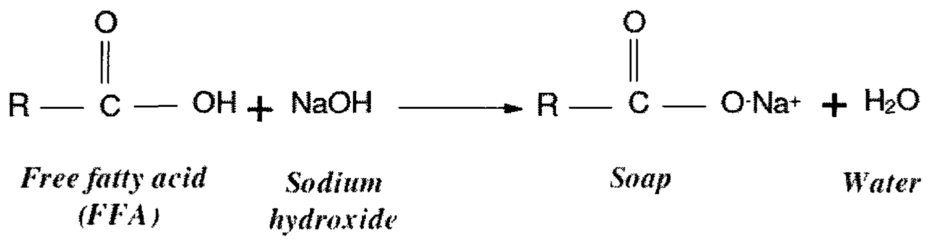


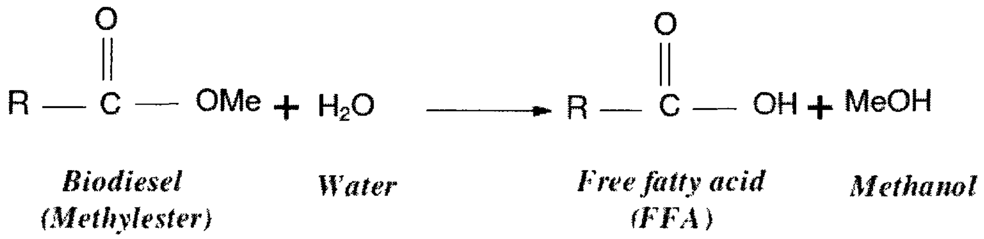
Figure 1.3 Overall transesterification reaction.

Transesterification is an equilibrium reaction and therefore, to achieve high final conversions an excess amount of alcohol is often used. As a result of the high reaction rate for the base-catalyzed compared with the acid-catalyzed reaction, most commercial biodiesel is made using alkaline catalysts such as sodium methoxide and potassium hydroxide. Although the alkaline-catalyzed reaction is preferred industrially, its application greatly depends on the nature of the feedstock. The total free fatty acid (FFA) content in the feedstock must not exceed 0.5 wt.% (Freedman et al., 1984; Liu, 1994; Canakci & Gerpen, 1999; Zhang et al., 2003a). Otherwise, the formation of soaps (see Figure 1.4) reduces the biodiesel yield and increases separation costs. Moreover, the alcohol and catalyst should be anhydrous (the total water content must be 0.1-0.3 wt.% or less) (Haas, 2004) because the presence of water in the feedstock promotes hydrolysis of the biodiesel to FFAs (see Figure 1.5). In general, these feedstock specifications enforce the use of high-quality feedstock that accordingly increases the final cost of biodiesel production.



$$R-\overset{\overset{\displaystyle O}{\|}}{C}-OH + NaOH \longrightarrow R-\overset{\overset{\displaystyle O}{\|}}{C}-O^-Na^+ + H_2O$$

Figure 1.4 Soap formation.



$$R-\overset{\overset{\displaystyle O}{\|}}{C}-OMe + H_2O \longrightarrow R-\overset{\overset{\displaystyle O}{\|}}{C}-OH + MeOH$$

Figure 1.5 Hydrolysis of biodiesel.

Although biodiesel has been known as a suitable substitute for petroleum-derived diesel fuel as blends or in neat form, the high production cost of biodiesel limits its commercial use. Biodiesel's overall cost consists of feedstock[3] production and its processing, biodiesel processing, and product distribution. Some studies reported that the feedstock cost, mainly allocated to the refining of fats/oil, contributes to the greatest component of biodiesel's overall cost (i.e., >70%) (Zhang et al., 2003a; Haas et al., 2006). In order to reduce biodiesel's manufacturing cost, the use of low-grade feedstocks with high amounts of FFAs (e.g., waste cooking oils, unrefined oils, non-edible oils, and fats) is recommended. Accordingly, Zhang et al.'s study showed that virgin oil (high-grade quality) costs 2-3 times more than waste cooking oil which leads to substantial increases in the final biodiesel's cost.

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[3] feedstock includes fats/oil, alcohol, and catalyst

Although the use of low grade feedstock is economically advised for biodiesel production, the low grade feedstock is not considered appropriate for the base-catalyzed process due to its high FFA content which results in the production of soaps as unwanted by-products. Hence to eliminate soap formation, the biodiesel process is generally carried out using either an acid-catalyst or in a two-stage acid/alkali catalyzed process. In the acid-catalyzed process, the transesterification of TG along with the esterification of FFA occur simultaneously. On the other hand, in the first stage of the two-stage acid/alkali process, acid catalyst is employed to convert the excess amount of FFAs to methyl esters. Subsequently, the base catalyst is applied to convert the remaining triglycerides to methyl esters. In spite of the fact that the acid-catalyzed process is not very popular in industry due to its low reaction rate and its need for corrosive-resistant equipment, it has many advantages over the two-stage process, both technologically and economically (Zhang et al., 2003a; Zhang et al., 2003b)

Vegetable oils (mainly soybean, rapeseed and canola oil), animal fats and waste cooking oils are commonly utilized for biodiesel production in industry. However, biodiesel from these conventional feedstocks can provide ~1% of current demand for transportation fuels (Brennan & Owende, 2010). Oil crop production for biodiesel cannot be increased because it induces a land use competition between biodiesel and food, and consequently escalation in food prices. In addition, increasing oil crop production may have negative environmental impacts caused by the use of fertilizers and pesticides. On the other hand, animal fats and waste cooking oils are limited resources. They marginally increase the biodiesel production volume. Moreover, biodiesel from animal fats is not suitable for extreme cold temperatures, because the high level of saturated fat in feedstock leads to poor cold flow properties of biodiesel and poor engine performance (Behzadi & Farid, 2007). Considering all the above-mentioned limitations, an alternative feedstock is required to produce the large volumes of biodiesel required by public demand and government legislation.

Microalgae have been recently considered as a promising alternative feedstock for biodiesel production. Microalgae are microscopic photosynthetic organisms with a photosynthetic mechanism similar to that in plants. Microalgae have a simple cellular structure and are submerged in an aquatic environment that provides them easy access to water, CO_2 and other nutrients (Minowa et al., 1995; Miao & Wu, 2006). Therefore, they have a higher photosynthetic efficiency, higher biomass production and faster growth compared with land crops. The biochemical composition of microalgae consists of proteins, carbohydrates, lipids and nucleic acids and it varies with the type of algae and the cultivation environment.

Although the oil content of 10-30% based on dry weight is mostly common for many algae under normal growth conditions, it can be doubled or tripled by manipulating culture conditions (e.g., *Botryococcus braunii* 25-75%, *Schizochytrium sp*, 50-77%) (Schenk et al., 2008). Moreover, the fatty acid chains can be adjusted through manipulation of the cultivation environment (e.g., light and nutrients) (Schenk et al., 2008). As a result, it is assumed that biodiesel from algae can be customized to meet geographical requirements. Although microalgae have been potentially considered as an appropriate feedstock for the production of large quantities of biodiesel, algae oil contains water which inhibits the transesterification process. Therefore, a pre-treatment process is essential to remove excess water for biodiesel production.

1.3 Biodiesel from the extracted algal/NAs oil

Microalgae have long been recognized as a promising alternative feedstock for biodiesel production. Recently, the concept of biodiesel from algae has gained renewed attention as microalgae have the potential to meet a significant amount of transportation fuel demands. The application of microalgae is not limited to biodiesel production. The use of algae for the treatment of domestic, industrial or agricultural wastewaters is called phytoremediation. Given the fact that tailing pond waters in Alberta's oil sands contain very toxic components (mostly NAs), their remediation is required after ceasing the surface mining operation. The use of algae for remediation of tailing pond waters coupled with biodiesel production has the potential to be a profitable cleanup process.

The use of microalgae for tailing waters treatment has both economic and environmental benefits. Firstly, microalgae are able to reduce the amount of CO_2 released from oil sands operation via the exhaust gas. Secondly, their residual biomass after biodiesel production will be utilized for land remediation on site. Research shows that various algal species such as Chlorophyta (*Botryococcus braunii*) and Cyanobacteria (*Merismopedia*) not only were able to grow in the tailing waters of the Athabasca's oil sands with greater than 20 mg/L of naphthenates (Leung et al., 2001) but also *Botryococcus braunii* was able to accumulate large amounts of lipids and hydrocarbons. It is also worth mentioning that *Botryococcus braunii* is recognized as a potential source of hydrocarbon fuels (Banerjee et al., 2002).

Algae are generally cultivated in open pond or closed photobioreactor systems (Chisti, 2007). The choice of production system relies on the intrinsic properties of the selected algae strain such as their growth rates as well as their culture density (Chisti, 2007). After cultivation of algae, the algae and accumulated NAs will be harvested from tailing pond waters to recover microalgae biomass. The extracted algal/NA oil can be esterified to produce biodiesel and its residual biomass can be utilized as a soil additive for land remediation after oil sands mining.

The algal triglycerides and FFAs along with NAs can be converted to their corresponding esters (biodiesel) through an acid-catalyzed transesterification/esterification reaction. The esterification of NAs at elevated temperatures (i.e., >90 °C) was previously investigated in some corrosion studies (Cirin-Novta et al., 2006; Wang et al., 2007; Wang et al., 2008a; Wang et al., 2008b). The NAs are very corrosive components causing equipment failures and leading to high maintenance costs in petroleum refineries. Therefore, oil refineries have been trying to remove NAs or lower their acidity through different techniques such as adsorption and esterification (Silva et al., 2007; Wang et al., 2008b). In summary, phytoremediation of tailing pond waters combined with biodiesel production is proposed as an eco-friendly, economical technique to deal with the large toxic tailing pond waters in Alberta's oil sands.

1.4 Objectives

The objectives of this thesis were to perform an empirical study of acid-catalyzed transesterification from extracted algal/NAs oil and a comprehensive study of the effects of alcohol:oil ratio, temperature, catalyst concentration and catalyst type on the reaction rate. The primary goal was to apply the extracted algal/NAs taken from Alberta's tailing ponds. In this study, we did not have access to extracted algal/NA oil samples and we therefore used canola oil and commercial NAs. In order to investigate the transesterification of canola oil/NAs, we decoupled the esterification of NAs from transesterification of canola oil, and developed a characterization method to calculate the conversion rate. The acid-catalyzed transesterification of canola oil/NAs was investigated afterwards.

1.5 Thesis Outline

This thesis is divided in 4 chapters. Chapter 2 consists of a manuscript for submission to the journal of Energy and Fuels. It describes the first ever study of the esterification of NA with methanol at the temperature in the range 70-90°C. In Chapter 3, NA is combined with a model triglyceride (i.e., canola oil) in an investigation of the effects of various factors on the esterification/transesterification of NA and canola oil. The contents of this chapter are to be submitted to the journal of Environmental Science and Technology for publication. A general discussion and conclusions are presented in Chapter 4. The thesis also includes an appendix which presents the safety information on chemicals used in this study and challenges with respect to the monitoring of NAs esterification reaction.

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Chapter 2

Paper I

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Acid–Catalyzed Esterification of Naphthenic Acids

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Abstract

The reclamation of mining operation tailings water is of major concern to the oil sands industry. Naphthenic acids (NAs) are recognized as the major toxic component in the tailings water. The study of the acid-catalyzed esterification of commercial NAs to biodiesel was performed as a means of utilizing these toxic compounds. In a series of batch acid-catalyzed esterification reactions, the influence of reaction temperature, catalyst type, catalyst concentration, and methanol to oil ratio on the esterification was investigated. It was found that the NA esterification reactions were positively dependent on temperature and catalyst concentration. The large excess of methanol to oil ratio had a negative effect on the NA esterification reaction. Finally, sulfuric acid was identified as a preferred catalyst for the esterification compared to *p*-toluenesulfonic acid.

2.1 Introduction

Oil sands production in Northeastern Alberta has increased throughout the past decade due to technological advances and the decline of global conventional oil supplies. Oil sands operations utilize the Clark hot water process, using hot water (80°C) and NaOH, to extract bitumen from oil sands (FTFC, 1995a). The extraction procedures produce a large volume of tailings water. Naphthenic acids (NAs), natural components of crude oils, become dissolved and concentrated in tailings water as result of the Clark extraction method. These tailings are then accumulated in large settling ponds on site due to a "zero discharge policy".

NAs are complex mixtures of alkyl-substituted acyclic and cycloaliphatic carboxylic acids with the general formula C_nH_{2n+Z} O_2, where n indicates the carbon number and Z specifies a homologous series (Brient et al., 1995). NAs have been known as the most abundant toxic material in tailing ponds with a 20-120 mg/L concentration (Holowenko et al., 2001). NAs have acute and chronic toxicity to aquatic organisms (Clemente & Fedorak, 2005). An aquatic toxicity study reported 50% lethality (LC 50) after 96 hours exposure using sodium naphthenate concentrations at 25 mg/L, 50 mg/L, and 75 mg/L for chum salmon, sturgeon, and roach, respectively. Furthermore, alterations in leukocyte and glycogen levels were observed in fish exposed to NAs (Dokholyan & Magomedov, 1983).

The oil sands industry is committed to reducing the toxicity of tailing waters after ceasing oil sands operations as part of their lease agreements (FTFC, 1995b). The concentration of NAs should be reduced below toxic levels. NAs are known to be persistent to degradation. An aerobic degradation study showed that commercial NAs were completely degraded in 14 days, whereas only about 25% of NAs native to tailing waters were removed after 40-49 days (Scott et al., 2005). It is hypothesized that the microbial population in tailings water selectively degrades the least alkyl substituted NAs, leaving behind more recalcitrant, highly branched NAs (Scott et al., 2005). Photodegradation studies indicated that NAs from tailing ponds were not completely degraded by natural sunlight and fluorescent radiation with or without TiO_2 (McMartin et al., 2004; Headley et al., 2009). Headley et al. (2009) reported that the degree of degradation of NA mixtures varied with the structure and size of the compounds.

A definitive means of remediating tailing waters has yet to be determined for the oil sands (FTFC, 1995b; Rogers et al., 2002). Algae are known to sorb contaminants from aquatic environments and enhance degradation of organic compounds (Headley et al., 2008). Little information is available on the phytoremediation of crude oil and polycyclic aromatic hydrocarbons (Safonova et al., 2004). Some algal species such as Cyanobacteria (*Merismopedia ssp*), Chlorophyta (*Botryococcus braunii*) have been shown to grow under toxic conditions within tailing ponds in Northern Alberta's oil sands (Leung et al., 2001). Therefore, algae can potentially be utilized for NA removal from tailing ponds. Algae can possibly accumulate, sorb and utilize the NAs within tailing waters as a primary source of carbon.

The application of algae is not limited to phytoremediation. Microalgae are known as a promising alternative feedstock for biodiesel production. Microalgae have a higher photosynthetic efficiency, higher biomass production and faster growth compared with land crops (Minowa et al., 1995; Miao & Wu, 2006). Therefore, the cultivation of algae in tailing ponds might provide an eco-friendly means to remediate NA contaminated water. The extracted algal/NA oil obtained from filtered algae can potentially be used in biodiesel production via a transesterification/esterification reaction. The algal biomass can be utilized as a soil additive for land remediation on site afterwards. Since little information is available on NA esterification, and NAs are a mixture of carboxylic acids; it is of interest to investigate the NA esterification reaction.

Esterification is an important reaction in the chemical engineering industry due to wide application of ester products in the production of solvents, plasticizers, and surface active agents. The esterification reaction is also a main route to produce biodiesel from low-cost feedstocks with a high content of free fatty acids (FFA). Esterification of carboxylic acids with alcohol is generally carried out using sulfuric acid. Since the esterification reaction is an equilibrium reaction, a high yield of methyl esters primarily depends on the reaction temperature and on the initial alcohol to carboxylic acids ratio. The esterification reaction between carboxylic acid groups of NAs and methanol can be represented by the following:

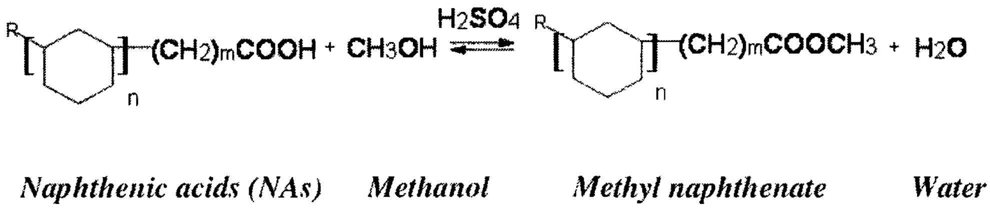

Figure 2.1 Esterification of naphthenic acid.

The acid-catalyzed esterification of NAs[4] assisted with microwave irradiation was studied with various alcohols and two different catalysts (Cirin-Novta et al., 2006). The outcomes of this study showed that the microwave procedure accelerated reactions, and the ester yields obtained with sulfuric acid were higher than those using *p*-toluenesulfonic acid (*p*-TsOH).

Veliev et al. (2009) studied the acid-catalyzed esterification of NAs[5] with acetylenic alcohols at different temperatures in the range of 80-110°C. They examined the effect of different catalyst types in the esterification reaction at an alcohol/oil molar

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[4] NAs isolated by alkine extraction form the commercial fraction of atmospheric gas oil of the Vojvodina crude oil “Velebit”.

[5] NAs obtained from kerosene-gas oil fractions of Baku crude oils (Binagadinsk oil fields).

ratio of 3:1. The results demonstrated that higher ester yields were attained using sulfuric acid compared with using p-TsOH, H_3PO_4, and KU-2x8(a type of cation-exchange resin).

Wang et al. (2008b) studied the esterification of NAs[6] with SnO at elevated temperatures (i.e., 200-320°C). The effects of several variables such as reaction temperature, the amount of catalyst, and the amount of methanol on reaction rate were examined. The results showed that the ester yields increased with increasing temperature, the amount of catalyst, and the amount of methanol. The optimal reaction conditions were determined as 300°C, methanol/oil: 5 wt%, and SnO 4 wt% (on the basis of NAs).

The purpose of this study was to investigate the acid-catalyzed esterification of NAs using methanol at different temperatures in the range 70-90°C, the typical temperature range used in biodiesel production. The effects of methanol/oil molar ratio, reaction temperature, catalyst type, and the amount of catalyst were examined on esterification reaction rate.

2.2 Experimental Section

2.2.1 Materials

Anhydrous methanol (99.8%, Sigma-Aldrich), naphthenic acids (Fluka), p-toluene sulfonic acid monohydrate (98.5%, Sigma-Aldrich) and sulfuric acid (98%, Fisher Scientific) were used as received. Molecular sieves (UOP type 3A°, Sigma-Aldrich), and benzene-d_6 (99.6 atom%, D Cambridge Isotope Laboratories Inc.) were used without further treatment for sample analysis.

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[6] NAs was the second vacuum fraction processed by Befar Corporation of China.

2.2.2 Apparatus

All experiments were carried out in a 5-L stainless-steel jacketed reactor (see Fig. 2.2). The reactor was equipped with a thermocouple port, a sampling port, a pressure gauge, and a relief valve. The heating system consisted of a 6-L heating bath, with a digital temperature controller, circulating silicon oil through the jacketed reactor at the required temperature. The reactants were mixed by a circulating pump during the course of the experiments. The pipes in the circulating circuit were insulated to minimize heat loss.

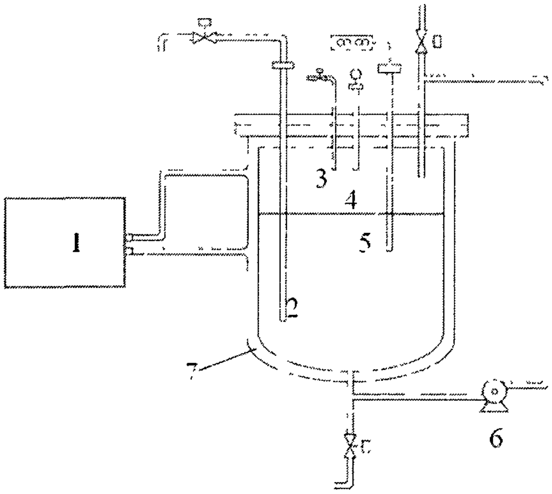

Figure 2.2 Reactor schematic: 1-heating bath; 2-sampling port; 3-relief valve; 4-pressure gauge; 5-thermocouple; 6-circulating pump; 7-reactor jacket.

2.2.3 Reaction and Sampling Procedures

The following procedure for the reactions at a given temperature (70, 80, 90°C) was used. Initially, a predetermined amount of methanol and sulfuric acid, as shown in Table 1, were mixed at room temperature and charged into the reactor. The specified amount of naphthenic acids (NAs) (see Table 2-1) was then added to the reactor (see Appendix A-1). The circulating pump and the heating bath were turned on. Temperature was raised to the desired temperature over a period of 1 h and maintained at this temperature for an additional 5 h (see Appendix A-2). The reactor pressure gradually increased during the experiments. The pressure varied from 15 to 20 psig (103.42 to 137.89 kPa), 23 to 28 psig (158.58 to193.05 kPa), and 32 to 37 psig (220.63 to 255.11 kPa) for the 70, 80, and 90°C runs, respectively.

Table 2-1 Feed compositions

MeOH (mol %)	NA (mol %)	*Catalyst (mol %)	MeOH:NA:Acid (molar ratio)	Volume (L)
93	6.64	0.35	14:1:0.05	1.6
92.7	6.6	0.7	14:1:0.1	1.6
98.4	1.28	0.35	77:1:0.274	1.69
98	1.3	0.7	77:1:0.55	1.69

*Sulfuric acid/ *p*-toluene sulfonic acid

Samples of 10 mL were taken from the reaction mixture at regular intervals. The reactions in the samples were immediately quenched by diluting with 5 mL distilled-deionized water. Time was allowed for phase separation in the sample vials. The upper layer mostly contained methyl naphthenate and unreacted NAs; while the excess methanol, catalyst, and water formed the lower layer. The upper layer was separated and transferred to a new vial. Since NAs are completely miscible in methanol, the unreacted NAs were distributed between the two phases. In order to prevent the loss of unreacted NAs in the separation process, the lower layer was heated at 50°C on a hot plate to evaporate the methanol. The oil phase was then retained. After the completion of the separation process, the ester mixture layer was washed 4 times with 5 mL distilled-deionized water in order to neutralize the sample. The samples were then put in a fume hood overnight to remove any residual methanol.

2.2.4 Product Analysis

The extent of esterification of NAs was determined by ^{1}H-NMR spectroscopy (see Appendix A-3). Fifty milligrams of each sample was dried over a few molecular sieve beads and dissolved in 6 mL of benzene-d_6. ^{1}H-NMR spectra were obtained at room temperature on a Bruker AVANCE 400 MHz; the final spectrum was the average of 16 scans. The relevant absorption peaks were chosen for integration. The carboxylic acid proton (-COOH) in NAs appeared as a broad peak (10.5-13 ppm), while the methoxy protons ($-COOCH_3$) in methyl naphthenate emerged as a singlet (3.4-3.5 ppm) as shown in Figure 2.3. The conversion was calculated directly from the integrated areas of these peaks using:

$$^{7}\text{Conversion} = \frac{(\text{COOCH}_3/3)}{[(\text{COOCH}_3/3)+\text{COOH}]}\times 100 \qquad (1)$$

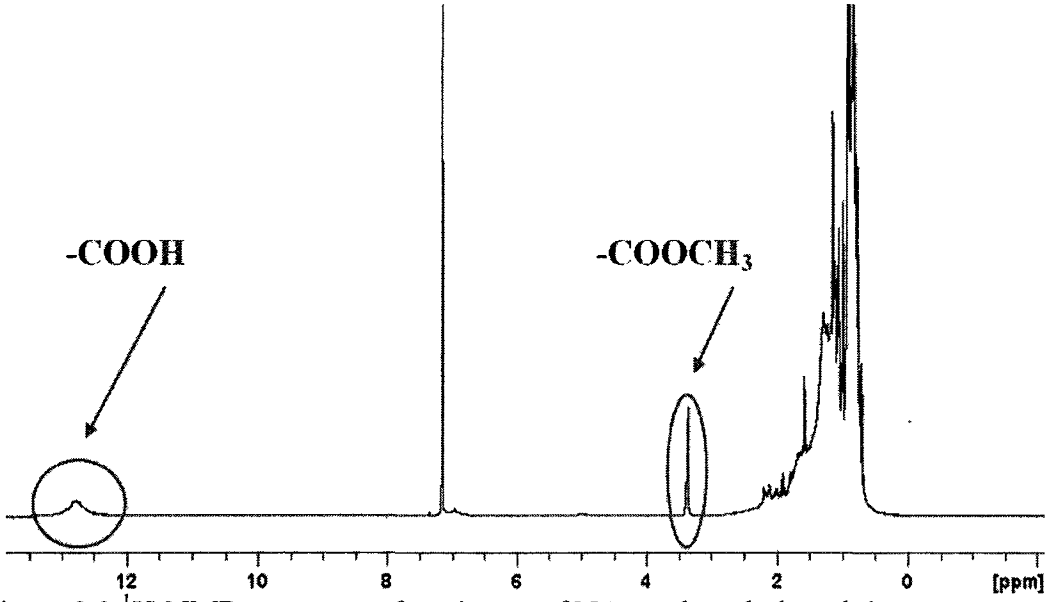

Figure 2.3 ^{1}H-NMR spectrum of a mixture of NAs and methyl naphthenate.

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[7] A 3% error, solely based on the analytical errors, was considered in all calculations.

2.3 Results and Discussion

The effect of reaction temperature on NA esterification was investigated at 70, 80 and 90°C while keeping the molar ratio of methanol to NAs at 14 and the sulfuric acid concentration at 0.7 mol%. Figure 2.4 shows the experimental conversion profile for each temperature. As expected, raising the temperature increased the esterification reaction rate. By increasing the temperature from 70 to 80°C, the reaction rate increased substantially and the final conversion increased from 76 to 95%. However, no significant change in the reaction rate was noted when increasing the temperature from 80 to 90°C, with a final conversion of 97% after 6 h in the 90°C run. This trend can be attributed to the complexity of molecular structure of NAs. It is likely that the short-chain NAs and simple cyclic NAs were converted to methyl esters easily throughout the first hour of reaction while the highly branched and cyclic NAs required higher temperatures than 90°C for methyl transformation. Therefore, all subsequent experiments were carried out at 80°C.

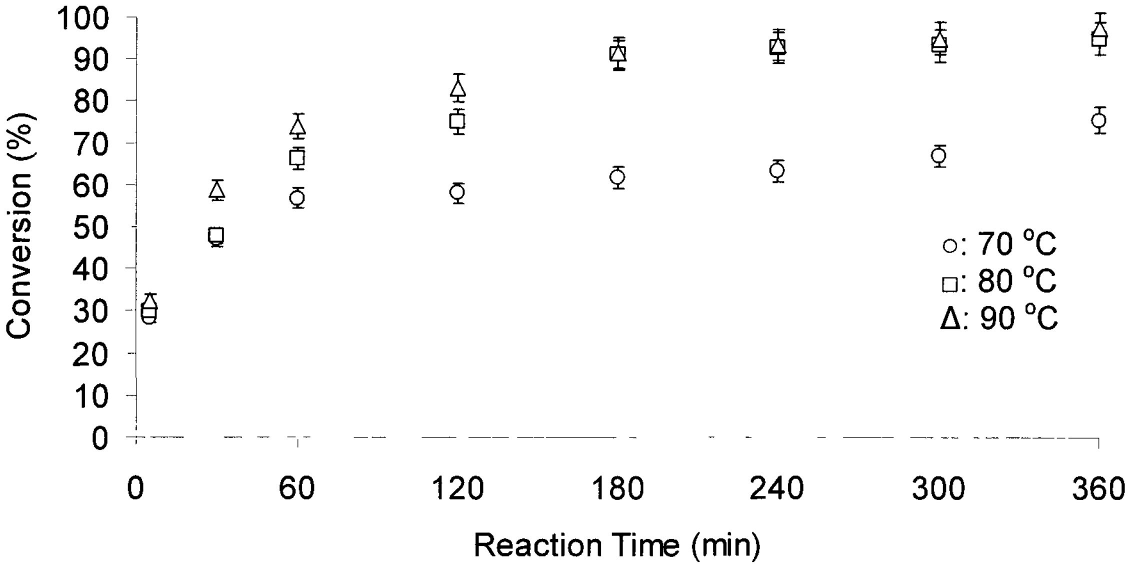

Figure 2.4 Effect of reaction temperature on NA esterification reaction: methanol/NAs=14, [sulfuric acid] =0.7 mol%.

The effect of catalyst concentration on the esterification reaction is shown in Figure 2.5. To investigate the influence of catalyst concentration, two different sulfuric acid concentrations (0.35 and 0.7 mol%) were tested at 80°C along with a molar ratio of methanol to NA of 14/1. After a 6 h reaction time, 73 and 95% conversions were obtained for the 0.35 and 0.7 mol% sulfuric acid concentrations, respectively. The yield of ester was positively affected by increasing the amount of catalyst. The acidic proton released from sulfuric acid catalyzed the esterification reaction through protonation of the carboxylic moiety of the NAs. Therefore, increasing the sulfuric acid concentration produced more H^+ available to catalyze the reaction.

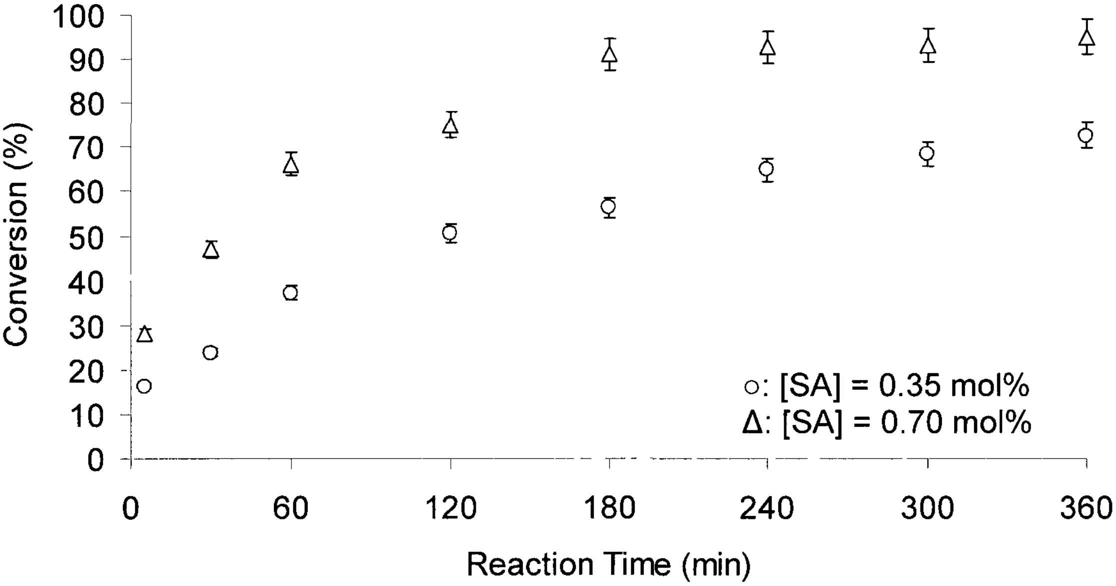

Figure 2.5 Effect of sulfuric acid concentration on NA esterification reaction: T=80°C, methanol/NAs=14.

Tesser et al. (2005) studied the esterification of oleic acid with methanol. They found that the esterification reaction rate was directly proportional to the catalyst concentration. Many studies also suggest that the esterification reaction rate constant is linearly dependent on catalyst concentration (Altiokka & Citak, 2003; Ali et al., 2007; Lucena et al., 2008). The data shown in Figure 2.5 are consistent with these reports.

Para-toluene sulfonic acid (*p*-TSA) is utilized as a strong organic acid catalyst in esterification reactions, and it has advantages over mineral catalysts. Unlike sulfuric acid, *p*-TSA catalyzes the esterification reaction without oxidation and discoloration (Scott & Gaulding, 1976). The esterification of NAs was studied at two different *p*-TSA concentrations (0.35 and 0.7 mol%) while keeping the molar ratio of methanol to NAs at 14 at a reaction temperature of 80°C. Figure 2.6 shows the conversion of NAs as a function of reaction time for both catalyst concentrations. The final conversion with 0.35 mol% *p*-TSA was 55% after 6 h while that with 0.70 mol% *p*-TSA reached 58%. This two-fold increase in *p*-TSA content did not greatly affect the esterification. This was likely due to the moisture content of the *p*-TSA. The *p*-TSA used in this study was a monohydrate. It is likely that the water content in the *p*-TSA deactivated the reaction. Thus, increasing the amount of *p*-TSA also increased the amount of moisture in the reaction mixture and, not surprisingly, the esterification rate did not increase. Similar effects were reported by (Hu et al., 1993) who showed that in the reaction of propionic acid and isobutyl alcohol, anhydrous phosphotungstic acid was three times more effective than polyhydrate phosphotungstic acid as catalyst.

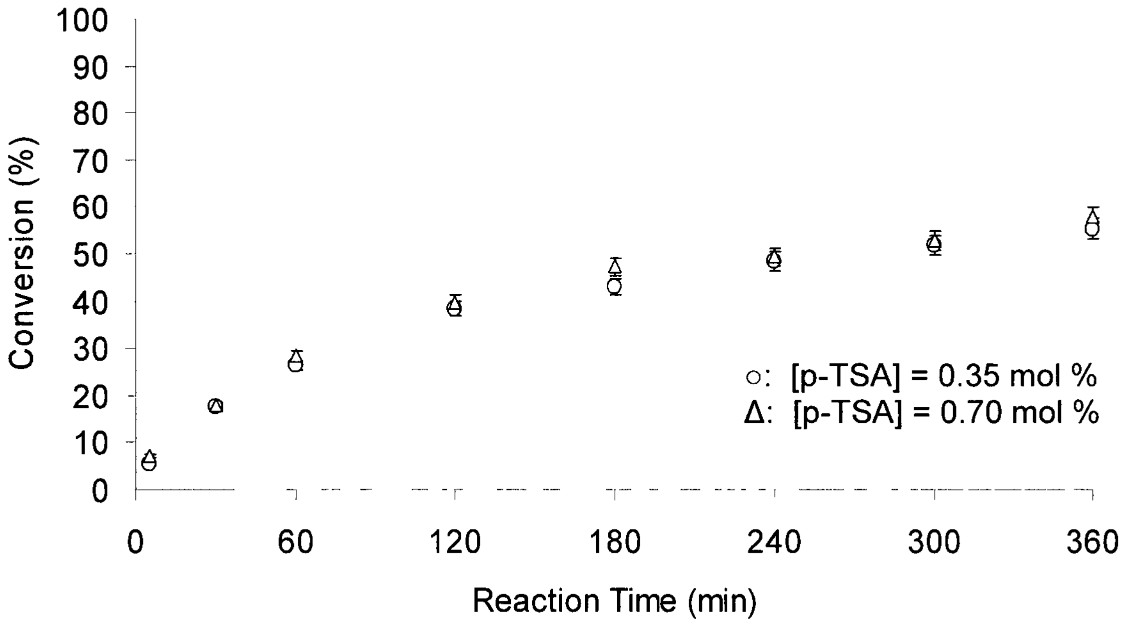

Figure 2.6 Effect of p-TSA concentration on NA esterification reaction: T = 80°C; methanol/NA = 14.

A non-catalyzed experiment was performed at a methanol to NA molar ratio of 14:1 at 80°C. This was done to compare the esterification reaction rate of the non-catalytic with the catalytic reactions. After 6 h, conversion reached only 2% and it can be concluded that for all intents, the esterification did not proceed without catalyst at the tested conditions (see Figure 2.7).

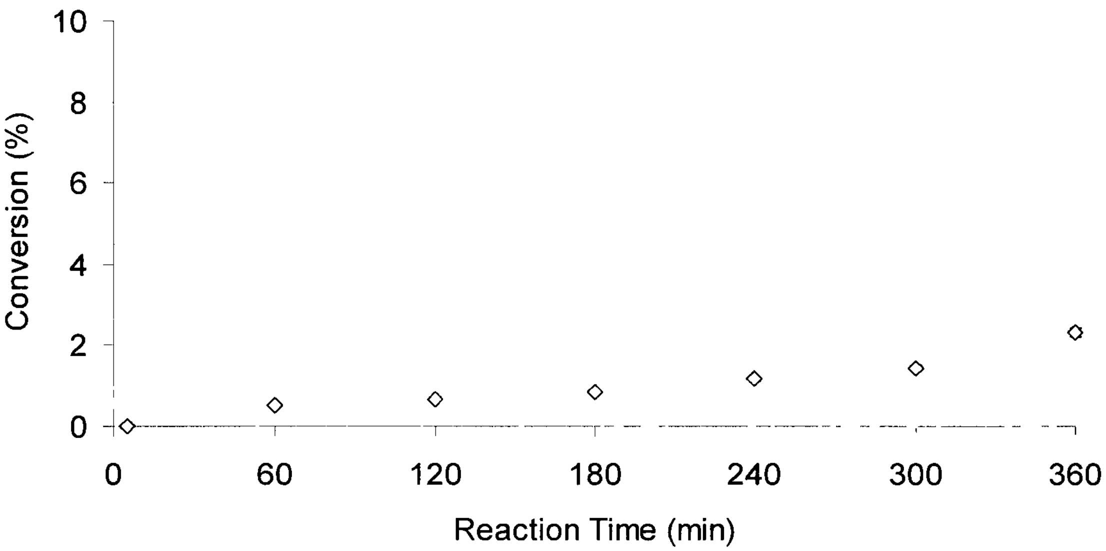

Figure 2.7 Non-catalyzed esterification of NAs with anhydrous methanol; T = 80°C, methanol/NA = 14.

Comparing the results of the NA esterification using equimolar amounts of sulfuric acid and *p*-TSA, as shown in Figures 2.8 and 2.9, we observe that the sulfuric acid-catalyzed esterification achieved a higher conversion than *p*-TSA. The reason is that sulfuric acid releases H^+ at concentrations twice that of *p*-TSA. Some studies reported that in the acid-catalyzed esterification reaction, the amount of catalyst should be determined by considering the number of acidic protons that each catalyst bears (Tmenov et al., 1977; Sepulveda et al., 2005; Cardoso et al., 2008). Furthermore, Cirin-Novta et al. (2006) studied the esterification of NAs using a microwave technique. They found that the final ester yield obtained by p-TSA was lower than that obtained by sulfuric acid. Veliev et al. (2009) studied the esterification of NAs with acetylenic alcohols using different heterogeneous and homogenous catalysts. They also found that sulfuric acid was more effective than *p*-TSA. In summary, Table 2.2 shows that the esterification of NAs is highly dependent on the amount of catalyst and the number of acidic protons released by the catalyst.

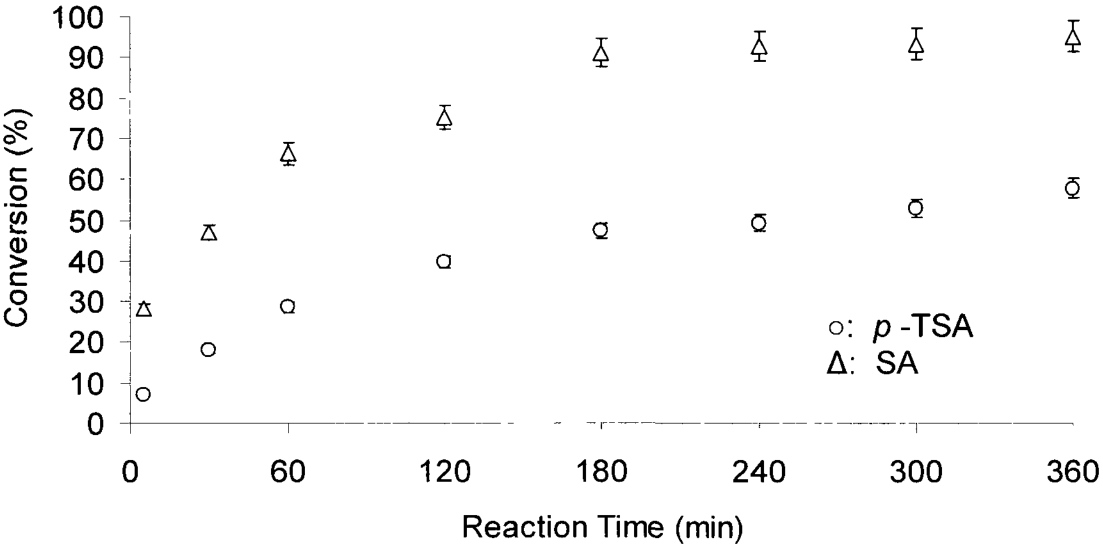

Figure 2.8 Effect of catalyst type on NA esterification reaction: T = 80°C; catalyst concentration = 0.7 mol%; methanol/NA = 14.

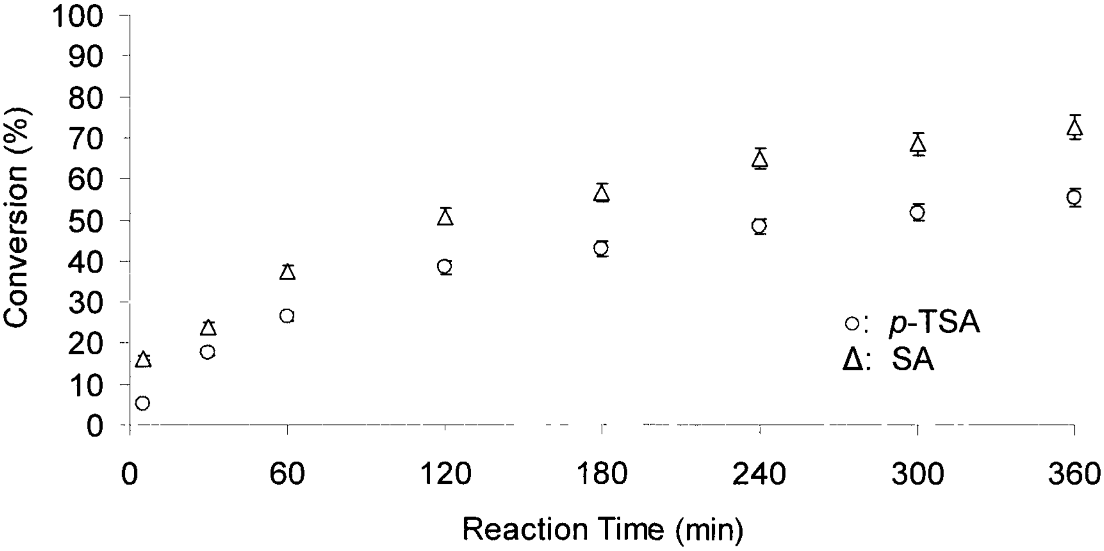

Figure 2.9 Effect of catalyst type on NA esterification reaction: T = 80°C; catalyst concentration = 0.35 mol%; methanol/NA = 14.

Table 2-2 The effect of catalyst concentration and catalyst type on the esterification of NAs

	Ester yields at 5 min (%)	Ester yields at 360 min (%)
Non catalytic	0	2
Sulfuric acid 0.35%	16	73
p-TSA 0.35%	5	55
Sulfuric acid 0.7%	28	95
p-TSA 0.7%	7	59

The effect of the molar ratio of methanol to NA on the esterification reaction was studied at two different sulfuric acid concentrations (0.35 and 0.7 mol%) at a reaction temperature of 80°C. The molar ratio of methanol to NA was increased from 14 to 77 for these experiments. As expected, the NA conversion increased with increasing sulfuric acid content as shown in Figure 2.10. The final conversion with 0.35 mol% sulfuric acid was 50% while that with 0.7 mol% sulfuric acid was 64%. The results of these experiments along with those experiments performed at methanol/NA molar ratios of 14 are shown in Figures 2.11 and 2.12. The results indicate that the NA conversion decreased by increasing the molar ratio of methanol to NA. In the NA esterification, all reactant components were fully miscible and reaction occurred in a single phase. Therefore, increasing the molar ratio of methanol to NA decreased the interactions between acidic protons and NA molecules (essentially, a dilution effect) resulting in a lower conversion.

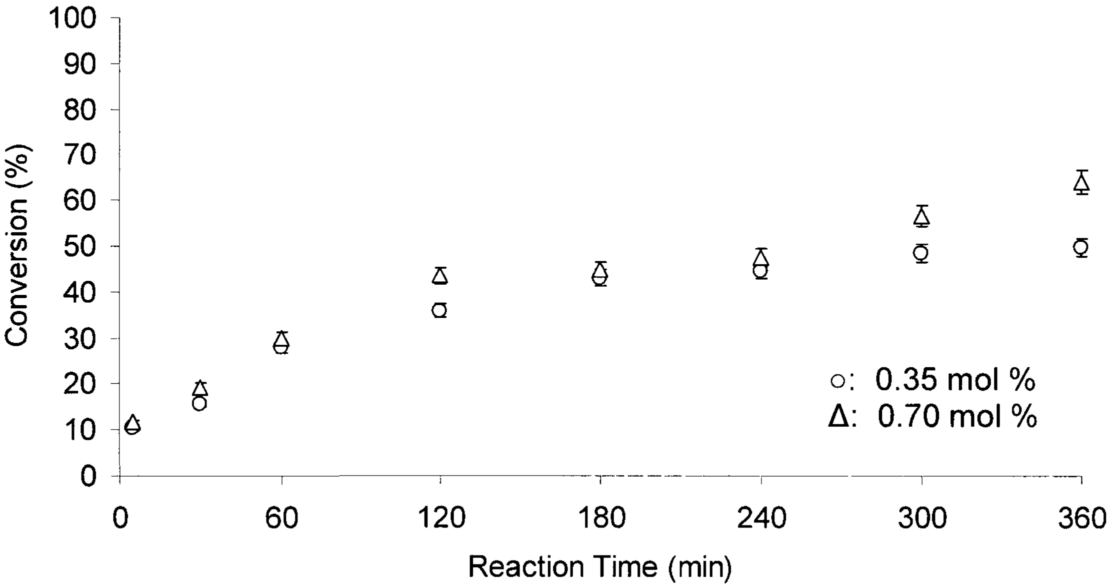

Figure 2.10 Effect of catalyst concentration on NA esterification reaction: T = 80°C; methanol/NA = 77.

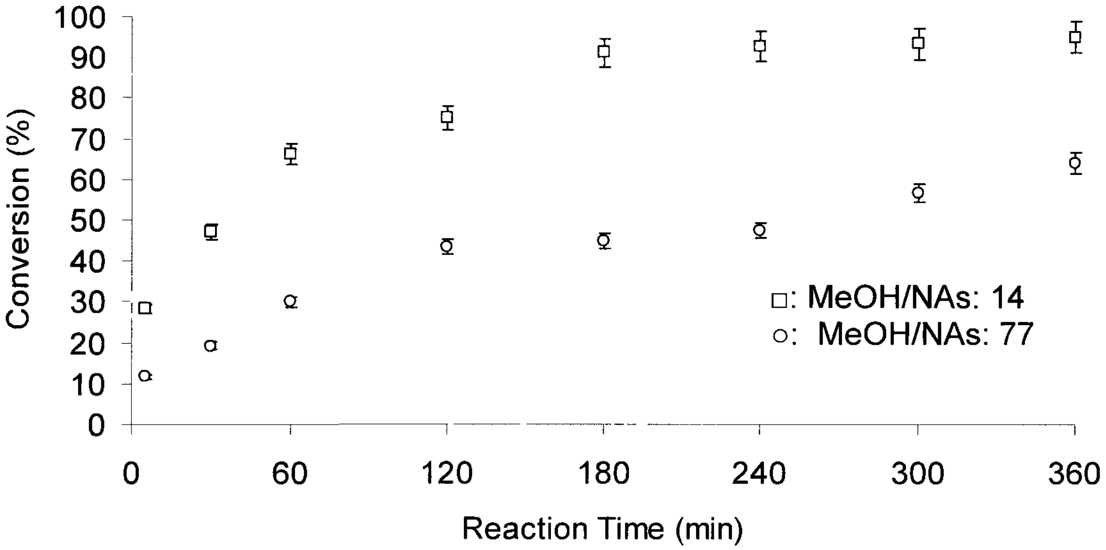

Figure 2.11 Effect of molar ratio of methanol to NA on esterification reaction: T = 80°C; [sulfuric acid] = 0.7 mol%.

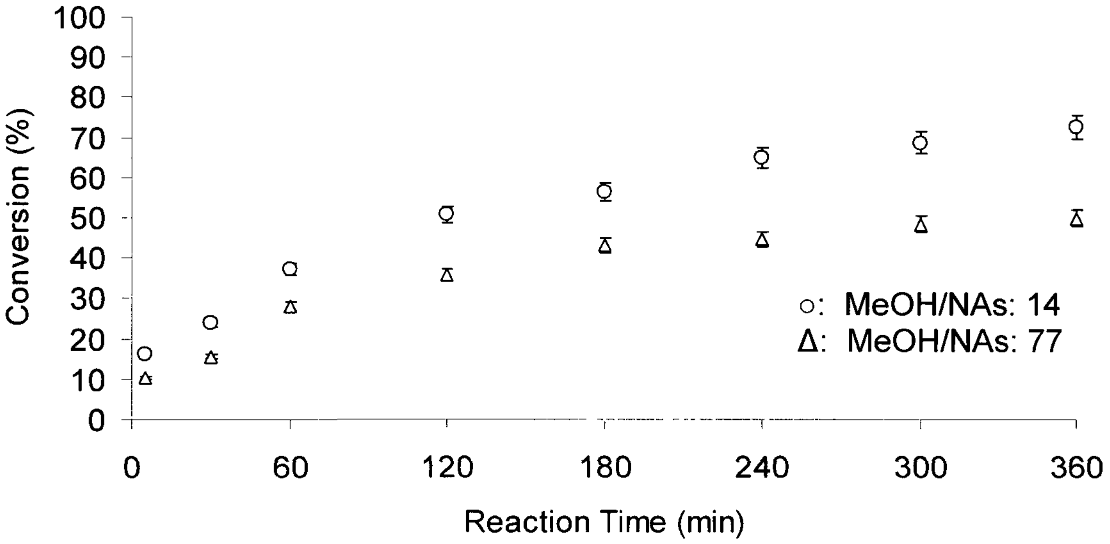

Figure 2.12 Effect of molar ratio of methanol to NA on esterification reaction: T = 80°C; [sulfuric acid] = 0.35 mol%.

2.4 Conclusions

The acid-catalyzed esterification reaction of NAs with methanol was carried out in a batch reactor at different operating conditions. The operating conditions varied were reaction temperature, catalyst concentration, catalyst type, and methanol:oil ratio. The influences of these variables were examined on the NAs esterification.

As expected, the reaction rate increased with temperature and the final ester production at 90°C was substantially higher than that at 70 °C. The experimental results also showed that NA esterification was insensitive to temperature beyond 80°C (up to 90°C) likely due to the higher activation energy of cyclic NA compounds. Temperatures higher than 90°C are likely required to convert the highly branched/cyclic NAs to their corresponding esters.

The sulfuric acid concentration had a significant impact on the NAs conversion rate and the yield of ester product, while *p*-TSA did not. This was due to the fact that *p*-TSA released fewer acidic protons than sulfuric acid to catalyze the esterification. Moreover, an increase in *p*-TSA concentration did not increase the esterification rate and final conversion due to its moisture content.

Increasing methanol to NA ratio had a negative influence on the esterification reaction rate due to the solubility of NAs in methanol. In conclusion, the operating conditions at 80°C with 0.7 mol % sulfuric acid and 14:1 methanol:oil ratio was considered as preferred conditions for NAs esterification reaction under the conditions studied. Given the relatively high conversions achieved, the esterification of NAs can be performed simultaneously with a transesterification reaction of vegetable or algal oils for the purposes of biodiesel production.

Acknowledgements

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Chapter 3

Paper II

Manuscript to be submitted to journal of Environmental Science and Technology

Transesterification/esterification of canola oil/naphthenic acids for methyl ester production

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Abstract

Algae have been recognized as a new alternative feedstock for biodiesel production due to their remarkable growth rate. The use of algae for remediation of surface mining tailings water is potentially an eco-friendly cleanup process. The primary contaminant in the tailings water is naphthenic acids (NAs) that could be extracted from tailing ponds using algae. The extracted algal/NAs oil can be used as a feedstock for biodiesel production. In this work, the study of biodiesel production from model compounds (canola oil/commercial NAs) was performed.

The batch acid-catalyzed transesterification/esterification of oil mixtures (canola oil/NAs) with methanol was investigated at different reaction temperatures, catalyst concentrations and methanol:oil ratios. The results showed that increasing each of the above variables had favourable effects on reaction rates and yield of the final ester product. The operating conditions at 80°C with 0.7 mol% sulfuric acid and 28:1 methanol:oil ratio were identified as preferred conditions for the transesterification/esterification reaction among the conditions studied.

3.1 Introduction

Alternative energy sources such as solar, geothermal, and biofuels have been considered increasingly due to the depletion of petroleum fuel reserves and greenhouse gas emissions. Biodiesel (liquid biofuel) is recognized as a sustainable, environmentally friendly substitute for transportation fuels. Biodiesel can be used in regular engines (as a blend or in neat form) without further engine modification. It prolongs engine life due to its lubricant characteristics (Chisti, 2007). It also has a higher flash point than petroleum-based oil which makes it safer fuel to use, transport, and store (Schwad et al., 1987; Shay, 1993).

Biodiesel is produced by the transesterification of vegetable oils/animal fats with methanol or by the esterification of free fatty acids (FFA). Currently, biodiesel can meet approximately 1% of global transport fuel demands (Brennan & Owende, 2010). Increasing oil crop production for biodiesel is infeasible due to its deleterious environmental impact and potential to escalate food prices. On the other hand, animal fats and yellow grease are limited resources that are not sufficient to meet liquid transportation fuel needs. Therefore, an alternative feedstock is required to satisfy future transportation fuel demands.

Algae have been recently recognized as a promising substitute feedstock for biodiesel production. They have a higher photosynthetic efficiency, higher biomass production and faster growth compared with land crops (Minowa et al., 1995; Miao & Wu, 2006). Moreover, large-scale production of algae, produced by open ponds or photobioreactors, has no negative impact on food prices (Chisti, 2008). Apart from the possible use of algae in biodiesel production, the application of algae in wastewater treatment has been investigated (Oswald W.J, 1988). The combination of algal wastewater treatment with biodiesel production can be considered as a profitable and eco-friendly cleanup process.

Wastewater treatment is an important concern for the oil sands industry in the Northeastern Alberta, Canada. Oil sands operations extract bitumen from oil sands through the Clark hot water technique, in which oil sands ore is mixed with steam, warm water (40-80°C) and sodium hydroxide as a conditioner (FTFC, 1995a). During the extraction process, naphthenic acids (NAs), natural components of crude oil, dissolve and release in the mixture. The process water is held in the tailing ponds due to a "zero discharge policy" (FTFC, 1995b).

The oil sands industry is committed to remediate process water tailing ponds. Some field studies have shown that NAs are the most abundant toxic component in the tailing ponds at concentrations of 20-120 mg/L (Holowenko et al., 2001). NAs have acute and chronic toxicity to aquatic organisms. An aquatic toxicity study showed that 96 hours exposure of fish to sodium naphthenate caused 50% lethality (LC 50) at 25 mg/L, 50 mg/L, and 75 mg/L for salmon, sturgeon, and roach, respectively (Dokholyan & Magomedov, 1983).

NAs are complex mixtures of alkyl-substituted acyclic and cycloaliphatic carboxylic acids. They are recognized to be recalcitrant to degradation. Although some reclamation options for tailing ponds have been investigated by the oil sands industry, no definitive means of remediation have been found to date (FTFC, 1995b; Rogers et al., 2002). Photodegradation studies indicate that NAs from tailing ponds are not completely degraded by natural light and fluorescent radiation with or without TiO_2 (McMartin et al., 2004; Headley et al., 2009). Headley et al. (2009) pointed out that the degree of degradation of NAs mixture was varied with the structure and size of the compound.

Although NAs are known for their commercial use as antimicrobial agents such as wood preservatives, some studies have indicated that microbial activity in laboratory cultures decreases the NAs toxicity (Herman et al., 1994). It is unclear whether or not NAs can be utterly degraded by microbes in the indigenous microbial population. It is believed that the microbial population selectively degrades the NAs based on their structure (Scott et al., 2005). A study reported that some algal species such as Cyanobacteria (*Merismopedia ssp*) and Chlorophyta (*Botryococcus braunii*) were able to grow under toxic conditions within tailing ponds in Northern Alberta's oil sands (Leung et al., 2001).

Although there is limited information about phytoremediation of crude oil and polycyclic aromatic hydrocarbons, some studies indicated that algae degraded phenol (Semple & Cataldo, 1996), naphthalene (Cerniglia et al., 1980), and crude oil (Raghukumar et al., 2001). Other studies also reported the removal of heavy metals by algae (Wilde & Benemann, 1993). It is believed that algae can remove NAs from tailing ponds through accumulation and/or a degradation process. If algae remove NAs from tailing waters, the extracted algal/NAs oil can be potentially utilized for biodiesel production. The algal biomass can be used as a soil additive for on site land reclamation afterwards. This proposed cleanup process for tailing ponds in the Athabasca oil sands can provide immense economic and environmental benefits.

Biodiesel is produced by transesterification. This reaction consists of transforming triglyceride into fatty methyl ester (FAME), in the presence of an alcohol (e.g., methanol,

ethanol) and a catalyst (e.g., alkali, acid) with glycerol as a major by-product. Most commercial biodiesel is made using alkaline catalysts such as sodium methoxide and potassium hydroxide due to the high reaction rate of the base-catalyzed transesterification. The base-catalyzed transesterification requires high-grade feedstock absent of water and free fatty acids (FFA) to prevent soap formation that leads to the hydrolysis of biodiesel, lower yields and significant downstream purification problems.

The use of high-quality feedstock (e.g., virgin canola oil) noticeably increases biodiesel cost (Freedman et al., 1984; Liu, 1994; Canakci & Gerpen, 1999; Zhang et al., 2003a). In order to reduce biodiesel cost, Zhang et al. (2003) recommended the acid-catalyzed transesterification of low-grade feedstock (with FFA > 6 wt%) given that virgin oil costs 2-3 times more than waste cooking oil. The acid-catalyzed transesterification reaction requires a longer reaction time and a higher temperature than the base-catalyzed reaction (Harrington, 1985; Schwad et al., 1987; Ma & Hanna, 1999). An economic assessment study, (Zhang et al., 2003) showed that the acid-catalyzed procedure with low-cost feedstock (e.g., waste cooking oil) was technically feasible, more economical, and less complex than the base-catalyzed procedure.

The variables affecting the acid-catalyzed transesterification have been investigated in several studies (Freedman et al., 1984; Canakci & Gerpen, 1999; Crabbe et al., 2001; Zheng et al., 2006). Canakci and Van Gerpen (1999) examined the acid-catalyzed transesterification of soybean oil with alcohols at temperatures below the boiling point of alcohols. The results showed that the ester yield increased with alcohol:oil ratio and 98.4% ester yield was obtained at the highest alcohol:oil ratio (30:1). Crabbe et al. (2001) studied the acid-catalyzed transesterification of palm oil at temperatures from 70 to 95°C with a methanol:oil ratio ranging from 3:1 to 23:1. By extrapolation, they claimed that 100% conversion required high molar ratios within the range of 35:1-45:1. In a study of the acid-catalyzed transesterification with waste frying oils at 70 and 80°C, results indicated that even higher molar ratios of methanol:oil in the range of 74:1 to 245:1 were required to complete transesterification (Zheng et al., 2006).

Temperature is, of course, a dominant variable in acid-catalyzed transesterification reactions. Freedman et al. (Freedman et al., 1984) investigated the butanolysis of soybean oil using 1 wt% sulfuric acid at temperatures at the range of 77-117°C. They showed that near complete conversion was achieved in a 3 h reaction time at 117°C, while comparable conversions required 20 h at 77°C. Several other studies reported a positive effect of reaction temperature on the ester product (Canakci & Gerpen, 1999; Crabbe et al., 2001). Crabbe et al. (2001) demonstrated that near complete conversion (99.7%) was achieved after 9 h at 95°C while methylation at 70°C was incomplete after 24 h.

Amongst homogeneous acid catalysts, sulfuric acid has been widely utilized in esterification reactions. In acid-catalyzed procedures using sulfuric acid, typical catalyst concentrations range from 1 to 5 wt% (Formo, 1954; Freedman et al., 1984; Freedman et al., 1986; Goff et al., 2004). Although the reaction rate depends on the catalyst concentration, very high catalyst concentrations may also promote ether formation by alcohol dehydration (Lotero et al., 2005).

The purpose of this study was to investigate the potential for acid-catalyzed transesterification/esterification of extracted algal/NA oil taken from mining operation tailing ponds. Since we did not have access to extracted algal/NA oil samples, we used canola oil and commercial NA. Corresponding to a feasibility study of NA esterification conducted by (Dastjerdi and Dubé, 2010), the methylation procedure was performed in the presence of sulfuric acid at different temperatures in the range 70-90°C. The effects of variables including methanol/oil molar ratio, reaction temperature, and the amount of catalyst on reaction rate were examined.

3.2 Experimental Section

3.2.1 Materials

Anhydrous methanol (99.8%, Sigma-Aldrich), naphthenic acid (Fluka), sulfuric acid (98%, Fisher Scientific), petroleum ether (Sigma-Aldrich), and canola oil ("no-name" brand purchased from Loblaws™ grocery store) were used as received. Molecular sieve (UOP type 3A°, Sigma-Aldrich), and benzene-d_6 (99.6 atom%, D Cambridge Isotope Laboratories Inc.) were used without further treatment for sample analysis.

3.2.2 Apparatus

All experiments were carried out in a 5-L stainless-steel jacketed reactor (see Fig. 3.1). The reactor was equipped with a thermocouple port, a sampling port, a pressure gauge, and a relief valve. The heating system consisted of a 6-L heating bath with digital temperature controller circulating silicon oil through the jacketed reactor at the required temperature. The reactants were mixed by a circulating pump during the course of the experiments. The pipes in the circulating circuit were insulated to minimize heat losses.

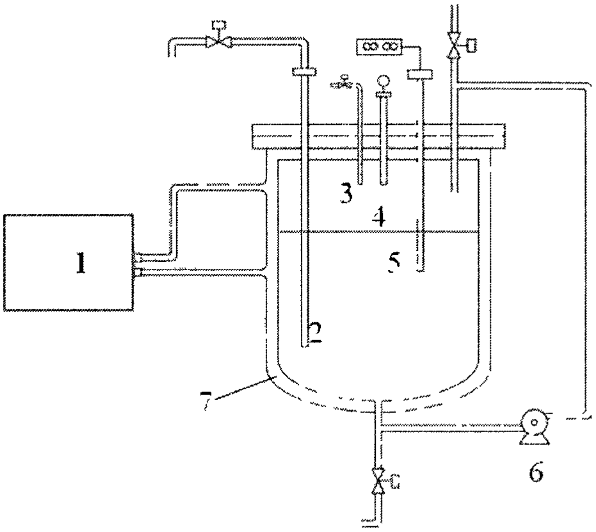

Figure 3.1 Reactor schematic: 1-heating bath; 2-sampling port; 3-relief valve; 4-pressure gauge; 5-thermocouple; 6-circulating pump; 7-reactor jacket.

3.2.3 Reaction and Sampling Procedures

The following procedure for reactions at a given temperature (70, 80, 90°C) was used. Initially, a pre-determined amount of methanol and sulfuric acid, as shown in Table 1, were mixed at room temperature and charged into the reactor. The specified amounts of canola oil and naphthenic acid (see Table 1) were then added to the reactor (see Appendix A-1). The circulating pump and the heating bath were turned on. Temperature was raised to the desired temperature in a period of 1 h and maintained at this temperature for an additional 3 h (see Appendix A-2). The reactor pressure gradually increased during the experiments. The pressure varied from 15 to 20 psig (103.42 to 137.89 kPa), 23 to 28 psig (158.58 to193.05 kPa), and 32 to 37 psig (220.63 to 255.11 kPa) for the 70, 80, and 90°C runs, respectively.

Table 3-1 Feed Compositions

MeOH (mol %)	*Oil (mol %)	Sulfuric acid (mol %)	MeOH:oil:acid (molar ratio)	Volume (L)
96.2	3.42	0.35	28:1:0.10	1.99
95.87	3.43	0.7	28:1:0.21	2
95.07	3.39	1.5	28:1:0.45	2
92.6	6.6	0.7	14:1:0.11	1.42
97.5	1.74	0.7	56:1:0.41	3.14

*Oil: mixture of canola oil and NAs (5 wt%)

Samples of 5 mL were taken from the reaction mixture at regular intervals. The reactions in the samples were immediately quenched by diluting with 2 mL distilled-deionized water. Petroleum ether (5 mL) was then added to each sample to facilitate phase separation. Each sample vial was shaken by hand and allowed to stand for 30 min to complete phase separation. The ester mixture and remaining oil formed the upper layer; while the excess methanol, sulfuric acid, glycerol, and water formed the lower layer. The upper layer was retained and then washed 3 times with 2 mL distilled-deionized water in order to neutralize the sample. The samples were then put in a fume hood overnight to allow the evaporation of the petroleum ether and any residual methanol.

3.2.4 Product Analysis

Proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy was employed to determine the completeness of the transesterification reaction (see Appendix A-3). Fifty milligrams of each sample was dried over a few molecular sieve beads and was dissolved in 6 mL of benzene-d_6. ^{1}H-NMR spectra were obtained at room temperature on a Bruker AVANCE 400 MHz; the final spectrum was the average of 16 scans. The relevant peaks in the spectrum were chosen for integration. The glyceridic protons of the triglycerides resonated two distorted double-doublets at 4.04-4.34 ppm while the methoxy protons of the methyl esters resonated a sharp singlet at 3.4 ppm as shown in Fig. 3.2.

The glyceridic protons of the mono- and diglycerides resonated at the same region as that of the triglycerides (Gelbard et al., 1995; Knothe, 2000). The NA was a minor component in the reactant mixture, and its concentration was not high enough to be detected in the spectrum. This fact was supported by (Guillen & Ruiz, 2001) in a study of edible oils and fats. Therefore, the total conversion of canola oil along with 5 wt.% NA to their corresponding methyl esters was calculated directly from the integrated areas of glyceridic protons of the triglycerides and methoxy protons of the methyl esters using:

$$^{8}\text{Conversion} = \frac{\left(\frac{COOCH_3}{3}\right)}{\left[\left(\frac{COOCH_3}{3}\right) + 3\left(\frac{TG}{4}\right)\right]} \times 100 \qquad (1)$$

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[8] A 3% error, solely based on the analytical errors, was considered in all calculations.

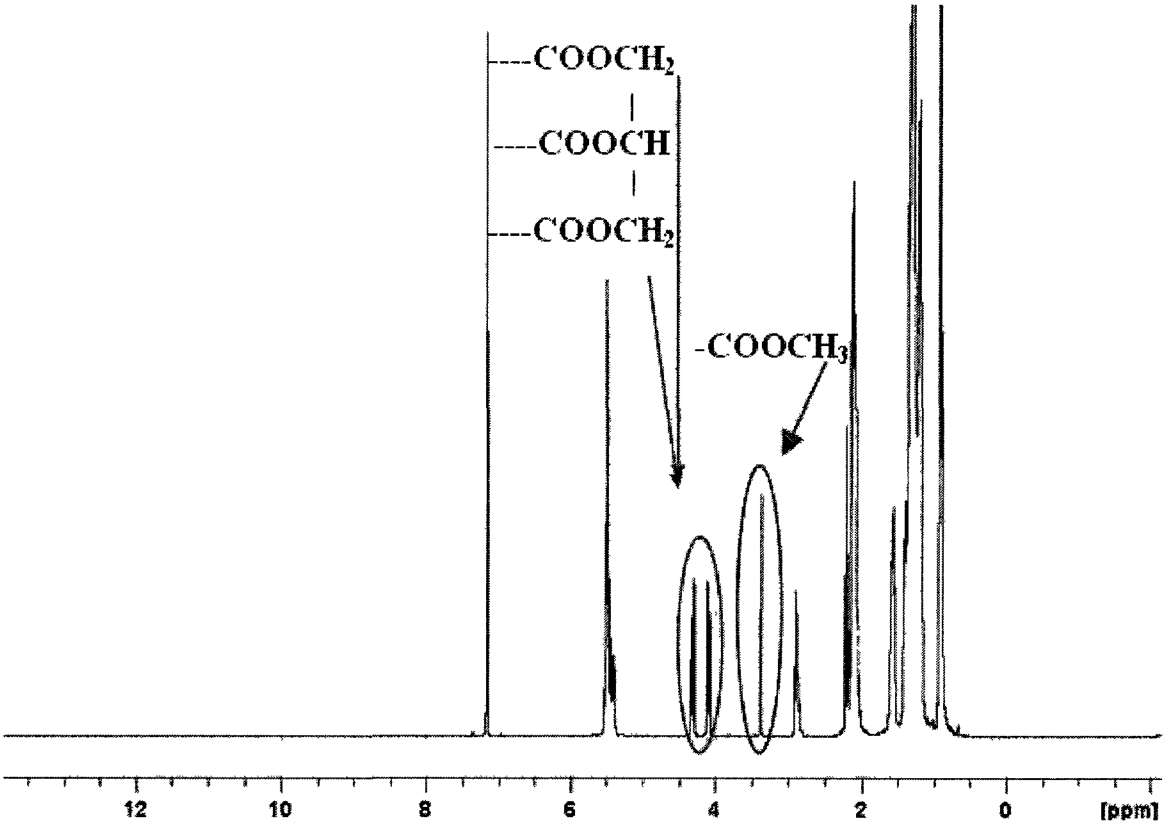

Figure 3.2 Typical ^{1}H-NMR spectrum of a transesterification reaction (spectrum recorded after 1h reaction time at 80°C).

3.3 Results and Discussion

The effect of reaction temperature on the ester yield was investigated at 70, 80 and 90°C while keeping the molar ratio of methanol to oil mixture (canola oil and 5 wt.% NA) at 28 and the sulfuric acid concentration at 0.7 mol%. Fig. 3.3 shows the experimental conversion profile for each temperature. The reaction rate increased substantially as the reaction temperature was increased; 100% conversion was obtained after 3 h at 90°C while the same conversion was obtained at 80°C after 4 h. The transesterification/esterification reaction at 70°C was incomplete, and conversion reached

55% after a 5 h reaction time. Aside from the typical kinetic effect of the temperature increase on reaction rate, the mass transfer across the boundary between the polar and non-polar phases was improved due to the higher solubility of the reactants at elevated temperatures (Noureddini & Zhu, 1997; Lotero et al., 2005). Thus, the reaction rate and the ester yield increased by increasing the temperature from 70 to 90°C.

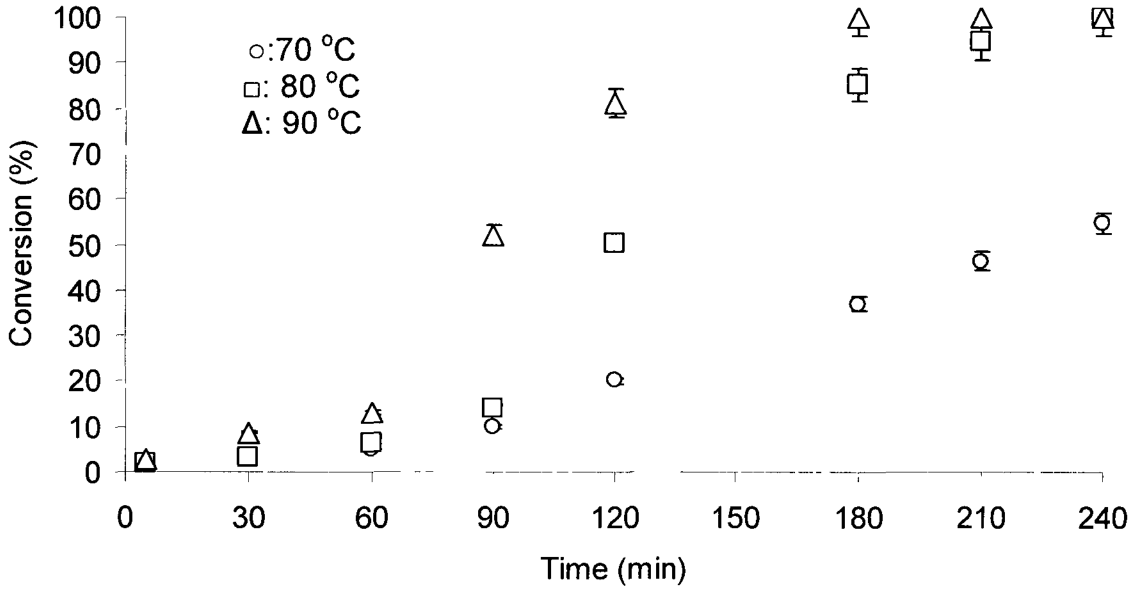

Figure 3.3 Effect of temperature on the ester yield: methanol:oil mixture = 28:1 molar, [sulfuric acid] = 0.7 mol%.

The effect of catalyst concentration on the ester yield is shown in Fig. 3.4. To investigate the influence of catalyst concentration, three different sulfuric acid concentrations (0.35, 0.7, and 1.5 mol%) were tested at 80°C using a molar ratio of methanol:oil of 28:1. In this study, reaction rate was observed to increase with catalyst concentration. The run with 1.5 mol% sulfuric acid was the fastest reaction, and it reached 100% conversion after 2 h while 100% conversion was obtained for 0.7 mol% sulfuric acid at 4 h. The run with 0.35 mol% reached 88% conversion in 4 h of reaction time. The catalyst concentration is considered as one of the most important factors for transesterification/esterification reactions (Freedman et al., 1984; Canakci & Gerpen, 1999; Zheng et al., 2006). The acidic protons of sulfuric acid catalyzed the transesterification/esterification of oil molecules (mixture of canola oil and NA) through protonation of carbonyl moieties in the oil mixture. With increasing sulfuric acid concentration, more proton acids were available to contact with carbonyl moieties resulting in the increased ester yield.

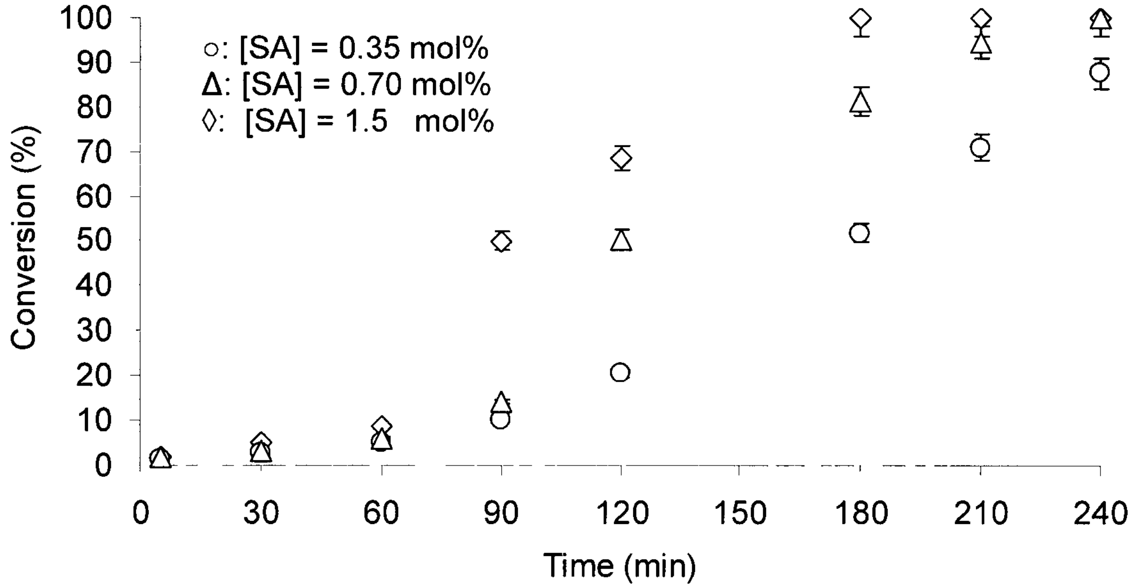

Figure 3.4 Effect of catalyst concentration on ester yield: T= 80°C, methanol:oil mixture = 28:1 molar.

Canakci and Van Gerpen (1999) studied the acid-catalyzed transesterification of yellow grease with methanol. The results showed that the reaction rate was improved by increasing the catalyst concentration, and the ester yield increased from 72.7 to 95.0% as the sulfuric acid concentration increased from 1 to 5 wt%. Goffe et al. (2004) investigated the transesterification of soybean oil with sulfuric acid at 100°C. They found that the experiment with 0.5% sulfuric acid reached considerably higher conversions in a shorter time than an experiment with 0.1% sulfuric acid.

The effect of methanol/oil molar ratio on the ester yield is indicated in Fig. 3.5. The transesterification reactions were performed at 14:1, 28:1 and 56:1 methanol:oil molar ratios while keeping the temperature at 80°C and sulfuric acid concentration at 0.7 mol%. As shown in Fig. 3.5, the ester yield increased as the percentage of methanol increased. The run with 56:1 methanol/oil molar ratio was nearly complete (99.4% conversion) in 3 h reaction time while the experiment with a 28:1 molar ratio reached 100% conversion after 3.5 h. The run with a 14:1 methanol:oil molar ratio was incomplete and 58% conversion was obtained after 4 h of reaction time.

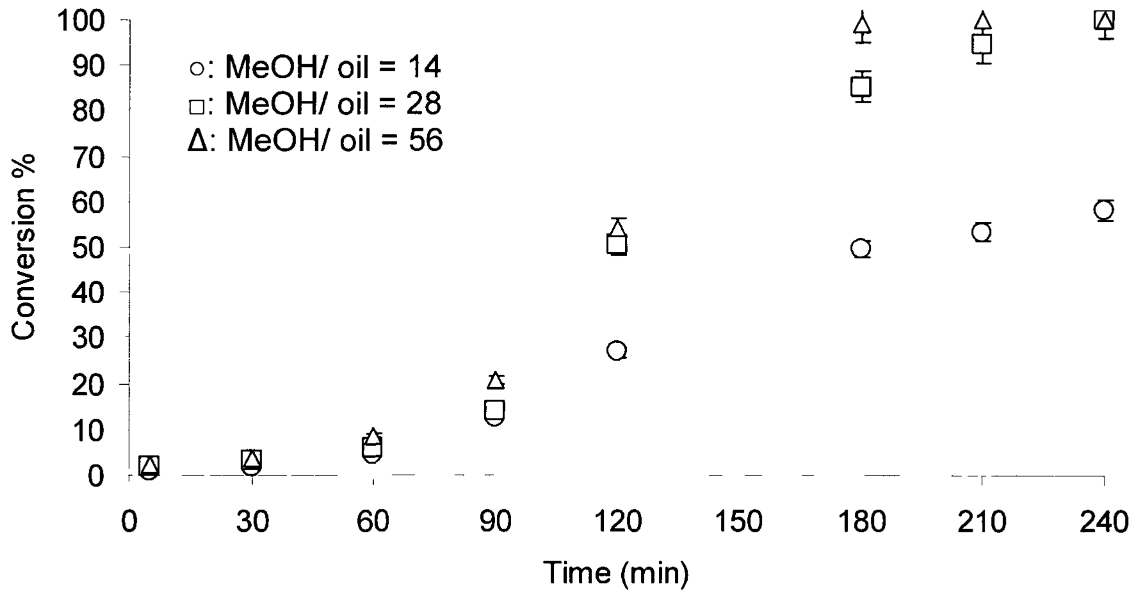

Figure 3.5 Effect of methanol:oil molar ratio on ester yield: T = 80°C, [sulfuric acid] = 0.7% mol.

The methanol:oil molar ratio is one of the most important factors affecting the methyl ester yield in transesterification reaction. The stoichiometric ratio for transesterification requires 3 mol of alcohol and 1 mole of triglyceride to yield 3 moles of methyl ester and 1 mol of glycerol. Since the transesterification is an equilibrium reaction, the excess amount of alcohol increases the ester production while shifting the equilibrium to the right. Therefore higher molar ratios result in greater ester yields in a shorter time (Freedman et al., 1984; Canakci & Gerpen, 1999; Zhang et al., 2003a).

The molar ratio is associated with the type of catalyst used in the transesterification reaction (Freedman et al., 1984; Canakci & Gerpen, 1999; Crabbe et al., 2001). Freedman et al. (Freedman et al., 1984) reported that an acid-catalyzed reaction required a 30:1 methanol/oil ratio, while an alkali-catalyzed reaction needed a 6:1 molar ratio to achieve the same conversion at a given reaction time. Mio and Qingyu (2005) investigated the acid–catalyzed transesterification of microalgal oil with methanol. The results indicated that a 56:1 molar ratio was optimal for the transesterification of microalgal oil.

3.4 Conclusions

Batch acid-catalyzed transesterification/esterification of oil mixtures (canola oil along with 5 wt% NA) with methanol was performed. Several parameters affecting methyl ester formation including reaction temperature, catalyst loading and methanol:oil ratio were investigated. Not surprisingly, increasing temperature had a favourable influence on methyl ester yield. Complete conversion was attained at 90°C after 3 h while the reaction at 70°C reached 55% conversion after 4h. The reaction was also sensitive to sulfuric acid catalyst loading. Complete conversion was achieved from the experiment with 1.5 mol% after 3 h whereas the run with 0.35 mol% reached 88% after 4 h. The influence of methanol:oil ratio as an important variable was confirmed in the present study. Increases in methanol loading resulted in higher methyl ester yields. It was found that nearly complete conversion was attained from the run with 56:1 methanol/oil ratio after 3 h reaction time while only 58% conversion was obtained from the run with 14:1 methanol/oil ratio after 4 h reaction.

As a result of these findings, the feasibility of using algal oils with NAs from tailing ponds for biodiesel production is confirmed. This approach could therefore provide immense economic and environmental benefit to the oil sands industry.

Acknowledgements

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Chapter 4

General Discussion and Conclusion

Chapter 4- General Discussion, Conclusions, Recommendations

A rapidly expanding oil sands industry in Alberta, Canada produces large volumes of tailings water containing high concentrations of NAs. NAs, a complex mixture of naturally occurring cyclic or acyclic carboxylic acids, are known as the most toxic components in the process tailings water. Reclaiming the tailings water has become a critical issue for the oil sands industry. Up to now, there is no definitive method to remediate the large settling ponds. The use of algae in the remediation of tailing waters coupled with biodiesel production might provide an eco-friendly way to reduce the environmental threat posed by NA toxicity and the oil sands operations in Northeastern Alberta, Canada. The extracted algal/NAs oil can be utilized for biodiesel production. Subsequently, the algal biomass can be used as a soil additive for land remediation after oil sands mining. Since we did not have access to algal/NAs oil collected from Athabasca's tailings water during the time of this study, we used mixtures of canola oil and commercial NAs.

4.1 General Discussion

The objectives of this thesis were to study the acid-catalyzed transesterification/esterification of canola oil/commercial NAs mixtures and to investigate the variables affecting the transesterification/esterification reaction. The question we wished to answer is: Is it feasible to convert algae oils containing NAs to biodiesel? Because NA is a complex mixture of cyclic and acyclic carboxylic acids and little information is available on NA esterification, we decided to examine NA question we wished to answer is: Is it feasible to convert algae oils containing NAs to biodiesel? Because NA is a complex mixture of cyclic and acyclic carboxylic acids and little information is available on NA esterification, we decided to examine NA esterification separately. The transesterification/esterification of an oil mixture was studied afterwards.

The influence of temperature as an important factor was tested in the NAs esterification and transesterification/esterification of oil mixture. Both reactions were carried out with methanol in the presence of sulfuric acid at the temperature in the range of 70-90°C. It was found that the temperature had a favourable impact on reaction rates of both NAs esterification and transesterification/esterification of oil mixture. The final conversions increased substantially with temperature from 70 to 90°C. In transesterification/esterification of oil mixtures, the positive influence of temperature can be attributed to the typical kinetic effect of the temperature and the solubility enhancement of reactants at elevated temperatures. However, increasing temperature from 80 to 90°C in the NAs esterification had an insignificant effect on the final conversion of NAs to esters likely due to the cyclic molecular structure of the NAs. It is likely that cyclic NAs molecules with more ring numbers (greater Z in C_nH_{2n+Z}) required a higher temperature than 90°C to break their bonds and form new bonds to make ester products.

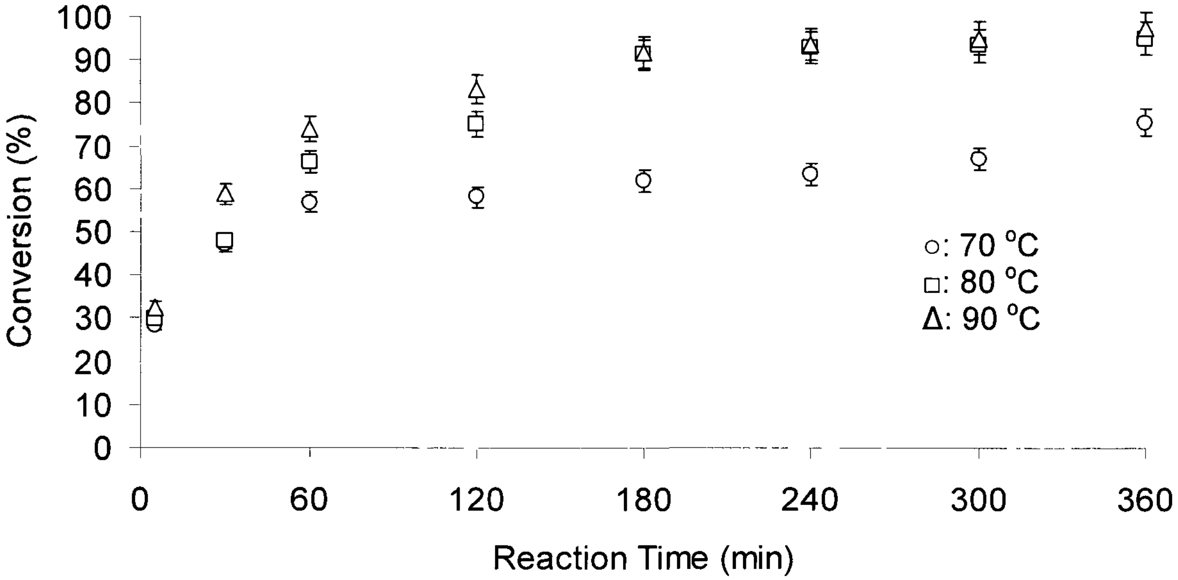

Figure 4.1 Effect of reaction temperature on NA esterification reaction: Methanol/NAs=14, [sulfuric acid] = 0.7 mol%.

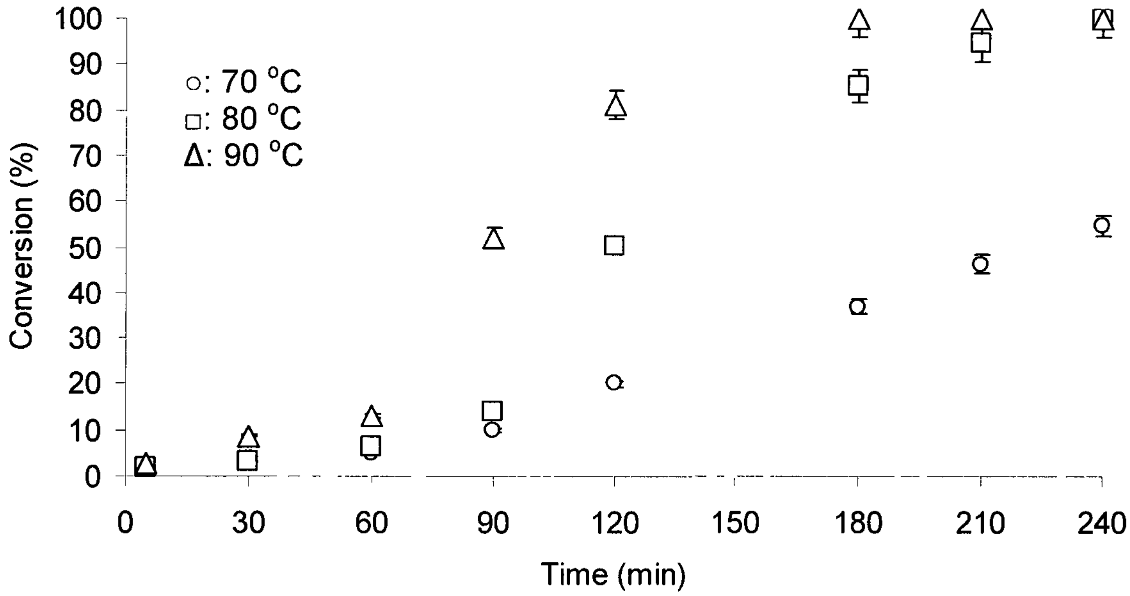

Figure 4.2 Effect of temperature on the ester yield: methanol:oil mixture = 28:1 molar, [sulfuric acid] = 0.7 mol%.

The variations in the sulfuric acid concentrations showed that NAs esterification and transesterification/esterification of oil mixtures were sensitive to the amount of catalyst. The reaction rates and the final ester products were increased as catalyst concentration increased in both reactions. The acidic protons released by sulfuric acid catalyzed esterification/transesterification through protonation of carbonyl moieties in the NAs and triglycerides molecules. Thus, increasing sulfuric acid concentration made more H^+ available to catalyze reactions.

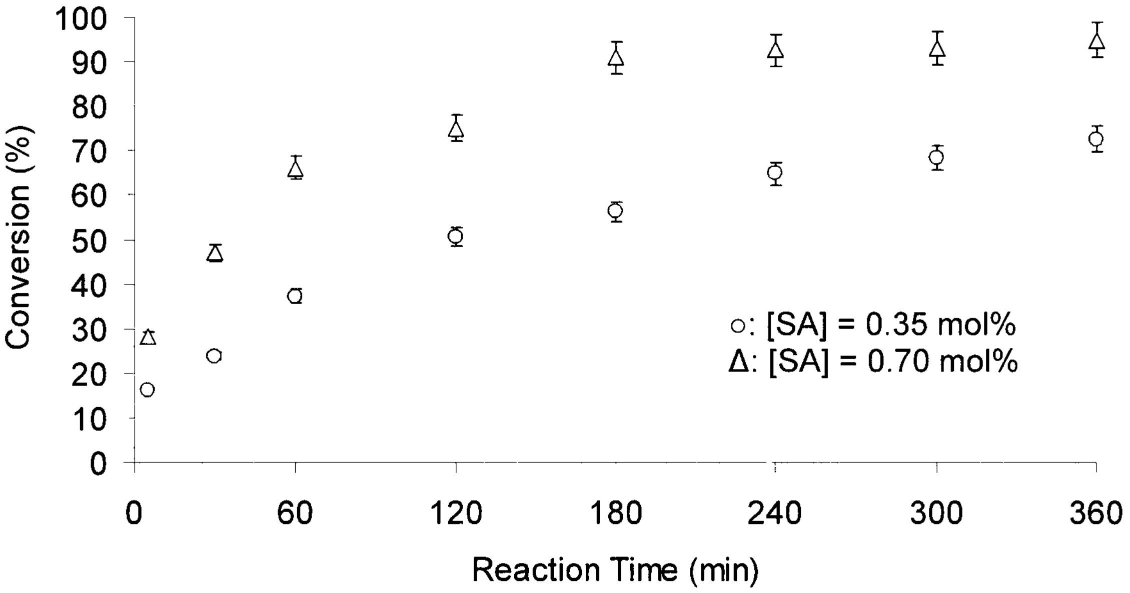

Figure 4.3 Effect of sulfuric acid concentration on NA esterification reaction: T = 80°C, methanol/NAs = 14.

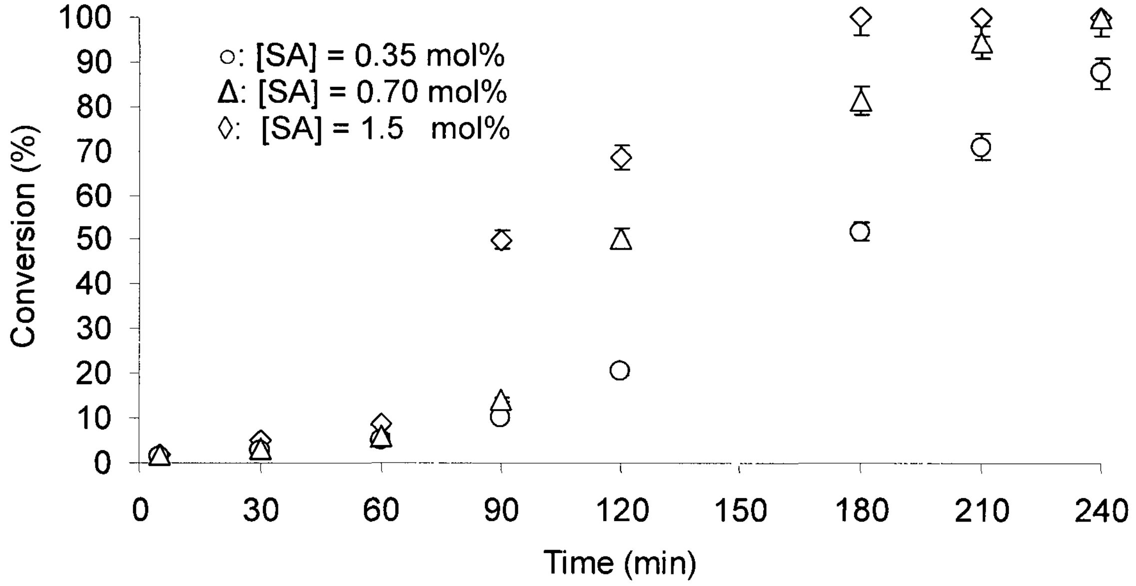

Figure 4.4 Effect of catalyst concentration on ester yield: T= 80°C, methanol:oil mixture = 28:1 molar.

In order to study the influence of methanol percentages in the NAs esterification and transesterification/esterification of oil mixtures, the methanol to oil ratio was varied. Esterification and transesterification are equilibrium reactions and therefore, to achieve high final conversions an excess amount of alcohol is required. The results of acid-catalyzed transesterification/esterification of oil mixtures showed that reaction rate and the final conversion were highly dependent on the amount of methanol loadings. The transesterification/esterification with higher methanol percentages were completed faster than those with lower methanol loadings, and the reaction with the lowest methanol percentage was not complete after 4 h reaction time. Experimental results of NAs esterification showed that increasing the methanol:oil ratio from 14:1 to 77:1 decreased the methyl ester yield considerably. NAs were dissolved in methanol. Consequently, the interactions between NAs and methanol decreased with increasing methanol concentration. The dilution effect in the system was found to be the main reason for decreasing the methyl ester yield.

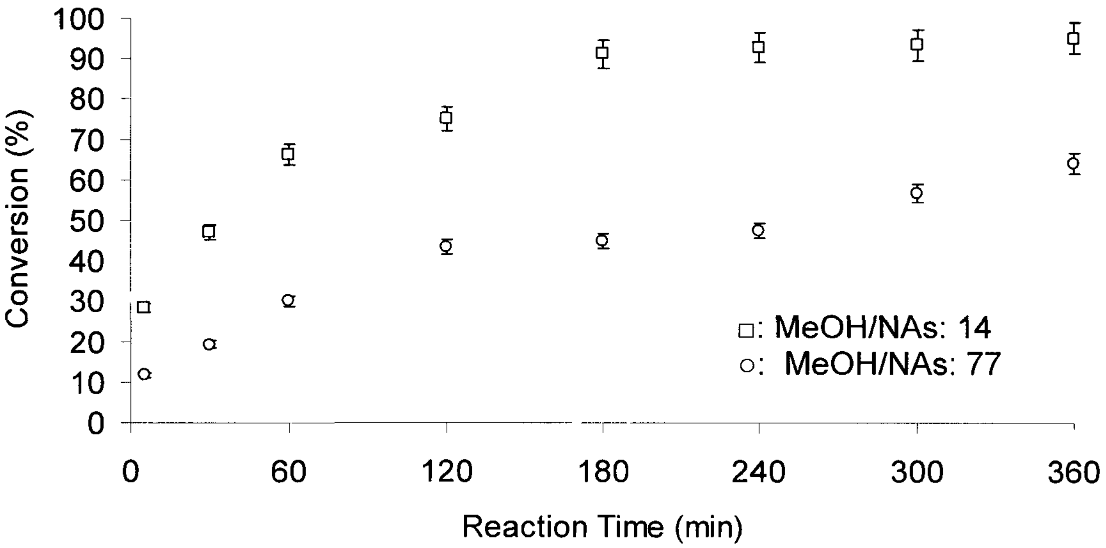

Figure 4.5 Effect of molar ratio of methanol to NA on esterification reaction: T = 80°C; [sulfuric acid] = 0.7 mol.

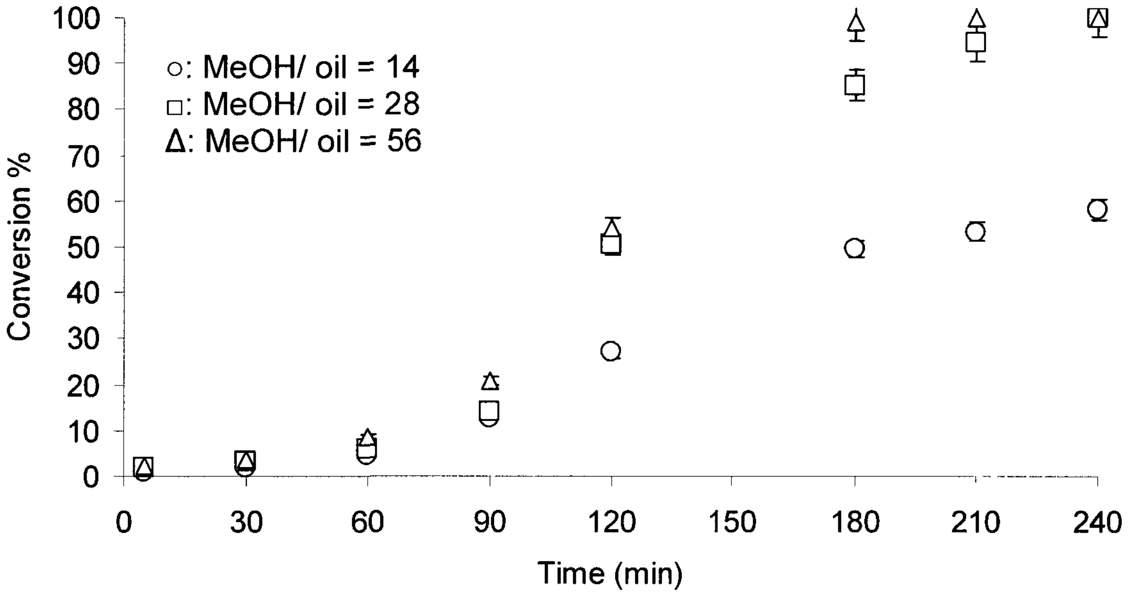

Figure 4.6 Effect of methanol:oil molar ratio on ester yield: T = 80°C, [sulfuric acid] = 0.7% mol.

4.2 Conclusions

The outcomes of this research confirm the feasibility of using algal oils with NAs from tailing ponds for biodiesel production. Apart from biodiesel production from algae, algae are able to decline the amount of CO2 released from oil sands operation via the exhaust gas. In addition, the residual biomass of algae after biodiesel production can be utilized for land remediation on site. Therefore, the biodiesel production from extracted NAs/algal oils could provide immense economic and environmental benefit to the oil sands industry.

4.3 Recommendations

The following recommendations are proposed for future study.

- As discussed, NAs are complex mixtures of several compounds. This made some of the interpretation of the results and characterization of the products exceedingly difficult. Therefore, a study of NAs characterization should be carried out to construct a calibration curve using model compounds.

- Given our comments that the conversion of cyclic NA compounds would likely require higher temperatures and the reports of the use of such temperatures in the literature, a study of acid-catalyzed esterification of NAs should be completed at elevated temperatures ($>100^{o}$C) using a long-chain alcohol such as n-butanol.

- As discussed in Chapter 2, the *p*-TSA moisture content was potentially playing a role in the reaction. Thus, we recommend that a pre-treatment of the *p*-TSA should be undertaken and the anhydrous form should also be used to investigate the effect of moisture content on catalyst activity.

- Our experiments were limited due to time constraints. It would be of interest, given the strong influence of methanol concentration on reaction rate, to find an optimal methanol percentage for the NA esterification reaction. A study of acid-catalyzed esterification of NAs using methanol:oil ratios of 3:1 and 28:1 should be carried out.

- At the beginning of this project, our collaborators were to have provided us with algal oil/NA mixtures taken directly from the tailing ponds in Alberta. These were not provided early enough for us to perform such a study. Thus, the present study should be extended to include actual algal/NAs oil mixtures obtained from tailings water.

Appendix A

A.1 Important information regarding handling and storage[9]

Naphthenic acid

CAS NO	1338-24-5
Handling	Avoid contact with skin and eyes. Avoid inhalation of vapour or mist. Normal measures for preventive fire protection.
Storage	Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.
Respiratory protection	Where risk assessment shows air-purifying respirators use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirator and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).
Hand protection	Handle with gloves
Eye protection	safety glasses

Methanol

CAS NO	67-56-1
Handling	Avoid contact with skin and eyes. Avoid inhalation of vapour or mist. Keep away from sources of ignition - No smoking. Take measures to prevent the build up of electrostatic charge.
Storage	Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage. Store in cool place.
Respiratory protection	Where risk assessment shows air-purifying respirators use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirator and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).
Hand protection	Handle with gloves
Eye protection	Face shield and safety glasses

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[9] Information was obtained from Material Safety Data Sheet

Sulfuric acid

CAS NO	7664-93-9
Handling	Avoid inhalation of vapour or mist. Normal measures for preventive fire protection.
Storage	Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.
Respiratory protection	Where risk assessment shows air-purifying respirators use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirator and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).
Hand protection	Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.
Eye protection	Tightly fitting safety glasses. Faceshield (8-inch minimum).Use equipment for eye protection tested and approved under appropriate government standard such as NIOSH (US) or EN 166 (EU).

p-toluenesulfonic acid monohydrate

CAS NO	6192-52-5
Handling	Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.
Storage	Keep container tightly closed in a dry and well-ventilated place.
Respiratory protection	Where risk assessment shows air-purifying respirators use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirator and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).
Hand protection	Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.
Eye protection	Face shield and safety glasses

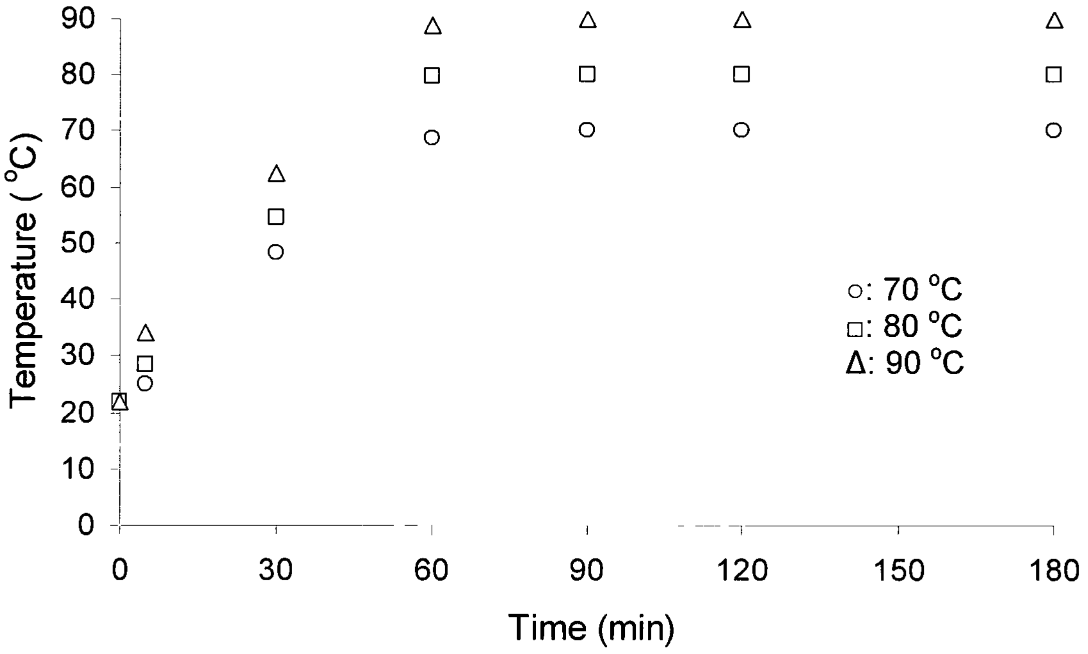
Petroleum ether

CAS NO	64742-49-0
Handling	Avoid contact with skin and eyes. Avoid inhalation of vapour or mist. Normal measures for preventive fire protection.
Storage	Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.
Respiratory protection	Where risk assessment shows air-purifying respirators use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirator and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).
Hand protection	Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.
Eye protection	Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU)

Benzene-D6

CAS NO	1076-43-3
Handling	Avoid contact with skin and eyes. Avoid inhalation of vapour or mist. Use explosion-proof equipment. Keep away from sources of ignition. No smoking. Take measures to prevent the build up of electrostatic charge.
Storage	Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage. Store in cool place. Handle and store under inert gas. hygroscopic
Respiratory protection	Where risk assessment shows air-purifying respirators use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirator and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).
Hand protection	Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.
Eye protection	Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU)

A.2 Temperature profile of heating bath



A-3 Monitoring of NAs esterification

In order to monitor biodiesel production, researchers have applied various analytical methods such as gas chromatography (GC), thin layer chromatography (TLC), and liquid chromatography-gas chromatography (Lechner et al., 1997) (Freedman et al., 1984; Plank & Lorbeer, 1995; Lechner et al., 1997; Knothe, 2001). They have tried to develop a precise, fast, and effortless analytical method to measure the yield of biodiesel production. High performance liquid chromatography (HPLC) (Noureddini & Zhu, 1997; Holcapek et al., 1999), infrared spectroscopy (IR) (Verleyen et al., 2001; Dube et al., 2004), and proton nuclear magnetic resonance (^{1}H-NMR) (Knothe, 2000; Morgenstern et al., 2006; Jin et al., 2007; Wahlen et al., 2008) have also been considered as convenient and fast techniques for monitoring the transesterification reaction.

HPLC has been widely used to analyze the transesterification reaction mixture. It can be applied without derivatization, which consumes time and reagent. HPLC provides necessary information for kinetic studies by identification and quantification of reaction components including biodiesel, triglycerides, di-/mono glycerides. Generally, a mixture of components is separated based on their hydrodynamic volume which is proportional to their molecular weights. We applied the HPLC technique to monitor the NAs esterification reaction. The chromatograph of pure NAs and reaction mixture of NA esterification is shown in Figs A.3.1 and A.3.2. A comparison of these two figures shows that there was no detectable peak related to the presence of naphthenic acid methyl esters (NAME) or to the disappearance of NAs in the reaction mixture. The retention time of pure NAs was between 28-32 min (see Fig. A.3.1) which was overlapped with the retention time of NAs/NAME (see Fig. A.3.2). Therefore, we found that HPLC was not an appropriate method to analyze the NAs esterification reaction in this study.

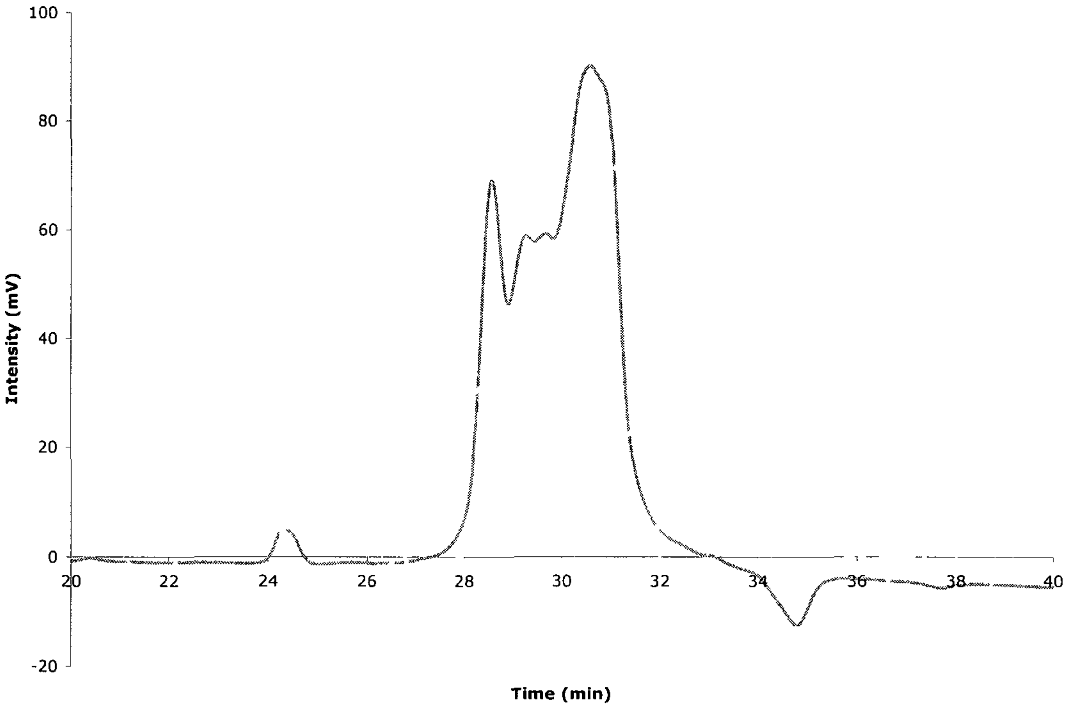


Figure A.3.1 The chromatograph of pure NA.

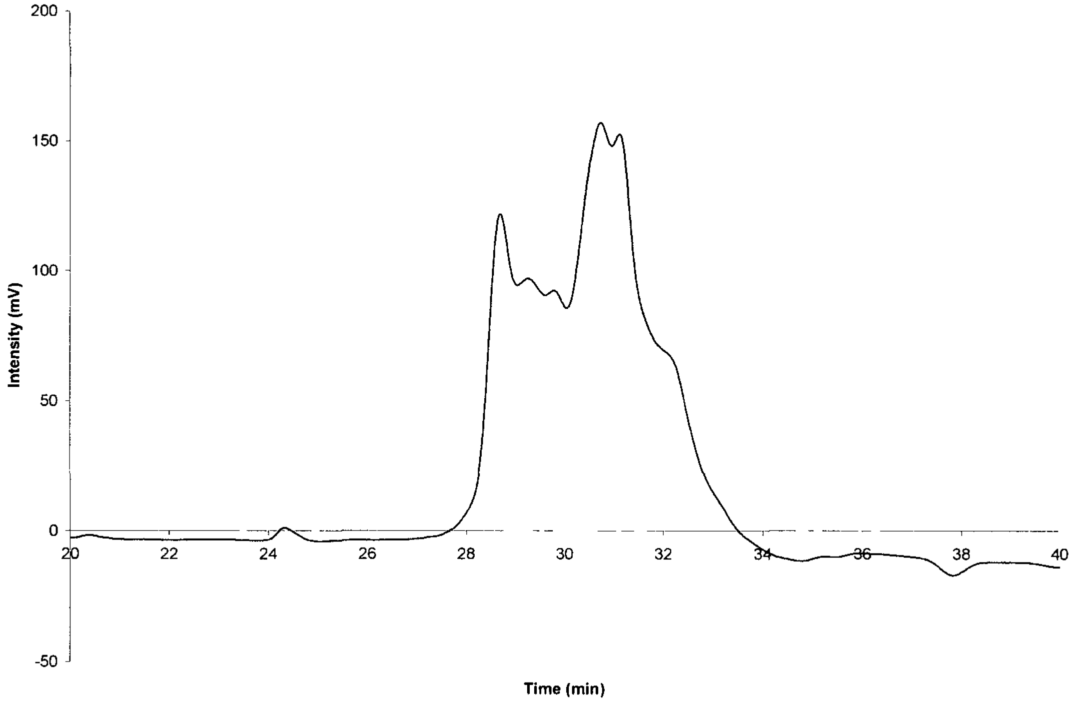


Figure A.3.2 The chromatograph of the NAME esterification reaction.

IR spectroscopy has been used to monitor the transesterification reaction by identification of characteristic peaks related to the functional groups of reaction components. According to Beer's law, the concentration of components is proportional to absorbance which can be measured by the peak height. The spectra of pure NAs along with NAs/NAME are shown in Fig. A.3.3. The sharp peak at 1699 cm^{-1} represents the C=O bond of NAs' carboxylic acid group; and the absorbance in the range of (2750-3000 cm^{-1}) is attributed to O-H bond of NAs' carboxylic acid group. Whereas the relatively broad peaks between (1735-1750 cm^{-1}) and (3000-3300 cm^{-1}) could be associated to the C=O bond of NA/NAME mixture and O-H bond of NAs' carboxylic acid group respectively. As a result, I assume there is no correlation between peaks assigned to pure NA and peaks related to NA/NAME mixture. Considering this inconsistency, we did not apply IR spectroscopy to analyze the NAs esterification in this study.

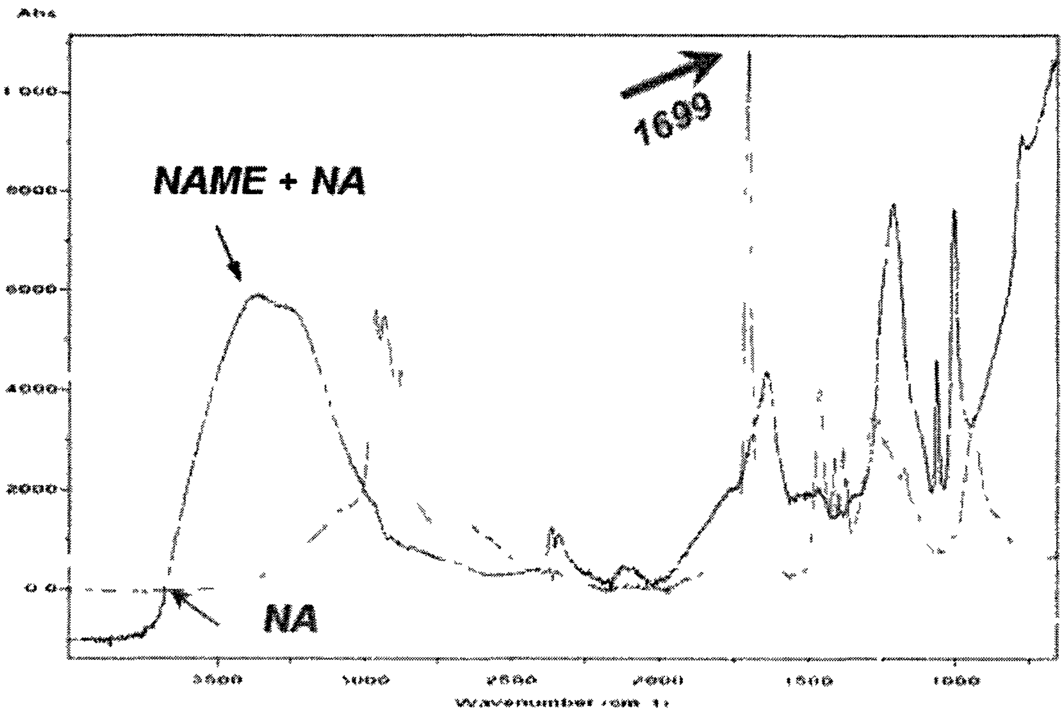


Figure A.3.3 IR spectra of NA and NA/NAME.

^{1}H-NMR spectroscopy has been used to determine the yield of transesterification reactions. In ^{1}H-NMR, the quantitative analysis is based on the fact that the amplitude of a proton nuclear magnetic resonance is proportional to the number of hydrogen nuclei contained in the molecule. The area of ^{1}H-NMR peaks is dependent on the number of protons. Figure A.3.4 shows the ^{1}H-NMR spectrum of the NA esterification reaction mixture. The yield of esterification was obtained by comparing the integration of peaks related to the carboxylic acid group in NAs to the methyl ester protons in the product. As a result, we found that the ^{1}H-NMR technique was suitable to measure the yield of NAs esterification.

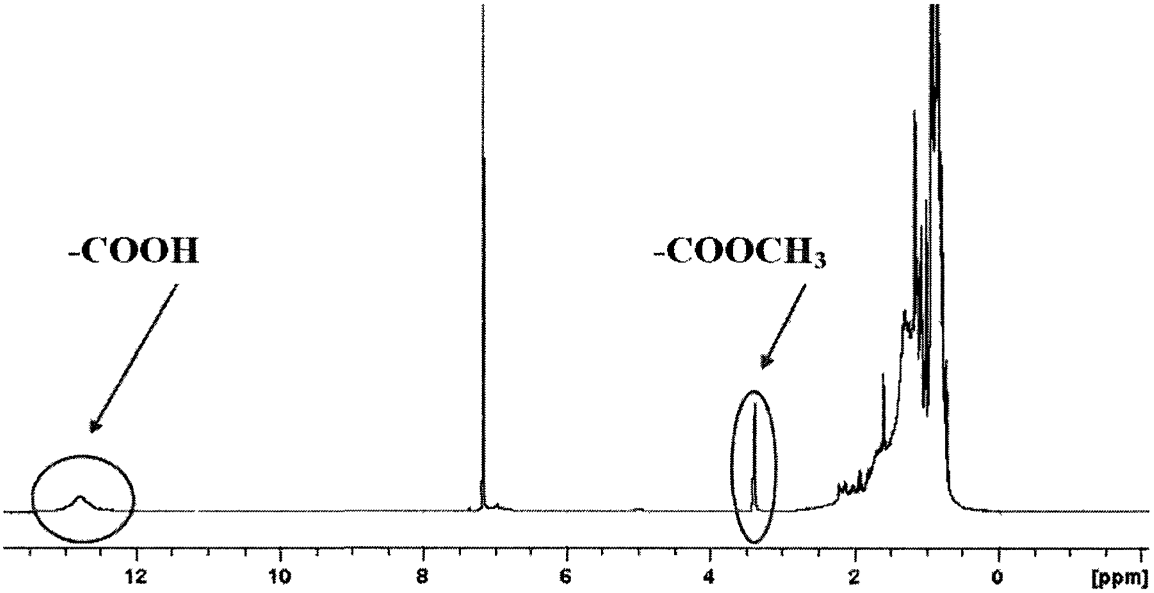


Figure A.3.4 ^{1}H NMR spectrum of a mixture of NAs and methyl naphthenate.

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