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Muhammad Nurunnabi Siddiquee

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PRODUCTION OF BIODIESEL FROM THE SOLID SLUDGE OF WASTEWATER TREATMENT PLANT

(Spine title: Production of Biodiesel from the Solid Sludge of Wastewater Treatment
Plant)

(Thesis Format: Integrated Article)

By:

Muhammad Nurunnabi Siddiquee

Graduate program in Engineering Science
Department of Chemical and Biochemical Engineering

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Engineering Science

School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

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THE UNIVERSITY OF WESTERN ONTARIO
School of Graduate and Postdoctoral Studies

CERTIFICATE OF EXAMINATION

Supervisor

Dr. Sohrab Rohani

Supervisory Committee

Dr. Amarjeet Bassi

Examiners

Dr. Amarjeet Bassi

Dr. Argyrios Margaritis

Dr. Ernest K. Yanful

The thesis by

Muhammad Nurunnabi Siddiquee

entitled:

Production of Biodiesel from the Solid Sludge of Wastewater Treatment Plant

is accepted in partial fulfilment of the
requirements for the degree of
Master of Engineering Science

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Abstract

Biodiesel, a promising renewable fuel, is produced mainly via catalytic transesterification and/or esterification reaction from various lipid sources. Primary and secondary sludges of municipal wastewater treatment facilities are potential lipid sources. In this study, factorial experimental analyses were used to study the influence of different variables on the lipid extraction and biodiesel production from primary and secondary sludges (Adelaide Pollution Control Plant, London, Canada). The maximum extracted lipids from the primary and secondary sludge sources were 14.46 (wt/wt) % and 10.04 (wt/wt) % (on the basis of dry sludge), respectively. The maximum biodiesel yield from extracted lipid by using homogeneous catalyst was 57.12 (wt/wt) % (on the basis of lipid) at 60°C for 14h of reaction. The biodiesel yield from the lipid of wastewater sludge by using mesoporous heterogeneous catalyst, SBA-15 impregnated with heteropolyacid (15% PW₁₂), was 30.14 (wt/wt) % (on the basis of lipid) at 135°C and 135psi for 3h of reaction.

Keywords: Lipid, biodiesel, wastewater sludge, free fatty acid (FFA), fatty acid methyl ester (FAME), homogeneous catalyst, SBA-15, mesoporous heterogeneous catalyst.

Statement of Co-authorship

Chapter 2: The original draft of this chapter was prepared by the author. It was reviewed by Dr. Sohrab Rohani, who also provided a series of revision steps for improvement. The recommendations were incorporated into the chapter by author. A version of this chapter has been published in the following journal:

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Chapter 3: The original draft of this chapter was prepared by the author. It was reviewed by Dr. Sohrab Rohani, who also provided a series of revision steps for improvement. The recommendations were incorporated into the chapter by author. A version of this chapter has been reviewed for possible publication in Fuel Processing and Technology.

Chapter 4: The original draft of this chapter was prepared by the author. It was reviewed by Dr. Sohrab Rohani, who also provided a series of revision steps for improvement. The recommendations were incorporated into the chapter by author. A version of this chapter has been submitted in Chemical Engineering and Technology.

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I have worked with many people in Biofuel and Catalyst Testing Laboratory, Control and Crystallization of Pharmaceutical Laboratory (CCPL), and Zeolitic & Nano Materials Laboratory (ZNML), The University of Western Ontario, during my education. Their contribution in all stages of the research deserves special mention. It is a pleasure to convey my gratitude to them.

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Notation

Adj R-squared	Adjusted Coefficient of Correlation
ASTM	American Society for Testing and Materials
B5	Biodiesel blend with diesel fuel containing 5% biodiesel
B20	Biodiesel blend with diesel fuel containing 20% biodiesel
B100	Pure Biodiesel
BD	Biodiesel
BET	Brunauer- Emmett- Teller method
CCPL	Control and Crystallization of Pharmaceutical Laboratory
FAME	Fatty Acid Methyl Ester
FFA	Free Fatty Acid
FID	Flame Ionization Detector
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
LCFA	Long Chain Fatty Acid
P	Phosphorous
PW	Phosphorous -tungsten
PEG	poly ethylene glycol
PPG	poly propylene glycol
Pred R-squared	Predicted Coefficient of Correlation
PRESS	Predicted residual error sum of squares
PS	Primary Sludge
R-squared	Coefficient of Correlation
SBA	Santa Barbara Amorphous
SBA-15	A highly ordered mesoporous silica
SEM	Scanning Electron Microscopy
Std. Dev.	Standard Deviation
TGA	Thermo gravimetric analysis
UFO	Used frying oil
UWO	University of Western Ontario

W	Tungsten
WWTP	Waste Water Treatment Plant
ZNML	Zeolitic & Nano Materials Laboratory
X_1	Solvent to sludge ratio, ml/g
X_2	Temperature, °C
X_3	Extraction time, h
X_4	Methanol to lipid ratio, ml/g
X_5	Reaction time, h
X_6	Temperature, °C
X_7	% Acid catalyst, ml/ml
X_8	Natural Zeolite, mg
X_9	Temperature, °C
X_{10}	Methanol to sludge ratio, ml/g
X_{11}	% Acid catalyst, ml/ml
XRD	X-Ray Diffraction
β_0	Constant term
β_i	The linear coefficient of factor X_i
β_{ij}	The interactive effect coefficient for factors X_i and X_j
β_{ijk}	The interactive effect coefficient for factors X_i , X_j and X_k
ε	The random error term having a normal distribution with mean zero and variance σ^2
σ^2	Variance

Chapter One

Introduction

1.1 Background

Energy plays an important role in the socio-economic development by raising the global standard of living and the quality of life. There are two available types of primary energy sources - renewables and nonrenewables. The reserves for nonrenewable energies are limited, and distributed unevenly around the world, for example, 63% global fossil fuel reserves are in Middle East [1]. Most of the energy we use nowadays comes from fossil fuels—a non-renewable energy source. Furthermore, the exploration, the refining, and the use of fossil fuels: petroleum, coal, and natural gas, cause environmental problems, i.e. air pollution and greenhouse gas emissions, and natural resource depletion, etc. To meet the crisis, there is utmost need of alternative energy resources which are cheap, renewable, technically acceptable, and environmentally viable [2]. Extensive research is being conducted around the globe to develop alternative fuel sources, such as biodiesel, alcohol, biomass, biogas, synthetic fuels. Among them biodiesel is in prominent position as it is considered an alternative to diesel fuel that is used in pure form (B 100) or as a blend with diesel fuel (B5, B20, etc). Other renewable fuels need some sort of modification before they are used as substitute of conventional fuels [3]. Although Rudolf Diesel invented the vegetable oil driven engine in 1900s, a full exploration of vegetable oil based fuel – biodiesel- started in the 1980s as a result of immense interest in renewable energy sources [4]. Biodiesel (BD) is mainly produced produced from various lipid sources by transesterification reaction in the presence of a base, acid, enzyme or solid catalyst. The most commonly used lipid sources for biodiesel production are vegetable oils such as rapeseed oil, soybean oil, canola oil, mustard oil, palm oil, sunflower oil, hemp oil, etc. The use of edible vegetable oils for biodiesel

production has recently been of great concern because they compete with food materials. Moreover, expensive raw materials like vegetable oils has made biodiesel production cost higher. Some species of plants yielding non-edible oils, e.g. jatropha, castor, neem, karanja, and tall oil may play a significant role in providing the lipid sources for biodiesel production. But these sources are not the unique solution as cultivation of these requires huge land. Recently, algae have shown great promises as a source of lipid for biodiesel production; however, these require cultivation in ponds or photobioreactors. So, research is continuing to find possible alternative sources for biodiesel production.

Municipal wastewater treatment plant (WWTP) produces large amounts of sludge which have become a major concern in operation of the WWTP. In London, ON, Canada, six wastewater treatment plants produced approximately 7.6×10^3 metric ton dry wastewater sludge in 2008 [5]. The wastewater treatment facilities in United States produced approximately 6.2 million metric tons (dry mass) of sludge per year [6]. Due to the increasing population, urbanization, and industrialization, the amount of sewage sludge is expected to increase in the future. Therefore, economic and environmental challenges have to be faced in sludge handling, treatment, and safe disposal.

A wastewater treatment facility employing an activated sludge process produces two main types of sludges- primary sludge and secondary sludge. A conventional wastewater treatment plant is shown in **Fig.1.1**. The primary sludge consists of floating oil, grease and solids, and collected from the bottom of the primary settling tank of a wastewater treatment plant after screening and grit removal. The secondary sludge, also known as activated sludge, is collected from the secondary clarifier/ settling tank and consists mainly of the microbial cells and suspended solids produced during the aerobic

biological treatment of wastewater. The composition of the primary and secondary sludges are shown in **Table 1.1**.

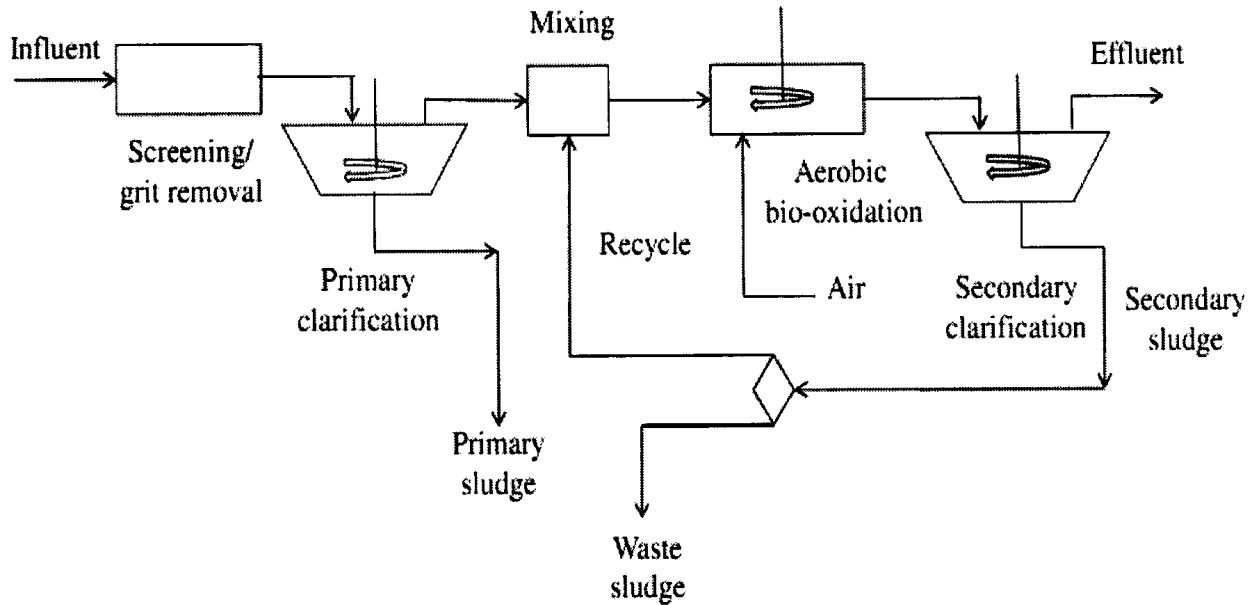


Figure 1.1: Schematic diagram of a conventional municipal wastewater treatment plant [6].

Table 1.1: Composition of primary and secondary sludge (as %weight of dry mass) [7].

Component	Primary Sludge			Secondary Sludge	
	(1)	(2)	(3)	(4)	(5)
Volatile fraction	79.7	73.5	75.0	59-75	79.0
Lipids	18.6	21.0	10.3	5-12	5.8
Cellulose	18.2	19.9	32.2	7	9.7
Hemicellulose			2.5		
Proteins	17.2	28.7	19.0	32-41	53.7

Municipal primary sludge contains considerable amount of lipids e.g. fats, oil and grease which can be extracted and converted into biodiesel. Additionally, the cell wall

membrane of microorganism is mostly consisted of phospholipids, which can also be extracted and converted to biodiesel. As municipal wastewater sludge is readily available at practically no cost or even with an incentive, biodiesel production cost may be reduced, along with mitigating the sludge treatment and disposal cost. For this reason, municipal WWTP sludge is considered as a potential feed stock for biodiesel production. However, the same processes which exist for extracting oils and converting them to biodiesel from oil containing seeds do not necessarily apply to primary and secondary sludges. Therefore, new lipid extraction and biodiesel production technologies must be developed for wastewater sludges which are efficient and cost effective on large scales.

1.2 Objectives of the Thesis

The objective of this research is to optimize the biodiesel production method from the sludge of wastewater treatment plant. The specific objectives are first to investigate the pre-treatment method of raw sludge for efficient lipid extraction, secondly, experimental analysis of lipid extraction from sludges using organic solvents, and characterize the extracted lipid. Subsequently, statistical analysis of biodiesel production by using homogeneous acid catalyst, and characterize the biodiesel. Finally, investigate the potential of heterogeneous acid catalyst for the biodiesel production from the lipid of wastewater sludge.

To achieve the goals, the specific objectives are sought in various chapters of the thesis.

1.3 Organization of the Dissertation

This dissertation has been summarized to present the originalities of the research work. It is composed of five chapters as shown in Fig. 1.2.

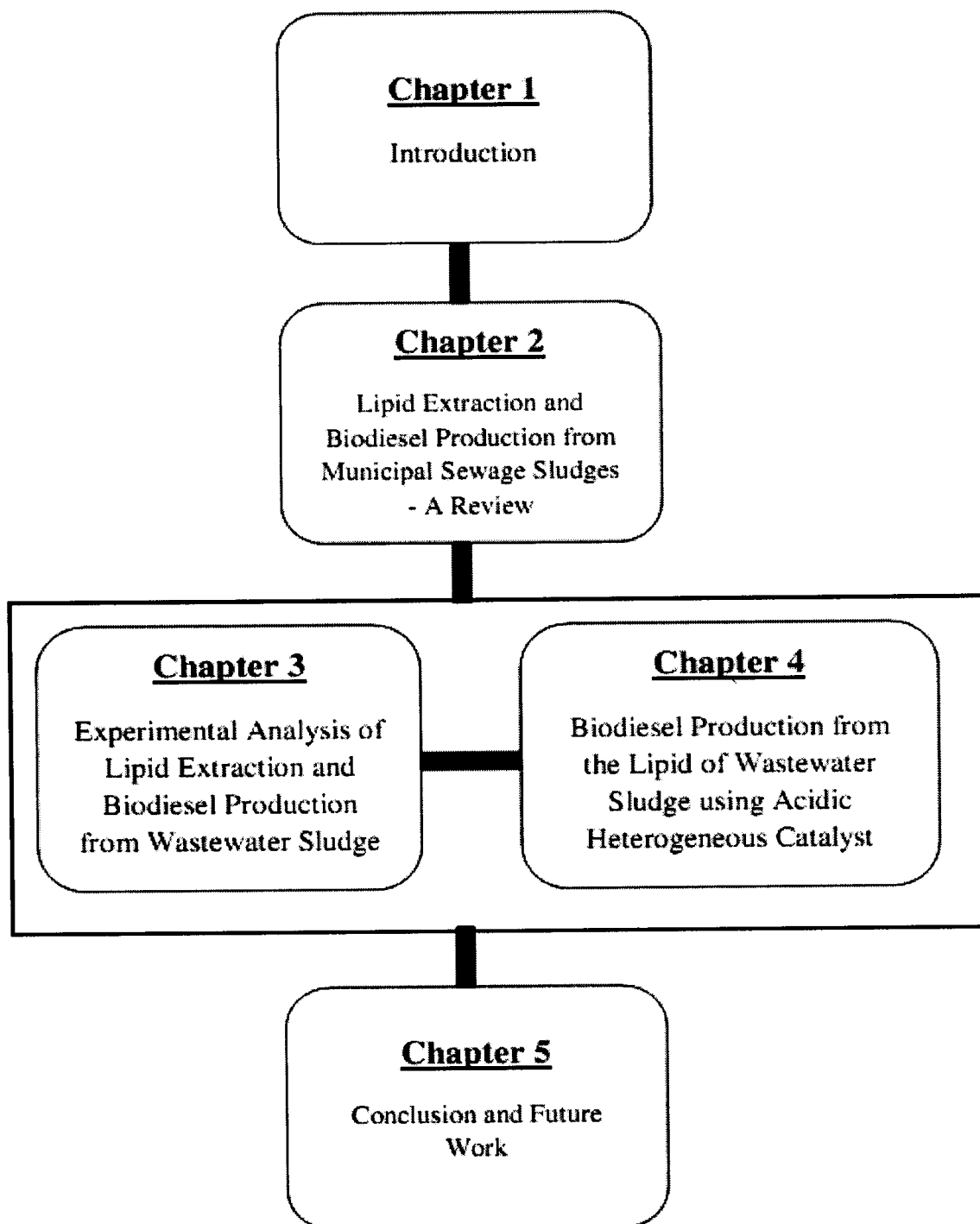


Figure 1.2: Organization of the dissertation.

Brief accounts for each chapter are summarized as follows.

In Chapter 1, a brief background of the biodiesel production, possible sources, wastewater sludge, and the potential of wastewater sludge as a raw material of biodiesel are discussed. The objective of the current research and the layout of the dissertation are also included in this chapter.

In Chapter 2, a comprehensive review of the latest development in biodiesel production, and challenges of biodiesel production from the wastewater sludge are presented. A version of this chapter has been published in the Journal of Renewable and Sustainable Energy Reviews.

In Chapter 3, lipid extraction and biodiesel production from the wastewater sludge by applying factorial experimental design approach are discussed. The empirical models derived from data analysis by using Minitab Software version 15, characterization of lipid and biodiesel are also mentioned in this chapter. A version of this chapter has been reviewed for possible publication in the Journal of Fuel Processing Technology.

In Chapter 4, synthesis of mesoporous heterogeneous catalyst, namely a mesoporous ordered silica (SBA-15) impregnated with heteropolyacid $\text{H}_3\text{PO}_4 \cdot 12\text{WO}_3 \cdot x\text{H}_2\text{O}$ (PW_{12}), and biodiesel production from the extracted lipid by using that catalyst are presented. A version of this chapter has been submitted for possible publication in the Journal of Chemical Engineering and Technology.

In Chapter 5, conclusions, contributions and limitations of current work are discussed and future works are proposed.

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Chapter Two

Lipid Extraction and Biodiesel Production from Municipal Sewage Sludges- A Review

2.1 Introduction

Worldwide rapidly increasing fuel demand (85 million barrels of liquid fuel per day in 2006 and projected to increase to 107 million barrels of liquid fuel per day in 2030) and reducing fossil fuel reserve (1,342 billion barrels oil as of January 2009) act as a driving force behind the search for alternative fuels [1]. The majority of all energy consumed worldwide is now coming from fossil fuel sources. Fossil fuel sources are non-renewable, and will be exhausted in near future [2]. Presently, there is an urgent need for alternative cheap and renewable energy resources with little or no environmental impact. The alternative fuel sources being developed around the globe include, such as biodiesel, alcohol, biomass, biogas, synthetic fuels. Among them biodiesel can be used directly, while others need some sort of modification before they are used as substitute of conventional fuels [3]. Biodiesel is renewable, biodegradable, less toxic, and safer for storage and handling, has excellent lubricity and could provide similar energy density to diesel [1, 4, 5]. It burns much cleaner than petroleum diesel as it contains oxygen and reduces most emissions (CO_2 , CO, particulate, except NO_x) [6]. Biodiesel does not require new refueling stations, new parts inventories or expensive engine modifications [7].

Chemically, biodiesel consists of fatty acid methyl esters that can be produced from various lipid sources by transesterification reaction with alcohol in the presence of a base, acid, enzyme or solid catalyst [1, 5, 8, 9]. Depending upon the climate and soil conditions, different countries are looking for different types of vegetable oils as substitutes for diesel fuels. For example, soya bean oil in the US, rapeseed and sunflower oils in Europe, palm oil in south-east Asia (mainly Malaysia and Indonesia) and coconut

oil in the Philippines are being considered [10]. A major economic challenge for the commercialization of biodiesel production is the high cost of pure vegetable or seed oils, which constitutes between 70% and 85% of the overall biodiesel production cost [1, 11, 12]. Moreover, the use of edible vegetable oils for biodiesel production has recently been of great concern. Used frying oil (UFO) for biodiesel production introduces other challenges due to the broad properties of UFO that may affect the biodiesel production consistency [1, 9, 13]. Biodiesel can also be produced from algae, which are produced naturally all over the world [10, 14]. Besides, some species of plants yielding non-edible oils, e.g. jatropha, castor, neem, karanja, etc may play a significant role in providing alternative raw materials [1, 15]. But cultivation of these alternative raw materials requires huge land. Thus, in order to reduce the cost per gallon of biodiesel, alternative feedstocks that are readily available in large quantities and at low cost must be considered.

Sewage sludge is an abundant organic waste or by-product generated in wastewater treatment plant (WWTP) facilities after primary and secondary treatment processes [16, 17, 18]. A wastewater treatment facility having an activated sludge process produces two main types of sludge- primary sludge, a combination of floating grease and solids, and secondary sludge, composed mainly of microbial cells and suspended solids produced during the aerobic biological treatment. The primary sludge is collected at the bottom of the primary clarifier and the secondary sludge, known also as activated sludge, is collected in the secondary clarifier [12, 17]. The management (handling, treatment, and disposal) of sludge is a complex challenge for any WWTP and contributes to 20-60% of total operating costs of WWTP [19].

Municipal wastewater treatment plants produce huge amounts of sludge per year that is readily available at free of cost or even with an incentive. Wastewater treatment plant facilities in USA alone produced over 6.2 million metric tons of dry sludge every year [20]. Six wastewater treatment plants in London, ON, Canada produced approximately 7.6×10^3 metric ton dry wastewater sludge in 2008 [21]. The amount of sewage sludge is expected to increase in the future due to increasing urbanization and industrialization. The use of sludge as a fertilizer is restricted in many countries of world due to bad odor, the presence of heavy metals, toxic substances and pharmaceutical chemicals, while the sludge incineration results in emissions that contain heavy metals and dioxins [22, 23]. The disposal of sludge in landfills involves with inherent chemical energy loss and associated health problems. The ocean dumping of sludge locally interrupts the ecology of biosphere [24]. One viable alternative to sludge management and disposal challenge is to utilize the sludge as a source of lipid feedstock for biodiesel production.

Lipid is a natural mixture of triglycerides, diglycerides, monoglycerides, cholesterols, free fatty acids, phospholipids, sphingo-lipids, etc [25]. The municipal wastewater sludge contains a significant amount of lipid fraction that is a composite organic matrix (characterized as oils, greases, fats and long chain fatty acid) originating from the direct adsorption of lipids from domestic and industrial wastes in the sludge, and/or from the phospholipids in the cell membranes of microorganisms, their metabolites and by-products of cell lysis. Research has indicated that the lipids contained in sewage sludge are a potential feedstock for biodiesel [18, 20]. The overall biodiesel production scheme can be shown in **Fig. 2.1**. To avoid the interference in the biodiesel

synthesis, lipids are usually extracted from the sludge with organic solvents. Several options have been attempted. However, the lipid extraction and biodiesel production from municipal sewage sludge poses great challenges for commercial realization. The main challenges include: (1) the pre-treatment of raw sludge for efficient lipid extraction, (2) the lipid extraction from the sludge, (3) the biodiesel production methods from solid sludge, (4) the quality of biodiesel, and (5) process economics and safety.

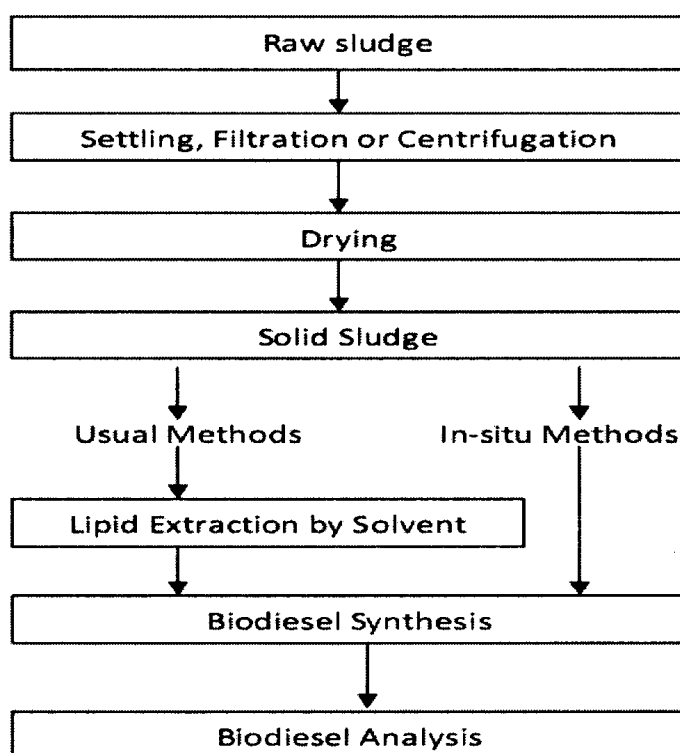


Figure 2.1: Overall biodiesel production scheme.

2.2 The Pre-treatment of Raw Sludge for Efficient Lipid Extraction

Raw primary sludge and secondary sludge contain 4-5 % (wt/v) and 1-2 % (wt/v) solids, respectively. The lipid extraction from the raw sludge requires huge amount of organic solvent and large vessels with stirring and heating system. Dewatered concentrate

sludge is sticky and hinders the lipid extraction process. But the lipid extraction from dried sludge is feasible.

Dufreche et al. [20] pre-treated the secondary sludge (2% solid) by gravity settling followed by centrifugation or pressure filtration. They performed centrifugation at 3000 rpm for 20 min and got 7-8 % (wt/wt) solids containing sludge. They also conducted pressure filtration by using 80 μm and 20 μm nylon filter and got 12-14 % (wt/wt) solids. They used Hydromatrix to absorb residual free water. But this technique is challenging for large scale pre-treatment of raw sludge. Mondala et al. [12] concentrated the raw primary and secondary sludge by gravity settling at 0 $^{\circ}\text{C}$ for 24 h. They further dewatered the sludge by centrifugation at 3000 rpm for 20 min and dried the sludge by freeze drying. Revellame et al. [1] recently used this technique for secondary sludge only. But this pre-treatment process is not feasible for large scale production. Boocock et al. [26] extracted lipids from dried sludge supplied by Wastewater Technology Centre of Environment Canada. Recently Pokoo-Aikins et al. [17] used the sewage sludge for lipid extraction and biodiesel production, but they did not mention the type of sludge they used. The pretreatment of raw sludge can significantly affect the lipid extraction process and consequently the yield of biodiesel production.

2.3 The Lipid Extraction and Lipid Analysis Methods

Lipid extraction is the first step for biodiesel production from wastewater treatment plant sludge. At present, several methods are available for lipid extraction from biological materials. Most of these methods use organic solvents, usually in mixtures, as in the Bligh and Dyer [27] and Folch et al. [28]. Boocock et al. [26] extracted 12 wt%

lipids by Soxhlet extraction method and 17– 18 wt% lipids by boiling solvent extraction from raw sewage sludge. They used sludge to solvent ratio of 1 to 6 with 300 ml solvent (chloroform or toluene) for 50 g dry sludge and 600 ml solvent (chloroform or toluene) for 100 g dry sludge in Soxhlet extraction method and boiling extraction method, respectively. They concluded that both chloroform and toluene are equally effective for lipid extraction, but preferred toluene based on cost and environmental considerations. Approximately 65 wt% of the extracts were found to be free fatty acids (C12-C18, mostly palmitic, stearic and oleic acid), 7 wt% were glyceride fatty acids and 28 wt% unsaponifiable material (C9 to C16 alkane).

Dufreche et al. [20] extracted lipids from activated sludge by using pure and/or mixture of hexane, methanol and acetone as solvents and obtained a maximum 27.43 ± 0.98 wt % lipid for three times extraction by using 60 v% hexane/ 20 v% methanol/ 20 v% acetone. They also used supercritical-CO₂ technique and supercritical-CO₂ with methanol co-solvent technique for lipid extraction and obtained 3.55 wt% and 13.56 wt% lipid respectively. They claimed that extraction of lipids by a mixture of n-hexane, methanol and acetone gave the largest conversion to biodiesel compared with other solvent systems.

Pokoo-Aikins et al. [17] extracted lipids from sewage sludge by using toluene, hexane, ethanol and methanol. They used sludge to solvent ratio 1:5 that was calculated according to Boocock et al. [26]. They separated the free fatty acids (FFA), triglycerides (oils) and the solvent (**Fig. 2.2**) and found the yield of FFA was 24.8 wt%, 24.9 wt%, 25.5 wt%, 25.5 wt% for toluene, hexane, methanol and ethanol, respectively. The maximum yield of triglyceride was 3.4 wt% for all four solvents.

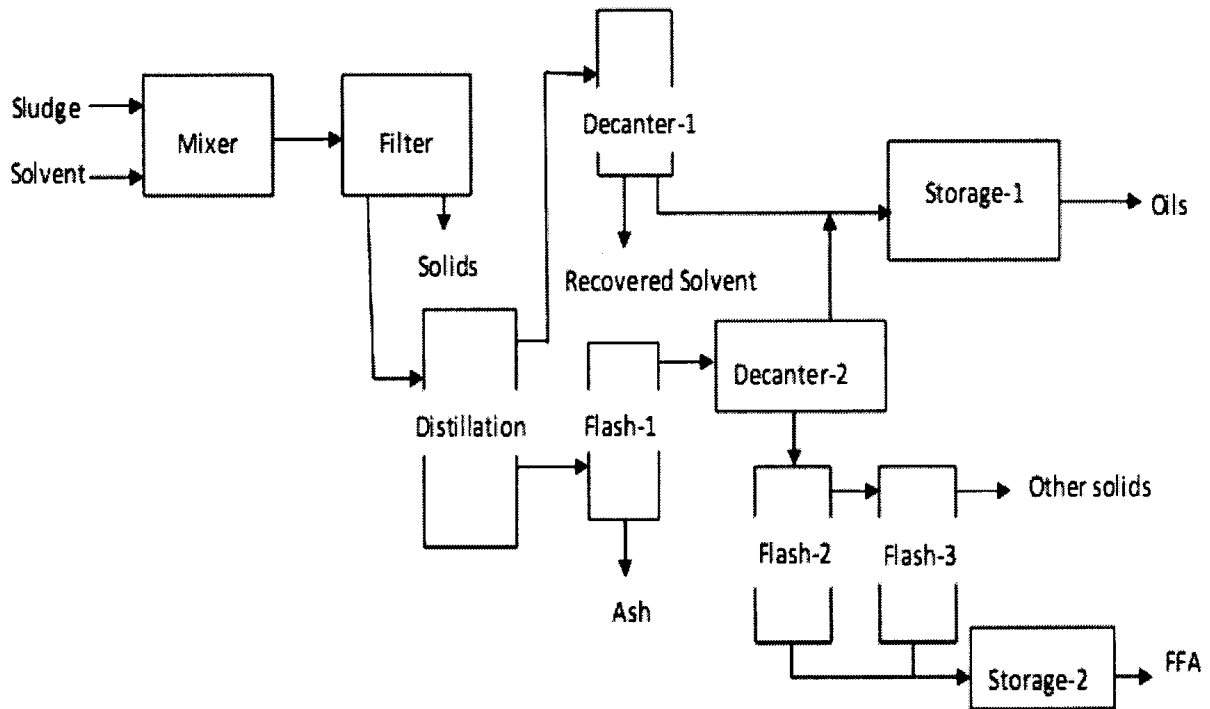


Figure 2.2: Sewage sludge extraction process with hexane or toluene as solvent [17].

2.4 The Biodiesel Production Methods from Solid Sludge

There are four primary techniques for biodiesel production- direct use and blending of raw oils, micro-emulsions, thermal cracking and transesterification [9]. The most commonly used method for biodiesel production is transesterification (also known as alcoholysis) reaction in presence of a catalyst. Transesterification is the process of exchanging the alkoxy group of an ester compound with another alcohol (**Fig. 2.3**), where R_1 , R_2 , and R_3 are long hydrocarbon chains, called fatty acid chains. This transesterification reaction consists of a series of consecutive, reversible reactions [30, 31, 32].

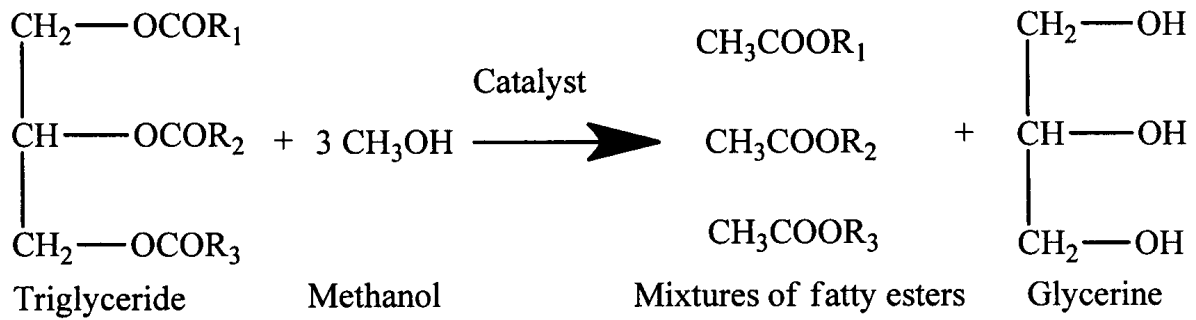
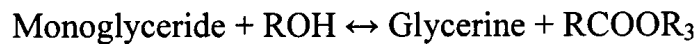
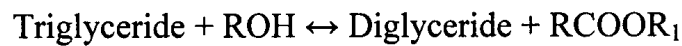


Figure 2.3: The general form of transesterification reaction [29].

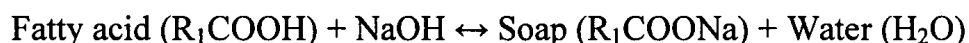
The triglyceride is converted stepwise by reacting with primary alcohol to diglyceride, monoglyceride and finally glycerol.



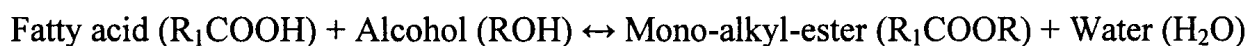
The primary alcohol used to form the ester is a major feedstock in biodiesel production process. Methanol is widely used as the alcohol for producing biodiesel because it is the least expensive alcohol. Besides, it has some advantages- (i) its reactivity is high (ii) it does not absorb water that interferes with transesterification reaction (iii) prevents soap formation (iv) its recovery is easier, as it does not form azeotrope like ethanol [33, 34]. Excess methanol (60-100% more methanol than required) is added in order to ensure total conversion of the vegetable oil or animal fat to its esters.

The yield of transesterification depends on several factors including the type of catalyst (base, acid, enzyme or heterogeneous), alcohol/vegetable oil molar ratio, temperature, and duration of reaction, water content, and free fatty acid content. Water can consume the catalyst and reduce catalyst efficiency.

Base catalyst transesterification is widely used commercially due to very fast reaction rate compared to other catalysts. Base catalyzed process is very sensitive to water and free fatty acids (FFA) present in lipid sources due to soap formation. It leads to catalyst consumption and soap formation that inhibits separation of the glycerol from the methyl esters and contributes to emulsion formation during the water wash.



On the other hand, acid catalyzed transesterification is 4000 times slower than base catalyzed transesterification and requires higher triglyceride to alcohol ratio [35]. But acid catalyst is able to catalyze both the esterification and transesterification and more biodiesel is produced. If water accumulates, it can stop the reaction. So, this approach requires a water management technique.



The use of heterogeneous catalysts has shown greater promise toward transesterification to obtain biodiesel. The commonly used heterogeneous catalysts are Mg/La mixed oxide, S-ZrO₂ sulfated zirconia, KOH/Nax zeolite, Li/CaO, CaO, KI/Al₂O₃, (ZS/Si) zinc stearate immobilized on silicagel, KNO₃/ Al₂O₃, SO₄²⁻/TiO₂-SiO₂, etc. Heterogeneous catalysts can be recovered conveniently from reaction products [9, 36]. The undesired saponification reactions can be avoided by using heterogeneous acid catalysts. This catalyst enables the transesterification of vegetable oils or animal fats with high contents of free fatty acid (FFA) [37]. Solid catalyst can be reused and provides the possibility for carrying out both transesterification and esterification reactions simultaneously [38]. However, the cost of solid catalyst is very high.

Enzymatic transesterification looks attractive and encouraging for reasons of ease of product separation, minimal wastewater treatment needs, easy glycerol recovery and the absence of side reactions [39]. However, enzymatic process has several technical difficulties such as slow reaction rate, contamination of the product with residual enzymatic activity, and high cost [10].

Kusdiana & Saka [31] developed a supercritical methanol technique in which biodiesel and glycerol were produced by the trans-esterification reaction of the raw oils and fats in absence of a catalyst. Biodiesel was also generated simultaneously by the esterification reaction of the free fatty acids, even if there was a high content of free fatty acids in the raw material oils and fats. Biodiesel was derived at high yield with no saponified products. Moreover, the separation and purification were easy because of the non-catalytic process. However, this method required extreme temperature and pressure conditions of 350 °C and 43 MPa, respectively, and induced breakdown of unsaturated fatty acids and trans-isomerization, leading to adverse effects on the fluidity of the fuel at low temperatures [40, 41]. The comparison of different technologies for biodiesel production is shown in **Table 2.1**.

Dufreche et al. [20] produced 4.41wt% biodiesel (based on total dry weight of activated sludge) from the solvent extracted lipids by acid catalyzed esterification-transesterification reaction. They also produced 6.23 wt% biodiesel from the dried secondary sludge by in-situ acid catalyzed transesterification. Mondala et al. [12] investigated the potential of primary and secondary sludge of municipal wastewater treatment plant as a lipid feedstock for biodiesel production by acid-catalyzed in situ transesterification process. They obtained at maximum FAME yield of 14.5 wt% and 2.5

wt% for primary and secondary sludge, respectively at 75 °C, 5% (v/v) H₂SO₄, and 12:1 methanol to sludge mass ratio.

Table 2.1: Comparison of different technologies for biodiesel production [42].

Variable	Base catalyst	Acid catalyst	Lipase catalyst	Supercritical alcohol	Heterogeneous catalyst
Reaction Temp. (°C)	60-70	55-80	30-40	239-385	180-220
Free fatty acid in raw materials	Saponified products	Esters	Methyl esters	Esters	Not sensitive
Water in raw materials	Interfere with reaction	Interfere with reaction	No influence		Not sensitive
Yield of methyl esters	Normal	Normal	High	Good	Normal
Recovery of glycerol	Difficult	Difficult	Easy		Easy
Purification of methyl esters	Repeated washing	Repeated washing	None		Easy
Production cost of catalyst	Low	Low	Relatively high	Medium	Potentially low

Revellame et al. [1] optimized the in-situ transesterification of activated sludge by full factorial design of four temperature levels (45, 55, 65, and 75 °C), six methanol to sludge ratio (5, 10, 15, 20, 25, and 30 v/wt) and five levels of catalyst concentration (0.5, 1, 2, 4, and 6 v%). They obtained 4.88 wt% and 4.79±0.02 wt% biodiesel, respectively, by numerical and experimental optimization at 55 °C, 25 methanols to sludge ratio, and 4

v% sulphuric acids. They concluded that biodiesel production decreases significantly at temperatures above 60 °C due to acid catalyzed polymerization of unsaturated fatty acids or their ester.

2.5 The Quality of Biodiesel

The biodiesel must satisfy the ASTM D 6751 Standard Specification for Biodiesel Fuel (**Table 2.2**) in order to be used in an engine without problems.

Table 2.2: ASTM D 6751 requirements [42].

Property	Method	Limits	Units
Flash point, closed cup	D 93	130 min	°C
Water and sediment	D 2709	0.050 max	v%
Kinematic viscosity, 40 °C	D 445	1.9-6.0	mm ² /s
Sulfated ash	D 874	0.020 max	wt%
Total sulfur	D 5453	0.05 max	wt%
Copper corrosion strip	D 130	No. 3 max	
Cetane number	D 613	47 min	
Cloud point	D 2500		°C
Carbon residue	D 4530	0.050 max	wt%
Acid number	D 664	0.80 max	mgKOH/g
Free glycerin	D 6584	0.020	wt%
Total glycerin	D 6584	0.240	wt%
Phosphorous	D 4951	0.0010	wt%
Vacuum distillation end point	D 1160	360 °C max, at 90% distilled	°C

Ensuring the quality of wastewater sludge biodiesel is a great challenge. In addition to triglyceride, fatty acids, phospholipids, bacterial lipids, sludge may also contain various chemicals like wax esters, steroids, terpenoids, polyhydroxyalkanoates, hydrocarbons, linear alkyl benzene, polycyclic aromatic hydrocarbons, pharmaceutical chemicals, etc [1, 18, 23, 43, 44]. These compounds may be extracted during the lipid extraction or in-situ biodiesel production and contribute to the overall gravimetric yield. Conversion of these contaminants to biodiesel via cracking process will significantly increase fuel yield from the wastewater sludge [1, 45, 46].

Dufreche et al. [20] , Mondala et al. [12], and Revellame et al. [1] reported that biodiesel from wastewater treatment plant sludge mainly contains methyl esters of palmitic acid(C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C 18: 2) that are similar to pure vegetable oil biodiesel composition.

Mondala et al. [12] also found the close similarity of the fatty acid composition of primary and secondary sludge by GC analysis. Therefore it might be feasible to combine the two sludge types into a single feedstock for biodiesel production. But lipid percentage of the mixed sludge will be changed and process parameters need to be changed accordingly.

2.6 Process Economy

Biodiesel is attractive due to environmental benefits and the fact that it is produced from renewable resources. However, the challenges are its cost and limited availability of fat and oil resources. The biodiesel production cost consists of two major cost items: the cost of raw material (fats and oils) and the operating costs. The cost of raw

materials accounts for 70–80% of the total cost of biodiesel fuel [1, 47]. Higher production cost has hindered the biodiesel growth and made it uncompetitive compared to diesel.

The biodiesel production from municipal sewage sludge can lower the cost significantly. Lipid extraction and biodiesel production from sewage sludge is associated with the use of organic solvents. But more than 99% of the solvents are recoverable [17]. The estimated biodiesel production cost from municipal primary and secondary sludge is USD 3.11 to USD 3.23 per gallon of biodiesel compared to USD 4.00 to USD 4.50 per gallon refined soy biodiesel and USD 3.00 per gallon for diesel (as of January 2010) [12, 17, 20, 48]. Pokoo-Aikins et al. [17] claimed that the overall biodiesel production cost from free sewage sludge is varied according to the solvent used in initial extraction step and is USD 3.39 per gallon for ethanol, USD 3.37 per gallon for methanol, USD 2.89 for hexane, and USD 2.79 per gallon for toluene used as the extraction solvent. Although toluene is cheaper, recovery of it is more energy intensive due to its higher boiling point. Mahamuni and Adewuyi [49] reported that the use of high-frequency ultrasound significantly reduces the biodiesel production cost.

Dufreche et al. [20] assumed 7 wt% overall biodiesel yield and estimated the production cost for sludge biodiesel (**Table 2.3**). Biodiesel production cost from sludge (USD 3.11 per gallon) is broken down to USD 2.06 per gallon for centrifuge, drying, and extraction processes and to USD 1.05 per gallon for other expenses. But they did not include the cost recovered by glycerol sale that will reduce the biodiesel production cost. After lipid extraction the valuable chemicals recovery from the sludge will be easier which may reduce the biodiesel production cost as well.

Table 2.3: Production cost estimate for sludge biodiesel [20].

	Cost per gallon (US \$)
Centrifuge O&M	0.43
Drying O&M	1.29
Extraction O&M	0.34
Biodiesel processing O&M	0.60
Labor	0.10
Insurance	0.03
Tax	0.02
Depreciation	0.12
Capital P&I service	0.18
Total cost	3.11

Assuming 7.0 wt % overall transesterification yield
 O&M operation and maintenance, P&I protection and indemnity

2.7 Conclusions

Biodiesel occupies a prominent position as a renewable liquid fuel. It has several benefits over diesel fuel. But higher production cost due to raw materials has made it uncompetitive compared to petro-diesel. Moreover, vegetable oils and animal fats which are the main raw materials for biodiesel production compete with food materials causing the cost of biodiesel to increase. Municipal sewage sludge is readily available and is a potential source of lipid for biodiesel production. But there are few challenges for biodiesel production from sludge.

First, the pre-treatment of raw sludge which includes collecting, dewatering and drying of sludge is quite costly. Freeze drying system to get dry sludge is energy and time consuming. Vacuum drying or other techniques can be used instead of freeze drying which will reduce the overall biodiesel production cost. Second, lipid extraction from sludge is also expensive and requires large volume of organic solvents. The amount of lipid depends on the sources and type of sludge. Solvent selection, sludge to solvent ratio, extraction time, temperature and solvent recovery are among the factors that affect lipid extraction efficiency and cost. Optimization of these factors is necessary for efficient lipid extraction. Lipid extraction by super critical CO₂ is not efficient due to higher operating costs. Microwave lipid extraction technique can be used which is rapid, safe, cheap and does not require samples devoid of water [50]. Third, biodiesel production methods are varied and often costly. The catalyst requirement for acid catalyzed in-situ transesterification is high. Acid catalyzed transesterification is slow. Moreover, water produced during esterification of FFA makes it even slower, and hence acid catalyzed esterification followed by base catalyzed transesterification- can be used for biodiesel production from sewage sludge. But optimization of the amount of alcohol, catalyst (both acid and base), catalyst neutralization, biodiesel washing and drying is necessary for acid catalyzed esterification followed by base catalyzed transesterification. Heterogeneous catalysts, enzymatic catalysts or non-catalytic supercritical methods which are not affected by FFA or water can also be used for biodiesel production. Extensive research work is necessary to get optimum method for biodiesel production from municipal sewage sludge and to make it profitable compared to other sources.

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Chapter Three

Experimental Analysis of Lipid Extraction and Biodiesel Production from Wastewater Sludge

3.1 Introduction

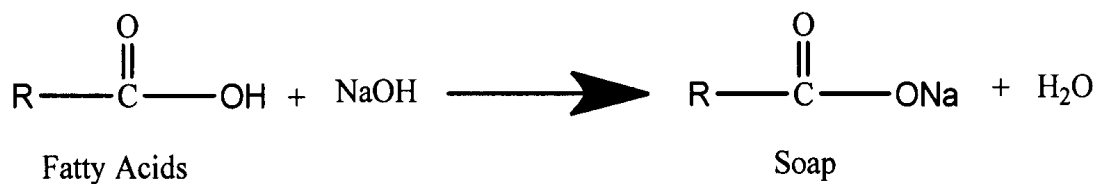
Nowadays most of the energy we use comes from fossil fuels— non-renewable energy sources which will be exhausted in the near future [1]. Furthermore, our dependence on fossil fuels as energy sources has caused serious environmental problems, i.e. air pollutants and greenhouse gas emissions, and natural resource depletion. Biodiesel is a promising green fuel to displace an appreciable amount of petroleum-based diesel fuel. It is renewable, biodegradable, less toxic, safe for storage and handling, provides similar energy density to diesel and can be used directly without any engine modification and does not require new refuelling stations [2- 5]. Chemically, biodiesel is a fatty acid alkyl ester, commonly known as fatty acid methyl ester (FAME) that is produced via esterification and/or transesterification of various lipid sources in the presence of a base, acid, enzyme or solid catalyst [2, 3, 6, 7]. Lipid is a natural mixture of triglycerides, diglycerides, monoglycerides, cholesterol, free fatty acids, phospholipids, sphingolipids, etc [8]. The production cost of biodiesel is high due to the fact that 75-85% of the total cost is related to the raw materials like vegetable oils and animal fats [2, 9, 10]. Therefore, there is an urgent need for alternative, cheap or available with incentive sources of lipid to produce biodiesel.

Wastewater treatment plant (WWTP) facilities produce plenty of primary sludge and secondary sludge (waste activated sludge). Six wastewater treatment plants in London, ON, Canada produced approximately 7.6×10^3 metric ton dry wastewater sludge in 2008 [11]. Wastewater treatment plant facilities in the USA alone produce over 6.2 million metric tons of dry sludge every year [12]. Highly complex sludge management

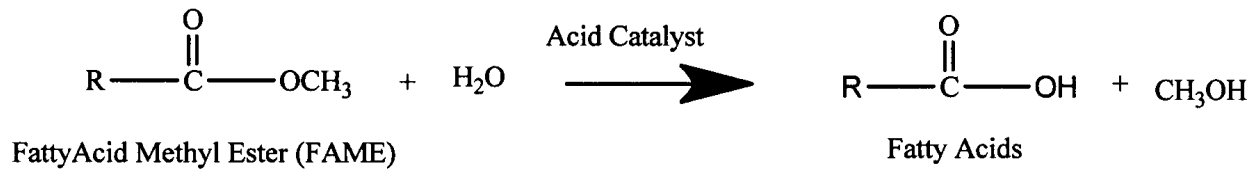
(handling, storage, transportation and disposal) system accounts for 20–60% of the total operating costs of WWTPs and is a matter of concern in many countries as sludge production is expected to increase in the next years due to the implementation of new WWTPs [13, 14, 15]. Therefore, optimization of sludge management is a key element to reduce WWTPs costs. Municipal sludge contains considerable amount of lipid which is a composite organic matrix originating from the direct adsorption of lipids from domestic and industrial wastes in the sludge, and/or from the phospholipids in the cell membranes of microorganisms, their metabolites and by-products of cell lysis [10]. Research has indicated that the lipids contained in sewage sludge are a potential feedstock for biodiesel [2, 10, 12, 16, 17]. Boocock et al. obtained 17–18 wt % lipids from dried sewage sludge by boiling solvent extraction using chloroform and toluene as solvents. Approximately 65 wt % of the extracts was found to be free fatty acids and 7 wt % were glyceride fatty acids [18]. However, toluene extracted lipid was not suitable for biodiesel production [12]. Moreover, these two solvents were not environment friendly and toluene recovery after lipid extraction was not economical due to higher boiling point. Dufreche et al. extracted lipid from secondary sludge by using organic solvents with different polarities or with supercritical carbon dioxide. They used sludge to solvent ratio 1: 40. Utilization of Hydromatrix was involved at high cost and might have also interfered with the gravimetric yield of the lipid along with the sludge residue as the authors neither filtered nor centrifuged the lipid containing solvent phase. However, they claimed that solvent extraction was more effective compared to expensive supercritical CO₂ technique. They also claimed that extraction of lipid by using the mixture of n-hexane, methanol and acetone gave the largest lipid amount compared with other solvent systems [12]. But the

recovery of the solvents from mixture of solvents was difficult. Pokoo-Aikins et al. separated the free fatty acids (FFA), triglycerides (oils) and the solvent from extracted lipids from sewage sludge by using toluene, hexane, ethanol, and methanol and found the yield of FFA was 24.8 wt%, 24.9 wt%, 25.5 wt%, 25.5 wt%, respectively. The maximum yield of triglyceride was 3.4 wt% for all four solvents [19]. Extraction of lipids from sludge may be influenced by many variables such as type of sludge, type and amount of solvent, extraction time, temperature, stirring rate, type of micro-organisms present in the sludge, etc. Although several researchers have demonstrated the lipid extraction from wastewater sludge, the effects of different parameters and their interactions have not been investigated.

At present, base catalyst transesterification is widely used commercially due to very fast reaction rate compared to other catalysts. But, base catalyzed process is highly sensitive to the presence free fatty acids (FFA) as FFA reacts with catalyst and form soap that consume catalyst, inhibits glycerol separation and facilitate emulsion formation during washing step.



Acid catalyzed transesterification is slower than base catalyzed transesterification and requires high lipid to alcohol ratio [20]. However, the main advantage of acid catalyst is the ability to catalyze both the esterification and transesterification to produce more biodiesel. As the presence of water can stop the reaction, this approach requires a water management technique.



Revellame et al., Mondala et al., and Dufreche et al. produced biodiesel from freeze dried primary and secondary sludge using acid-catalyzed in-situ transesterification process [2, 10, 12]. However, the use of freeze drying to remove water from sludges increases the required energy and time. Additionally, the solvent and acid catalyst requirements were also high. Moreover, water produced by in-situ esterification made the reaction rate slow. The use of commercial zeolite was reported in biodiesel production to absorb water produced by esterification reaction [21]. However, there was no report about the use of natural zeolite as a dehydrating agent for the production of biodiesel. In this study, we used natural zeolite to absorb water produced during the esterification reaction of FFA.

The yield of biodiesel production depends on several factors including the type of catalyst (base, acid, enzyme or heterogeneous), alcohol/lipid ratio, temperature, and reaction time, water and free fatty acid content, etc. Although several researchers have demonstrated biodiesel production from the lipid of wastewater sludge, the effects of various parameters have not been investigated.

An experimental design is a fast, economic, and effective way to systematically investigate the effect of several variables simultaneously (multivariate data analysis) [22]. In the experimental study of M variables and N experiments, an M x N matrix constitutes a variable space (X). A response variable (Y) for each experiment is necessary for the analysis of the experimental data. The motto of the multivariate data analysis is to

develop an empirical model to describe the relation between X and Y as well as possible [23, 24].

In the present work, a 2^3 two-level factorial design was applied to study three factors (solvent to sludge ratio (X_1), temperature (X_2) and extraction time (X_3)) which we believed could play an important role in the extraction of lipid using organic solvents. To reduce the number of experiments, stirring rate was kept constant during the extraction experiments and assumed that micro-organism types have no effect on lipid extraction. Hexane was used as a non-polar solvent and methanol was used a polar solvent. Acetone, toluene, and mixtures of solvents were not used due to the recovery problem after lipid extraction. The amount of FFA in each type of extracted lipids was determined by using gas chromatograph. Biodiesel was produced from each type of extracted lipids via acid catalyzed esterification-transesterification reaction. To increase the biodiesel yield, natural zeolite was used as a dehydrating agent. A half fraction 2^5 Plackett-Burman factorial design was applied to study the effects of five factors, namely methanol to lipid ratio (X_4), reaction time (X_5), temperature (X_6), amount of acid catalyst (X_7), and amount of natural zeolite (X_8), in the production of biodiesel from the lipid of primary sludge extracted by methanol. Stirring rate was kept constant during the experiments. This study also demonstrates a factorial experimental analysis of in-situ biodiesel production from dried primary sludge to compare the biodiesel yield with ex-situ biodiesel production.

3.2 Materials and Methods

3.2.1 Materials

High Performance Liquid Chromatography (HPLC)-grade methanol and hexane were purchased from Caledon Laboratories Ltd. (Georgetown, ON, Canada), sodium bicarbonate was purchased from Sigma-Aldrich Inc. (St. Louise, MO, USA), Sodium Chloride was purchased from EM Science (Gibbstown, NJ, USA), anhydrous sodium sulphate was purchased from BDH Inc. (Toronto, ON, Canada), H_2SO_4 was purchased from Mallinckrodt Baker Inc. (Phillipsburg, NJ 08856, USA), and all the gases (He, H_2 , and air) used for gas chromatography were ultra high purity grade and distributed by Praxair Canada Inc. (Mississauga, ON, Canada). Fatty acids standard were purchased from AccuStandard Inc. (New Haven, CT, USA). Commercial zeolite Y was purchased from Sigma-Aldrich Inc. (St. Louise, MO, USA). Synthesized zeolite was collected from Zeolitic & Nano Materials Laboratory (ZNML, UWO, Canada). Standard SuplecoTM standard 37component FAME mix- a 37 component reference mixture of fatty acid methyl ester in the range of C_4 to C_{24} including saturated, mono-unsaturated and poly unsaturated FAME- was purchased from Supleco (Bellefonte, PA, USA) and used to calibrate the GC for FAME analysis. All chemicals, standard, and gases were used as received. The glassware was washed with hot water and detergent, rinsed with deionised water and air dried.

3.2.2 Sample Collection and Preparation

The municipal primary and secondary sludge samples were collected from Adelaide Pollution Control Plant, London, ON, Canada on September 23, 2009 and same sludges were used during entire study. Raw sludge was allowed to settle for 24 hours at

0°C. After discarding the supernatant liquid, the resulting sludge was then centrifuged at 3000 rpm for 10 minutes using IEC Centra-HN centrifuge (International Equipment Company, Needham Heights, USA) for further dewatering. Dewatered sludge was spread on tray and put in a fume hood for 4 days to dry under vacuum at ambient temperature. The dried sludge contained approximately 95% solids, measured by drying the sludge at constant mass in an oven (Yamato DKN 400 Constant Temperature Oven, Yamato Scientific America Inc., Santa Clara, CA 95050, U.S.A.) at 120°C. Dried sludge was crushed in a mortar and pestle, homogenized and then stored in a freezer prior to use.

The natural zeolite used in this study was a clinoptilolite-rich tuff from Semnan region of Iran. The natural zeolite was pulverized, sieved and then washed with distilled water in order to remove all the soluble impurities and then dried at 323 K overnight using a Lindberg/Blue Mendel (Box Furnace, Thermo Scientific, USA) prior to use as a dehydrating agent in the production of biodiesel. The particle size (20-100 μ) and BET surface area (40.5861m²/g) were measured, respectively, by using a Mastersizer (Mastersizer 2000, Malvern Instruments Ltd., Malvern, UK) and a BET surface area and pore size analyzer (Micromeritics ASAP 2010, Micromeritics Instrument Corporation, Norcross, GA, USA) instrument.

3.2.3 Factorial Design of Lipid Extraction from Dried Primary & Secondary Sludge

The three extraction variables under investigation and their high and low experimental levels and center points are given in **Table 3.1** and **Table 3.2**, respectively for dried primary sludge (five gram) and secondary sludge (one gram). Two replicates were done for all treatment combinations.

Table 3.1: The experimental parameters and their high and low levels and center points for the lipid extraction from dried primary sludge.

Variables	Low level (-1)	High level (+1)	Center point (0)
X ₁ : Solvent to sludge ratio (ml/g)	10	25	17.5
X ₂ : Temperature (°C)	25	75	50
X ₃ : Extraction time (h)	0.5	4	2.25

Table 3.2: The experimental parameters and their high and low levels and center points for the lipid extraction from dried secondary sludge.

Variables	Low level (-1)	High level (+1)	Center point (0)
X ₁ : solvent to sludge ratio (ml/g)	10	30	20
X ₂ : Temperature (°C)	25	75	50
X ₃ : Extraction time (h)	0.5	4	2.25

The block diagram of lipid extraction from raw sludges is illustrated in **Fig. 3.1**. The extraction by using methanol and hexane were performed according to the scheme stated in **Table 3.3**. The yield of lipid extraction was determined according to modified version of Durfreche et al. and Boocock et al.[12, 18]. Assigned amount of the dried sludge was weighed into a round bottom flask and the assigned volume of the solvent was added and then the resulting mixture was heated to the desired temperature at ambient pressure by using VWR Hotplate/Magnetic stirrer (VWR International Mississauga, Ontario, Canada) and a magnetic stirring bar was used for mixing at 500 rpm. After assigned extraction time, the resulting slurry was immediately filtered using VWR filter paper (size 5.5 cm) and a Buchner funnel attached to vacuum. A solvent flash of 25 ml and 10 ml solvent were used for the lipid extraction from primary and secondary

sludge, respectively. The filtrates were centrifuged by using IEC Centra-HN centrifuge (International Equipment Company, Needham Heights, USA) at 3000 rpm for 4 minute to remove any residual particles. The resulting supernatants were transferred to a measuring flask. A 1.5 ml aliquot of lipid solution was pipetted into 2.0 ml Supelco PTFE lined capped vial (Supelco, Bellefonte, PA) for the determination of FFA. The remaining solvent phase was transferred to a 250 ml round bottom flask and the solvent was removed under vacuum using a Büchi Rotavapor R-200 (Büchi Labortechnik, Switzerland) at 45°C. After all the solvent was removed, the flask was flushed with air to remove any remaining solvent in the gas phase. The resulting lipid was weighed using Mettler Toledo analytical balance (model PG603-S, Mettler-Toledo Inc, Columbus, OH, 43240) and the yield of extracted lipid was expressed as gram of extractable lipid per gram of dry solid.

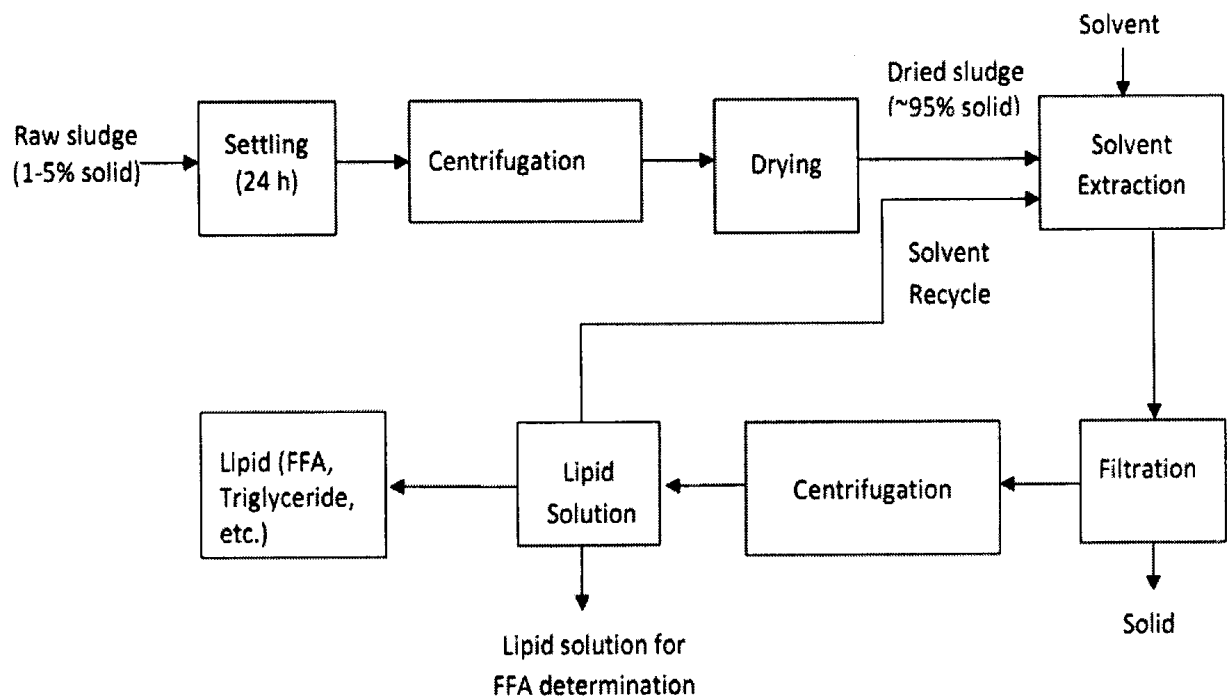


Figure 3.1: Block diagram of lipid extraction from the raw sludges of wastewater treatment plant.

Table 3.3: A 2^3 factorial designs, containing the main variables and the response variable for the models of lipid extraction from primary and secondary sludge.

Run number	X_1	X_2	X_3	Response (%wt/wt lipid on the basis of dry sludge)			
				Primary sludge		Secondary sludge	
				Methanol extraction	Hexane extraction	Methanol extraction	Hexane extraction
1	-1	1	1	11.54	8.38	6.64	1.64
2	1	-1	1	11.14	5.98	6.13	1.63
3	-1	-1	-1	6.96	4.06	2.83	1.05
4	-1	1	-1	8.88	5.66	5.56	1.21
5	1	1	-1	13.94	6.34	9.28	2.72
6	-1	1	-1	8.94	5.58	5.72	1.37
7	1	1	1	14.46	11.04	9.91	3.04
8	-1	1	1	11.44	8.28	6.79	1.71
9	1	-1	-1	8.62	5.14	3.50	1.31
10	1	1	-1	13.84	6.42	9.13	2.65
11	-1	-1	-1	7.04	4.16	2.97	1.14
12	1	-1	1	11.04	5.92	6.27	1.54
13	-1	-1	1	8.28	5.38	5.24	1.73
14	-1	-1	1	8.22	5.30	5.17	1.84
15	1	-1	-1	8.58	5.22	3.67	1.40
16	1	1	1	14.46	11.16	10.04	2.95

3.2.4 Determination of FFA in Extracted Lipids

The amount of FFA in lipid was determined using Varian CP-3800 gas chromatograph (Varian Inc., Lake Forest, CA) equipped with FID, and a 50m x 0.25 mm x 0.2 μ m Varian CP-Wax 58 (FFAP) CB capillary Column (Varian Inc., Lake Forest, CA). Helium was used as a carrier gas and the sample injection volume was 1.0 μ l with a split ratio of 100:1. The column flow was constant at 1.2 ml/min and column oven temperature was programmed to maintain at 100 °C for 1.0 min, increase from 100 °C to 200 °C at 15 °C /min and maintained for 1.0 min, then increased from 200 °C to 240 °C at 5 °C /min, and finally maintained at 240 °C for 25.33 min. The detector and injector temperature were set at 260 °C for the duration of the analysis.

3.2.5 Biodiesel Production from Extracted Lipids

The modified Christie's method was used for the conversion of extracted lipid to FAMES in presence of an acid catalyst (H_2SO_4) [25]. The free fatty acids (FFA) present in the extracted lipid were also esterified in presence of an acid catalyst in addition to transesterification of triglyceride (**Fig. 3.2**), & produced fatty acid methyl ester (FAME).

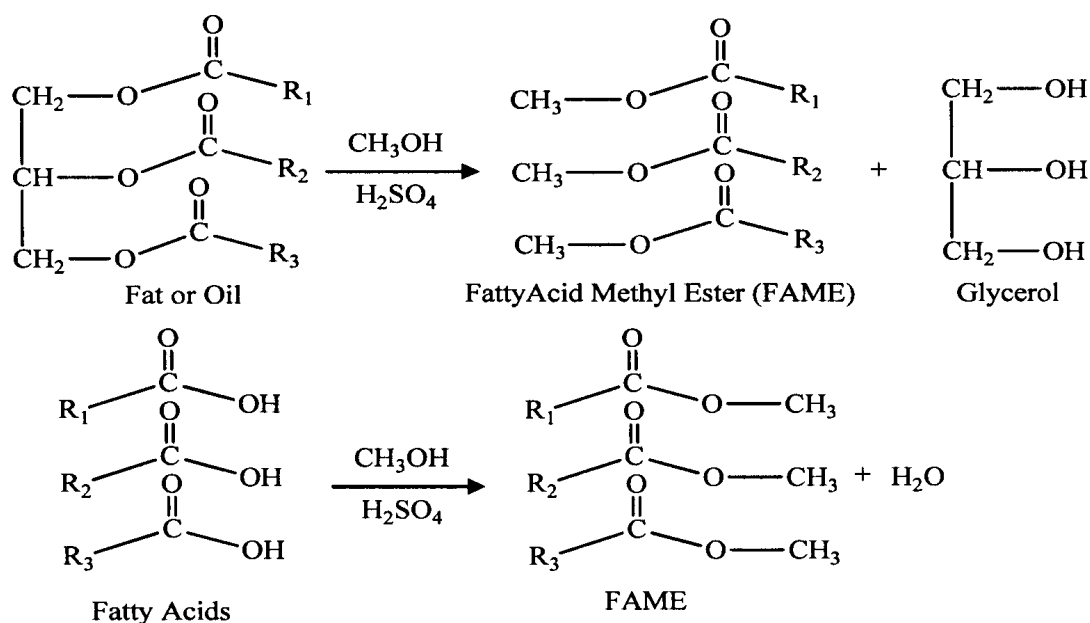


Figure 3.2: Acid-catalyzed transesterification of triglycerides and esterification of fatty acids.

Both the methanol and hexane extracted lipids from the primary and secondary sludge were treated following the same procedure (**Fig. 3.3**). Two hundred mg of extracted lipid was dissolved in 10 ml of hexane and transferred to a round bottom flask. Twenty ml of 2% H_2SO_4 in methanol was added to the flask. Resulting mixture was heated at 60°C for 24 hours by using VWR Hotplate/Magnetic stirrer (VWR International Mississauga, Ontario, Canada) and a magnetic stirring bar was used for mixing at 500 rpm. The loss of methanol due to evaporation was minimized by using a condenser with water at 20°C . The resulting mixture was allowed to cool to stop the reaction and the

flask contents were transferred to a separatory funnel and then 10ml aliquot of 5% NaCl in water was added. Twenty ml of hexane was added to the separatory funnel and shaken for 3 minutes to extract biodiesel. Extraction procedure was repeated 3 times.

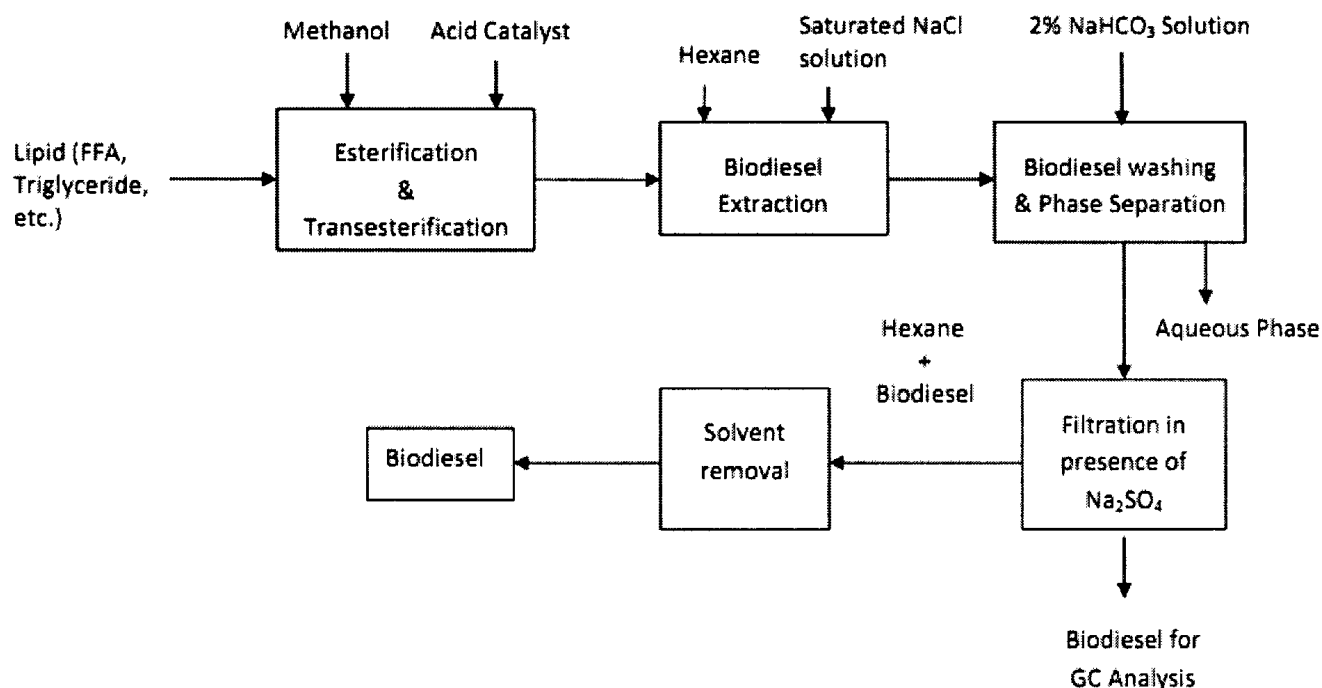


Figure 3.3: Block diagram of biodiesel production from the extracted lipid by using acid catalyzed esterification and transesterification.

Hexane layer was washed with 10 ml of 2 % NaHCO₃ followed by 10 ml of warm water and dried by passing through a Whatman filter paper (110 mm dia) containing anhydrous sodium sulphate and collected into a measuring flask. A 1.5 ml aliquot of hexane phase was pipetted into 2.0 ml Supelco PTFE lined capped vial (Supelco, Bellefonte, PA) for FAME analysis using GC equipped with FID (Flame Ionization Detector). The remaining hexane phase was transferred to a round bottom flask and the solvent was removed under vacuum using a Büchi R205 Rotary Evaporator (Büchi Labortechnik, Switzerland) at 40 °C to get biodiesel. The yield of FAME was determined from FAME analysis using GC.

3.2.6 Effect of Zeolite on Biodiesel Production from Extracted Lipid

The commercial zeolite Y, natural zeolite and laboratory synthesised zeolite Y were used to study the effect of zeolite on biodiesel production from the lipid of primary sludge, extracted by using methanol. The procedure of biodiesel production and the amount reactants were similar to the previous section 'Biodiesel production from extracted lipids' except 50 mg of zeolite was added to the reactant mixture. Zeolite was separated after the completion of reaction by using an IEC Centra-HN centrifuge (International Equipment Company, Needham Heights, USA) at 3000 rpm for 10 minutes.

3.2.7 Factorial Design of Biodiesel Production from the Lipid of Primary Sludge Extracted by Methanol

A half fraction 2^5 Plackett-Burman factorial design of biodiesel production from the lipid of primary sludge, extracted by using methanol, was conducted due to the higher lipid yield compare to others. Five variables under investigation and their high and low experimental levels and center points are given in **Table 3.4**. Two replicates were done for all treatment combinations.

Table 3.4: The experimental parameters of biodiesel production from the lipid of primary sludge and their high and low levels, and center points.

Factors	Low level (-1)	High Level (+1)	Centre Point (0)
X ₄ : Methanol to lipid ratio (ml/g)	50	200	125
X ₅ : Reaction time (h)	4	24	14
X ₆ : Temperature (°C)	45	75	60
X ₇ : % Acid catalyst (ml/ml)	1	5	3
X ₈ : Natural Zeolite (mg)	25	75	50

Table 3.5: A half fraction 2^5 factorial design, containing the main variables and the response variable for the model of biodiesel production from the lipid of primary sludge.

RunOrder	Methanol (X_4)	Time (X_5)	Temperature (X_6)	Acid (X_7)	N Zeolite (X_8)	Biodiesel Yield
1	1	1	-1	1	-1	42.6
2	1	-1	-1	-1	-1	41.7
3	1	1	1	1	1	50.5
4	0	0	0	0	0	57.1
5	-1	-1	1	-1	-1	42.8
6	1	-1	1	-1	1	44.5
7	-1	1	1	-1	1	45.1
8	-1	1	-1	1	1	42.7
9	-1	-1	-1	1	-1	54.6
10	1	1	-1	1	-1	42.6
11	-1	1	1	1	-1	49.3
12	1	1	1	-1	-1	47.4
13	-1	1	1	-1	1	45.5
14	-1	1	-1	-1	-1	48.1
15	1	1	1	-1	-1	47.1
16	1	-1	1	-1	1	44.6
17	-1	-1	1	-1	-1	43.3
18	-1	-1	1	1	1	43.7
19	1	-1	-1	1	1	35.6
20	-1	-1	-1	1	-1	54.7
21	1	-1	1	1	-1	37.5
22	1	1	1	1	1	49.6
23	1	-1	-1	-1	-1	42.3
24	-1	-1	-1	-1	1	51.2
25	-1	1	-1	1	1	41.8
26	-1	1	1	1	-1	50.1
27	1	1	-1	-1	1	44.6
28	1	-1	-1	1	1	35.7
29	-1	1	-1	-1	-1	48.4
30	-1	-1	-1	-1	1	49.3
31	-1	-1	1	1	1	44.1
32	1	-1	1	1	-1	37.7
33	0	0	0	0	0	56.8
34	1	1	-1	-1	1	45.4

The biodiesel productions from extracted lipid were performed according to the scheme stated in **Table 3.5** by using the modified version of Christie's method in presence of H_2SO_4 catalyst. Two hundred milligram of extracted lipid was dissolved in 10 ml of hexane and transferred to a round bottom flask. Assigned amount of natural zeolite, H_2SO_4 and methanol were added to the flask and the resulting mixture was heated at assigned temperature for the time mentioned in **Table 3.4** by using VWR Hotplate/Magnetic stirrer (VWR International Mississauga, Ontario, Canada) and a magnetic stirring bar was used for mixing at 500 rpm. The methanol loss due to evaporation was minimized by using a condenser with water. The mixture was allowed to cool to stop the reaction and then centrifuged using IEC Centra-HN centrifuge (International Equipment Company, Needham Heights, USA) for 5 minutes to remove the natural zeolite. The remaining part of biodiesel production was similar to the previous section 'Biodiesel production from extracted lipids'.

3.2.8 Factorial Analysis of in-situ Biodiesel Production from Dried Primary Sludge

Biodiesel was produced by in-situ esterification-transesterification from dried primary sludge according to the modified Mondala's method [10]. A block diagram of in-situ biodiesel production has been illustrated in **Fig. 3.4**. A 2^3 -level factorial design was applied to study the effect and interactions of three parameters namely, temperature (X_9), methanol to sludge ratio (X_{10}), and catalyst (H_2SO_4) concentration (X_{11}). The high and low experimental levels and center points of the parameters under are given in **Table 3.6**. Two replicates were done for all treatment combinations.

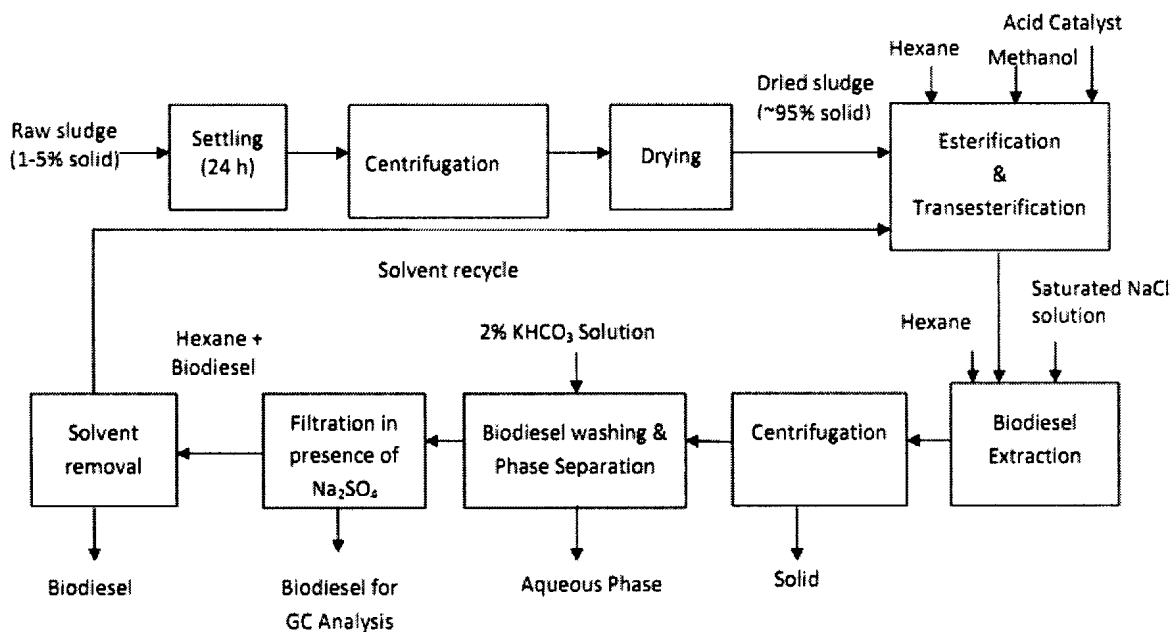


Figure 3.4: Block diagram of in-situ biodiesel production from the dried sludge by using acid catalyst.

Table 3.6: The experimental parameters of in-situ biodiesel production and their high and low levels, and center points.

Factors	Low level (-1)	High Level (+1)
X ₉ : Temperature (°C)	45	75
X ₁₀ : Methanol to sludge ratio (ml/g)	10	25
X ₁₁ : % Acid catalyst (ml/ml)	0.5	4

The biodiesel productions from dried primary sludge were performed according to the scheme stated in **Table 3.7**. Four gram of dried primary sludge was transferred to a 500 ml round bottom flask. The assigned amount of H₂SO₄ acid was mixed with assigned amount of methanol and the resulting mixture was added to flask that contained sludge. Twenty ml hexane was also added to the flask to facilitate the lipid extraction during the in-situ biodiesel production. A magnetic stirring bar was used for mixing. The mixture was heated at assigned temperature at ambient pressure for 24 h. The resulting slurry was

filtered using VWR filter paper (size 5.5 cm) and a Buchner funnel attached to vacuum. A flash of 30 ml hexane was used to recover any biodiesel remaining in filter cake. The supernatant was transferred to a separatory funnel and then 10ml aliquot of 5% NaCl in water was added. Fifty ml of hexane was added to the separatory funnel and shaken for 3 minutes to extract biodiesel. Extraction procedure was repeated two times.

Table 3.7: The 2^3 factorial designs, containing the main variables and the response variable for the model of in-situ biodiesel production from the dried primary sludge.

Run Order	Temperature (°C) X_9	Sludge to methanol ratio, (ml/g) X_{10}	Catalyst concentration X_{11} (ml/ml)	Biodiesel yield %(wt/wt) (on the basis of dry sludge)
1	-1	1	1	12.1
2	1	1	1	12.6
3	1	1	-1	7.7
4	-1	-1	-1	10.1
5	-1	1	-1	7.3
6	-1	1	-1	7.3
7	0	0	0	10.4
8	1	1	-1	7.8
9	1	-1	-1	11.3
10	1	-1	1	10.5
11	1	1	1	12.8
12	1	-1	1	10.6
13	-1	-1	1	9.8
14	-1	-1	-1	10.1
15	-1	-1	1	9.9
16	1	-1	-1	11.2
17	-1	1	1	12.16

Hexane phase was washed with 10 ml of 2 % NaHCO_3 followed by 10 ml of warm water. Aqueous layer was discarded and hexane layer was dried by passing through a Whatman filter paper (110 mm dia) containing anhydrous sodium sulphate and collected into a measuring flask. A 1.5 ml aliquot of hexane phase was pipetted into 2.0 ml Supelco PTFE lined capped vial (Supelco, Bellefonte, PA) for FAME analysis using

GC equipped with FID (Flame Ionization Detector). The remaining hexane phase was transferred to a round bottom flask and the solvent was removed under vacuum using a Büchi R205 Rotary Evaporator (Büchi Labortechnik, Switzerland) at 40 °C to get biodiesel. The yield of FAME was determined from FAME analysis using GC.

3.2.9 FAME Analysis

The FAME was analyzed using Varian CP-3800 gas chromatograph (Varian Inc., Lake Forest, CA) equipped with FID, and a 50m x 0.25 mm x 0.2 µm Varian CP-Wax 58 (FFAP) CB capillary Column (Varian Inc., Lake Forest, CA). Helium was used as a carrier gas and the sample injection volume was 1.0 µl with a split ratio of 80:1. The column flow was constant at 1.2 ml/min and column oven temperature was programmed to maintain at 100 °C for 1.0 min, increase from 100 °C to 200 °C at 15 °C /min, then increased from 200 °C to 240 °C at 5 °C /min, and finally maintained at 240 °C for 24 min. The detector and injector temperature were set at 260 °C for the duration of the analysis.

3.2.10 Statistical Analysis

All statistical analyses were done by using Minitab software version15 (Minitab Inc, State College PA, USA).

3.3 Results and Discussion

3.3.1 Factorial Design of Lipid Extraction from Dried Primary & Secondary Sludge

The normal probability plots for effects of lipid extraction from primary sludge and secondary sludge are shown in Fig. 3.5 and Fig. 3.6, respectively.

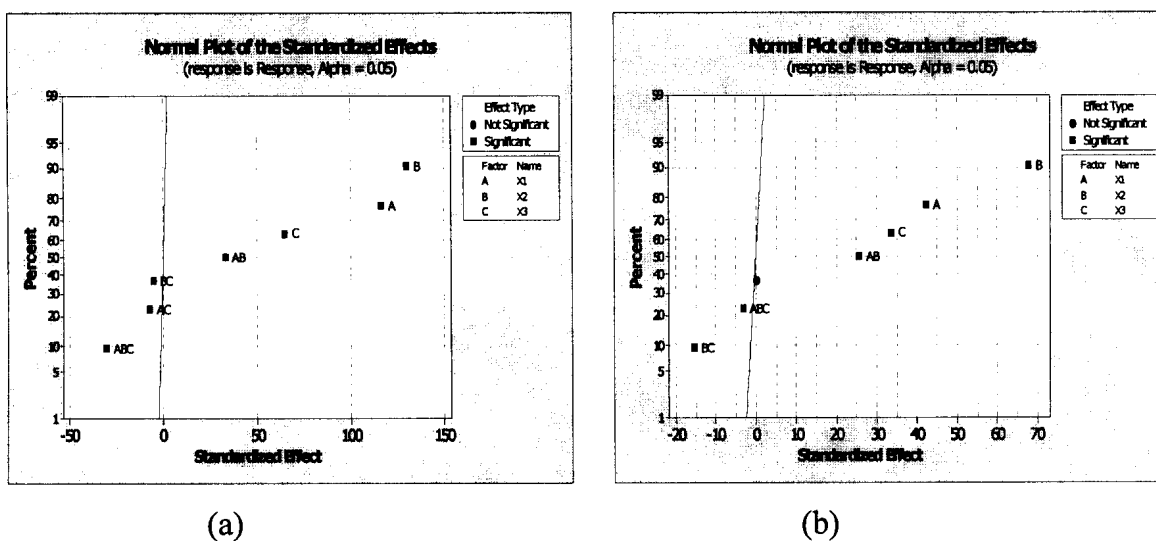


Figure 3.5: Normal probability plots of effects for lipid extraction from dried (a) primary sludge (b) secondary sludge using methanol as solvent.

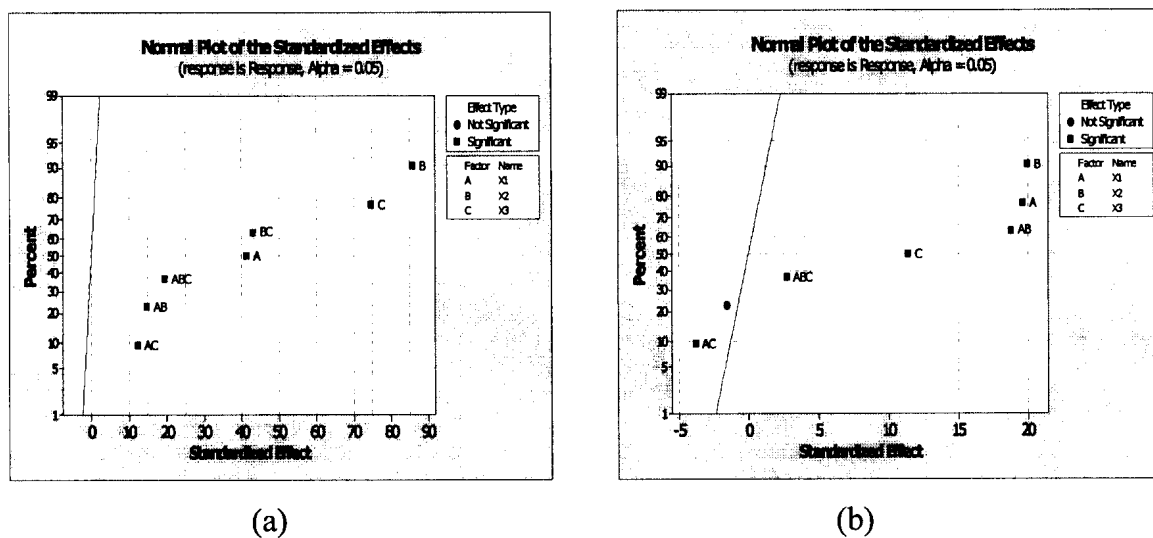


Figure 3.6: Normal probability plots of effects for lipid extraction from dried (a) primary sludge (b) secondary sludge using hexane as solvent.

The responses, the amount of lipid Y expressed in terms of wt/wt% on the basis of dry sludge, for each lipid extraction scheme are presented in **Table 3.3**. The underlying model for a three-factor-factorial experiment is given by

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 + \varepsilon \dots (3.1)$$

Here, β_0 is the constant term, β_i is the linear coefficient of factor X_i , β_{ij} is the interactive effect coefficient for factors X_i and X_j , β_{ijk} is the interactive effect coefficient for factors X_i , X_j and X_k , and ε is the random error term having a normal distribution with mean zero and variance σ^2 [26]. In the model, positive coefficients indicate that the high level of the variables increase the lipid yield. Based on the data analysis using Minitab, the empirical models are developed for each type of solvent and dried sludge. The coefficients of model are shown in **Table 3.8**.

Table 3.8: The coefficients of the model for 2^3 factorial designs of the lipid extraction from primary and secondary sludge using methanol and hexane as solvents.

Coefficients	Primary sludge		Secondary sludge	
	Extraction using methanol	Extraction using hexane	Extraction using methanol	Extraction using hexane
β_0	10.4613	6.5012	6.1781	1.8081
β_1	1.5488	0.6512	1.0631	0.3469
β_2	1.7263	1.3562	1.7056	0.3531
β_3	0.8612	1.1787	0.8456	0.2019
β_{12}	0.4387	0.2313	0.6431	0.3319
β_{13}	- 0.0962	0.1938	0.0000	- 0.0669
β_{23}	- 0.0738	0.6788	- 0.3844	0.0000
β_{123}	- 0.4062	0.3088	- 0.0769	0.0481

3.3.1.1 Lipid Extraction Using Methanol as Solvent

It can be seen from **Table 3.8** that, temperature (X_2) has the highest coefficient and is the most significant factor for the models of lipid extraction from primary and secondary sludge using methanol as solvent. The solvent to sludge ratio (X_1) is also an important factor for the model. As extraction time (X_3) is not important compare to other two factors, using the lower level X_3 that will reduce the production cost. This is an agreement with the findings for the model of lipid extraction from both the primary sludge and secondary sludge. This may be due to the fact that methanol is a smaller polar molecule. The maximum amount of extracted lipid using methanol as solvent were 14.46 wt/wt% (on the basis of dry sludge) that was the highest amongst all the extractions for primary sludge and secondary sludge. However, the maximum 10.04 wt/wt% (on the basis of dry sludge) lipid was extracted secondary sludge using methanol as a solvent; this result provided the highest amount of lipid extraction amongst all secondary sludge lipid extraction data. Methanol is expected to extract the polar lipids like fatty acids, phospholipids, etc. The statistical parameters for the models of lipid extraction from primary sludge and secondary sludge are shown respectively in **Table 3.9** and **Table 3.10**, respectively. In both cases, the predicted R-squared is in reasonable agreement with the adjusted R-squared.

Table 3.9: The statistical parameters from data analysis of the model for lipid extraction by using methanol.

Std. Dev.	0.05315	PRESS	0.0904
Mean	10.4613	R-squared	0.9998
Maximum	14.46	Adj R-squared	0.9996
Minimum	6.96	Pred R-squared	0.9991

Table 3.10: The statistical parameters from data analysis of the model for lipid extraction by hexane from primary sludge.

Std. Dev.	0.1002	PRESS	0.321
Mean	6.1781	R-squared	0.9991
Maximum	10.04	Adj R-squared	0.9982
Minimum	2.83	Pred R-squared	0.9962

3.1.1.2 Lipid Extraction Using Hexane as Solvent

Temperature (X_2) is the most significant factor for the models of lipid extraction from primary and secondary sludge using hexane as solvent. In case of primary sludge, all the factors and interaction terms among the factors are statistically significant at 5% level of significance with positive coefficients. This suggests using higher amount of hexane. Due to the non-reactive nature and larger molecular size compared to methanol, hexane takes longer time for lipid extraction. A maximum of 11.16 (wt/wt) % (on the basis of dry sludge) lipid was extracted from primary sludge using hexane as a solvent. Hexane is expected to extract triglycerides, diglycerides, monoglycerides and fatty acids of higher hydrocarbon due to its non-polar nature. However, for the lipid extraction from secondary sludge, all the factors and the interaction terms among the factors are not statistically significant. The temperature, X_2 , and solvent to sludge ratio, X_1 , are both almost equally significant. The interaction between X_1 and X_2 , and extraction time (X_3) are also important factors compare to others. This suggests using the higher level for all the factors to get high response. However, overall response for the model is lower. This is due to the fact that hexane may not be suitable to extract the phospholipids, the main component of the cell membrane of micro-organism. A maximum of 3.04 (wt/wt) % (on

the basis of dry sludge) lipid was extracted from secondary sludge using hexane as a solvent. This amount is very low compare to methanol extracted lipid. This is an agreement with the findings of Dufreche et al. for the lipid extraction from activated sludge [12]. The statistical parameters are shown in **Table 3.11** and **Table 3.12**, respectively, for the model of lipid extraction from primary and secondary sludge. The predicted R-squared of 0.9982 and 0.9754 are in reasonable agreement with the adjusted R-squared of 0.9991 and 0.9885, respectively, for primary and secondary sludge.

Table 3.11: The statistical parameters from data analysis of the model for lipid extraction by methanol from dried secondary sludge.

Std. Dev.	0.063	PRESS	0.1272
Mean	6.501	R-squared	0.9995
Maximum	11.160	Adj R-squared	0.9991
Minimum	4.060	Pred R-squared	0.9982

Table 3.12: The statistical parameters from data analysis of the model for lipid extraction by hexane from primary sludge.

Std. Dev.	0.07067	PRESS	0.1598
Mean	1.80813	R-squared	0.9938
Maximum	3.04	Adj R-squared	0.9885
Minimum	1.05	Pred R-squared	0.9754

3.3.2 Determination of FFA in Extracted Lipids

The lipid extracted from primary sludge by using methanol and hexane contained maximum 58.85(wt/wt) % and 51.18 (wt/wt) % FFA (on the basis of lipid), respectively. A maximum of 34.58 (wt/wt) % and 25.19 (wt/wt) % FFA (on the basis of lipid) were

contained in the lipid extracted from secondary sludge by using methanol and hexane, respectively. Among the free fatty acids, palmitic acid was in maximum amount in all four cases.

3.3.3 Biodiesel Production from Extracted Lipids

The FAME yield of the H_2SO_4 catalyzed esterification-transesterification of the hexane and methanol extracted lipids were 41.25 (wt/wt) % and 38.94(wt/wt) % (on the basis of lipid) for the primary sludge, and 26.89 (wt/wt) % and 30.28 (wt/wt) % (on the basis of lipid) for the secondary sludge. However, these yields may increase by optimizing the acid catalyzed esterification-transesterification reaction.

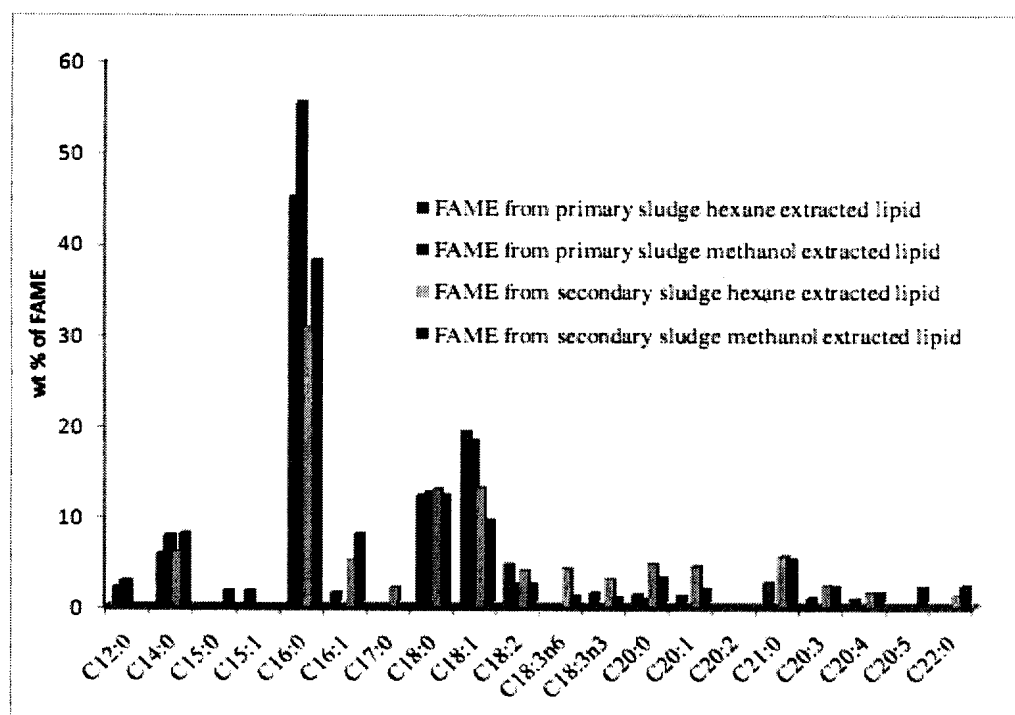


Figure 3.7: FAME analysis of biodiesel obtained via acid catalyzed esterification-transesterification of methanol and hexane extracted primary and secondary sludge lipid.

As shown in **Fig. 3.7**, both the primary and secondary sludge's biodiesel contain mainly the methyl ester of myristic acid (C 14:0), palmitic acid (C16:0), palmitoleic acid

(C 16:1) stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C 18:2). Methyl ester of palmitic acid (C 16:0) is present in greatest amount in the biodiesel produced from both the hexane and methanol extracted lipids. Biodiesel from the secondary sludge extracted lipid contains fatty acids of higher hydrocarbons as compared to biodiesel from primary sludge extracted lipids. These results are found to be in agreement with the findings of Mondala et al. by the in-situ transesterification of primary and secondary sludge [10]. Biodiesel has improved properties like oxidative stability, cetane number and cold flow mainly composed of oleic acid (71.3%) [27]. But Konthe claimed that for low temperature application methyl ester of palmitoleic acid (C16:1) is more suitable compared to methyl ester of oleic acid (C 18:0) [28]. Biodiesel from hexane extracted lipid contains more FAME of higher hydrocarbon and unsaturated acid composition compared to the biodiesel from methanol extracted lipid.

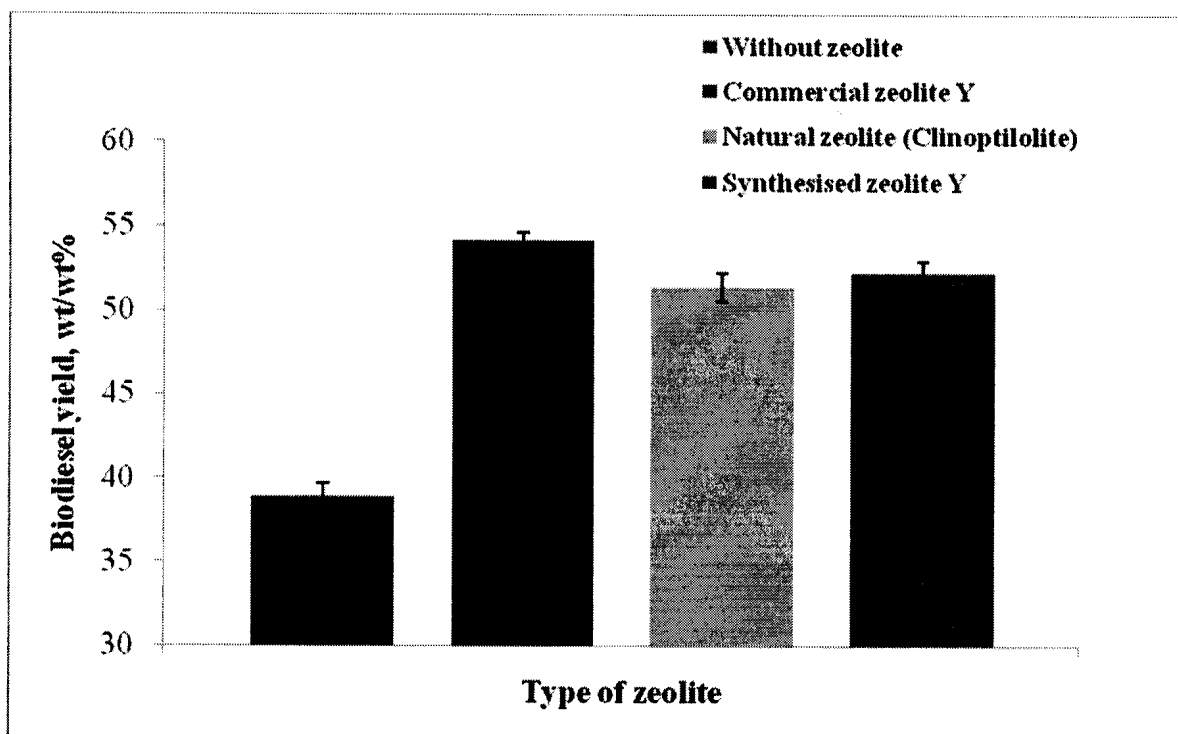


Figure 3.8: The effect of zeolite on biodiesel production via H_2SO_4 catalyzed esterification-transesterification of lipid.

3.3.4 Effect of Zeolite on Biodiesel Production from Extracted Lipid

The effect of zeolite on biodiesel production is shown in Fig. 3.8. The use of zeolite has significantly increased the biodiesel yield from 38.94 (wt/wt) % (on the basis of lipid) to 54.13 (wt/wt) %, 51.39 (wt/wt) %, and 52.21 (wt/wt) % (on the basis of lipid) for commercial zeolite Y, natural zeolite, and synthesised zeolite Y, respectively. As natural zeolite is cheaper compare to others, I used it for the experimental analysis of biodiesel production from the lipid of wastewater sludge.

3.3.5 Factorial Design for Biodiesel Production from the Lipid of Primary Sludge Lipid Extracted by Methanol

The normal probability plot for effects of biodiesel production from extracted lipid is shown in Fig. 3.9.

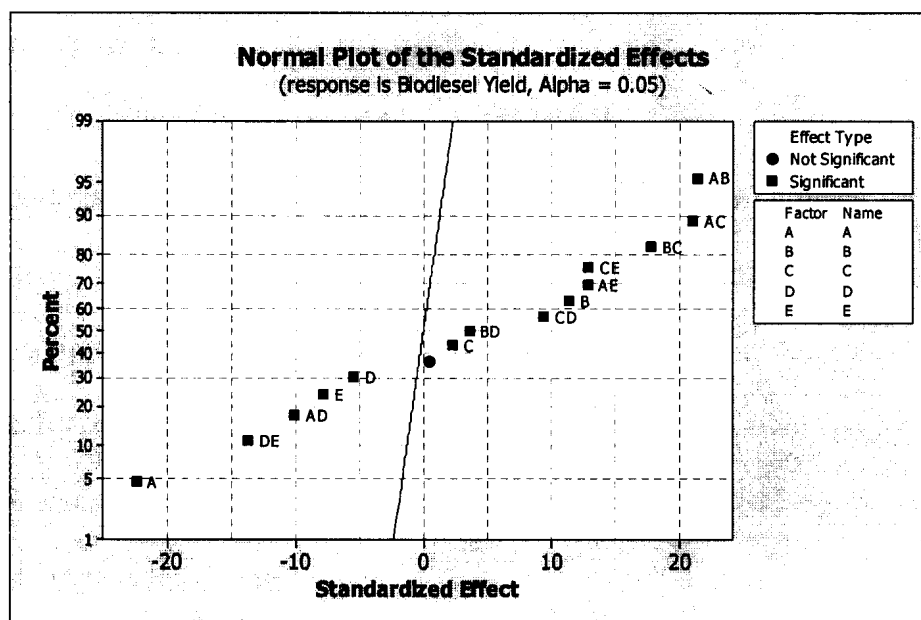


Figure 3.9: Normal probability plots of effects for biodiesel production via H_2SO_4 catalyzed esterification-transesterification of lipid.

The responses, the biodiesel yield, Y expressed in terms of wt/wt% on the basis of lipid, for is presented in **Table 3.5**. The maximum FAME yield of half fraction 2^5 Plackett-Burman factorial design was 57.12% (wt/wt) (on the basis of lipid). This biodiesel yield was the result of centre point run that was the optimum condition for biodiesel production from the lipid of primary sludge extracted by using methanol. The response for the model is the amount of biodiesel Y expressed in terms of wt/wt% (on the basis of lipid). The underlying model for a half fraction 2^5 Plackett-Burman factorial design is given by

$$Y = 45.311 - 1.941X_4 + 0.994X_5 + 0.202X_6 - 0.477X_7 - 0.676X_8 + 1.862X_4X_5 + 1.83X_4X_6 - 0.872X_4X_7 + 1.118X_4X_8 + 1.550X_5X_6 + 0.314X_5X_7 + 0.819X_6X_7 - 1.189X_7X_8 + \varepsilon \quad \dots(3.2)$$

Here, X_i is the factors influencing the biodiesel production namely methanol to lipid ratio (X_4), reaction time (X_5), temperature (X_6), amount of acid catalyst (X_7), and amount of natural zeolite (X_8); $X_i X_j$ is the interaction between the factors, and ε is the random error term having a normal distribution with mean zero and variance σ^2 [26]. According to the **Fig. 3.9** and the model (Eqn. 3.2), the interaction of methanol to lipid ratio (X_4) and reaction time (X_5) was turned out to be the most significant for the model. Reaction time, temperature, amount of natural zeolite, concentration of acid catalyst, methanol to lipid ratio and the some interactions among the variables were also found to be statistically significant. The R-squared of 0.9955 was in reasonable agreement with the adjusted R-squared of 0.9812 for the experimental analysis of biodiesel production from the lipid of wastewater sludge.

3.3.6 Effect of Time on Biodiesel Production

The effect of reaction time on biodiesel production from the lipid of primary sludge, extracted by using methanol, is shown in **Fig. 3.10**. The four temperature levels 40°C, 50°C, 60°C, and 70°C and the optimum amounts (**section 3.3.5**) of natural zeolite, lipid to methanol ratio and catalyst concentration, were used for the 48 h of study. The reaction was fast initially due to the esterification of the free fatty acid present in the lipid. However, the reaction was slow after 12 h that may be due to the slow acid catalyzed transesterification of lipid.

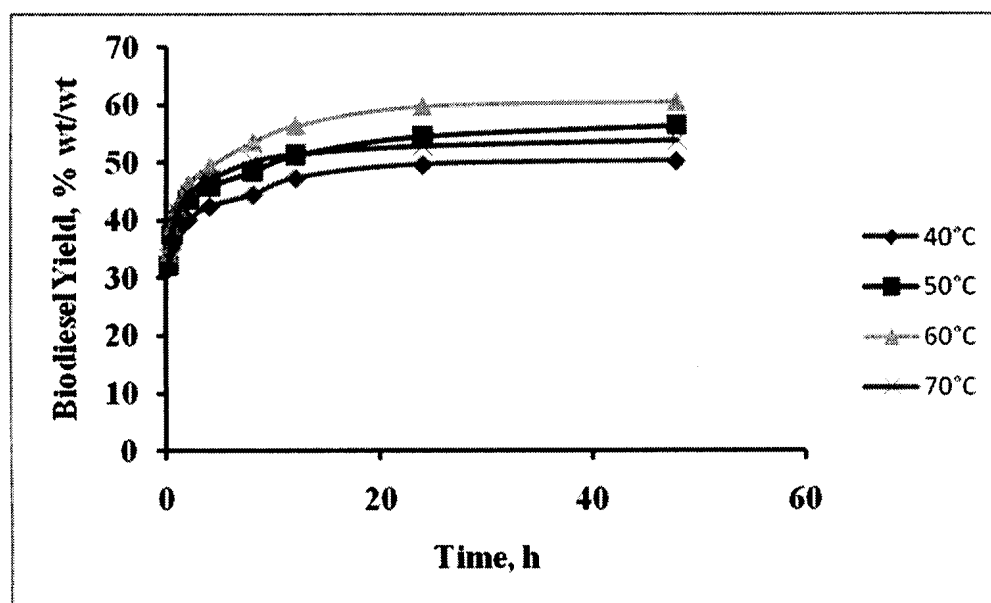


Figure 3.10: The effect of time on biodiesel production via H_2SO_4 catalyzed esterification-transesterification of lipid of the wastewater sludge.

3.3.7 Effect of Temperature on Biodiesel Production

The effect of temperature on biodiesel production from the lipid of primary sludge is shown in **Fig. 3.11**.

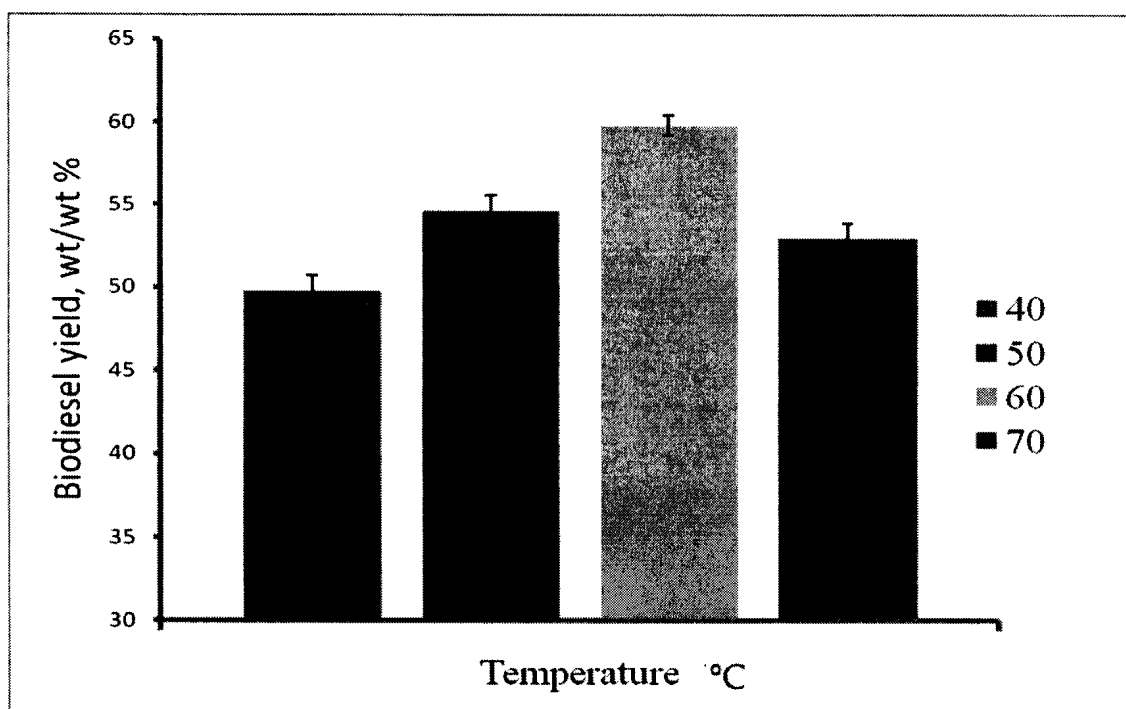


Figure 3.11: The effect of temperature on biodiesel production via H_2SO_4 catalyzed esterification-transesterification of lipid of the wastewater sludge.

The four temperature levels 40°C, 50°C, 60°C, and 70°C were used for the 24h reaction time. The yield of biodiesel production was increased with temperature upto 60°C. The maximum yield was obtained at 60°C that was approximately 60 wt/wt% (on the basis of extracted lipid). However, significant polymerization of unsaturated fatty acids and their derivatives might have caused the decrease of biodiesel yield at temperature above 60°C. Another, possible reason is the loss of methanol at higher temperature.

3.3.8 Factorial Analysis of in-situ Biodiesel Production from Dried Primary Sludge

The maximum FAME yield of 2^3 Plackett-Burman factorial designs was 12.81% (wt/wt) (on the basis of dry sludge). The normal probability plot for effects of biodiesel production from extracted lipid is shown in Fig. 3.12.

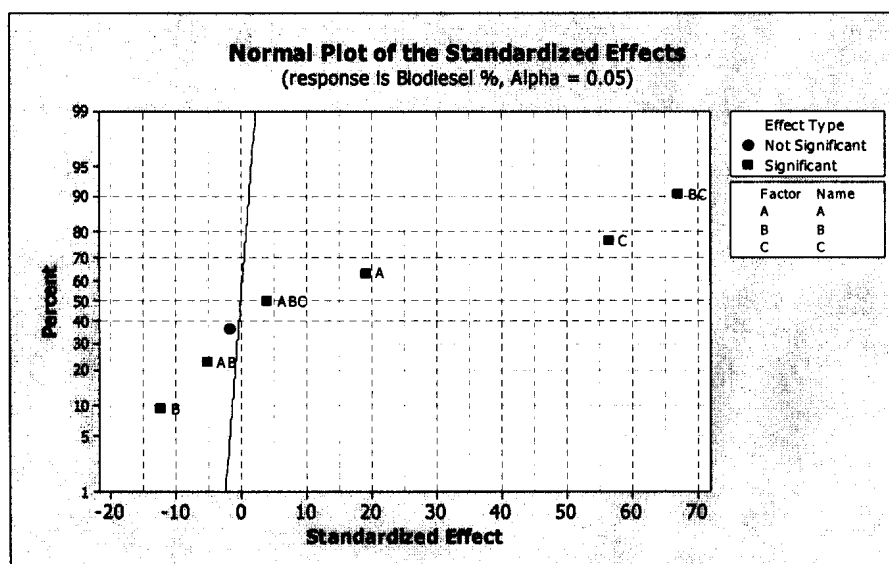


Figure 3.12: Normal probability plots of effects for in-situ biodiesel production from dried primary sludge.

The responses, the biodiesel yield, Y expressed in terms of wt/wt% on the basis dry sludge, is presented in **Table 3.7**. The underlying model for in-situ biodiesel production is represented as:

$$Y = 10.228 + 0.376X_9 - 0.241X_{10} + 1.112X_{11} - 0.103X_9X_{10} - 0.036X_9X_{11} + 1.321X_{10}X_{11} + 0.078X_9X_{10}X_{11} + \varepsilon \quad \dots\dots\dots (3.3)$$

Here, X_i is the factors influencing the in-situ biodiesel production namely temperature (X_9), sludge to methanol ratio (X_{10}), catalyst concentration (X_{11}); $X_i X_j$ is the interaction between the factors, and ε is the random error term having a normal distribution with mean zero and variance σ^2 [26]. According the model (Eqn. 3.3) and **Fig. 3.12**, the interaction of sludge to methanol ratio and catalyst concentration is the most significant for the model. Catalyst concentration is the single most significant variable for the in-situ biodiesel production. That means, higher percentage of acid catalyst is required to get maximum biodiesel yield. The highest coefficient of variation

(relative standard deviation) for the all treatment combinations was 7.87% indicating satisfactory data agreement between replicate runs. The predicted R-squared of 0.9952 was in reasonable agreement with the adjusted R-squared of 0.9981 for the experimental analysis of biodiesel production from the lipid of wastewater sludge.

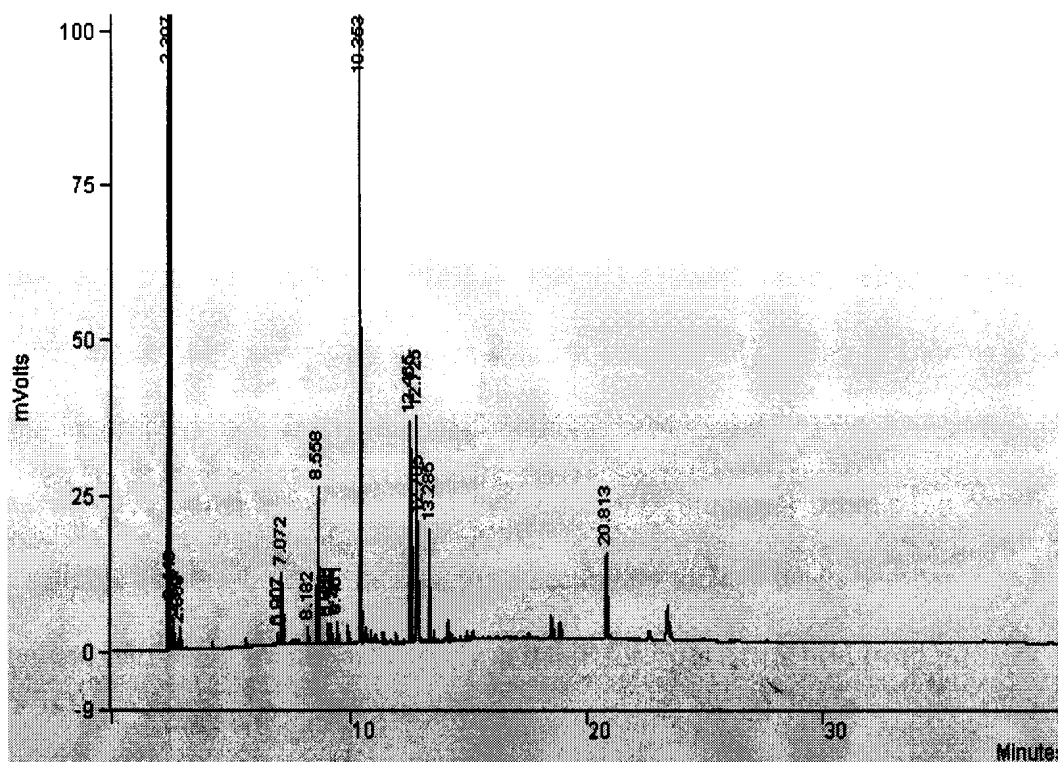


Figure 3.13: Gas chromatograph of FAME produced by in-situ esterification-transesterification of the dried primary sludge.

A gas chromatograph of in-situ biodiesel production from dried primary sludge is shown in **Fig. 3.13**. This figure illustrates the similarity of FAME compositions between ex-situ and in-situ biodiesel production. To increase the biodiesel yield, natural zeolite was also used to absorb the water produced by in-situ esterification. However, there was no increase of biodiesel yield that may be due to the presence of solid residues.

3.4 Conclusions

Models based on the response surface optimization are developed for lipid extraction from dried municipal primary and secondary sludge and biodiesel production. Among the three factors of lipid extraction, temperature proved to be the most significant at 5% level of significance for each of the performed extractions. Extraction using methanol resulted in a higher percentage of lipid, 14.46 (wt/wt) % and 10.04 (wt/wt) % (on the basis of dry sludge), from the primary and secondary sludge sources, respectively, as compared to 11.16 (wt/wt) % and 3.04 (wt/wt) % lipid (on the basis of dry sludge), from the primary and secondary sludge sources, respectively, using hexane as a solvent. The lipid extracted by using methanol contain higher amount of FFA compare to hexane extracted lipid. Considering the lipid extraction and biodiesel production, methanol is more suitable for the lipid extraction from secondary sludge when compared to hexane; hexane and methanol are almost equally suitable for lipid extraction from primary sludge sources. However, lipid extraction from dried sludge using methanol is more advantageous as methanol is the raw material for biodiesel production, moreover, it requires short extraction time. Fatty acid compositions of primary and secondary sludge were quite similar, and these findings were in agreement with the findings of Mondala et al. by the in-situ transesterification of primary and secondary sludge [10]. Hence, it is suitable to produce biodiesel from the mixed lipid of primary and secondary sludge. The use of natural zeolite has increased biodiesel yield from 38.94 (wt/wt) % to 51.39 (wt/wt) %. Among the factors under investigation for biodiesel production, the interaction of methanol to lipid ratio(X_4) and reaction time (X_5) was turned out to be the statistically most significant for the model at 0.05 level of significance. Amount of natural zeolite,

concentration of acid catalyst, methanol to lipid ratio, reaction time, temperature, and the some interactions among the variables were also found to be statistically significant at 5% level of significance. At the optimized conditions (center points) for biodiesel production from the methanol extracted lipid of primary sludge, biodiesel yield was 57.12 (wt/wt) % that was approximately 18% more biodiesel yield with a reduction of 10 h reaction time. The factorial analysis of in-situ biodiesel production clarified the requirement of higher amount of acid catalyst for better biodiesel yield. Hence, it is advantageous to produce biodiesel from the extracted lipid in presence of natural zeolite by using the existing biodiesel production facilities.

3.5 References

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Chapter Four

Biodiesel Production from the Lipid of Wastewater Sludge using Acidic Heterogeneous Catalyst

4.1 Introduction

Biodiesel has worldwide attraction and growing interest as a renewable, biodegradable, and clean fuel. It contains 10% to 11% oxygen by weight and has a higher cetane number compare to diesel fuel, no aromatics, and almost no sulfur. For this reason, it can significantly reduce CO₂, SO_x, and unburned hydrocarbons emission from motor vehicles. It provides similar energy density to petro-diesel and can be used in most diesel engines in pure form (B100) or may be blended with petroleum diesel at any concentration [1, 2, 3, 4, 5]. However, the biodiesel production from pure vegetable oil is a major economic challenge as 70% and 85% of the overall biodiesel production cost is associated with the raw-materials [2, 7, 8]. Moreover, these raw-materials for biodiesel production compete with food materials. Alternative raw-materials like algae, waste cooking oil, wastewater sludge, non-edible oil like jatropha, castor, neem, karanja, etc. are also being used for biodiesel production [4]. However, presence of large amount of free fatty acid (FFA) is an obstacle for biodiesel production from these sources. Thus, it is important to develop new catalytic processes which can produce biodiesel from the lipid sources containing higher FFA.

Chemically, biodiesel a fatty acid alkyl ester commonly known as fatty acid methyl ester (FAME), is mainly produced by esterification and/or transesterification reaction (**Fig.4.1**) of various lipid sources with alcohol in the presence of a homogeneous base, acid, enzyme or a heterogeneous catalyst. Base catalyst transesterification is very fast compared to other catalysts and widely used commercially. However, base catalyst is suitable for pure vegetable oil and is highly sensitive to the presence free fatty acids that react with base catalyst and form soap that consumes catalyst, inhibits glycerol separation

and facilitates emulsion formation during washing step [5]. Acid catalyzed process is slower than base catalyzed transesterification and requires high lipid to alcohol ratio [9]. However, acid catalyst is able to catalyze both the esterification and transesterification (Fig. 4.1) reactions to produce more biodiesel. This approach requires a water management technique as the presence of water can stop the esterification reaction.

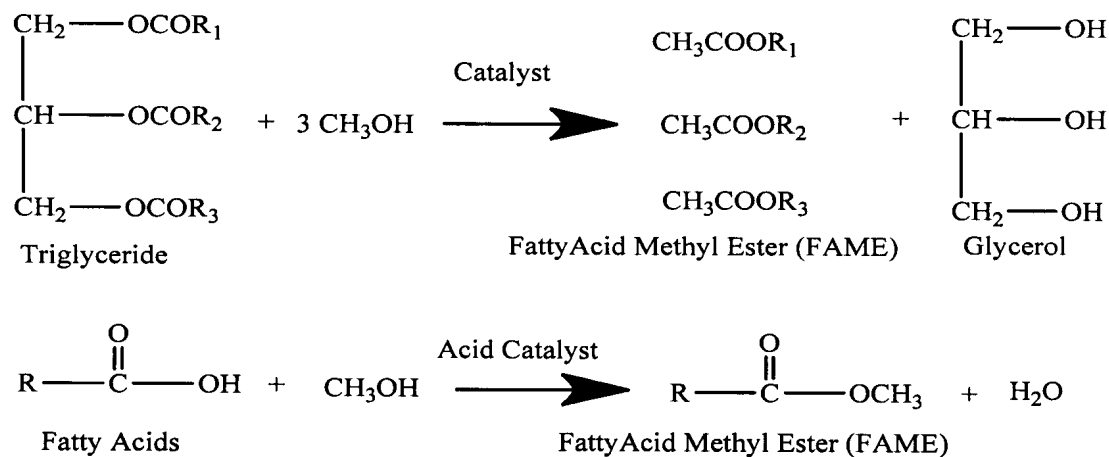


Figure 4.1: Acid-catalyzed transesterification of triglycerides and esterification of fatty acids.

Two-step method – an acid catalyzed followed by base catalyzed – has been reported for biodiesel production from waste or unrefined oils as raw materials [10, 11]. However, the process requires multiple reactions, washing and separation steps. Due to the corrosive nature of strong acid or base, catalysts must be removed from the biodiesel by multiple washing steps which requires significant amount water along with the loss of catalyst [1].

Heterogeneous catalysts have shown greater promise for biodiesel production. The commonly used heterogeneous catalysts are Mg/La mixed oxide, S-ZrO₂ sulfated zirconia, KOH/Nax zeolite, Li/CaO, CaO, KI/Al₂O₃, (ZS/Si) zinc stearate immobilized on silicagel, KNO₃/Al₂O₃, SO₄²⁻/TiO₂-SiO₂, ZnO-La₂O₃, etc. [1, 4, 12]. Heterogeneous

catalysts can be recovered conveniently from reaction products. Moreover, the undesired saponification reactions can be avoided by using heterogeneous acid catalysts. These catalysts enable the transesterification of vegetable oils or animal fats with high contents of free fatty acid (FFA) [13]. Solid catalysts can be reused and provide the possibility for carrying out both transesterification and esterification reactions simultaneously [14].

Wastewater sludge has been considered as potential feedstock for biodiesel production [15, 16, 17]. Lipids are usually extracted from the sludge with organic solvents to avoid the interference and subsequently, biodiesel is produced from the extracted lipids. Approximately 17–18 wt % lipids (on the basis of dry sludge) were extracted from dried sewage sludge by boiling solvent extraction using chloroform and toluene as solvents of which 65 wt % of the extracts were found to be free fatty acids and 7 wt % were glyceride fatty acids and 28 wt% were unsaponifiable material [18]. Pokoo-Aikins et al. used toluene, hexane, ethanol, and methanol to separate the free fatty acids (FFA), triglycerides (oils) and the solvent from the extracted lipids from sewage sludge and found the yield of FFA was 24.8 wt%, 24.9 wt%, 25.5 wt%, 25.5 wt%, respectively. For all the four solvents, the maximum yield of triglyceride was 3.4 wt% [15]. Very few researchers report biodiesel production from wastewater sludge, and there is no report of using heterogeneous catalyst for biodiesel production from the lipid of wastewater sludge.

In this study, a mesoporous heterogeneous catalyst, namely a mesoporous ordered silica, SBA-15, impregnated with different percentage of heteropolyacid $\text{H}_3\text{PO}_4 \cdot 12\text{WO}_3 \cdot x\text{H}_2\text{O}$, was synthesized, characterized, and used in the production of biodiesel from the lipid of wastewater sludge.

4.2 Materials and Method

4.2.1 Materials

Poly (ethylene glycol)-block-poly (propylene glycol)-block-poly (ethylene glycol) (i.e. PEG-PPG-PEG; P-123) and sodium bicarbonate were purchased from Sigma-Aldrich Inc. (St. Louise, MO, USA), sodium silicate was purchased from The PQ Corporation (Malvern, PA, USA). Supleco™ standard 37component FAME mix, a 37 component reference mixture of fatty acid methyl ester in the range of C₄ to C₂₄ including saturated, mono-unsaturated and poly unsaturated FAME, was purchased from Supleco (Bellefonte, PA, USA) and used to calibrate the GC for FAME analysis. HPLC- grade methanol and hexane were purchased from Caledon Laboratories Ltd. (Georgetown, ON, Canada); sodium chloride was purchased from EM Science (Gibbstone, NJ, USA); anhydrous sodium sulphate was purchased from BDH Inc. (Toronto, ON, Canada). All the chemicals were used without any treatment.

4.2.2 Extraction of Lipid from Sludge

The wastewater sludge, collected from Adelaide Pollution Control Plant, London, ON, Canada, on September 23, 2009 and same sludge was used during entire study. The sludge was allowed to settle for 24 hours at 0°C and the supernatant liquid was discarded. The resulting sludge was then centrifuged using IEC Centra-HN centrifuge (International Equipment Company, Needham Heights, USA) for further dewatering. Dewatered sludge was spread on tray and put in a fume hood to dry under vacuum at ambient temperature. Dried sludge was crushed in a mortar and pestle, homogenized and then stored in a freezer prior to use.

The lipid was extracted from dried sludge by using methanol as a solvent. The dried sludge was weighed into a round bottom flask and methanol was added and then the resulting mixture was heated to the 70°C temperature at ambient pressure. A magnetic stirring bar was used for mixing and the loss of methanol due to evaporation was minimized by using a condenser with water at 20°C. After assigned extraction time (1 h), the resulting slurry was immediately filtered using VWR filter paper (size 5.5 cm) and a Buchner funnel attached to vacuum. Any residual particles were removed by centrifuging the lipid solution. The methanol was removed from the resulting supernatants under vacuum using a Büchi Rotavapor R-200 (Büchi Labortechnik, Switzerland) at 45°C and then flask was flushed with air to remove any remaining methanol in the gas phase. The resulting lipid was stored in refrigerator prior to use.

4.2.3 Catalyst Preparation

Highly ordered mesoporous silica SBA-15, which was used as the catalyst substrate, was synthesized according to the procedure described in the literature [19] with minor changes of the procedure. Briefly, 6 g of triblock copolymer of poly (ethylene glycol)-block-poly (propylene glycol)-block-poly (ethylene glycol) (i.e. PEG-PPG-PEG; P-123) with an average molecular weight of ~5,800 was added to 180 ml of 2M HCl solution in a conical beaker. The mixture was vigorously mixed using a magnet stirrer at 35°C until all of the P-123 was dissolved (i.e. solution A). In another beaker, 15.5 g of sodium silicate solution (>27 wt% SiO₂) was diluted to 45 ml of deionized water (i.e. solution B). Then, solution B was added drop-wise to the solution A under vigorous stirring. Mixing of the resultant milky mixture was continued for 10 more min before switching to the static synthesis conditions of ageing at 35°C for 24 h. The SBA-15

mesoporous was crystallized at 100°C for 24 h under static condition. The SBA-15 product was then cooled to ambient temperature and separated from supernatant by means of vacuum filter using appropriate filter paper, rinsed with extra amount of deionized water to remove unreacted silicon precursor, dried at 60°C in an electrical oven for 5 h. In order to remove the organic molecules of the p-123 surfactant from the SBA-15 pores, the product was calcined at 500°C for 5 h under a controlled heating and cooling rate. Schematic diagram of SBA-15 synthesis is illustrated in **Fig. 4.2**.

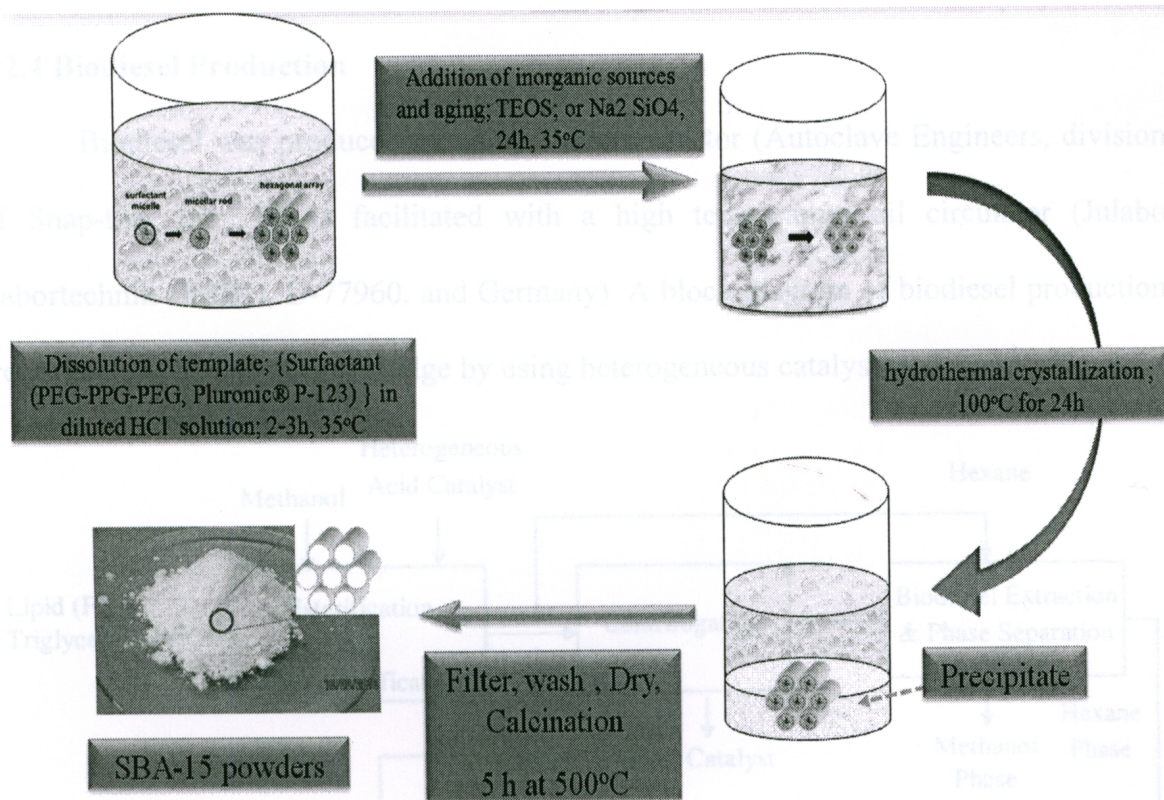


Figure 4.2: General synthesis scheme for the ordered mesoporous silica SBA-15.

To prepare acidic heterogeneous catalysts for biodiesel synthesis purposes, the mesoporous SBA-15 was loaded with different percentage of dodeca-tungstophosphoric acid (i.e. $\text{H}_3\text{PO}_4 \cdot 12\text{WO}_3 \cdot x\text{H}_2\text{O}$) as a super-acid (i.e. heteropolyacid; HPA) by means of an

“incipient wetness” impregnation method. To do this, appropriate amount of HPA (wt/wt) %, which was corresponding to 5%, and 15% of HPA loading with respect to SBA-15 support, and was dissolved in methanol to form a homogeneous solution. The HPA solution was then added to the measured quantity of mesoporous substrate, while it was mixed using a magnetic stirrer for 30 min. Later, the formed slurry was dried at 60°C and calcined at 170°C for 5 h under air atmosphere with controlled rate of heating and cooling.

4.2.4 Biodiesel Production

Biodiesel was produced by using a micro-reactor (Autoclave Engineers, division of Snap-tite, Inc., USA) facilitated with a high temperature oil circulator (Julabo Labortechnik GMBH, D-77960, and Germany). A block diagram of biodiesel production from the lipid of wastewater sludge by using heterogeneous catalyst is shown in **Fig. 4.3**.

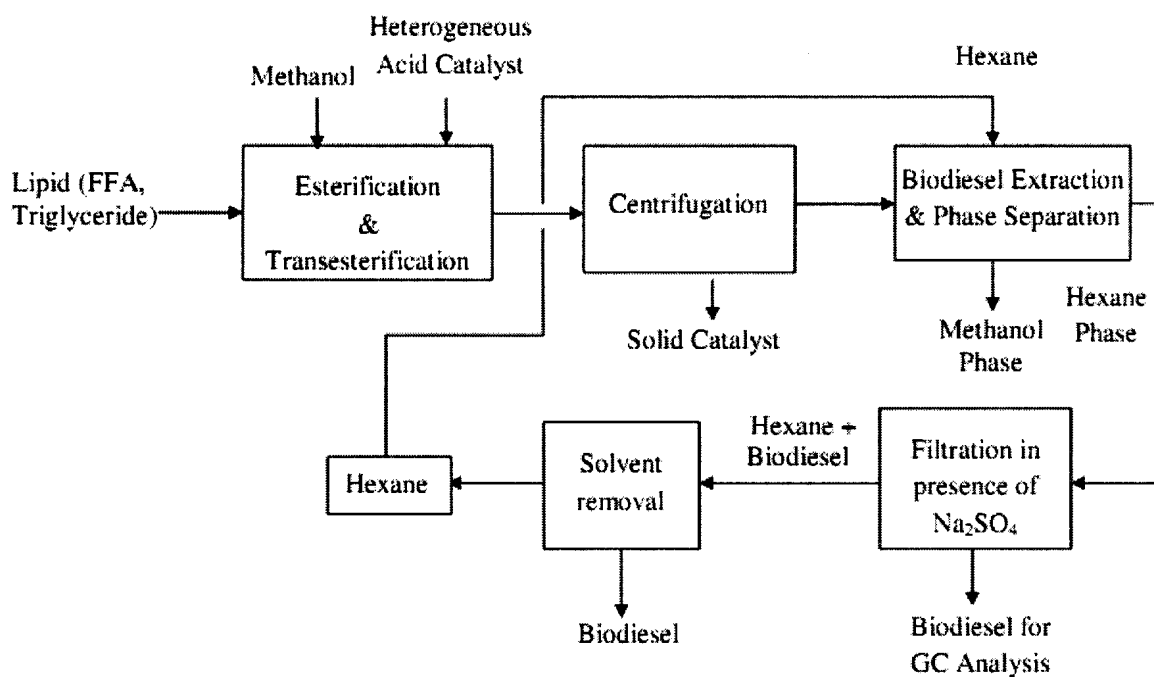


Figure 4.3: Block diagram of biodiesel production from the extracted lipid by using solid acid catalyzed esterification and transesterification.

Five hundred milligram of extracted lipid was mixed with 100 ml of methanol and assigned amount of acidic mesoporous heterogeneous catalyst namely, SBA-15 impregnated with different percentage of heteropolyacid. The resulting mixture was introduced into the reactor and heated at 135°C (corresponding pressure in the reactor was 135 psig) and allowed to react for 3h. After the reaction time, the mixture was allowed to cool and then centrifuged using IEC Centra-HN centrifuge (International Equipment Company, Needham Heights, USA) for 10 min at 3000 rpm to remove the solid materials. The supernatants were transferred to a separatory funnel and 50 ml of hexane was added to the separatory funnel and shaken to extract biodiesel. Extraction procedure was repeated twice. Hexane layer was passed through a Whatman filter paper (110 mm dia) containing anhydrous sodium sulphate and collected into a measuring flask. A 1.5 ml aliquot of hexane phase was pipetted into 2.0 ml Supelco PTFE lined capped vial (Supelco, Bellefonte, PA) for FAME analysis using GC. The remaining hexane phase was transferred to a round bottom flask and the solvent was removed under vacuum using a Büchi R205 Rotary Evaporator (Büchi Labortechnik, Switzerland) at 40°C to get biodiesel. The yield of FAME was determined from FAME analysis by using a GC.

4.2.5 FAME Analysis

Varian CP-3800 gas chromatograph (Varian Inc., Lake Forest, CA) equipped with FID, and a 50m x 0.25 mm x 0.2 µm Varian CP-Wax 58 (FFAP) CB capillary Column (Varian Inc., Lake Forest, CA) was used to analyzed the FAME. Helium was used as a carrier gas and the sample injection volume was 1.0 µl with a split ratio of 80:1. The column flow was constant at 1.2 ml/min and column oven temperature was programmed

to maintain at 100 °C for 1.0 min, increased from 100 °C to 200 °C at 15 °C /min, then increased from 200 °C to 240 °C at 5 °C /min, and finally maintained at 240 °C for 24 min. The detector and injector temperature were set at 260 °C for the duration of the analysis.

4.3. Results and Discussion

4.3.1 Catalyst Characterization

The as-synthesized SBA-15 was characterized by means of various instrumental techniques such as SEM, BET surface area, TGA, and XRD. The SEM image, which is shown in **Fig. 4.4**, was taken by means of JSM 600F model, Joel, Japan operating at 10 keV of acceleration voltage. SEM image of the fabricated mesoporous SBA-15 exhibits hexagonal cylinder-like morphologies, which is one of the typical morphologies known for this type of highly ordered mesoporous silica materials.

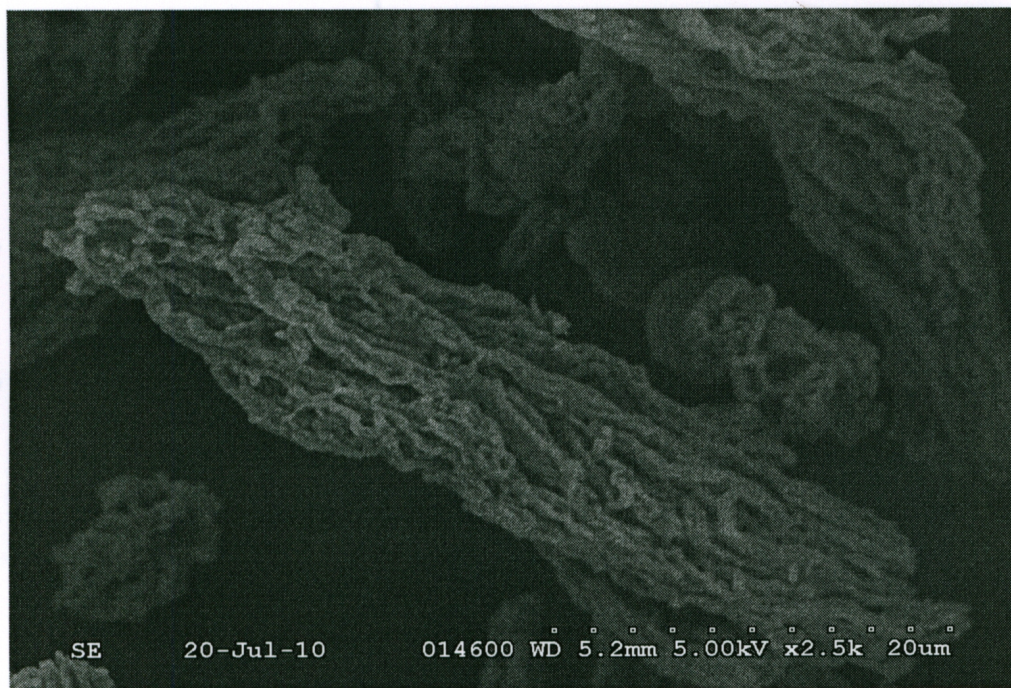


Figure 4.4: SEM micrograph of a calcined SBA-15 sample.

XRD of both the as-synthesized SBA-15, and SBA-15 impregnated with heteropolyacid (15% PW) were conducted by a Rigaku-Miniflex powder diffractometer (Carlsbad, CA, USA). XRD patterns of the as-synthesized SBA15, pure heteropolyacid and the SBA-15 sample impregnated with heteropolyacid (15% PW) are illustrated in Fig. 5. Since the main peaks of the mesoporous SBA-15 located at 2-theta smaller than 5° it does not show any major characteristic peaks at the scanned range (i.e. 2-theta 5-80 degrees), then it can be concluded that the peaks exhibited on the impregnated SBA-15 sample belonged to the heteropolyacid.

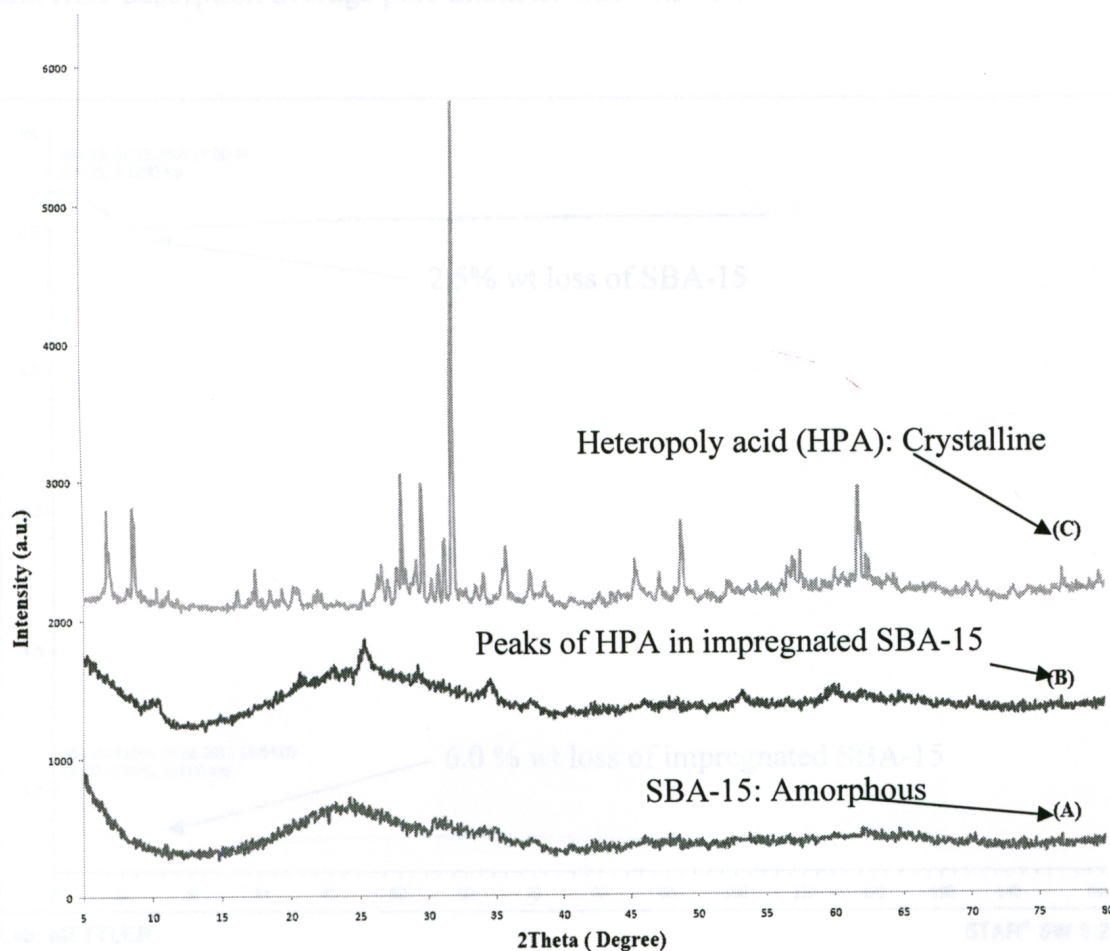


Figure 4.5: XRD patterns of (A) as-synthesized SBA-15, (B) SBA-15 impregnated with heteropolyacid (15% PW), and (C) pure heteropolyacid.

Surface area of the as-synthesized mesoporous was measured using a BET surface area and pore size analyzer (Micromeritics ASAP 2010) instrument. Prior to measurement of nitrogen adsorption-desorption isotherms, the sample was degassed under vacuum (10^{-5} Torr) at $125\text{ }^{\circ}\text{C}$ for 6 h. BET surface area of the calcined SBA-15 sample was $901.224\text{ m}^2/\text{g}$, specific surface area was $760.81\text{ m}^2/\text{g}$, and BET adsorption average pore diameter was 54.54 \AA , which are in close agreement with those reported in the relevant literatures [19]. However, for SBA-15 impregnated with heteropolyacid (15% PW) the BET surface area was $607.34\text{ m}^2/\text{g}$, specific surface area was $497.11\text{ m}^2/\text{g}$, and BET adsorption average pore diameter was 44.59 \AA .

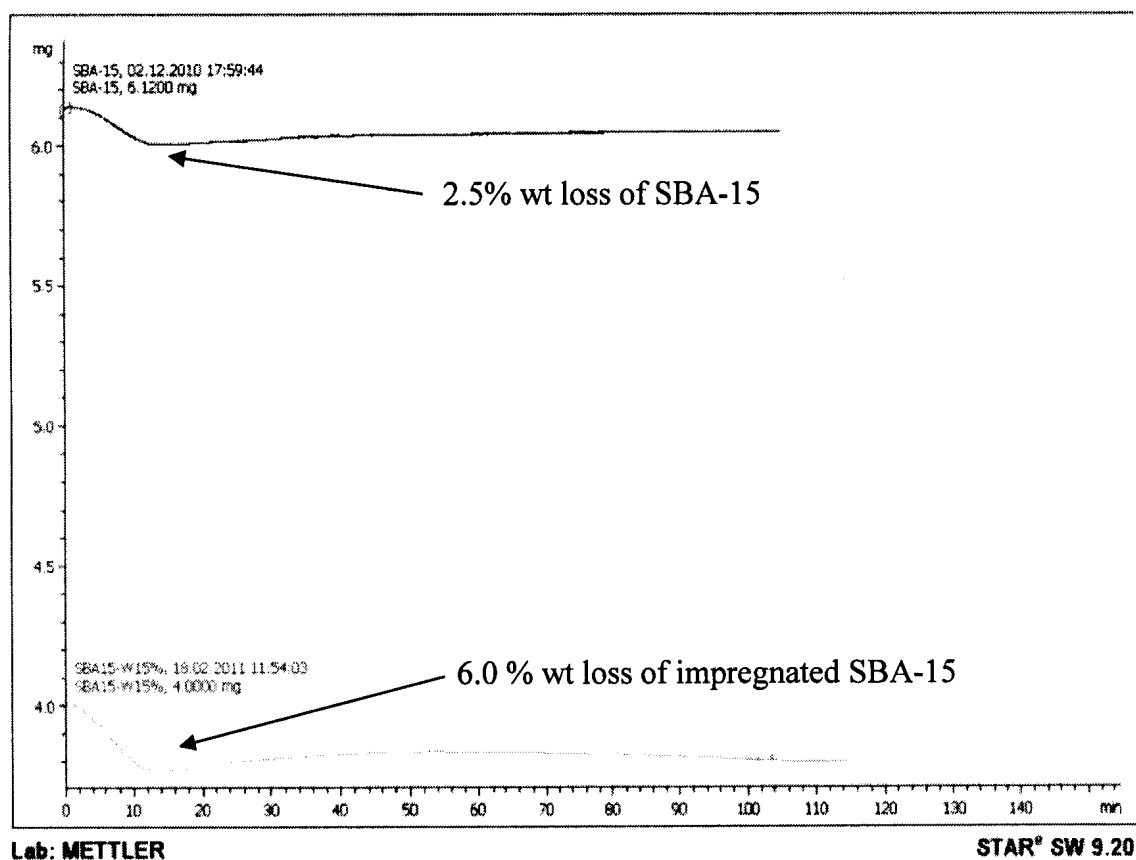


Figure 4.6: Comparison of the TGA thermograms of as-synthesized SBA-15, and SBA-15 impregnated with heteropolyacid (15% PW).

A thermogravimeter of type Mettler TGA/SDTA851e was used to compare the as-synthesized SBA-15, and SBA-15 impregnated with heteropolyacid (15% PW). Comparison of the TGA thermograms of as-synthesized SBA-15, and SBA-15 impregnated with heteropolyacid (15% PW) is shown in **Fig. 4.6**. Samples were heated from 25°C to 600°C at a heating rate of 5°C/min under nitrogen atmosphere. TGA results were indicated the higher percentage of weight loss in case of SBA-15 impregnated with heteropolyacid (15% PW) compare to SBA-15. That was due to the weight loss of impregnated heteropolyacid.

4.3.2 FAME Analysis

The maximum FAME yield was 30.14 (wt/wt) % (on the basis of lipid) that was obtained for 15% PW₁₂ at 135°C and 3h reaction time. The effect of PW₁₂ loading on the lipid conversion is illustrated in **Fig. 4.7**.

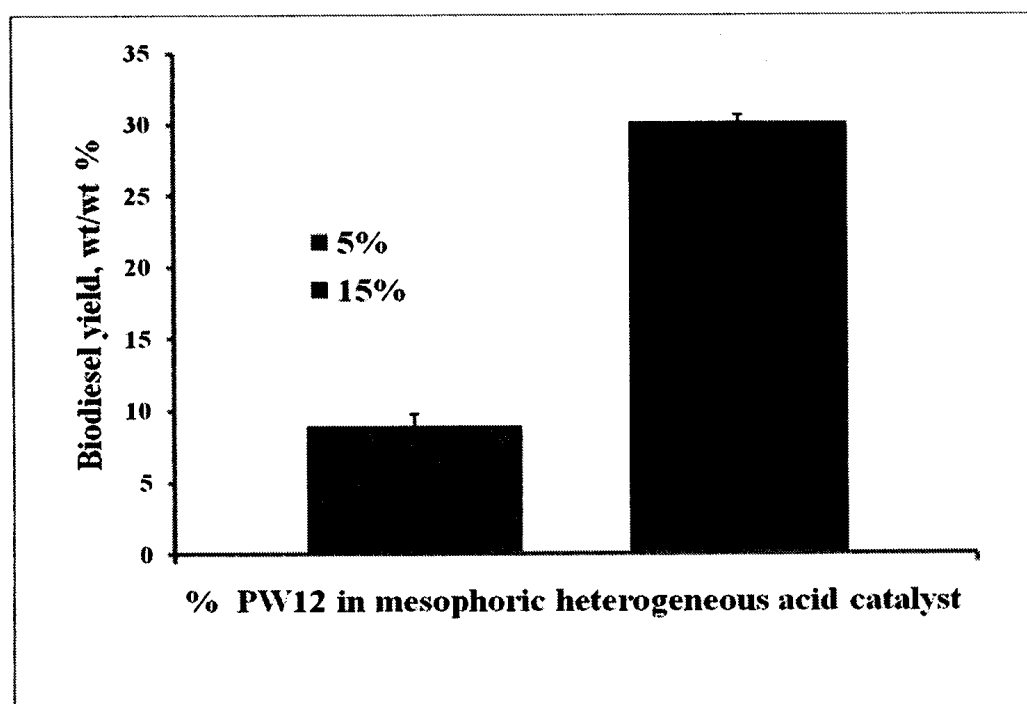


Figure 4.7: The effect of PW₁₂ loading in heterogeneous catalyst for biodiesel production from the lipid of wastewater sludge.

As shown in **Fig. 4.7**, biodiesel yield was increased by increasing the loading of PW_{12} . Although the maximum FAME yield was only 30.14 (wt/wt) %, reuse of catalyst, and the optimization of biodiesel production process may increase the overall biodiesel yield.

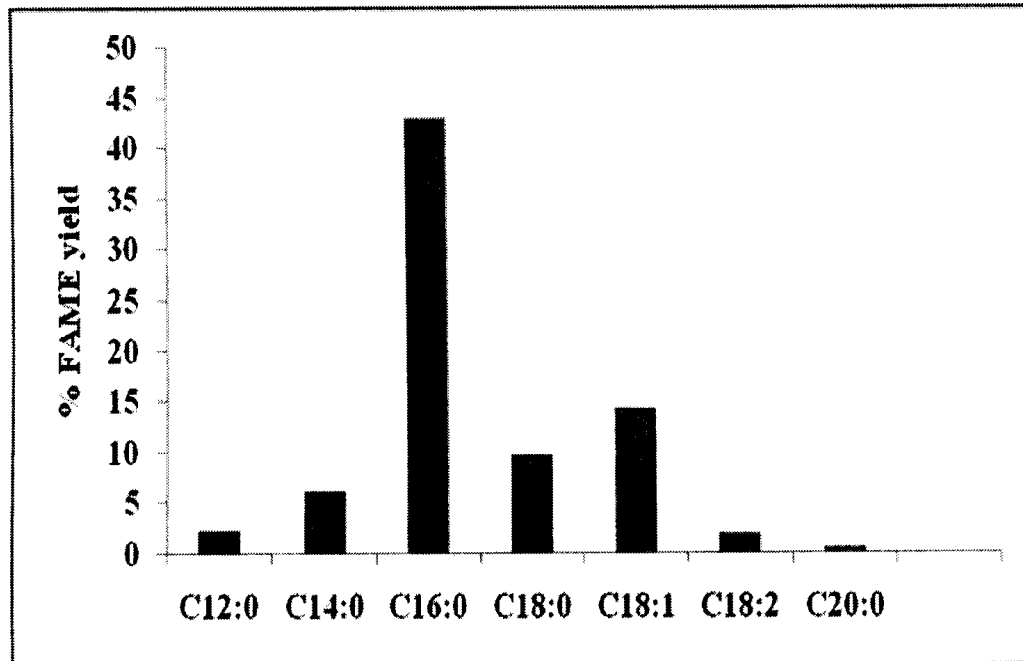


Figure 4.8: FAME analysis of biodiesel produced from the lipid of primary sludge by using solid acid catalyst.

The FAME analysis of biodiesel produced from the lipid of wastewater sludge by using solid acid catalyst is shown in **Fig. 4.8**. According to the **Fig. 4.8**, biodiesel contain mainly the methyl ester of myristic acid (C 14:0), palmitic acid (C16:0), palmitoleic acid (C 16:1), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C 18:2). Methyl ester of palmitic acid (C 16:0) is present in greatest amount in the biodiesel produced from the extracted lipid by using solid acid catalyst. These results are found to be in agreement with the findings of biodiesel produced by using homogeneous acid catalyst.

4.4 Conclusions

The potential of mesoporous ordered silica, SBA-15, impregnated with heteropolyacid $\text{H}_3\text{PO}_4 \cdot 12\text{WO}_3 \cdot x\text{H}_2\text{O}$ (PW_{12}) was investigated to produce biodiesel from the solid sludge from the wastewater treatment plants. Different analytical techniques including scanning electron microscopy (SEM) and BET surface analysis were applied to characterize the prepared mesoporous catalysts. The highest biodiesel yield at the examined conditions was 30.14 (wt/wt) % (on the basis of lipid) for the mesoporous catalyst impregnated with 15% PW_{12} . The optimization of biodiesel production by considering the various factors such as reaction time, temperature, catalyst loading, reuse of catalyst, etc. may increase the overall biodiesel yield. However, the FAME composition of biodiesel produced by using the homogeneous acid catalyst and heterogeneous acid catalyst are quite similar.

4.5 References

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Chapter Five

Conclusions and Recommendations

5.1 Lipid Extraction from Wastewater Sludge

Biodiesel, one of the most promising renewable green fuels, is produced from various lipid sources. Primary and secondary sludges of municipal wastewater treatment facilities are potential sources of lipids. Lipids are usually extracted from the sludge with organic solvents to avoid the interferences in the biodiesel production. In this work, a 2³ two-level factorial design was used to study the influence of three different variables (solvent to sludge ratio, temperature, and extraction time) on the lipid extraction efficiency from dried municipal primary and secondary sludge using methanol and hexane as organic solvents. The empirical models were developed for each type of solvent and sludge.

5.1.1 Conclusions

- The temperature turned out to be the most significant variable at 5% level of significance for lipid extraction by using methanol and hexane as solvents.
- Extraction using methanol resulted in a maximum of 14.46 (wt/wt) % and 10.04 (wt/wt) % lipid (on the basis of dry sludge), from the primary and secondary sludge sources respectively. A maximum of 11.16 (wt/wt) % and 3.04 wt/wt% lipid (on the basis of dry sludge) was extracted from the primary and secondary sludge sources, respectively, using hexane as a solvent.
- Methanol is more suitable for the lipid extraction from both dried primary and secondary sludge, and hexane works well only for the primary sludge. However, lipid extraction from dried sludge using methanol is more advantageous as methanol is the raw material for biodiesel production. Moreover, methanol requires shorter extraction time.

- Lipids extracted from wastewater sludges contain higher amounts of free fatty acids.

5.1.2 Limitations

- Although wastewater sludges are readily available without any cost or even with an incentive, dewatering and using organic solvents for lipid extraction are expensive.
- Health related risks and safety considerations should be considered extracting the lipid from wastewater sludge with possible pathogens.

5.1.3 Further Work

- Economic feasibility study for the large scale lipid extraction from the wastewater sludge should be carried out.
- Feasibility study to use microwave or pressurized vessels for the lipid extraction from wastewater sludge should be conducted.

5.2 Biodiesel Production from Wastewater Sludge

In this study, biodiesel was produced from the lipid of wastewater sludge by using homogeneous acid catalyst (H_2SO_4) as well as heterogeneous acid catalyst. A half fraction 2^5 Plackett-Burman factorial design was applied to study the effects of five factors, namely methanol to lipid ratio (X_4), reaction time (X_5), temperature (X_6), amount of acid catalyst (X_7), and amount of natural zeolite (X_8), in the production of biodiesel from the lipid of primary sludge extracted by methanol. A factorial experimental analysis was also used to produce biodiesel from dried primary sludge to compare the biodiesel yield with ex-situ biodiesel production.

5.2.1 Conclusions

5.2.1.1 Biodiesel Production from Extracted Lipids using Homogeneous Catalyst

- The biodiesel yield of hexane and methanol extracted lipids were 41.25 (wt/wt) % and 38.94(wt/wt) % (on the basis of lipid) for the primary sludge, and 26.89 (wt/wt) % and 30.28 (wt/wt) % (on the basis of lipid) for the secondary sludge.
- The biodiesel of both the primary and secondary sludge contain mainly the methyl ester of myristic acid (C 14:0), palmitic acid (C16:0), palmitoleic acid (C 16:1) stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C 18:2). Among these, methyl ester of palmitic acid (C 16:0) is present in greatest amount. Biodiesel from hexane extracted lipid contains more FAME of higher hydrocarbon and unsaturated acid composition compared to the biodiesel from methanol extracted lipid.
- The use of natural zeolite as a dehydrating agent significantly increased the biodiesel yield, approximately 18 (wt/wt) % (on the basis of lipid).
- The maximum FAME yield of half fraction 2⁵ Plackett-Burman factorial design was 57.12 (wt/wt) % (on the basis of lipid). The interaction of methanol to lipid ratio and reaction time turned out to be the most significant for the developed model.

5.2.1.2 Experimental Analysis of in-situ Biodiesel Production

The maximum FAME yield of 2³ Plackett-Burman factorial designs was 12.81 (wt/wt) % (on the basis of dry sludge). The interaction of sludge to methanol ratio and

catalyst concentration was the most significant for the model developed for the in-situ biodiesel production. Catalyst concentration was the single most significant variable for the in-situ biodiesel production. Higher percentage of acid catalyst and methanol were required to get maximum biodiesel yield.

5.2.1.4 Biodiesel Production from the Lipid of Wastewater Sludge using Heterogeneous Acid Catalyst

The mesoporous heterogeneous acid catalyst, SBA-15 impregnated with heteropolyacid $\text{H}_3\text{PO}_4 \cdot 12\text{WO}_3 \cdot x\text{H}_2\text{O}$ (PW_{12})), is a potential catalyst for biodiesel production from the lipid of wastewater sludge. The biodiesel yield for sample impregnated with 15% PW_{12} was 30.14 (wt/wt) % (on the basis of lipid) at temperature of 135°C, and pressure of 135psi for 3 h of reaction. The optimization of the process may increase the biodiesel production.

5.2.2 Limitations

- Water management technique is required for higher biodiesel yield from the lipid of wastewater sludge by using homogeneous acid catalyst. This is due to the esterification reaction in the presence of higher FFA in extracted lipid.
- In addition to known FAME composition, there were several small unidentified peaks that were not counted to calculate the overall biodiesel yield.
- Although solid catalyst provide better biodiesel yield at higher temperature, the maximum tolerable temperature for the micro-reactor used was 135°C.

5.2.3 Future work

- Economic feasibility study for large scale biodiesel production from wastewater sludge must be conducted.
- The quality of the biodiesel produced from the wastewater sludge must be further tested using the ASTM properties of biodiesel.
- A more comprehensive investigation is required to study the biodiesel production, from the lipid of wastewater sludge, by using solid acid catalyst.
- A flow reactor containing heterogeneous catalyst bed can be used for biodiesel production from the lipid of wastewater sludge.
- A comprehensive investigation to determine the P-content in the biodiesel produced from the phospholipid containing secondary sludge.

Appendices

1. Appendix A

1.1 Chromatogram of standard FAME

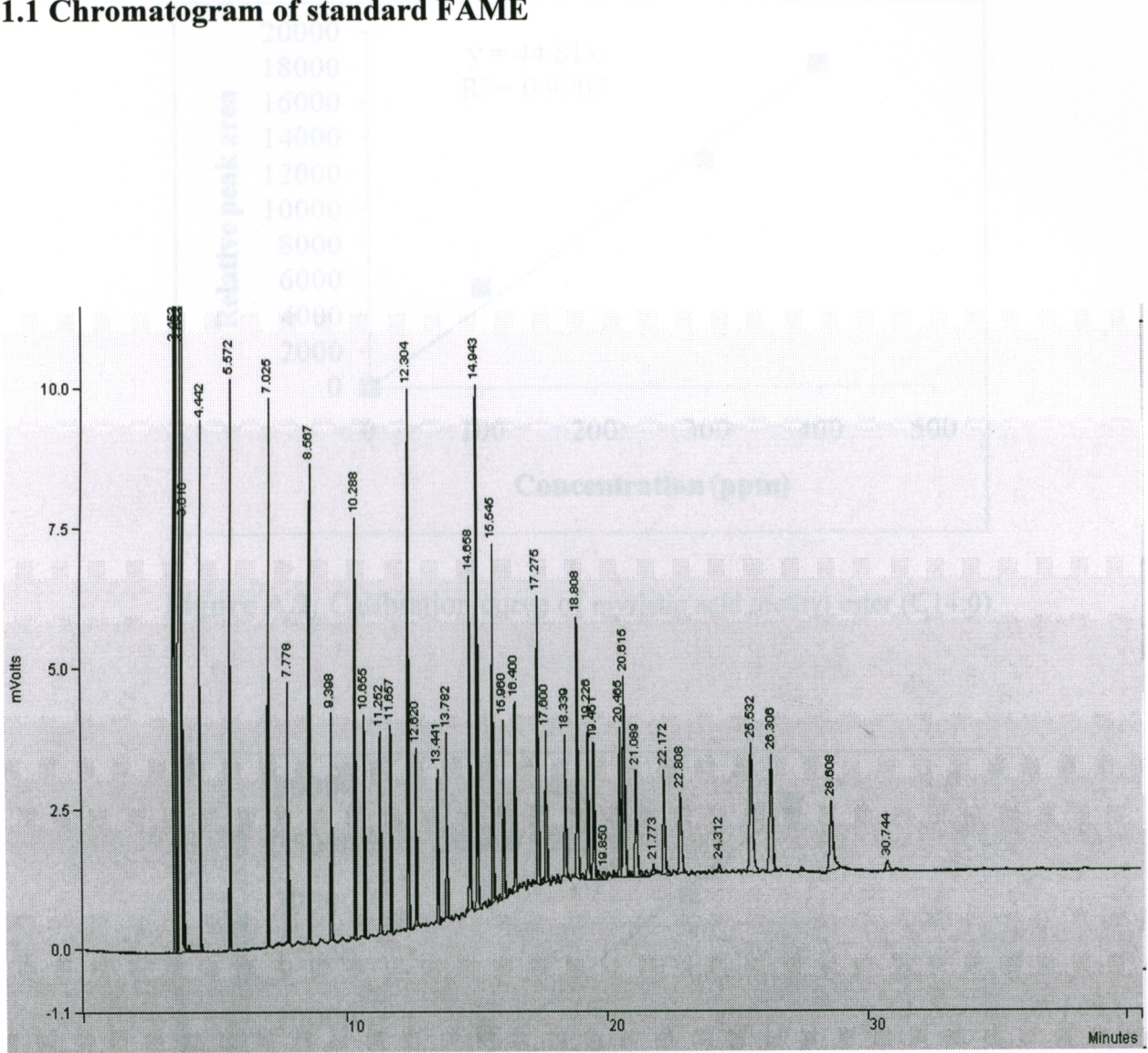


Figure A.1: A GC chromatogram of Supleco™ standard 37component FAME mix.

Figure A.3: Calibration curve of palmitic acid methyl ester (C16:0)

1.2 Calibration Curves of Important FAMES

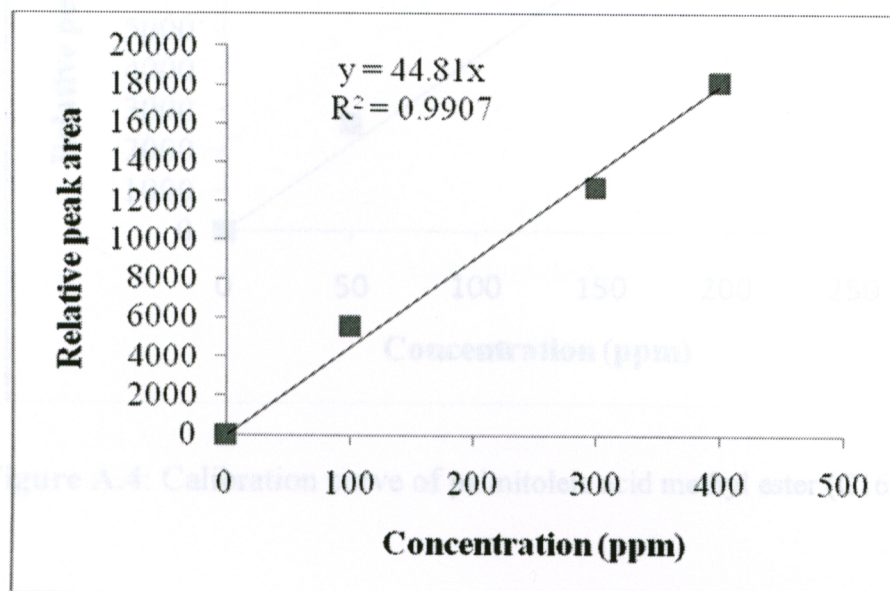


Figure A.2: Calibration curve of myristic acid methyl ester (C14:0).

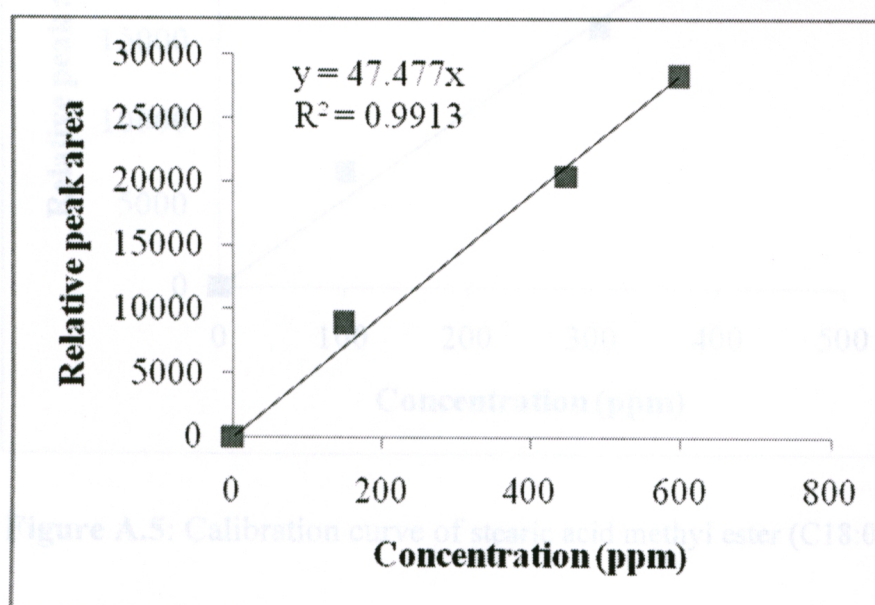


Figure A.3: Calibration curve of palmitic acid methyl ester (C16:0).

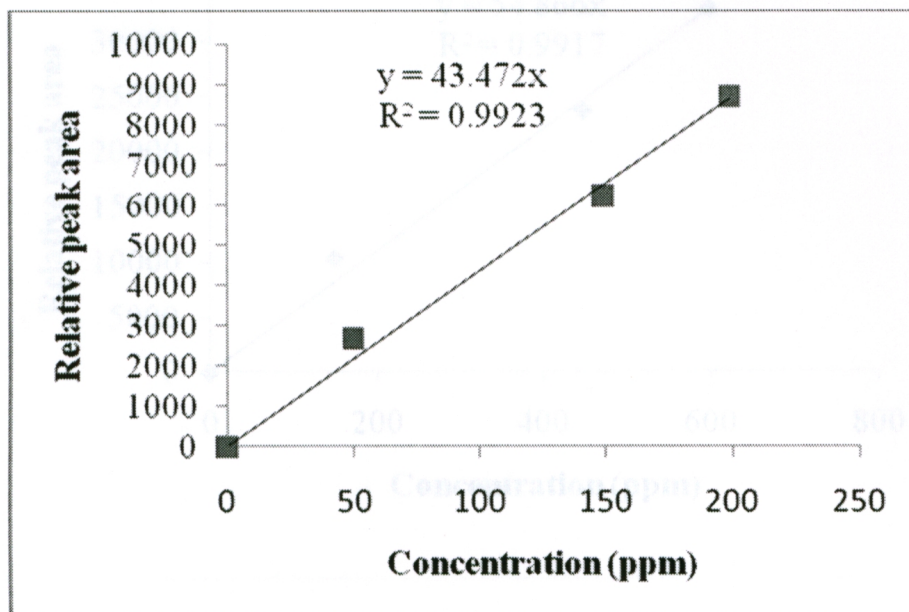


Figure A.4: Calibration curve of palmitoleic acid methyl ester (C16:1).

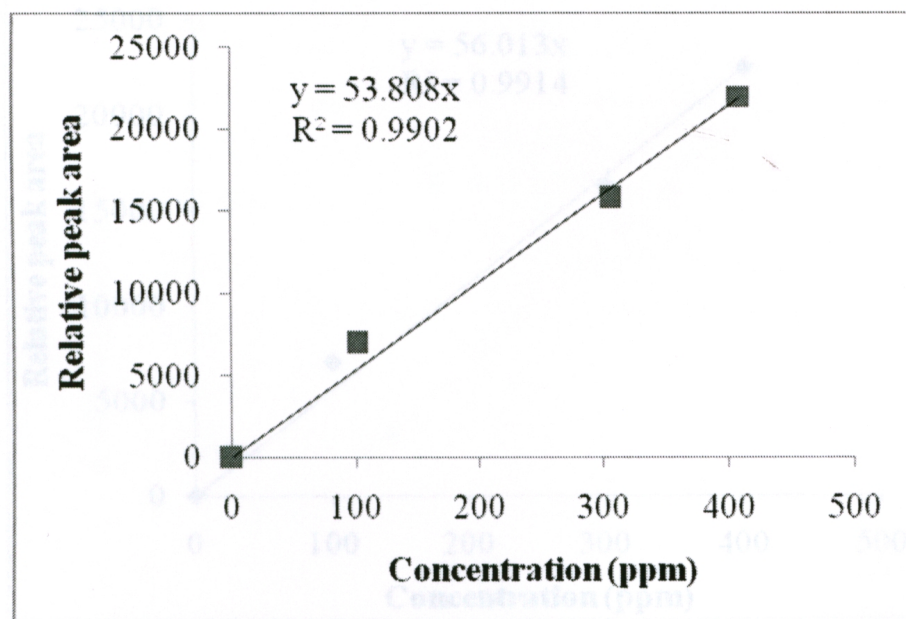


Figure A.5: Calibration curve of stearic acid methyl ester (C18:0).

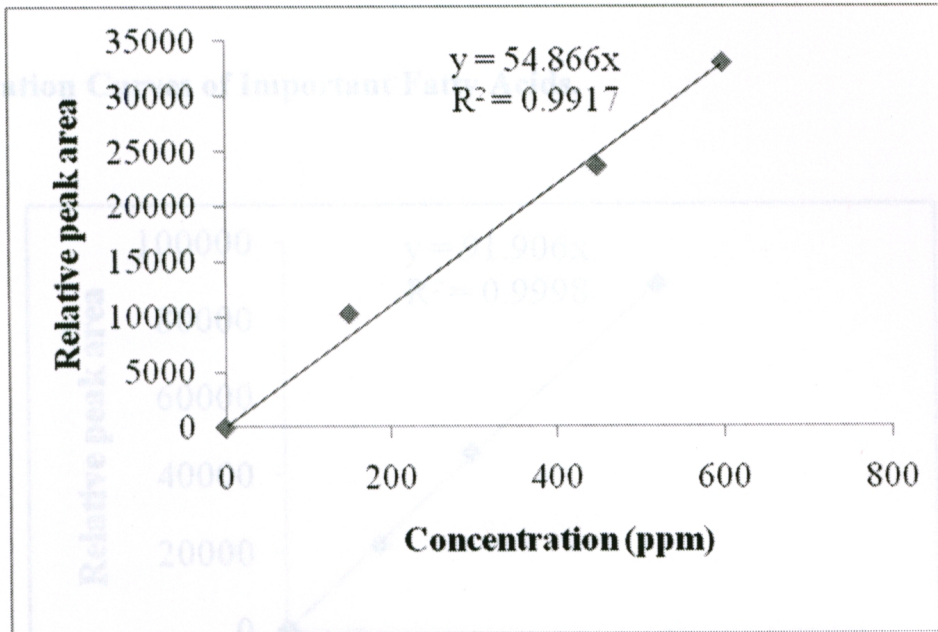


Figure A.6: Calibration curve of oleic acid methyl ester (C18:1).

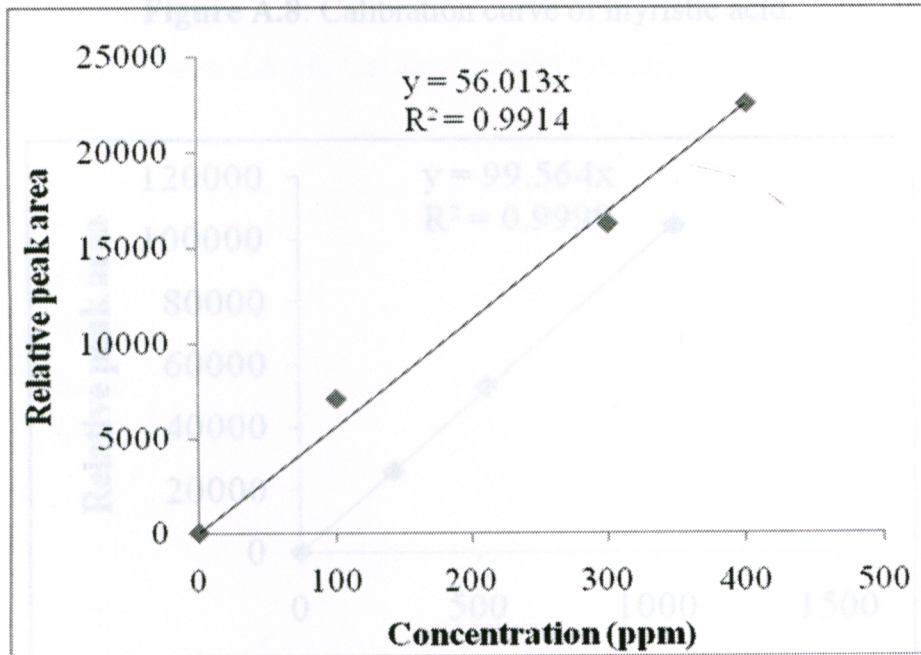


Figure A.7: Calibration curve of linoleic acid methyl ester (C18:2n6c).

Figure A.9: Calibration curve of palmitic acid.

1.3 Calibration Curves of Important Fatty Acids.

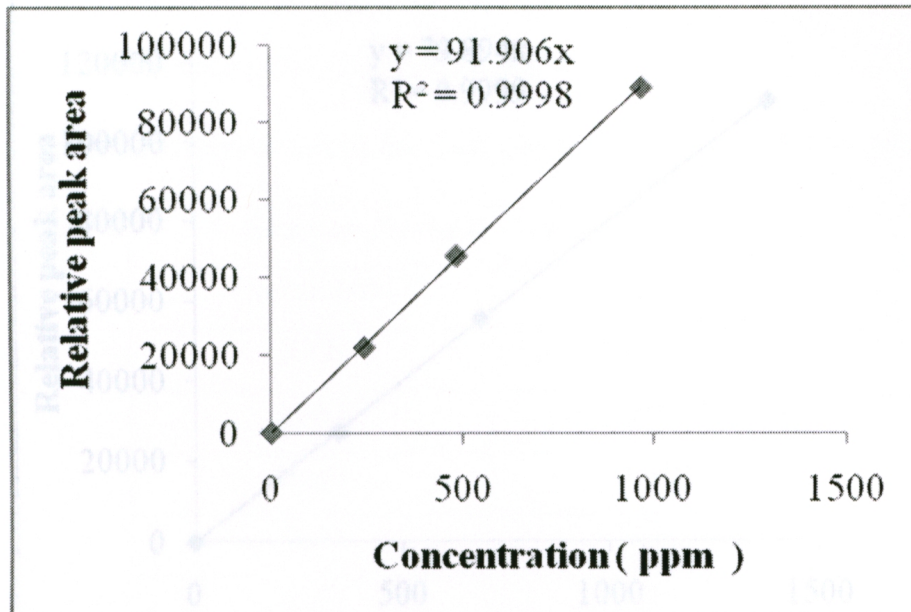


Figure A.8: Calibration curve of myristic acid.

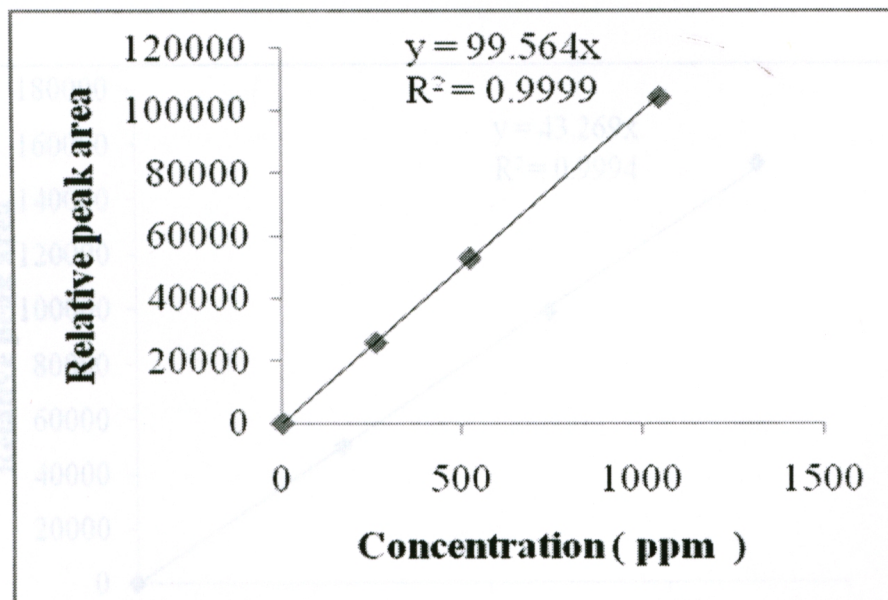


Figure A.9: Calibration curve of palmitic acid.

3.4 Chemical Composition, Particle size Distribution, XRD, SEM, and BET Surface Area of Natural Zeolite (Clinoptilolite).

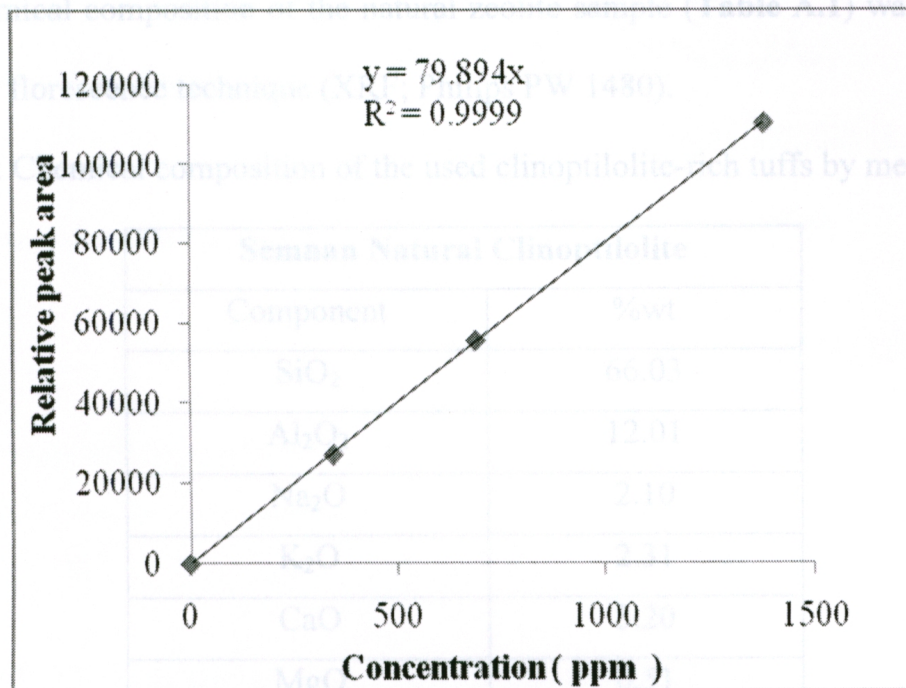


Figure A.10: Calibration curve of stearic acid.

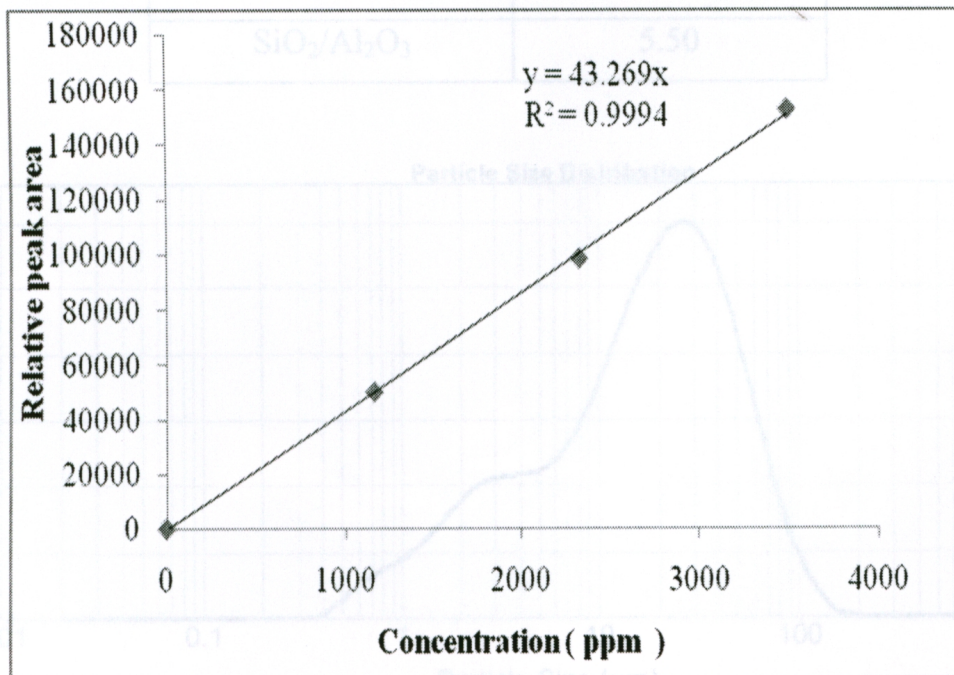


Figure A.11: Calibration curve of oleic acid.

1.4 Chemical Composition, Particle size Distribution, XRD, SEM, and BET Surface Area of Natural Zeolite (Clinoptilolite).

Chemical composition of the natural zeolite sample (**Table A.1**) was determined using X-ray fluorescence technique (XRF; Philips PW 1480).

Table A.1: Chemical composition of the used clinoptilolite-rich tuffs by means of XRF.

Semnan Natural Clinoptilolite	
Component	%wt
SiO ₂	66.03
Al ₂ O ₃	12.01
Na ₂ O	2.10
K ₂ O	2.31
CaO	3.20
MgO	0.51
Fe ₂ O ₃	1.25
TiO ₂	0.27
L.O.I	11.89
SiO ₂ /Al ₂ O ₃	5.50

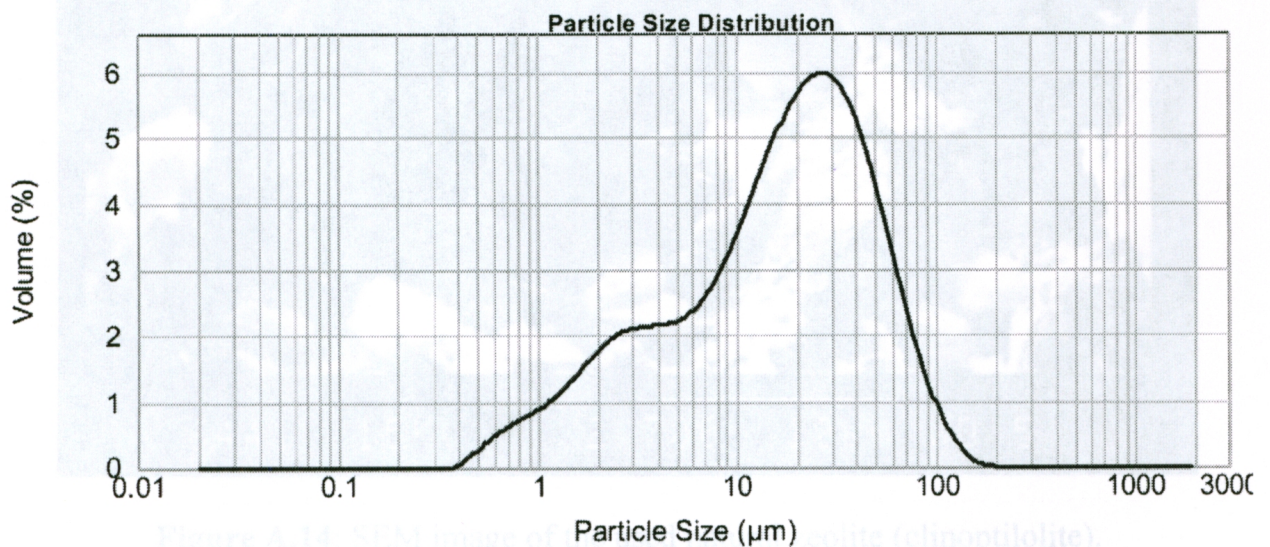


Figure A.12: Particle size distribution of Natural Zeolite (Clinoptilolite).

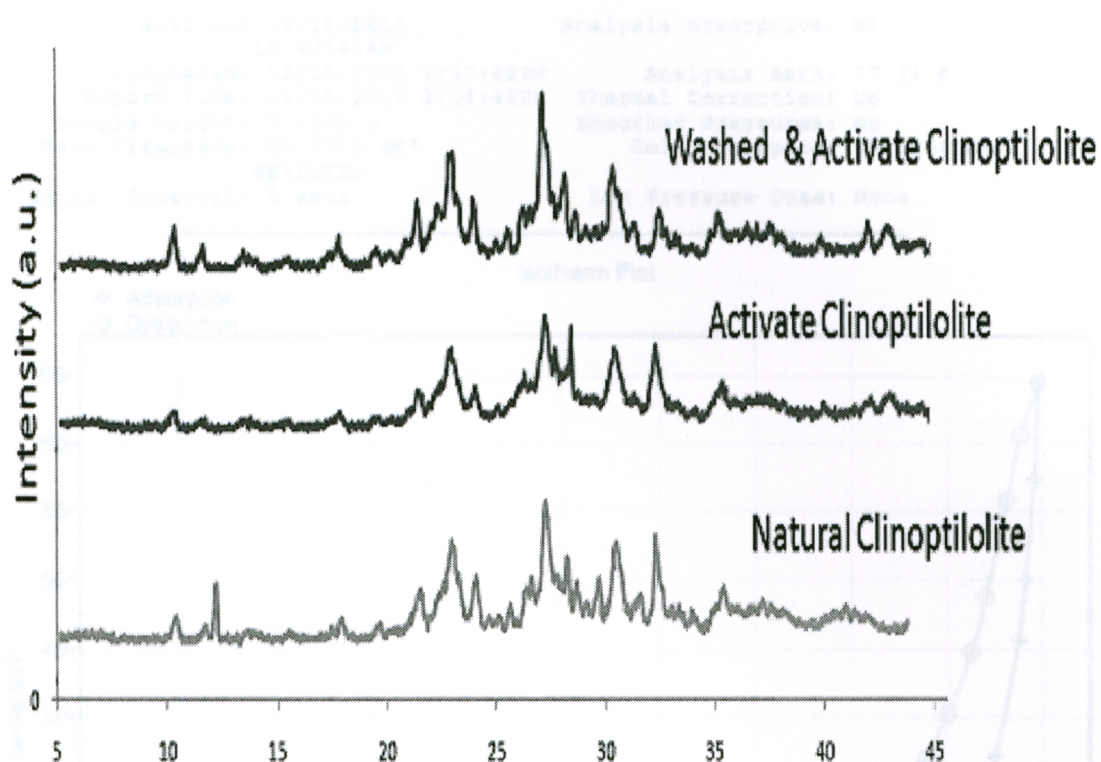


Figure A.13: XRD patterns of natural zeolite (clinoptilolite), natural zeolite activated at 300°C, and truly washed with deionized water and activated at 300°C.

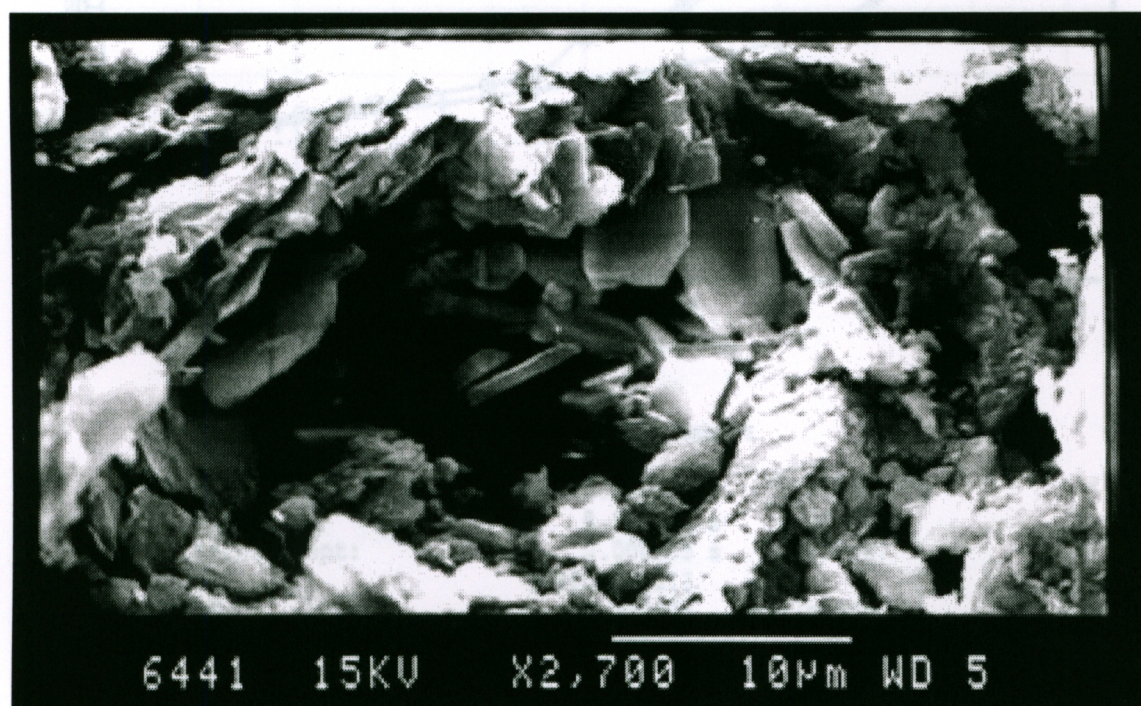
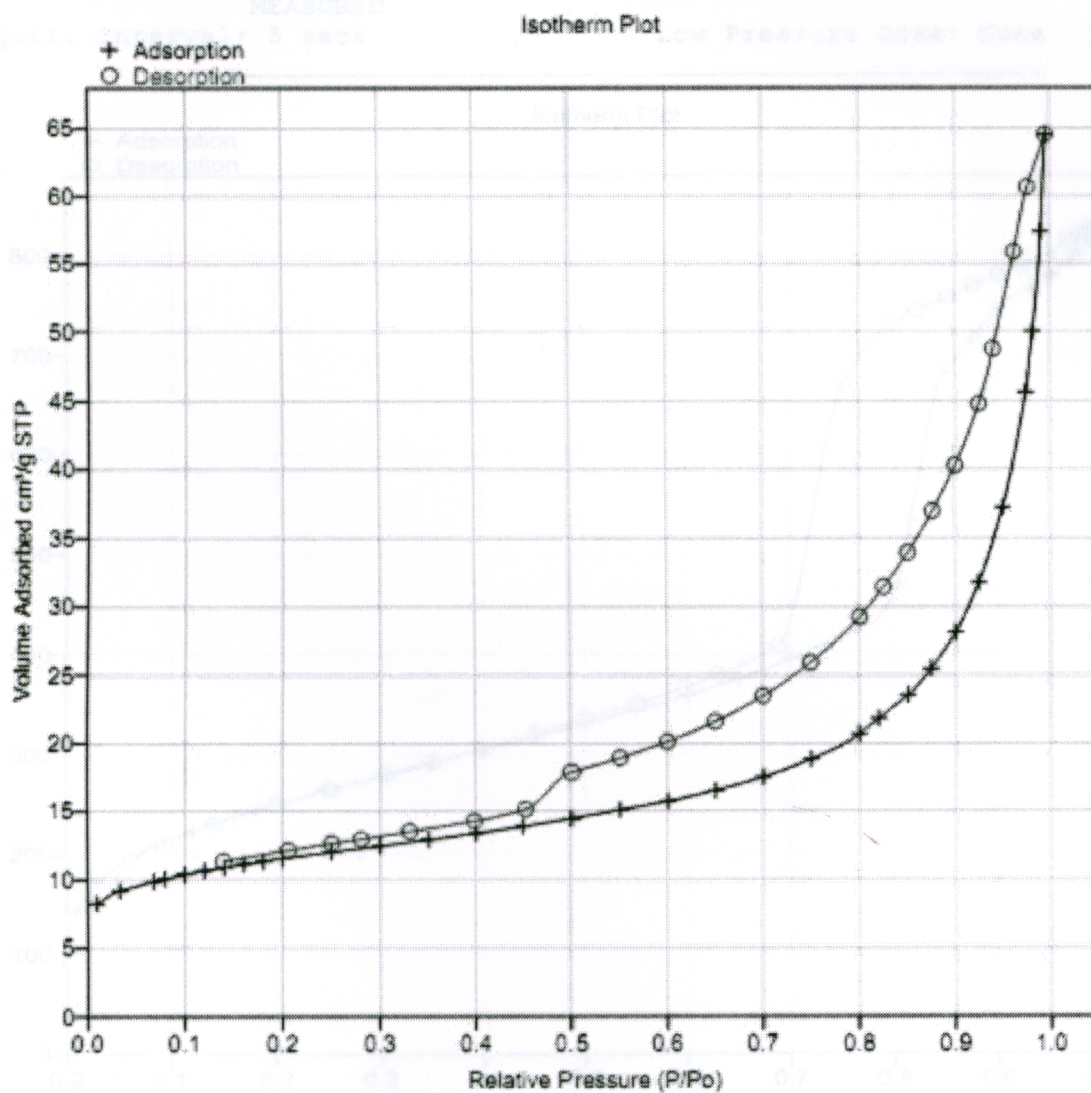


Figure A.14: SEM image of the used natural zeolite (clinoptilolite).

Started: 12/15/2010 10:43:41AM Analysis Adsorptive: N2
 Completed: 12/15/2010 1:47:48PM Analysis Bath: 77.35 K
 Report Time: 12/16/2010 3:06:49PM Thermal Correction: No
 Sample Weight: 0.1360 g Smoothed Pressures: No
 Warm FreeSpace: 26.0713 cm³ Cold FreeSpace: 88.6064 cm³
 MEASURED
 Equil. Interval: 5 secs Low Pressure Dose: None



BET Surface Area Report

BET Surface Area:	40.5861 ± 0.2963	m ² /g
Slope:	0.107254 ± 0.000776	
Y-Intercept:	0.000005 ± 0.000107	
C:	23382.703213	
VM:	9.323275	cm ³ /g STP
Correlation Coefficient:	9.998431e-01	
Molecular Cross-section:	0.1620	nm ²

Figure A.15: BET isothermal plot and surface area report of natural zeolite (clinoptilolite).

1.5 BET Isothermal Plot of SBA-15.

Started: 12/10/2010 9:46:05AM Analysis Adsorptive: N2
 Completed: 12/10/2010 7:17:43PM Analysis Bath: 77.35 K
 Report Time: 2/18/2011 3:03:07PM Thermal Correction: No
 Sample Weight: 0.1430 g Smoothed Pressures: No
 Warm Freespace: 27.9548 cm³ Cold Freespace: 87.7291 cm³
 MEASURED
 Equil. Interval: 5 secs Low Pressure Dose: None

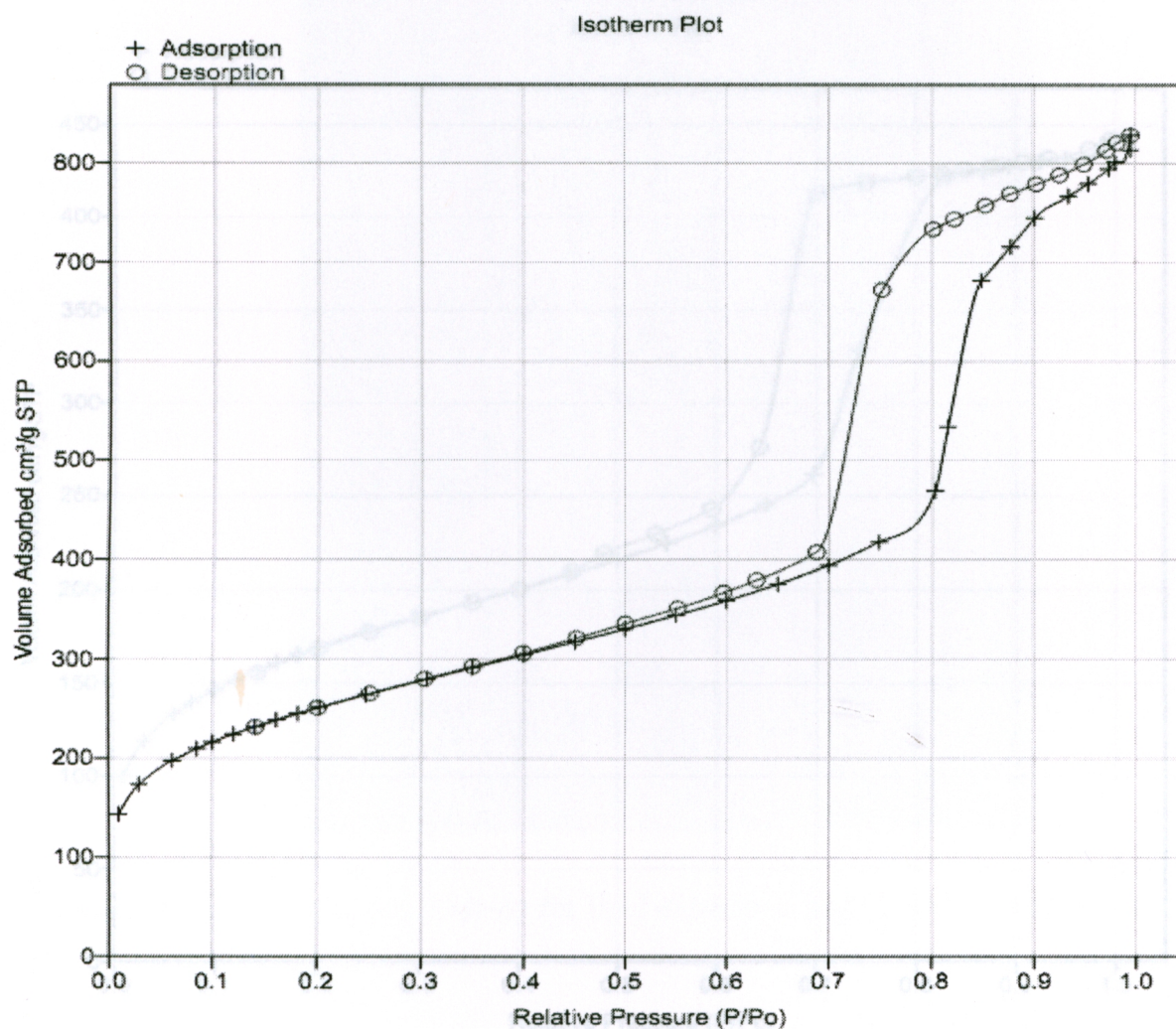


Figure A.16: BET isothermal plot and surface area report of SBA-15.

2. Appendix B: Photographs of Experimental Set-up

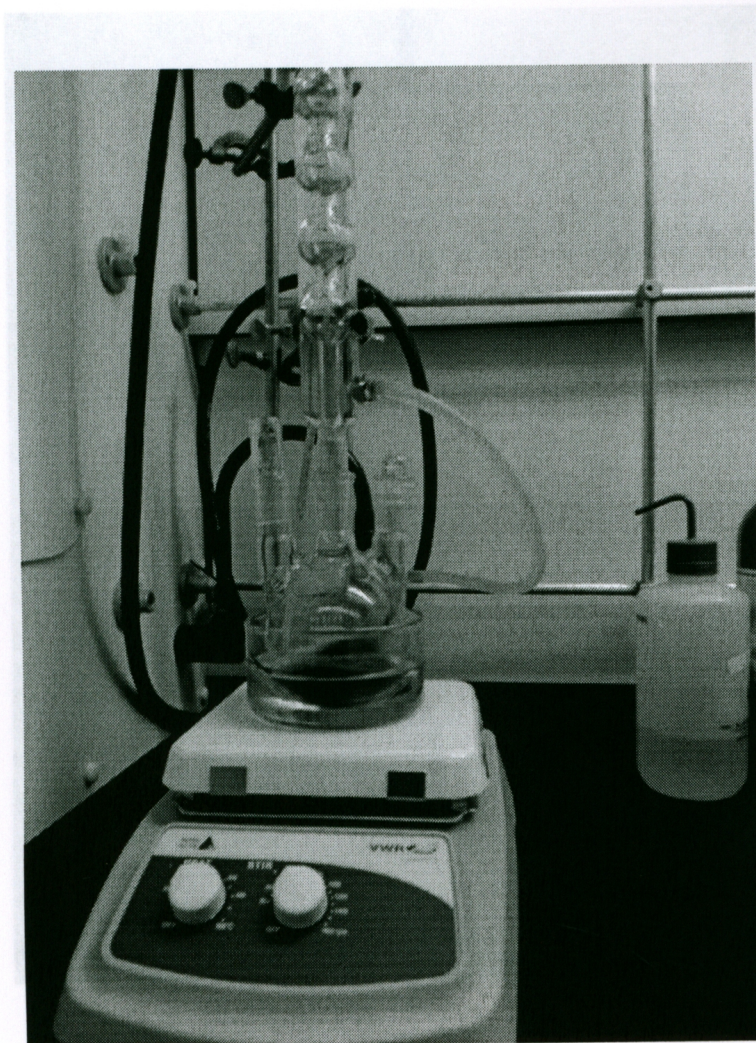


Figure B.2: Experimental set-up for biodiesel separation.

Figure B.1: Experimental set-up for lipid extraction and biodiesel production.

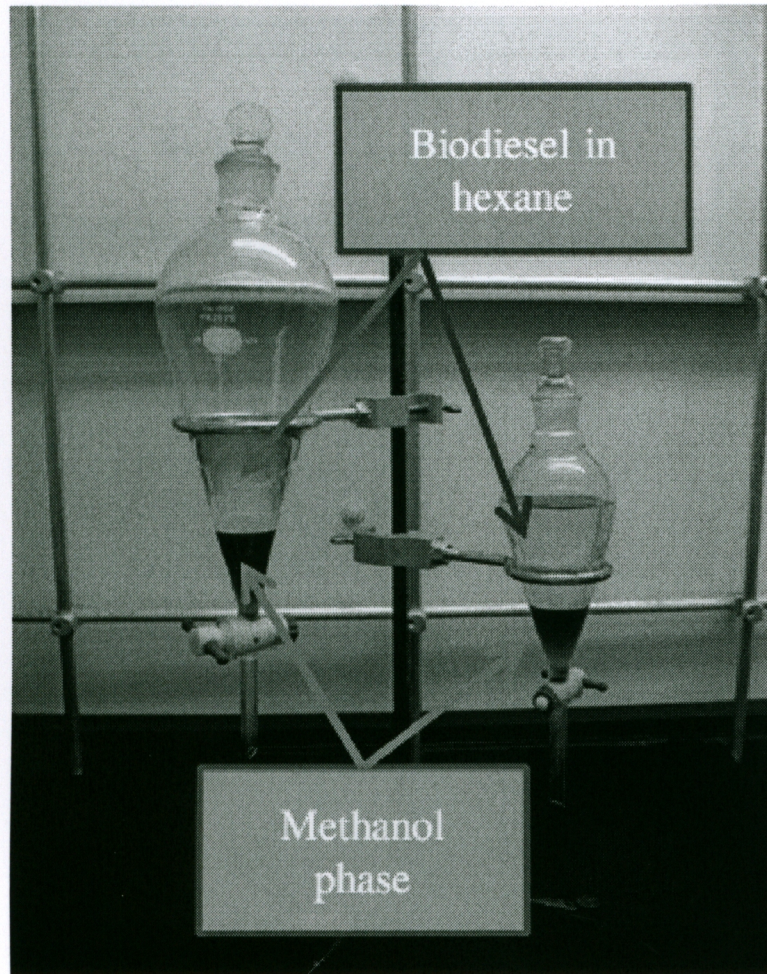


Figure B.2: Experimental set-up for biodiesel separation.

Figure B.3: (a) Biodiesel produced from an extracted lipid, and (b) Extracted lipid from wastewater sludge.

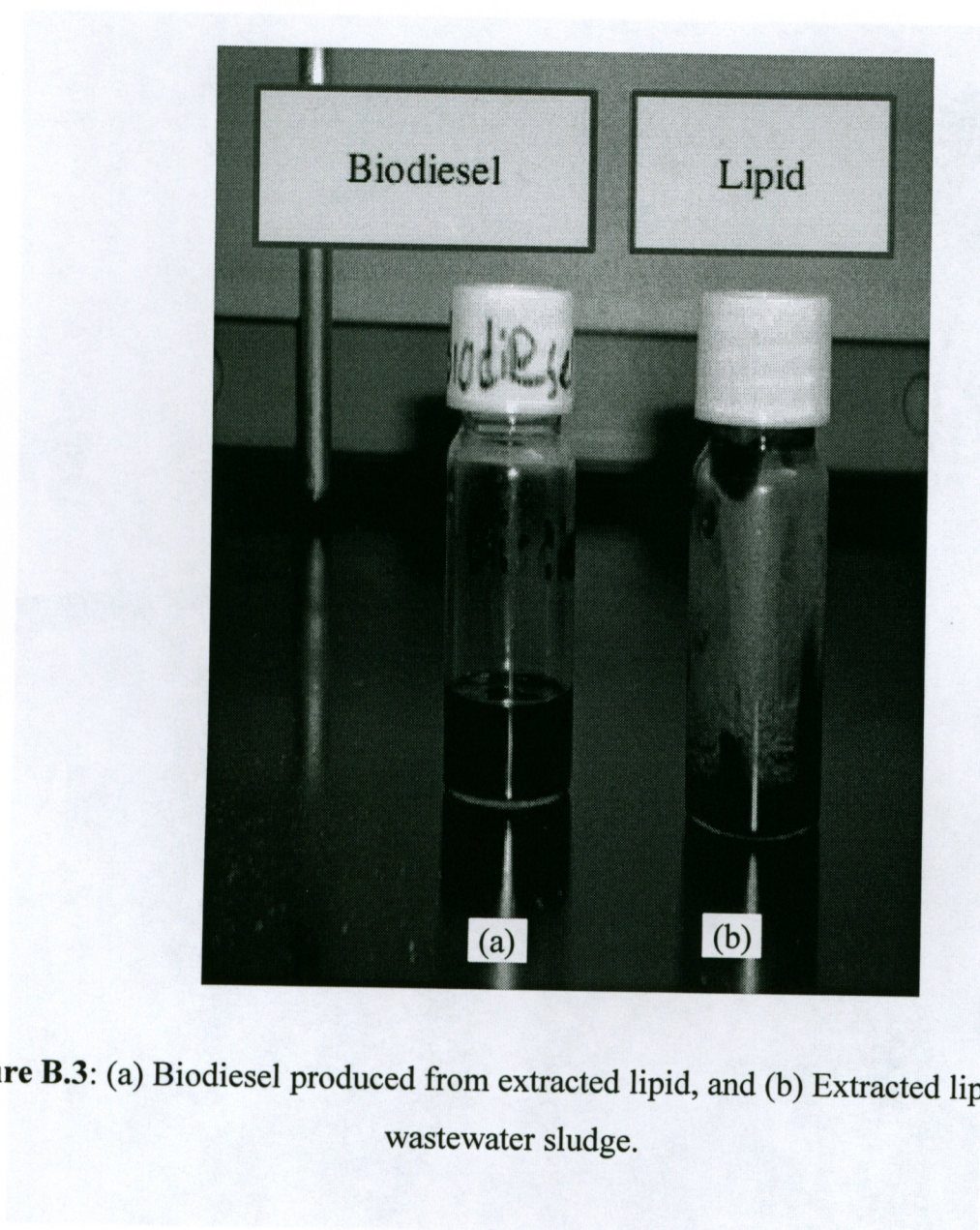


Figure B.3: (a) Biodiesel produced from extracted lipid, and (b) Extracted lipid from wastewater sludge.

Figure B.4: A micro-reactor (Associative Engineers, division of Snap-tite, Inc., USA) for the production of Biodiesel by using heterogeneous acid catalyst.

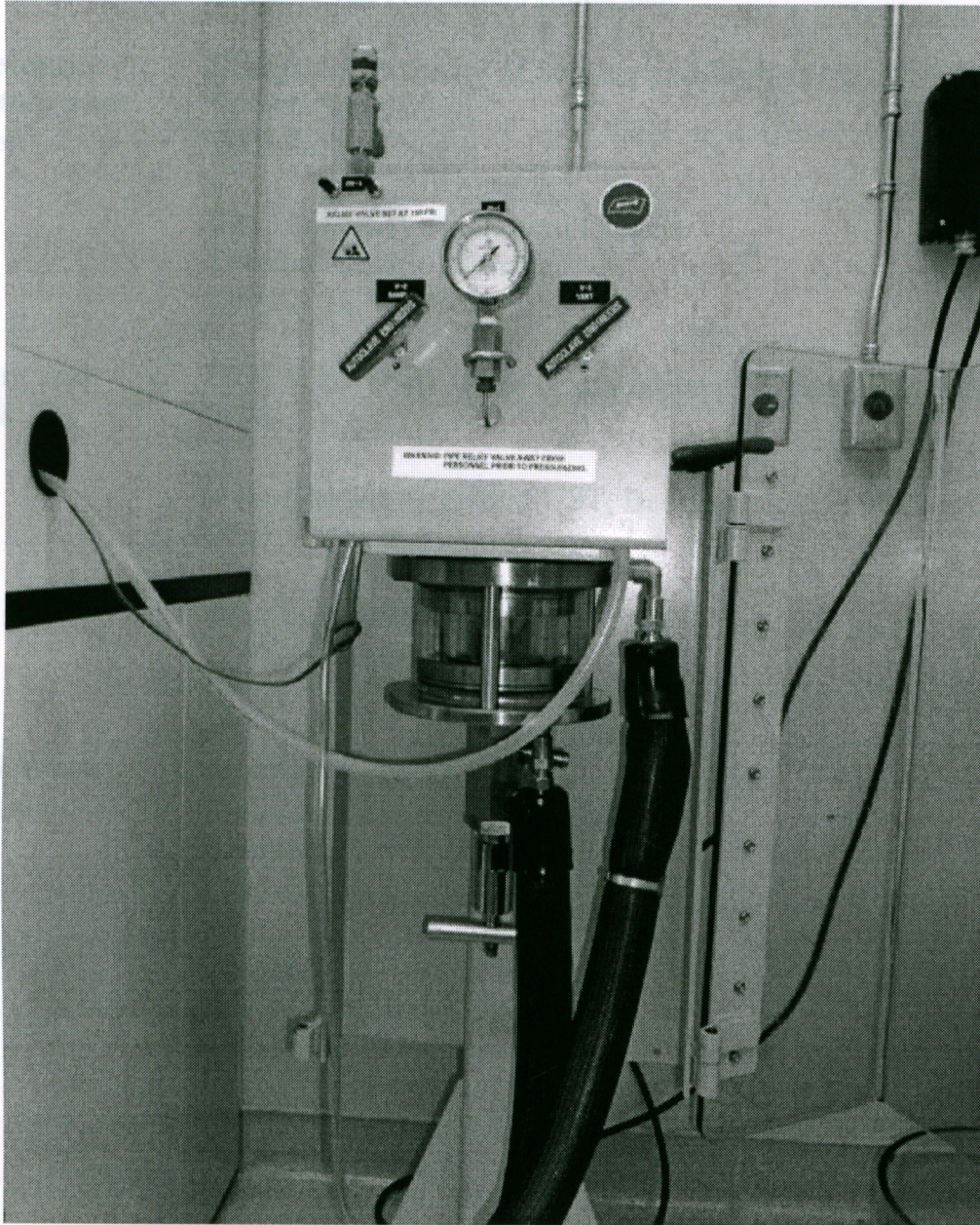


Figure B.4: A micro-reactor (Autoclave Engineers, division of Snap-tite, Inc., USA) for the production of biodiesel by using heterogeneous acid catalyst.

Shatiguse MN, Rahimi S. Lipid Extraction and Biodiesel Production from Municipal Sewage Sludges: A Review. *Renewable and Sustainable Energy Review* 2011; 15: 1067-1072